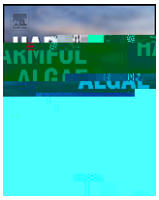

Progress in developing a new detection method for the harmful algal bloom species, Karenia brevis, through multiwavelength spectroscopy

Adam H. Spear*, Kendra Daly, Debra Huffman, Luis Garcia-Rubio

University of South Florida, College of Marine Science, 140 7th Avenue South, St. Petersburg, FL 33701, United States

1. Introduction

Karenia brevis, a species of toxic dinoflagellate, is known to cause harmful algal blooms (HABs) annually during the late summer and fall in the Gulf of Mexico

absorption thickness, and inferred index of refraction. The absorption thickness and refractive index were approximated from bulk properties of whole cells. Spectral properties were examined throughout the visible spectrum (412–730 nm) with a spectral resolution of 3.3 nm, and in some cases nine total wavelengths. Millie et al. (1997) and Kirkpatrick et al. (2000) developed a method which correlates the fraction of chlorophyll biomass contributed by K. brevis and the fourth derivative absorption-based similarity index. This absorption-only analysis of the visible spectrum allowed for the quantification of gyroxanthin-diester, a rare accessory pigment found in only a few dinoflagellate species, which was established as a consistent predictor when correlated with chlorophyll biomass. While previous research has shown significant progress in characterizing the optical properties of K. brevis, there is potentially a considerable amount of spectral information not yet reported to date. Several recent studies have proved to be extremely accurate in characterizing spectral properties of a complex microorganism by dividing the cell into multiple components, as well as combining scattering and absorption properties (Alupoaei,

(Van Der Hulst, 1957; Kerker, 1969):

$$\tau(\lambda_0) \quad \mathrm{Np}\, l\left(\frac{\Pi}{4}\right) x \int_0^{\infty} Q_{ext}(m(\lambda_0), D) D^2\, f(D)\, dD \tag{1}$$

where D is the effective particle diameter, $Q_{ext}(m(\lambda_0), D)$ corresponds to the Mie extinction coefficient, l is the path length, and Np is the number of particles per unit volume. The Mie extinction coefficient is a function of the optical properties of the particles and the suspending medium through the complex refractive index ($m(\lambda_0)$) given in Eq. (2):

$$m(\lambda_0) \quad \frac{n(\lambda_0) - i\kappa(\lambda_0)}{n_0(\lambda_0)} \tag{2}$$

where $n(\lambda_0)$ and $n_0(\lambda_0)$ correspond to the refractive index of the particles and the suspending medium, respectively. The absorption coefficient of the suspended particles is represented by $\kappa(\lambda_0)$. Eq. (1) can be written in matrix form by discretizing the integral with an appropriate quadrature approximation (Elicabe and Garcia-Rubio, 1988, 1990) given by Eq. (3) as

$$\underline{\tau} \quad A\, \underline{f} - \underline{\varepsilon} \tag{3}$$

where ε represents a composite of experimental errors, which are errors due to the model approximations and errors introduced by the discretization procedure (

component, specifically the chromophoric groups, have a sig-

chromophores. Whenever scattering was included in the description of spectral features, only the bulk properties of the cell were considered (Millie et al., 1997; Kirkpatrick et al., 2000; Mahoney, 2001). The approach of splitting the cell into multiple components allows for the spectral properties of a complex organism to be characterized at a much more detailed and therefore sensitive level. Utilizing the ultraviolet portion of the spectrum allows for an increased degree of sensitivity with the ability to characterize additional spectral features such as cell size, and nucleotide and protein concentration. The proposed model offers the possibility of exploring the sensitivity of the measurement to as many cellular components as determined to be necessary by the researcher. As a result, the potential for a high level of characterization of the cell increases the possibility of separating a similar species of the same genus. This is an important attribute due to the findings of Heil et al. (in press) which documented at least four other known Karenia species besides K. brevis and Karenia mikimotoi in a K. brevis dominated bloom in the Gulf of Mexico. All six species were found to co-occur during a 2005 bloom in the Gulf of Mexico, with K. brevis dominating total Karenia abundance from bloom initiation to termination, on average comprising over 81% of Karenia cells in each sample. The second most dominant species, K. mikimotoi, still reached significant concentration levels of 10^7

4. Discussion

The results described in the previous section demonstrate that despite the apparent difference between measured and predicted spectra, a considerable amount of progress has been made on the understanding of the spectral features of K. brevis. It is now possible to predict with some degree of accuracy what parameter, based on both absorption and scattering components, is responsible for each feature in the observed in situ spectra. Representation of pigment composition within the chloroplast, combined with physical features, show significant influence on the total optical density of the whole cell. Influence from the nucleotide content from the macrostructure and nucleus, combined with physical features, are also apparent in the total optical density.

This method which utilizes every spectral feature given by the interaction of light with the cellular components and their contribution to the total spectrum of K. brevis is reported for the first time. Previous optical studies of K. brevis were limited as a result of disregarding the ultraviolet wavelength portion of the spectrum and the use of only the absorption properties of the main