Optical discrimination of a phytoplankton species in natural mixed populations

Abstract

Developing optical detection techniques for discriminating particular phytoplankton species in mixed assemblages has long been a goal of aquatic scientists. Previously, a processing algorithm for phytoplankton absorption spectra was reported that suggested detection of the red tide dinoflagellate Gymnodinium breve was possible. The algorithm evaluated the fourth derivative of the particulate absorption spectrum of an unknown sample and compared it to a standard fourth derivative spectrum for G. breve using a similarity index. We report here the first-time application of this technique to the detection of G. breve in natural, mixed phytoplankton communities. Pigment and spectral absorption data were collected from natural blooms of G. breve in the eastern Gulf of Mexico. This dinoflagellate is the only species of phytoplankton in the Eastern Gulf of Mexico observed to contain the pigment gyroxanthin-diester, and it appears in constant proportion to cellular chlorophyll a in G. breve. The in vitro absorption spectrum of gyroxanthin-diester is nearly identical to other xanthophylls (including diadinoxanthin, lutein, and 19’-hexanoyloxyfucoxanthin) and is not singularly responsible for imparting a unique absorption signature. Quantifying gyroxanthin-diester and Chl a allowed us to estimate the fraction of the biomass in mixed populations associated with G. breve. Subsequent regression of the G. breve similarity indexes to the G. breve biomass fractions yielded a significant linear correlation. Finally, the liquid waveguide capillary cell appears to be a promising technology for automating this technique.

Harmful algal blooms pose a threat that requires efforts to reduce or eliminate their negative impacts and consequences (Boesch et al. 1996). In the Gulf of Mexico, toxic blooms of the dinoflagellate Gymnodinium breve Davis regularly lead to untimely restrictions on commercial and recreational shellfish harvesting and deleterious effects on tourism and public health (Steidinger et al. 1973). Mitigation of some harmful effects from these toxic blooms of G. breve can be achieved by early detection of this species in mixed phytoplankton communities. Currently, microscope examination of discrete water samples is the principal detection method for G. breve. Unfortunately, this method is slow, labor intensive, and intermittent. Significant benefits can be gained from an automated detection method, and optical methods hold promise for such applications (Cullen et al. 1997).

Over the past two decades, oceanographers have developed optical instrumentation that can collect data in a non-intrusive manner. Optical techniques are amenable to a variety of platforms (satellites, aircrafts, mooring, and profiling instrumentation), allowing researchers to design multiplatform sampling networks capable of collecting data over ecologically relevant scales (Smith et al. 1987; Dickey 1993). Many integrated observing systems currently are under development by the oceanographic community (Glenn et al. 1998). Although promising, optical approaches have been criticized because they provide only bulk composite signals for a given water mass, and the signatures for distinct phytoplankton species are difficult to discriminate (Garver et al. 1994).

Laboratory work suggests that partial discrimination of algal species from cellular absorption is possible. For example, Johnsen et al. (1994), using stepwise discriminant analyses 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 c₁ and/or c₂, chlorophyll c₃, and no accessory chlorophyll), differentiated toxic chlorophyll c₃-containing dinoflagellates and prymnesiophytes from taxa not having this pigment. However, problematic and toxic taxa could not be further separated from other chlorophyll
was collected off Sarasota Florida (denoted by a star). In the Gulf of Mexico (bold line). The cruise consisted of a spatially extensive sampling stations along the west Florida coast and continental shelf between August 1995 and August 1997 (Fig. 1).

Environments ranged from estuarine to offshore, oligotrophic waters. The samples were grouped into those collected in the Sarasota area over the entire 2-yr period and those collected during a cruise of the OSV Anderson covering shelf waters from Charlotte Harbor to Apalachicola in the last week of August 1997. Aliquots were filtered, under low vacuum (<10 cm Hg), through GFF (Whatman) glass-fiber filters to concentrate the particles for pigment and absorption determinations. No special effort was made to acquire a predetermined amount of particulate matter. These filters were processed, as described below, either immediately or after storage in liquid nitrogen. In addition, unfiltered water samples were used for particulate absorption determinations in a liquid waveguide capillary cell (LWCC, World Precision Instruments) during the cruise of the OSV Anderson.

Photosynthetic and photoprotective pigment components were determined using high-performance liquid chromatography (HPLC). Pigment analyses were conducted according to Wright et al. (1991) using a C-18 Hypersil reverse-phase column. Chromatographic peaks were detected by a photodiode array UV-VIS detector (SPD-M6A, Shimadzu) and identified by retention time and comparison of absorbance spectra with spectra of pigments from standard microalgal cultures.

Particulate absorption spectra were measured using the quantitative filter technique (QFT) (Kiefer and Soohoo 1982; Kishino et al. 1986; Mitchell and Kiefer 1988; Bricaud and Stramski 1990). Sample and reference filters were placed directly in front of the detector windows of a scanning dual-beam spectrophotometer (DMS80, Varian) to minimize scattering loss. Optical density spectra were acquired by a desktop computer interfaced to the analog output of the spectrophotometer. These spectra were corrected for path-
length amplification (Mitchell 1990) and then normalized to the mean value between 400 and 700 nm (Roesler et al. 1989).

During August 1997, on the OSV Anderson cruise, absorption spectra also were measured using a 0.5-m path-length LWCC coupled to a fiber optic spectrometer (SD2000, Ocean Optics) and a fiber optic incandescent light source (F-O-Lite, World Precision Instruments). The spectrometer was interfaced to a notebook computer through a PCMCIA A/D converter (DAQCard-700, National Instruments) and controlled through software provided by the spectrometer manufacturer (OOLIBase, Ocean Optics). The resulting absorption spectra were also normalized to the mean absorption between 400 and 700 nm.

Absorption spectra were compared using a similarity index (from Shimazu Scientific Instruments) described previously by Millie et al. (1997). Briefly, fourth derivative spectra (Butler and Hopkins 1970) initially were computed for the normalized absorption spectra described above. This yielded spectra detailing the wavelength position and magnitude of curvature in the parent absorption spectra (Millie et al. 1995, 1997). The spectral similarity index was determined by computing the angle between the vectors comprising the fourth derivative spectra of a standard G. breve sample (culture or monospecific bloom sample) and the unknown natural, mixed-population sample as

$$SI = 1 - \frac{2 \times \arccos \left( \frac{A_{std} A_{unk}}{|A_{std}| \times |A_{unk}|} \right)}{\pi}$$

where $A_{std}$ and $A_{unk}$ are the normalized particulate absorption spectra of the standard and unknown samples respectively. The arc-cosine transformation and division by $\pi/2$ converts from a nonlinear result (cosine of the angle) to a linear result between zero and one. All empirical relationships presented are model II regressions (cf. Laws 1997).

Fig. 3. Ratio of gyroxanthin-diester to chlorophyll $a$ in whole water. At low G. breve cell counts other (non-gyroxanthin-diester) species contributed chlorophyll $a$, yielding lower ratios. However, at high G. breve cell counts the ratio represents the cellular ratio for near monospecific G. breve blooms. The asymptote of the fitted exponential curve matches the ratio observed in G. breve cultures and previous monospecific blooms.

A linear relationship existed ($r^2 = 0.99$, $n = 36$, $r$ significant at $p < 0.01$) between whole water concentrations of the carotenoid, gyroxanthin-diester and G. breve cell number (Fig. 2). A similar observation was made for cultures and natural assemblages by Millie et al. (1997). A constant cellular ratio has been previously noted between gyroxanthin-diester and chlorophyll $a$ (Chl $a$) in both natural blooms (when the assemblage was nearly monospecific) and cultures of G. breve (Millie et al. 1997). During this study a positive linear relationship was observed for G. breve cell number and low values of the ratio gyroxanthin-diester to Chl $a$ (Fig. 3). This largely reflected the variable amount of Chl $a$ associated with G. breve and other algal species present within these mixed natural assemblages. At high gyroxanthin-diester to Chl $a$ ratios, where G. breve was the dominant species present, the ratio showed little variability, consistent with laboratory studies. The high ratio values observed in the field populations (0.05) were very similar to laboratory cultures of G. breve (0.05–0.06, Millie et al. 1995). Given this the carotenoid pigment, gyroxanthin-diester appears to be a reliable indicator of the presence of the red tide organism G. breve in the eastern Gulf of Mexico. The constant ratio of gyroxanthin-diester to Chl $a$ in G. breve also is a fortuitous feature that allows for the estimation of G. breve biomass in mixed populations. Estimates of the fraction of Chl $a$ biomass contributed by G. breve in this study were based on this constant ratio between gyroxanthin-diester and Chl $a$ applied to pigment complements from the natural phytoplankton communities. Although gyroxanthin-diester was a reliable indicator for G. breve, its analytical detection by
The distinct absorption properties of locations.

Absorption spectra were also measured using the LWCC at 50 locations during the August 1997 cruise in the Eastern Gulf of Mexico. Those locations had G. breve cell counts that ranged from zero cells L\(^{-1}\) to 5 \times 10^6 cells L\(^{-1}\) and Chl \(a\) concentrations that ranged from 0.4 \(\mu\)g L\(^{-1}\) to 4.4 \(\mu\)g L\(^{-1}\). The fraction of the Chl \(a\) biomass that was attributable to G. breve ranged from zero to 0.91. The similarity index was linearly related to the fraction of Chl \(a\) biomass contributed by G. breve (\(r^2 = 0.38, n = 41, r\) significant at \(p < 0.01\)) (Fig. 5).

The LWCC was successful in discriminating G. breve from other phytoplankton in natural assemblages. Discrimination results derived from the LWCC were less robust than those derived from the QFT, which was not unexpected since it was the first test of a prototype configuration. During post-processing it was discovered that the manufacturer’s software was set to truncate absorbance values to just three decimal places. This approach substantially degraded the resolution of the absorbance data collected during the cruise, but notably did not nullify their application to discriminating G. breve. Additionally, the tungsten-halogen light source was deficient in violet and blue light, which resulted in loss of absorbance signal in a critical portion of the spectrum. Further effort on refining this instrument will yield a very useful field survey system capable of spectral absorption measurements even for oligotrophic waters. The great advantage of the LWCC is that the long pathlength of the system (0.25 m and up) results in sufficient sensitivity to measure absorption spectra even under ambient conditions in the oligotrophic waters of the Gulf of Mexico.

In conclusion, considering the wide range of bloom conditions sampled over 2 yr by a number of personnel using several different techniques, the significant correlation between the fraction of chlorophyll biomass contributed by G. breve and the absorption-based similarity index suggests this is a robust approach for discriminating this toxic red tide species. Also, the LWCC is a promising new technology that lends itself to conducting this species-discrimination method in an automated unattended mode that could be used to provide early warning to the presence of G. breve.

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References

blooms in coastal waters: Options for prevention, control and mitigation. NOAA Coastal Ocean Program Decision Analysis Series No. 10. NOAA Coastal Ocean Office.

Received: 29 March 1999
Accepted: 18 November 1999
Amended: 29 November 1999

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