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Use of Discriminant and Fourth-Derivative Analyses With High-resolution Absorption Spectra for Phytoplankton Research: Limitations at Varied Signal-to-Noise Ratio and Spectral Resolution

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Future management efforts aimed at inhibiting harmful algal blooms will require extensive temporal and spatial monitoring of phytoplankton community composition. A cost-effective approach to delineating phytoplankton community composition may be through analysis of absorption spectra, measured in situ with instruments deployed on moorings or by remote sensing. Classification techniques relying on absorption spectra include discriminant and fourth-derivative analysis. We investigated how well these techniques performed theoretically at varied signal-to-noise ratio and spectral resolution representative of a new absorption and attenuation instrument called HiStar. Our findings suggest that discriminant analysis of absorption spectra is a highly useful technique for categorizing green algae, cyanobacteria, noxious bloom-forming dinoflagellates, diatoms, and other chrysophytes. For the purposes of discriminating dinoflagellates from the other algae groups, discriminant analysis worked well with either low- or high-resolution spectral data. The discriminant analysis technique was able to delineate a noxious bloom-forming dinoflagellate species, *Prorocentrum minimum*, at signal-to-noise ratios as low as ~17. The current noise level in the HiStar, however, is ~28-fold too high to allow correct classification of this dinoflagellate at concentrations where shellfisheries are closed. Improvements to the discriminant analysis (e.g., inclusion of scatter properties) or to the HiStar must be accomplished before this technique becomes useful for harmful algal bloom management applications. Fourth-derivative analysis of absorption spectra, also a useful classification technique and a possible approach to assess physiological state of some algae, required at least 4 nm spectral resolution for assessment of chlorophylls *a* and *b*. The spectral resolution of HiStar (3.3 nm) meets this requirement.

Harmful algal blooms (HABs) are detrimental to commercially important fisheries and disrupting to local economies. They are also a hazard to human health, even causing death in some cases (Paerl, 1988; Shumway, 1990; Villac et al., 1993; Anderson and Garrison, 1997). Furthermore, the frequency and magnitude of HABs may be on the rise (Smayda, 1990). Over recent years, HABs in the Gulf of Mexico have been documented frequently (Buskey et al., 1997; Tester and Steidinger, 1997). Consequences of these blooms include widespread fish kills, degradation of estuarine habitat, marine mammal mortalities, and human ailments (Buskey and Stockwell, 1993; Beauchamp et al., 1996; Anderson, 1999).

Current objectives of broad agency initiatives addressing HABs include understanding bloom triggering mechanisms, biology and ecology of noxious phytoplankton species, and predictability of bloom behavior (see ECOHAB, 1994). Increased knowledge from these initiatives will better shape our understanding of HABs in the context of existing conceptual models (Tilman, 1977; Sommer et al., 1986; Odum et al., 1995). In turn, these models will become better tools for design of HAB management schemes (Roelke, 1997; Roelke et al., 1997, 1999) and for development of forecasting mathematical models as called for in recent broad agency announcements (i.e., HyCODE and Harbor Processes by the Office of Naval Research).

There is a strong need for widespread monitoring of phytoplankton communities to protect current fisheries and consumers and to support future HAB management efforts. Current techniques employed to delineate phytoplankton community composition include microscopy (see Hallegraeff et al., 1995), high-performance liquid chromatography (Mantoura and Llewellyn, 1983; Millie et al., 1993), and biomolecular techniques (Scholin et al., 1994). These methods are appealing because they can be species specific. Their cost in equipment and required expertise, however, prohibits them from being used extensively. In addition, these methods require field collec-

tion of discrete water samples. As with any field exercise, trade-offs exist between the spatial resolution of collected data and the total sampling area, as well as between the temporal resolution of collected data and the sampling duration.

Another approach to delineating phytoplankton community composition unbound by the confines of a field exercise may be through decomposition and analysis of the inherent optical properties (IOPs) of a water body, i.e., the absorption and scatter properties. For example, the use of absorption spectra from a diverse range of phytoplankton taxa showed that noxious bloom-forming dinoflagellate species could be delineated from other algae through discriminant analysis (Johnsen et al., 1994; Millie et al., 1997). In addition, fourth-derivative analysis of absorption spectra, often used to detect presence of chlorophylls and phycobilins (Bidigare et al., 1989), was used to determine previous light history of an algal culture with reference to its physiological state (Millie et al., 1995a). Similarly, scattering properties relative to those of absorption have also been used to determine the physiological state of phytoplankton (Maillet et al., 1998). These applications of IOPs to phytoplankton research have been demonstrated only under laboratory settings; they have yet to be performed in natural environments.

With the development of new technologies, optical approaches to HAB issues in natural environments may be feasible. For example, a new instrument (HiStar) capable of long-term deployment can measure total hyperspectral absorption and attenuation properties of a water body in situ, from which scatter properties can be estimated (Roelke et al., 1998). Similarly, new airborne (AVIRIS, CASI) and satellite (COIS) sensors are capable of gathering hyperspectral imagery from which total absorption and scatter properties can be derived (Carder et al., 1993; Jupp et al., 1994; Davis and Carder, 1998). Direct measurement of IOPs from moorings and derived estimations of IOPs from aircraft and satellite data can be achieved over much broader temporal and spatial scales at much less cost than traditional field sampling (Yentsch, 1989; Millie et al., 1995b). The absorption and scatter properties of pigment-containing constituents can then be unmixed from the bulk IOPs, provided the contribution of colored dissolved organic matter, i.e., gelbstoff, and nonphotosynthetic particles, i.e., tripton, are understood.

Delineation of phytoplankton community composition through decomposition and analysis of IOPs is taxonomically less detailed than the methodologies mentioned previously. This approach, however, offers insight to the physiological state of some algae. Regarding monitoring of some HABs, species-specific identification may not be necessary. As mentioned previously, noxious bloom-forming dinoflagellates as a group can be discriminated from other algae (Johnsen et al., 1994; Millie et al., 1997). Concerning model predictability, knowledge of an alga's physiological state is crucial to model results, especially under conditions of disequilibrium (Grover, 1992; Roelke et al., 1999). For these reasons, use of IOPs for rapid detection of some HABs and initialization of forecasting models may be the best approach for large-scale monitoring and management efforts. A combination of discriminant and fourth-derivative analysis on absorption spectra, coupled to analysis of scatter and absorption ratios, may be the most useful approach.

The mentioned phytoplankton research with discriminant, fourth-derivative, and ratio analysis of IOPs was performed on data collected in the laboratory with benchtop spectrophotometers where the signal-to-noise ratio (SNR) and spectral resolution are not typically a problem. The present research addresses how well these optical techniques perform, in theory, at SNR and spectral resolution representative of HiStar when deployed into a natural environment. Specifically, this paper investigates how well discriminant analysis of absorption spectra correctly classifies a noxious bloom-forming dinoflagellate, *Prorocentrum minimum,* under varied SNRs and how well fourth-derivative analysis of absorption spectra resolves chlorophylls *a* and *b* under varied spectral resolutions. We do not investigate the scatter and absorption ratio analysis in this manuscript because we are unaware of available data detailing scatter properties over a wide range of physiological states of specific algae.

For the purposes of this manuscript, we focus on the in situ measurement of absorption instead of the airborne estimates. In our opinion, at present, in situ measurement of absorption is a more practical approach to delineating phytoplankton community composition. Our opinion is based on the following reasons. First, data collected from airborne sensors must remove the signal obtained from the atmosphere, a large portion of the total signal, and then, through a mathematical inversion, estimate the absorption from the irradiance re-

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flectance. Both calculations add uncertainty to the determination of absorption. Second, when deploying in situ sensors, the contribution of gelbstoff, a large part of the total in situ absorption signal in coastal waters, can be measured directly by deploying two instruments, one equipped with a filter covering the water inflow. An algorithm must then be applied to unmix the tripton signal from the pigment signal (Roesler et al., 1989).

Methods

Access to a phytoplankton culture facility was not possible during this research. Instead, reliance was placed on previously reported absorption spectra, where spectra were obtained by both suspension and filter pad techniques in adapted spectrophotometers (Table 1). Forty in vivo absorption spectra were digitized at 1-nm increments from published figures that represented a wide range of taxonomic groups. Some of the absorption spectra were duplicates of species but under different culture conditions (i.e., nutrient and irradiance levels) and so were included. For the purpose of this research, we assumed that the obtained spectra were noise free. Each absorption spectrum was normalized to its spectral mean to minimize cell packaging effects (Roesler et al., 1989). All of the dinoflagellate spectra used were representative of noxious bloom-forming species.

Discriminant analysis is a statistical technique that utilizes linear combinations of independent variables to form a basis for a classification scheme. For this application to phytoplankton research, the independent variables are the wavelength-specific absorption values for each of the spectra. Discriminant analysis has two very useful applications. First, it will identify a set of independent variables that are needed to discriminate between known groups. In regard to phytoplankton research, sets of wavelengths can be identified that are necessary to discriminate between known phytoplankton groups. Second, the analysis can be used to classify an unknown sample (within a certain probability) into a known group. For application to phytoplankton research, an absorption spectrum of unknown phytoplankton composition can be classified into one of the known algal groups.

With the use of commercial software (SPSS, Inc., 1994), discriminant analyses of the 40 absorption spectra were conducted with all wavelengths between 440 and 650 nm, which represented a total of 211 independent variables. Wavelengths below and above this range, where the spectral characteristics are mostly attributable to chlorophyll *a*, were excluded (Bidigare et al., 1989; Millie et al., 1997). Chlorophyll *a* is not a useful discriminating factor because it is found in all algae.

Previous research with discriminant analysis for phytoplankton studies employed stepwise techniques (Johnsen et al., 1994; Millie et al., 1995a, 1997), which maximize group coefficients for function 1 of the discriminant analysis output. Stepwise techniques are particularly useful when discrimination between two groups is desired. For our purposes, however, we wanted discrimination between five algal groups (green algae, cyanobacteria, noxious dinoflagellates, diatoms, and other chrysophytes), which is better accomplished with two functions of the discriminant analysis output. Consequently, we opted for a standard discrimination method where all independent variables are entered simultaneously into the analysis. By using a standard method, group coefficients for function 1 are not as great as stepwise methods, but group coefficients for function 2 are greater, which, in this case, resulted in better overall discrimination between the five algal groups.

The discriminant analysis was repeated with absorption values from only nine of the spectral wavelengths used in the previous analysis (440, 444, 449, 464, 539, 543, 547, 553, and 557). Nine wavelengths were chosen because of the commercial availability of a nine-channel absorption and attenuation meter (Wetlabs, Inc.). This allowed us to compare the classification ability of the discriminant analysis with both high- and low-resolution absorption spectra. Our selection of which nine wavelengths to include in the analysis was based on two factors: first, the weightings of each spectral wavelength as identified in the previous discriminant analysis with high-resolution absorption data and second, the limitations for success identified by the fourth-derivative analysis experiment, i.e., $\leq$4 nm separation, which is detailed latter in the manuscript.

To investigate the influence of varied SNR on the performance of the discriminant analysis, two experiments were performed with the high-resolution absorption spectra (assumed to be noise free). The first experiment applied 10 different SNRs to an absorption spectrum of *P. minimum* (Table 1, culture 79A, high light). The amount of noise applied to each wavelength-specific absorption value was dependent on a set ratio of the maximum wavelength-specific absorption value as follows:

TABLE 1. Absorption spectra obtained from the literature used for the discriminant analysis.

Algal grouping	Genus species	Irradiance (μmol photons m^{-2} s^{-1})	Reference
Diatom (division Chrysophyta class Bacillariophyceae)	*Chaetoceros curvisetum*	400	Bricaud et al., 1988
	Chaetoceros didymum	400	Sathyendranath et al., 1987
	Chaetoceros gracile	50	Schofield et al., 1990
	Chaetoceros lauderi	400	Bricaud et al., 1988
	Chaetoceros protuberans	400	Hoepffner and Sathyendranath, 1991
	Skeletonema costatum	400	Sathyendranath et al., 1987
	Skeletonema costatum	75	Johnsen et al., 1992
Dinoflagellate (division Pyrrhophyta)	*Gymnodinium breve*	60	Millie et al., 1997
	Gymnodinium galatheanum	170	Johnsen and Sakshaug, 1993
	Gymnodinium galatheanum	30	Johnsen and Sakshaug, 1993
	Gyrodinium aureolum	170	Johnsen and Sakshaug, 1993
	Gyrodinium aureolum	30	Johnsen and Sakshaug, 1993
	Heyerocapsa pygmaea	500	Johnsen et al., 1997
	Heyerocapsa pygmaea	35	Johnsen et al., 1997
	Prorocentrum minimum	—	Schofield et al., 1996
	Prorocentrum minimum (79A)	500	Johnsen and Sakshaug, 1993
	Prorocentrum minimum (79A)	35	Johnsen and Sakshaug, 1993
	Prorocentrum minimum (EXUV)	500	Johnsen et al., 1997
	Prorocentrum minimum (EXUV)	35	Johnsen et al., 1997
Cyanobacteria (division Cyanophyta)	*Synechococcus* sp.	20	Bricaud et al., 1988
	Synechocystis sp.	16	Bricaud et al., 1988
	Synechocystis sp.	200	Bricaud et al., 1988
Other chrysophytes (division Chrysophyta)	*Aureococcus* sp.	—	Yentsch and Phinney, 1989
	Chrysochromulina polylepis	75	Johnsen et al., 1992
	Coccolithus huxleyi	20	Bricaud et al., 1983
	Emiliania huxleyi	50	Schofield et al., 1990
	Hymenomonas elongata	200	Bricaud et al., 1983
	Hymenomonas elongata	400	Hoepffner and Sathyendranath, 1991
	Pavlova lutheri	400	Bricaud et al., 1988
	Pavlova lutheri	250	Johnsen et al., 1994
	Pavlova lutheri	35	Johnsen et al., 1994
	Pavlova pingius	400	Bricaud et al., 1988
	Prymnesium parvum	400	Bricaud et al., 1988
Green algae (division Chlorophyta)	*Dunaliella euchlora*	—	Perry and Porter, 1989
	Dunaliella marina	400	Sathyendranath et al., 1987

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TABLE 1. Continued.

Algal grouping	Genus species	Irradiance (μmol photons m^{-2} s^{-1})	Reference
	Dunaliella salina	400	Bricaud et al., 1988
	Platymonas sp.	200	Bricaud et al., 1983
	Platymonas suecica	400	Hoepffner and Sathyendranath, 1991
	Pyramimonas parkeae	60	Millie et al., 1997
	Tetraselmis maculata	200	Bricaud et al., 1983

$$Noise = \frac{a_{\lambda max}}{SNR} Random,$$

where $a_{\lambda max}$ was the maximum spectral absorption value ($a_{\lambda max}$ = 440 nm for the selected *P. minimum* spectrum), *SNR* was a preselected signal-to-noise ratio, and *Random* had a value between zero and one that was determined with a random number generator (Matlab, Inc.). Ten SNRs were used in this experiment: 200*, 175*, 150*, 125*, 100*, 75*, 50*, 25*, 10*, and 5*, where the * denotes reference to λ = 440 (refer to columns 3–12 in Table 2). For each SNR investigated, referred to as test cases for the remainder of the manuscript, 100 different spectra of *P. minimum,* with added noise, were generated. Each spectrum within a test case was unique because the amount of noise added at each spectral wavelength was a function of a random number generator. For this first discriminant analysis experiment, 1,000 different absorption spectra were used.

The second discriminant analysis experiment also addressed classification accuracy at varied SNRs and focused on *P. minimum.* For this experiment, however, composite absorption spectra were generated that comprised a contribution from *P. minimum* and a contribution from other algae. Five scenarios were investigated in the experiment where the contribution of *P. minimum* to the total absorption spectra was 0, 20, 40, 60, or 80% of the total chlorophyll *a* (note that a 100% scenario was investigated in the previous experiment). The remaining portion of the composite absorption spectra were determined by selecting 2–10 other phytoplankton species listed in Table 1 that were not dinoflagellates. For a single composite spectrum in a given scenario, the number of other species to mix with *P. minimum,* the selection of which other species to mix with *P. minimum,* and the percentage contribution of a selected species (relative to other selected species) were all determined with a random number generator. For each scenario investigated in the second discriminant analysis experiment, 100 spectra were generated. Again, each spectrum is unique because of the use of a random number generator. A representative spectrum from each scenario, without added noise, is shown in Figure 1 along with the 100% scenario, which is simply the absorption spectrum from the *P. minimum* culture (79A, high light).

Noise was then added to the spectra of each scenario in the second discriminant analysis experiment according to the 175*, 125*, 75*, and 50* test cases from the first discriminant

TABLE 2. Wavelength-specific signal-to-noise ratio for the different test cases with absorption spectra from a dinoflagellate culture, *Prorocentrum minimum* (refer to Table 1, culture 79A, high light). The first column lists the 22 spectral wavelengths identified by the discriminant analysis as useful for discrimination between the five algae groups. The wavelengths are listed in accordance to their importance as discriminating factors along function 1 of the analysis, i.e., the absolute value of their discriminant score, as shown in the second column. Columns 3–12 list the signal-to-noise ratios for the 22 spectral wavelengths for each of the test cases investigated. The signal-to-noise ratios are greatest at $\lambda = 440$ for each test case because the absorption coefficient, which controls the amount of signal, at this wavelength is greatest over the wavelength range used in the discriminant analysis.

Absorption wavelength	Discriminant score, function 1	Test case[a]									
		200*	175*	150*	125*	100*	75*	50*	25*	10*	5*
543	15.75	34.5	30.2	25.9	21.6	17.2	12.9	8.6	4.3	1.7	0.9
540	−9.71	36.4	31.9	27.3	22.8	18.2	13.7	9.1	4.6	1.8	0.9
547	8.57	30.1	26.3	22.6	18.8	15.0	11.3	7.5	3.8	1.5	0.8
548	−7.34	29.5	25.8	22.1	18.4	14.8	11.1	7.4	3.7	1.5	0.7
553	−6.84	24.1	21.1	18.1	15.1	12.0	9.0	6.0	3.0	1.2	0.6
541	6.14	35.0	30.7	26.3	21.9	17.5	13.1	8.8	4.4	1.8	0.9
557	−4.97	19.2	16.8	14.4	12.0	9.6	7.2	4.8	2.4	1.0	0.5
559	4.79	18.0	15.7	13.5	11.2	9.0	6.7	4.5	2.2	0.9	0.4
542	−4.75	34.5	30.2	25.9	21.6	17.2	12.9	8.6	4.3	1.7	0.9
544	−4.07	32.0	28.0	24.0	20.0	16.0	12.0	8.0	4.0	1.6	0.8
440	3.57	200.0	175.0	150.0	125.0	100.0	75.0	50.0	25.0	10.0	5.0
546	−3.35	30.9	27.0	23.2	19.3	15.4	11.6	7.7	3.9q	1.5	0.8
464	3.16	184.6	161.5	138.4	115.3	92.3	69.2	46.1	23.1	9.2	4.6
545	2.90	31.4	27.5	23.6	19.7	15.7	11.8	7.9	3.9	1.6	0.8
449	−2.51	186.5	163.2	139.9	116.6	93.2	69.9	46.6	23.3	9.3	4.7
539	2.05	38.1	33.3	28.6	23.8	19.0	14.3	9.5	4.8	1.9	1.0
450	−1.98	185.4	162.2	139.0	115.9	92.7	69.5	46.3	23.2	9.3	4.6
550	1.44	27.1	23.7	20.3	16.9	13.6	10.2	6.8	3.4	1.4	0.7
444	−1.32	193.1	169.0	144.8	120.7	96.6	72.4	48.3	24.1	9.7	4.8
551	−0.75	25.9	22.7	19.4	16.2	13.0	9.7	6.5	3.2	1.3	0.6
560	0.38	17.4	15.2	13.0	10.8	8.7	6.5	4.3	2.2	0.9	0.4
555	0.06	22.2	19.5	16.7	13.9	11.1	8.3	5.6	2.8	1.1	0.6

[a] * Denotes maximum wavelength-specific signal-to-noise ratio per test.

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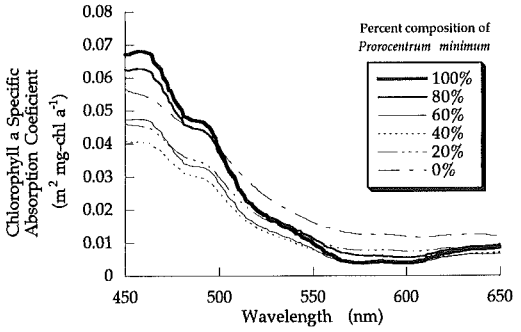

Fig. 1. Representative absorption spectra are shown from each of the scenarios of various *Prorocentrum minimum* contributions that were used in the second discriminant analysis experiment. In each of the scenarios, 100 unique spectra were generated with absorption spectra reported previously.

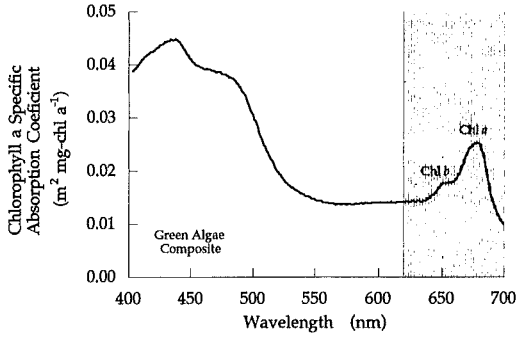

Fig. 2. Absorption spectra of a green algae composite with distinct chlorophyll *a* and *b* curvature. The shaded region indicates the spectral range over which the fourth-derivative analysis focused.

analysis experiment. The selection of these test cases was based on the results of the first discriminant analysis experiment, where the spectral wavelength receiving the heaviest weighting was identified, and the known noise level of the HiStar at this spectral wavelength. In summary, the second discriminant analysis experiment comprised five scenarios that represented composite absorption spectra with various amounts of *P. minimum* (with 100 unique noise-free spectra in each scenario). To each scenario, random noise was added at five different levels, i.e., five test cases within each scenario. In all, 25 discriminant analyses were performed with a total of 2,500 different absorption spectra.

Derivative analysis on absorption spectra with respect to wavelength is a numerical technique used to detect chlorophylls and phycobilins as well as to determine previous light history of phytoplankton (Bidigare et al., 1989; Millie et al., 1995a). Whereas discriminant analysis accentuates differences between spectra, derivative analysis is sensitive to the shape of a spectral curve. The fourth-derivative is preferred over others because of its low noise level (Butler and Hopkins, 1970). A composite of absorption spectra was created with phytoplankton species from the green algal group (Fig. 2) and normalized to its spectral mean (Roesler et al., 1989). Green algae were selected because this phytoplankton group contains chlorophylls *a* and *b*, two pigments with absorption peaks in the near-red region of the spectrum. Figure 2 illustrates how the presence of these two pigments affects the shape of the absorption spectrum. Fourth-derivative analyses (Butler and Hopkins, 1970) were repeated with spectral resolution ranging from 1 to 10 nm. For each analysis, the wavelength $\lambda = 675$ (chlorophyll *a* maximum in the near-red; Millie et al., 1995a) was included. The remaining incremental wavelengths were determined below and above λ_{675} according to the spectral resolution under investigation.

Results

The standard discriminant analysis with the high-resolution noise-free absorption spectra from the 40 species listed in Table 1 worked well for distinguishing the five algal groups. Only two spectra were misclassified. The diatom *Chaetoceros lauderi* was placed in the "other chrysophytes" group, and one of the *Hymenomonas elongata* spectra was placed in the green algae group instead of the "other chrysophytes" group. All 12 dinoflagellate spectra were correctly classified within a probability of 0.99 ± 0.01. Of the 211 absorption wavelengths, or independent variables, only 22 were identified as useful for discrimination between the five algal groups (see first column of Table 2). Absorption values corresponding to 543 nm had the highest discriminant score on the primary discriminant function (see second column of Table 2), which comprised 72% of the total variance (Fig. 3).

With absorption values from only nine spectral wavelengths, a classification scheme was produced that was very similar to the first discriminant analysis. All 12 dinoflagellate spectra were again correctly classified (0.98 ± 0.03). The only difference of note was that the probabilities of successful classification for the other phytoplankton groups were slightly less (data not shown).

For the purpose of this research, we assumed

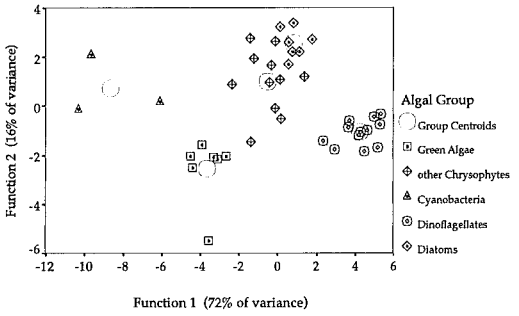

Fig. 3. Canonical discriminate functions with a standardized discriminant analysis with absorption spectra from 22 wavelengths. Five groups were classified. All of the dinoflagellates shown were noxious bloom-forming species.

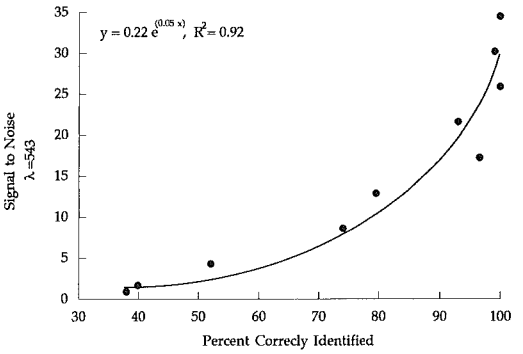

Fig. 4. Deterioration of the discriminant analysis results with decreasing signal-to-noise ratio. A monospecific bloom of *Prorocentrum minimum* was classified in the dinoflagellate group at a ≥90% rate at signal to noise ratios ≥~17.

that HAB management efforts will require successful classification rates of at least 90% for spectra representative of a phytoplankton community dominated by *P. minimum*. As stated previously, absorption values at λ = 543 were weighted heavier than other spectral wavelengths by the discriminant analysis. Therefore, this wavelength was emphasized in the study. For the first discriminant analysis experiment, where the contribution to the total chlorophyll *a* was solely from *P. minimum*, the lowest SNR for λ = 543 meeting the 90% successful classification rate threshold was ~17, which occurred in the 100* test case (Fig. 4; Table 2). Note that the SNR is lower at λ = 543 than at λ = 440 because the absorption coefficient, which controls the amount of signal, at λ = 543 for *P. minimum* is less than the absorption coefficient at λ = 440. This is true for all 22 wavelengths reported in Table 2.

As the contribution of *P. minimum* to the total chlorophyll *a* declined, the ability of the discriminant analysis to classify *P. minimum*-containing spectra into the dinoflagellate group became unreliable. For example, with the test cases with no added noise, the technique successfully classified all of the mixed spectra representative of communities containing >80% *P. minimum* into the dinoflagellate group. But for mixed spectra representative of communities containing ≤60% *P. minimum*, classification into the dinoflagellate group was well below the 90% success rate threshold (Fig. 5).

The addition of noise greatly reduced the performance of the discriminant analysis within the 90% success rate threshold. For instance, this technique successfully classified spectra representative of communities containing >80% *P. minimum* into the dinoflagellate group at SNR as low as ~33 at λ = 543. When the SNR ≤ ~24, however, the discriminant analysis failed to classify the same set of spectra into the dinoflagellate group within the 90% success rate threshold (Fig. 5).

The addition of noise also increased the likelihood of the discriminant analysis returning a false positive, that is, classification of a composite spectrum into the dinoflagellate group that contained no dinoflagellates. For example, the technique yielded a false positive at a rate of 6% on spectra with no *P. minimum* contribution when the SNR was 16 (Fig. 5).

Our investigation with the fourth-derivative analysis showed that the ability of the technique to resolve chlorophylls *a* and *b* deteriorated as spectral resolution decreased. The contribution of chlorophylls *a* and *b* to the

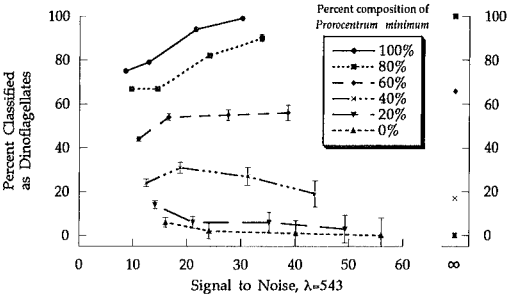

Fig. 5. Deterioration of the discriminant analysis results with decreasing *Prorocentrum minimum* contribution and signal-to-noise ratio. A mixed phytoplankton community containing *Prorocentrum minimum* was classified in the dinoflagellate group at a ≥90% rate when *P. minimum* comprised ≥80% of the total chlorophyll *a*. With the addition of noise, spectra containing ≥80% *P. minimum* were classified into the dinoflagellate group when the signal-to-noise was ≥33.7.

composite spectra of green algae was high (Fig. 2). Spectral resolutions from 1 to 4 nm were very similar in the fourth-derivative showing distinct peaks for chlorophylls *a* and *b*. At 5-nm resolution, however, the chlorophyll *a* peak began to deteriorate. The chlorophyll *b* peak became less distinct at 7-nm resolution (Fig. 6).

Discussion

With the exception of two spectra, we were able to distinguish five major phytoplankton groups by the discriminant analysis. It is well known that individual phytoplankton species alter their pigment composition in response to variations in irradiance magnitude and quality and that the shifts in pigment composition result in shifts in the absorption spectra (Kirk, 1994). It is also well established that phytoplankton members within a single group have varied pigment composition, which also results in varied absorption spectra within the group. The spectra we used in our discriminant analysis were representative of different species at varied irradiance levels as well as the same species at varied irradiance levels. Our results indicate that variability between major phytoplankton groups is greater than the variability between members of the same phytoplankton group as well as variability found within a single species.

Depending on the level of detail desired for phytoplankton classification, hyperspectral measurement of IOPs might not be necessary. For example, the information obtained from HiStar could also be attained with a nine-channel absorption and attenuation meter with well-placed band-pass filters. Indeed, phytoplankton spectra have been classified into general taxonomic groups, although not as many as five, by discriminant analysis with absorption values from fewer than nine spectral wavelengths (Johnsen et al., 1994; Millie et al., 1997).

For management applications, the detection threshold in terms of cells per liter where a monospecific algal bloom of *P. minimum* or a mixed algal bloom dominated by *P. minimum* can be correctly classified by the discriminant analysis approach is of interest. The detection threshold can be calculated if the following are known: the approximate chlorophyll *a*-specific absorption coefficient, the SNR limitation of the discriminant analysis, and the noise level of the instrument being used, in this case the HiStar. For this study, the chlorophyll *a*-specific absorption coefficient at $\lambda = 543$ for *P. minimum* was 0.0125 m^2 mg chl a^{-1} (Johnsen and Sakshaug, 1993). The version of the HiStar currently available has a noise level of $\sim 1 \times 10^{-2}$ m^{-1} over the 500–600-nm spectrum (Wetlabs, pers. comm.). With application of a SNR of 17.2 (the minimum ratio meeting the ≥90% successful classification rate requirement, estimated from Fig. 4), a monospecific bloom of *P. minimum* could be successfully classified at ≥13.8 mg chl *a* m^{-3}, or $\geq 1.38 \times 10^7$ cells $liter^{-1}$ with the conversion of 1 pg chl *a* $cell^{-1}$ (John-

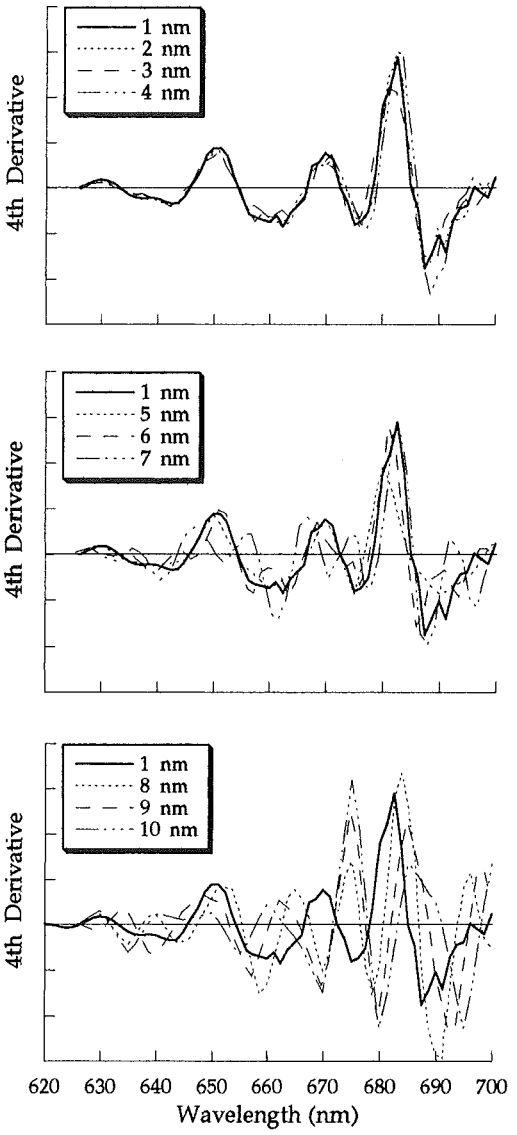

Fig. 6. Deterioration of the fourth-derivative analysis (unitless) results with increasing spectral resolution. Chlorophyll *a* and *b* peaks in the fourth-derivative from a green algae composite were very similar from 1 to 4 nm spectral resolution but deteriorated at spectral resolutions ≥5 nm.

sen and Sakshaug, 1993). Mixed spectra containing 80% *P. minimum* required a SNR of ~34 for classification into the dinoflagellate group. With application of the same chlorophyll *a*-specific absorption coefficient and noise level of the HiStar, successful classification would occur at $\geq \sim 2.7 \times 10^7$ cells liter^{-1}. Current management practices, however, close fisheries when *Prorocentrum* spp. are $\geq 5 \times 10^5$ cells liter^{-1} (Shumway et al., 1995). At present, the noise level in the HiStar is too high for the instrument to be useful for monitoring of *P. minimum* blooms.

This is not to say that this optical approach to monitoring phytoplankton communities will not work. This classification technique will improve as the sensitivity of in situ instrumentation continues to develop, e.g., the current HiStar is only a second-generation instrument, and our ability to optically differentiate between algal groups increases. In the case of monitoring for *P. minimum,* the discrepancy between the current cell concentration detection threshold and the cell concentration at which fisheries are closed is ~28-fold. In our opinion, a 28-fold increase in the utility of this classification technique is feasible, especially with inclusion of species-specific scatter properties along with species-specific absorption characteristics in discriminate analysis. Scattering properties may be distinct among phytoplankton groups. If so, inclusion of scatter properties in the discriminant analysis will result in greater distances between algal group centroids, i.e., better discrimination between algal groups.

As mentioned before, fourth-derivative analysis may be very useful when coupled with discriminant analysis both for management applications and for forecasting model initialization. The minimum spectral resolution where fourth-derivative analysis is still able to resolve chlorophylls and phycobilins must be understood. These results show that 4 nm spectral resolution is sufficient to resolve chlorophylls *a* and *b*. A spectral resolution of 4 nm may be sufficient to resolve other pigments as well. Data collected from the HiStar have a spectral resolution of ~3.3 nm and are adequate for this application of fourth-derivative analysis.

Conclusions

The present study concentrated on limitations of discriminant and fourth-derivative analysis on absorption spectra for application to phytoplankton research at varied SNR and spectral resolution. Use of the discriminant analysis was highly successful at classifying algae into five general groups but required input of absorption values from 22 spectral wavelengths, some of which were 1 nm resolution. Discrimination of the dinoflagellate group from the other phytoplankton groups was still possible, however, with only nine spectral wavelengths with ≥4 nm resolution. The trade-off incurred by decreasing the number of spectral wavelengths used in the discriminant analysis was a slight decrease in the probability of successful classification of the other phytoplankton groups. Hyperspectral measurement of IOPs may not be necessary at a level of classification relevant to some HAB management efforts, i.e., monitoring for noxious dinoflagellates, but it may be necessary if more detailed classification of algae is desired.

For in situ phytoplankton monitoring purposes, the utility of discriminant analysis of absorption spectra was greatly diminished with the addition of noise to the spectra. At present, the discriminant analysis technique will be successful only when phytoplankton cell concentrations are high and the community is dominated by a single species or multiple species of closely related taxa and physiology. Consequently, use of discriminant analysis of absorption spectra to monitor phytoplankton community composition is not likely to replace traditional phytoplankton monitoring methods, such as field sampling followed by microscopy. Rather, the discriminant analysis technique may be used to complement traditional methods, e.g., times and locations of when and where field sampling should focus can be identified. Fourth-derivative analysis works well at the spectral resolution offered by current technologies. More work detailing how the shape of absorption spectra relates to physiological state of individual species is needed before further investigation of this research approach.

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