Taxonomic recognition of plankton using optics

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Optical techniques for detection of micro-plankton species

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The Problem:

In general cells with Diameter<20μm look very similar.
Hard to differentiate species based on morphology.
Differentiation between cells is possible based on functionality (e.g. Nitrogen fixing ability), presence of specific organelles, presence of specific pigments, or genetic information.

Methods based on the optical properties of cells:

SINGLE PARTICLE ANALYSIS:
Flow cytometry (forward and side scattering, fluorescence), imaging cytometry, flow-CAM, multi-angle light scattering.

BULK PARTICLE ANALYSIS:
Spectral absorption and fluorescence of specific pigments, multi-angle light scattering of specific morphology and internal structure, remotely sensed reflectance.

1. Imaging Cytometry (fluorescence+microscopy

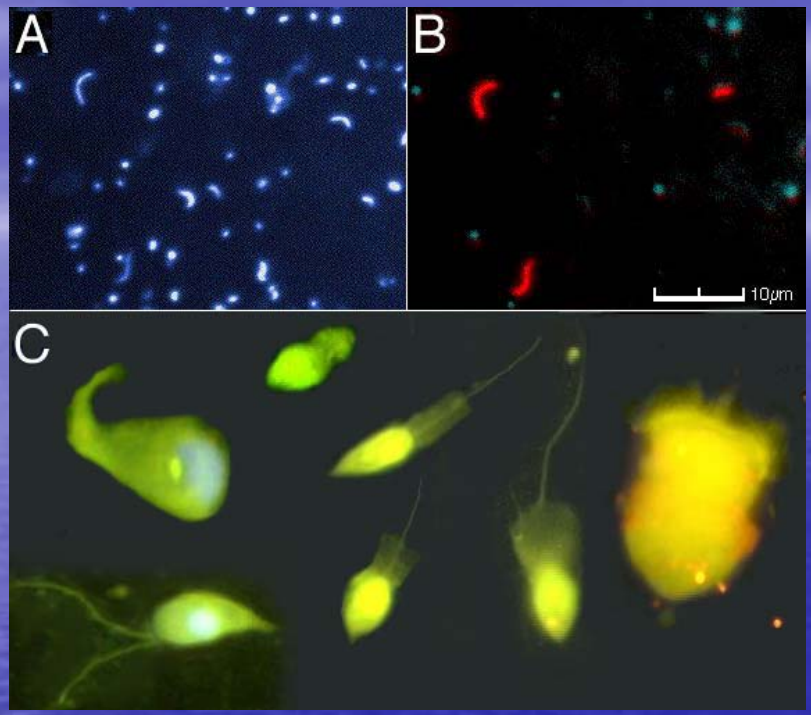
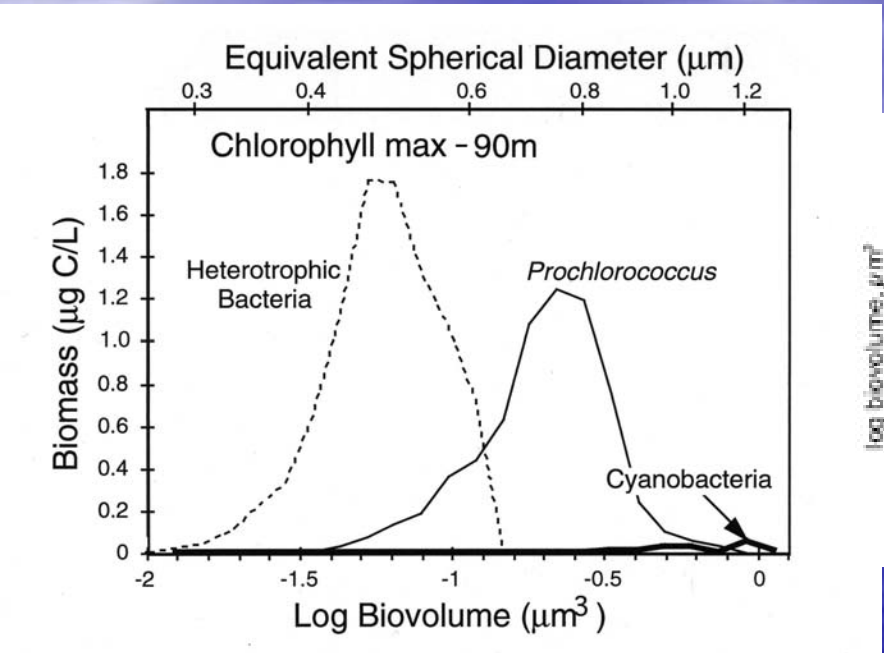


Figure 1. Imaging Cytometry Digital image analysis of epifluorescence microscope images is ideal for analysis of prokaryotes and heterotrophic protists from natural marine samples. Such imaging systems provide rapid determination of cell abundance and sizes for calculating size spectra and biomass. A) DAPI stained bacteria from a Sargasso Sea sample. B) The same field imaged with infrared fluorescence optics shows aerobic, anoxygenic photoheterotrophs containing bacteriochlorophyll (red). Chlorophyll-a containing Prochlorophytes appear cyan. C) Composite showing variety of small heterotrophic protists from Georges Bank waters imaged using the fluorochrome, proflavin. Cells shown include Comatium, Leucocryptos, choanoflagellates, a dinoflagellate and a ciliate. (See more images at http://www.bigelow.org/cytometry).



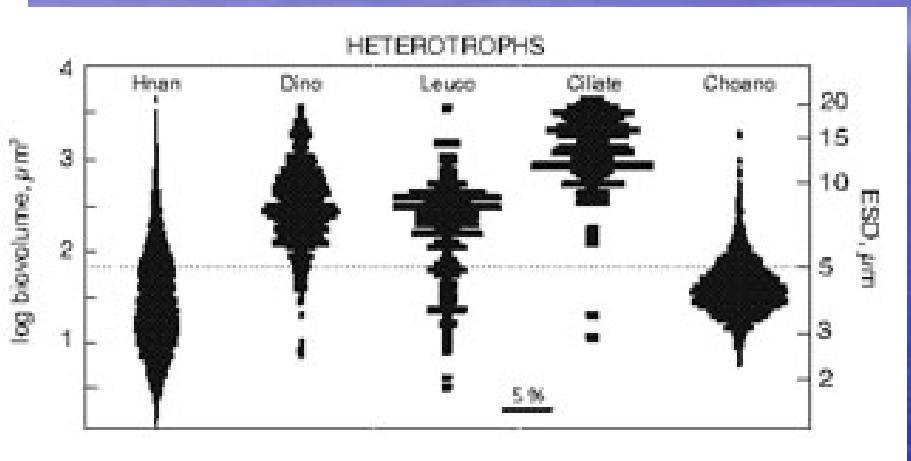


Figure 2. Imaging Cytometry Digital image analysis of epifluorescence microscope images is ideal for analysis of prokaryotes and heterotrophic protists from natural marine samples. Such imaging systems provide rapid determination of cell abundance and sizes for calculating size spectra and biomass. A) Size spectra of Sargasso Sea picoplankton (Sieracki et al. 1995, Sieracki and Viles 1998). B) Size spectra of Georges Bank heterotrophic protists, 2 - 20 mm.

2. Flow Cytometry (fluorescence+side and forwardscattering):

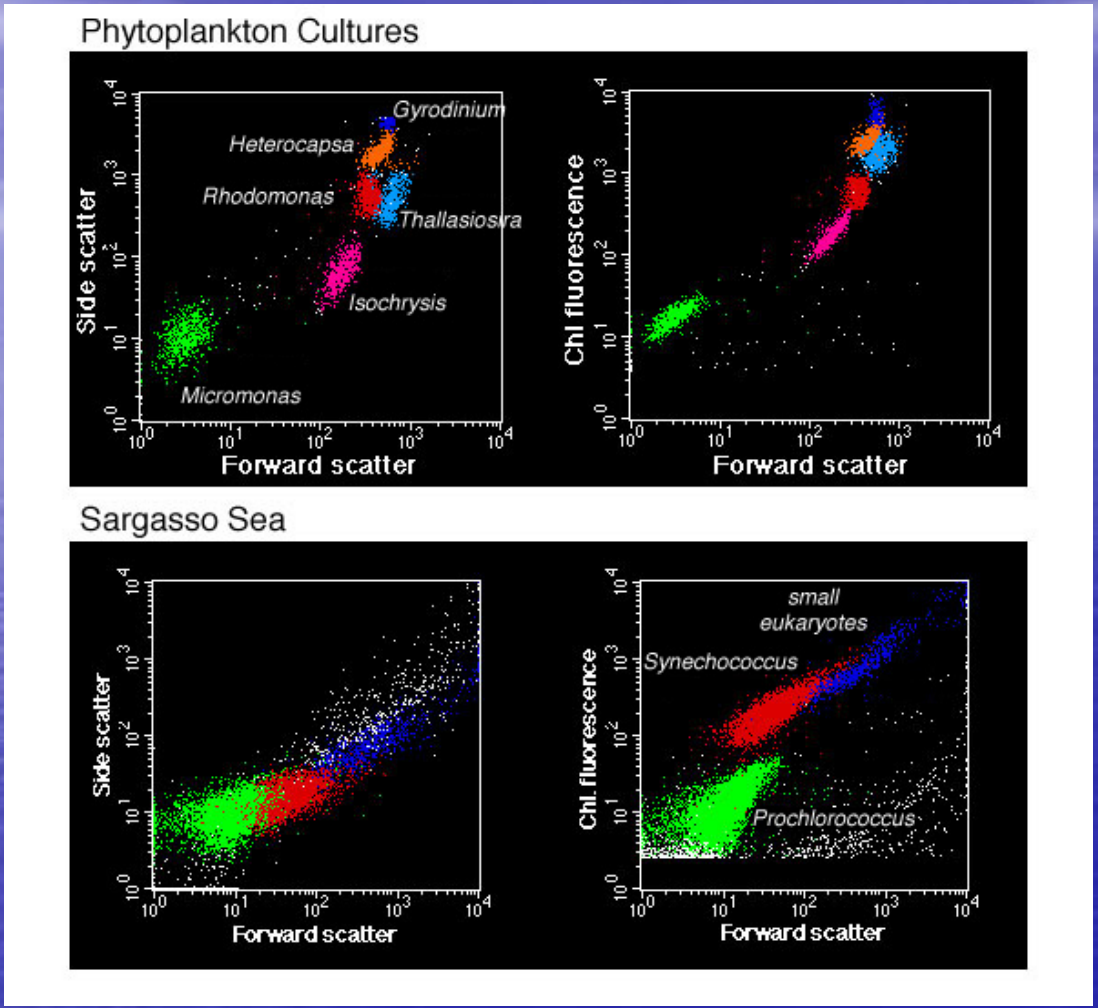


Figure 3. Flow cytometry is ideal for detecting and quantifying prokaryotes and pico- and nanophytoplankton from natural samples. These figures show "allometric analysis" in the left panels (side vs. forward light scatter) and "taxonomic analysis" (scatter vs. fluorescence) on the right panels for phytoplankton cultures and a Sargasso Sea sample (Phinney and Cucci, 1989).

3. Imaging in flow (microscopy):

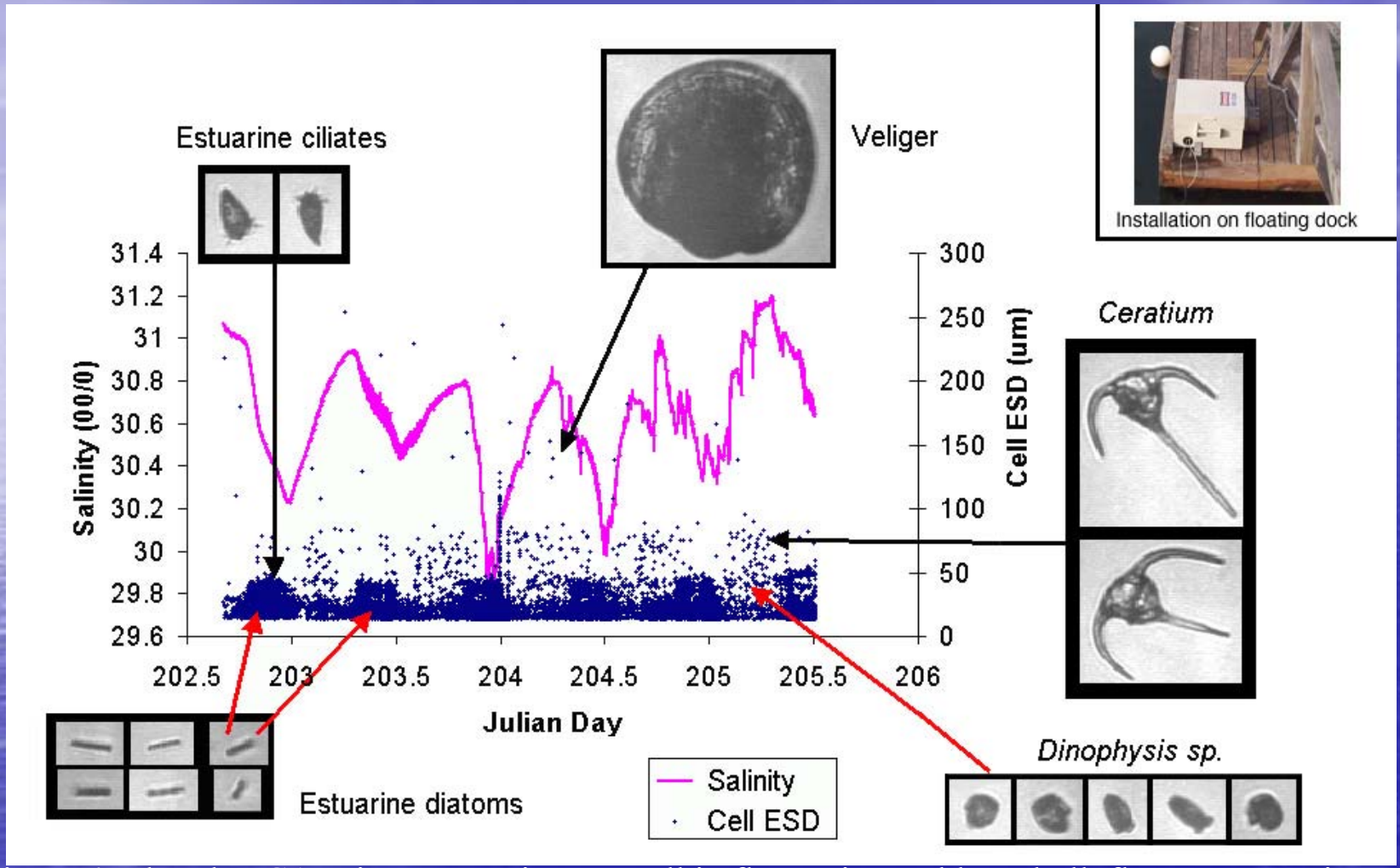


Figure 4. The FlowCAM instrument images cell in flow using a chlorophyll fluorescence trigger. Cell sizes are measured directly from the images. This instrument is ideal for analysis of microplankton (>20mm) including phytoplankton and ciliates. The instrument can be installed on a floating dock and run continuously or in the flow through system on a ship underway (Sieracki et al. 1998).

New deployment methodology: *in-situ* flow-cytometry:

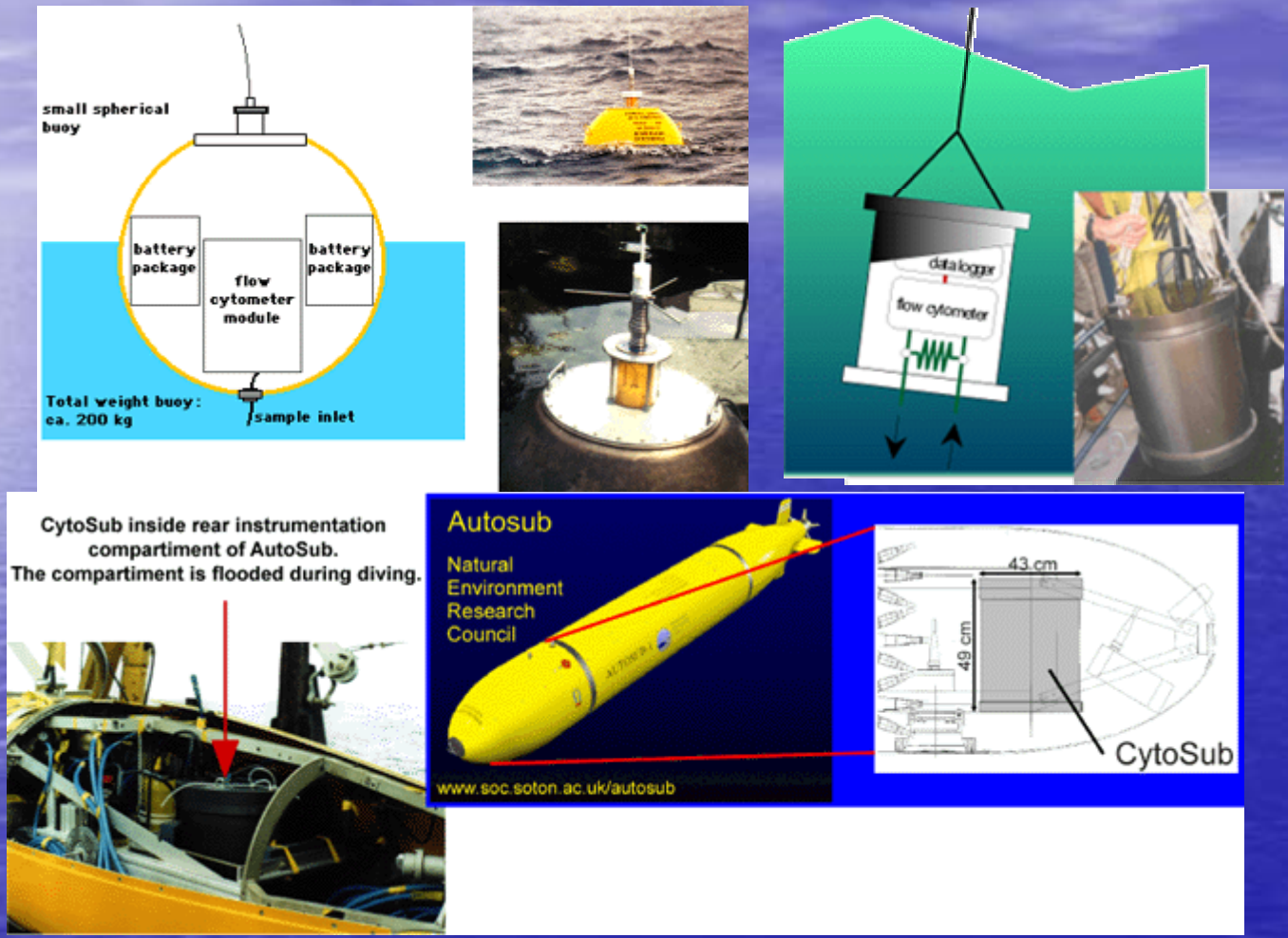
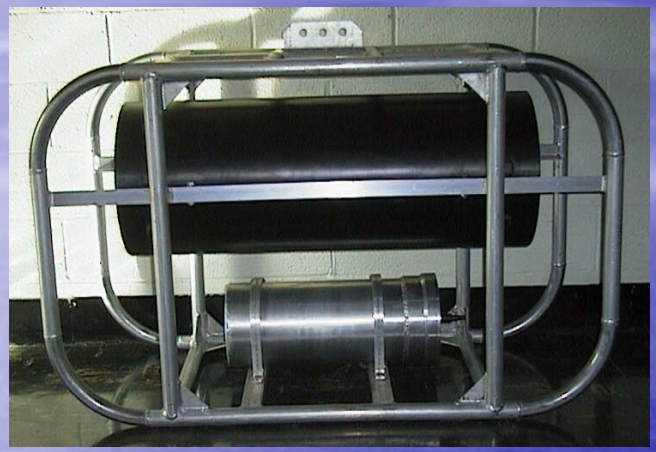
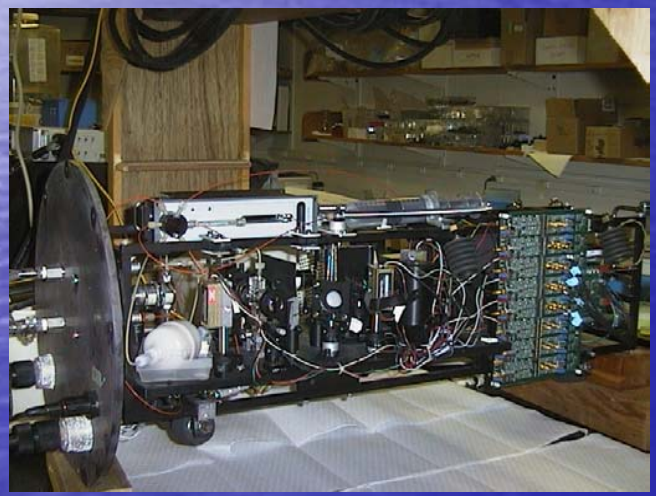
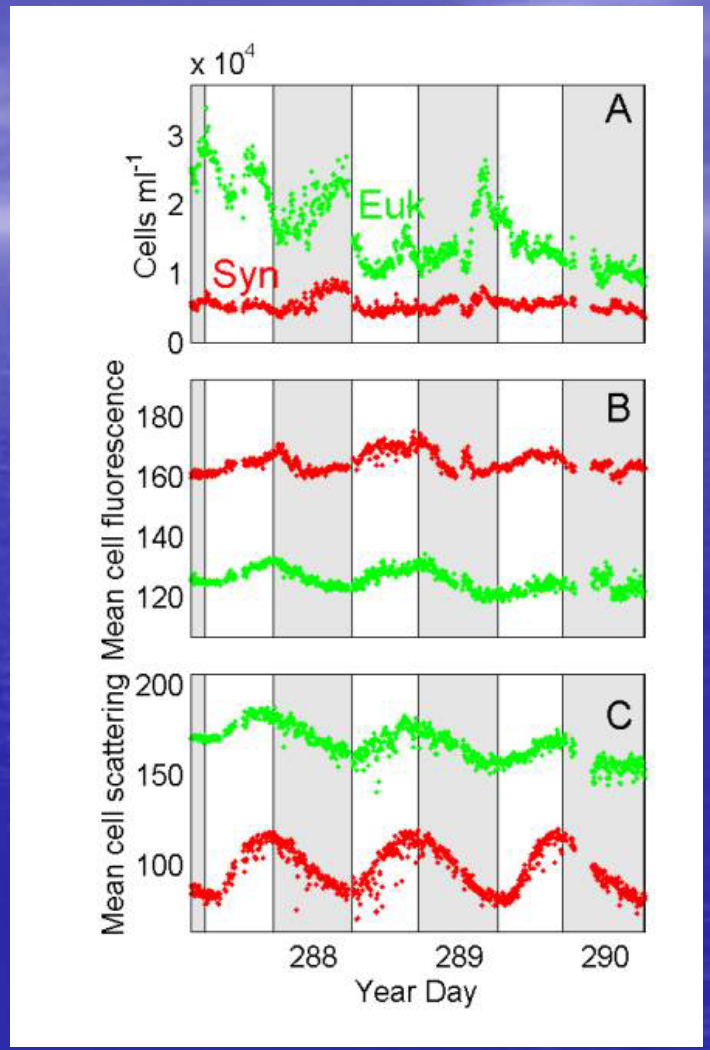


Figure 5. *In-situ* flow-cytometry. The Cyto-buoy flow-cytometer can be deployed from a boat, be moored or be sent on a submarine.

New deployment methodology: *in-situ* flow-cytometry:





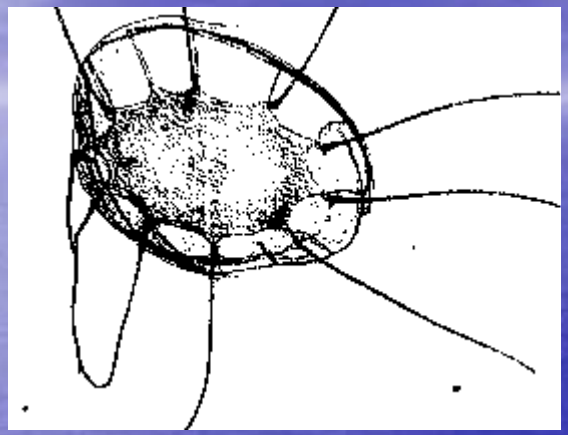


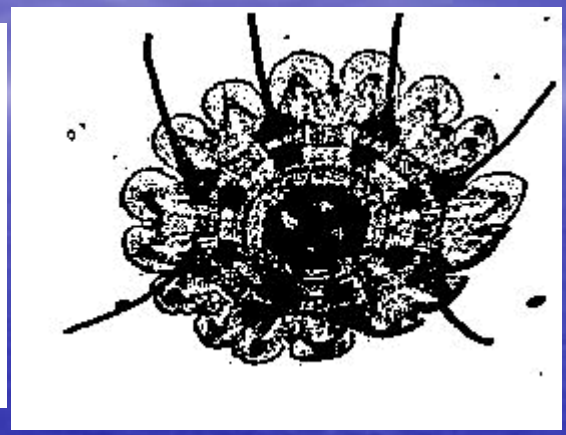
Olson and Sosik's (WHOI) prototype in-situ flow-cytometer (left) and data collected with it during a 3-day deployment at LEO XV in October 2001.

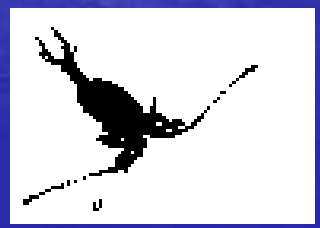
S.I.P.P.E.R.

Shadowed Image Particle Profiling and Evaluation Recorder

Chaetognath
Copepod
Decapod
Doliolids
Echinoderm
Euphausiids
Fish
Gelatinous
Heteropod
Larvaceans
Polychaete
Pteropod
Protoctista
Salps
Siphonophores
Squid
Trichodesmium








Example of images collected by SIPPER (USF) in the in-shore and off-shore waters of the Gulf of Mexico.

S.I.P.P.E.R.

Different deployment methods, AUV (left) and towed package for high resolution in-situ mesurements.

The Sieracki ‘Uncertainty’ Principle:

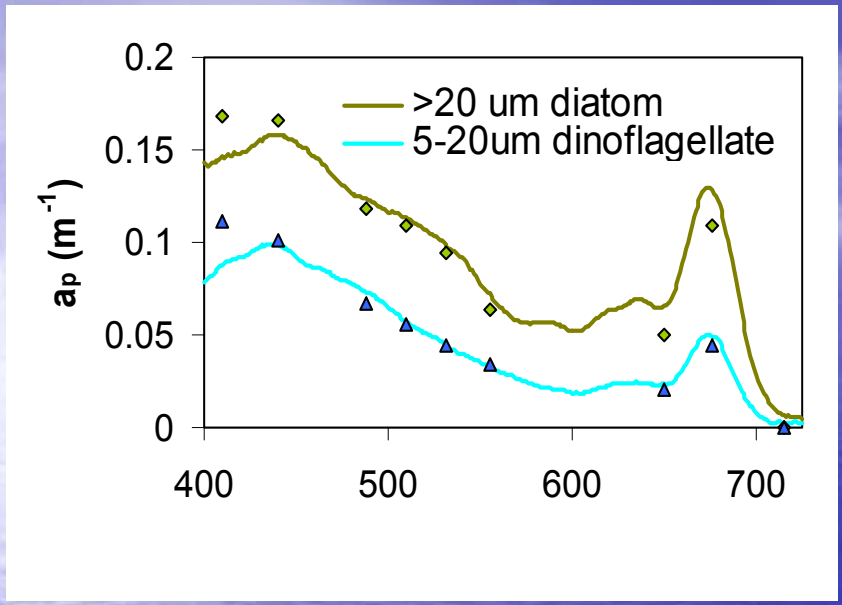
When analyzing complex plankton communities with limited resources there has to be a compromise between getting:

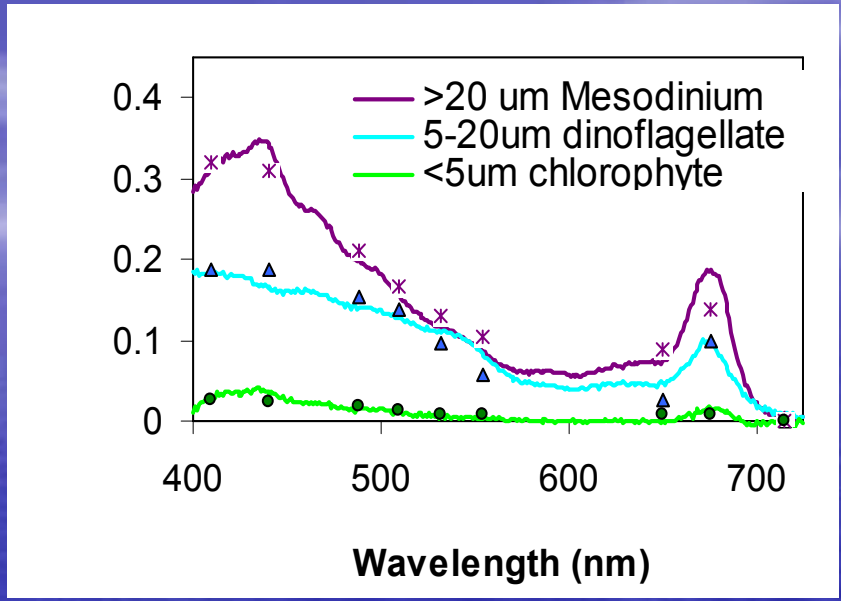
1) accurate population counts and cell measurements
and
2) accurate species identification.

Corollary:
It is very difficult to get high resolution measurements and taxonomic identification from an ecologically significant number of samples.

BULK PARTICLE ANALYSIS:

1. Size fractionated *in-situ* absorption spectroscopy:





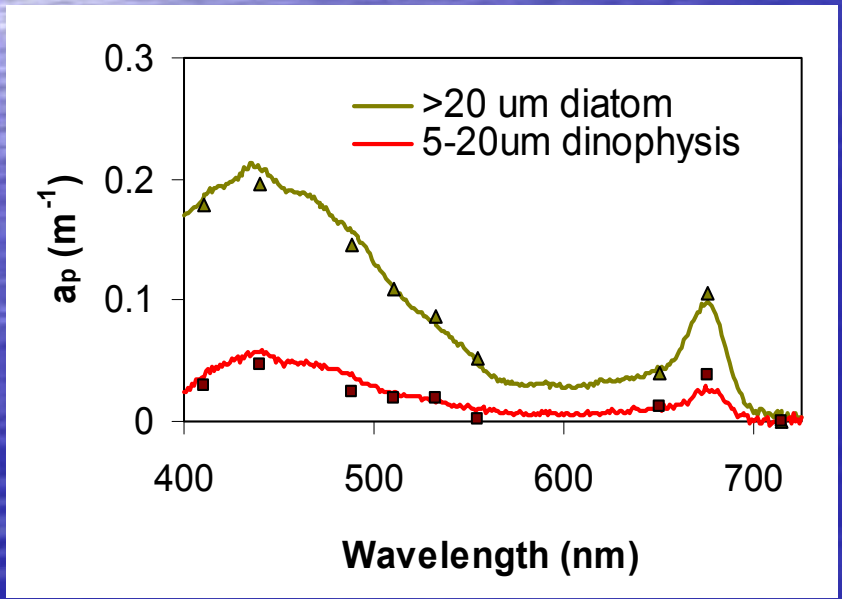
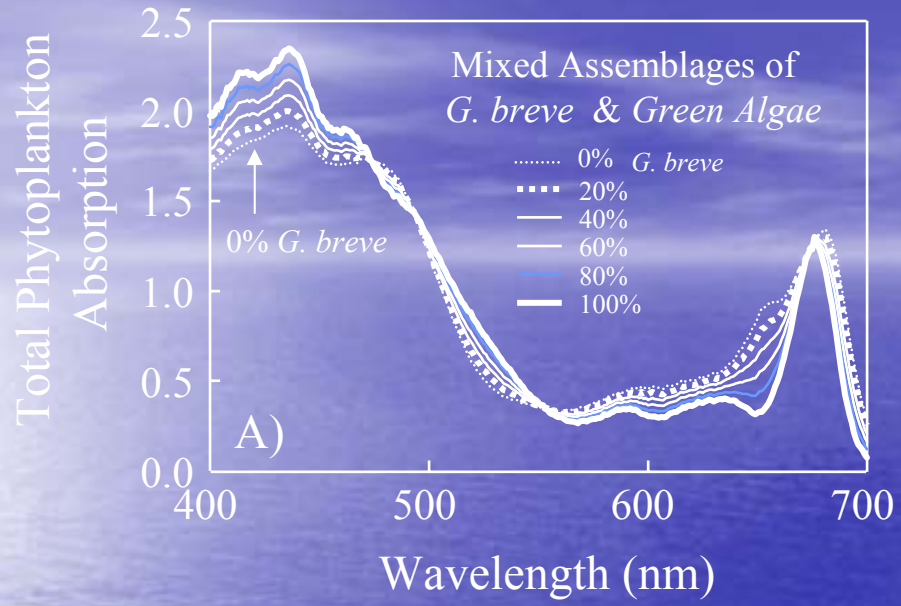
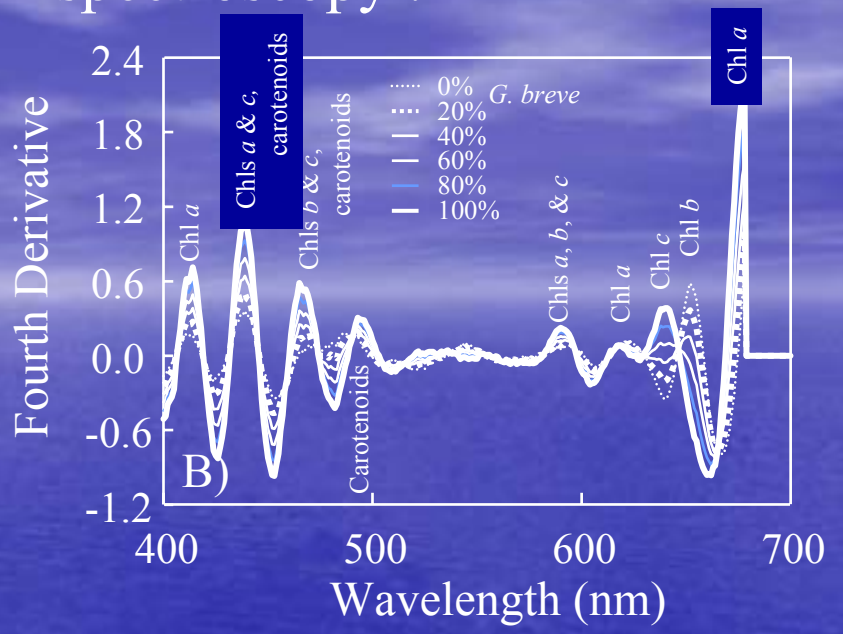
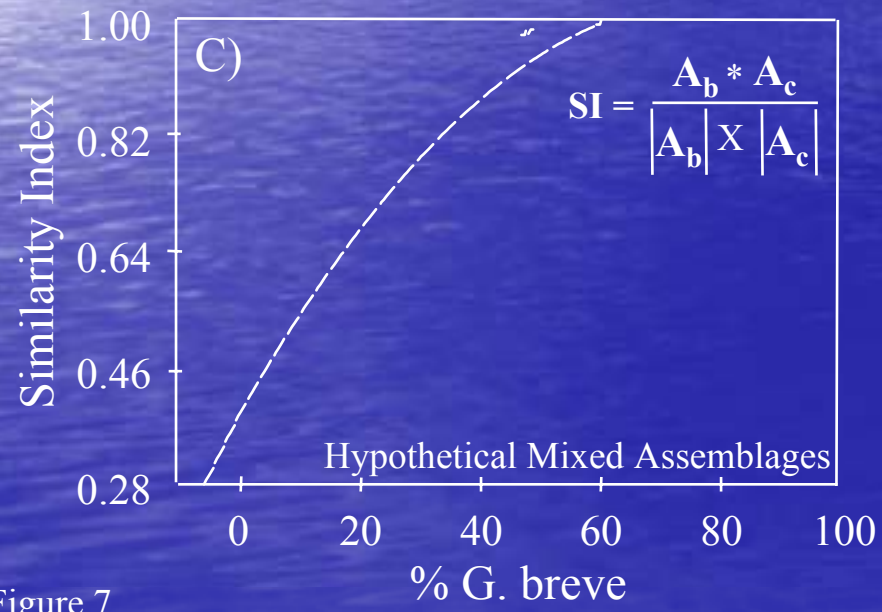


Fig. 6. Multiple casts were performed with ac9s *in-situ* sepctrophotometers configured with filters placed on the intake ports. Profiles of total, <20 um, <5 um, and <0.2 um fractions were made. Size fractions computed by difference (symbols). Water samples were collected, fractionated and analyzed spectrophotometrically (solid lines). Dominant species in each size fraction were identified microscopically. Spectral differences associated with distinct pigmentation confirmed species composition. Data from inshore of the Benguela upwelling front during expansive red tides characterized by variable species composition (Roesler, Etheridge, and Pitcher).

2. 4th derivative analysis of absorption spectroscopy :







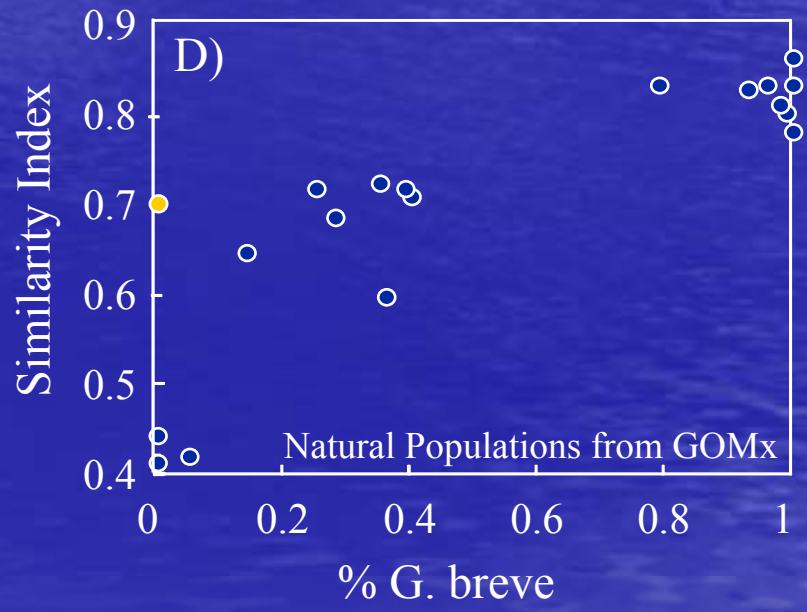


Figure 7

Fig. 7. (A) Absorption for a hypothetical assemblage of *D. tertiolecta* and *G. breve*. The individual spectra reflect various proportions of dinoflagellate and green algae ranging from 0% to 100% *G. breve*. (B) the 4th order derivative spectra for the mixed assemblage spectra presented in A. (C) The relationship between the similarity index versus the relative proportion of *G. breve* for a hypothetical mixed assemblages. (D) Similarity-index values associate with natural mixed phytoplankton population encountered in the Gulf of Mexico. *G. breve* is the only phytoplankton to contain gyroxanthin-diester and it appears in constant proportion to its chlorophyll *a*. Redrawn from Schofield et al. (1999).

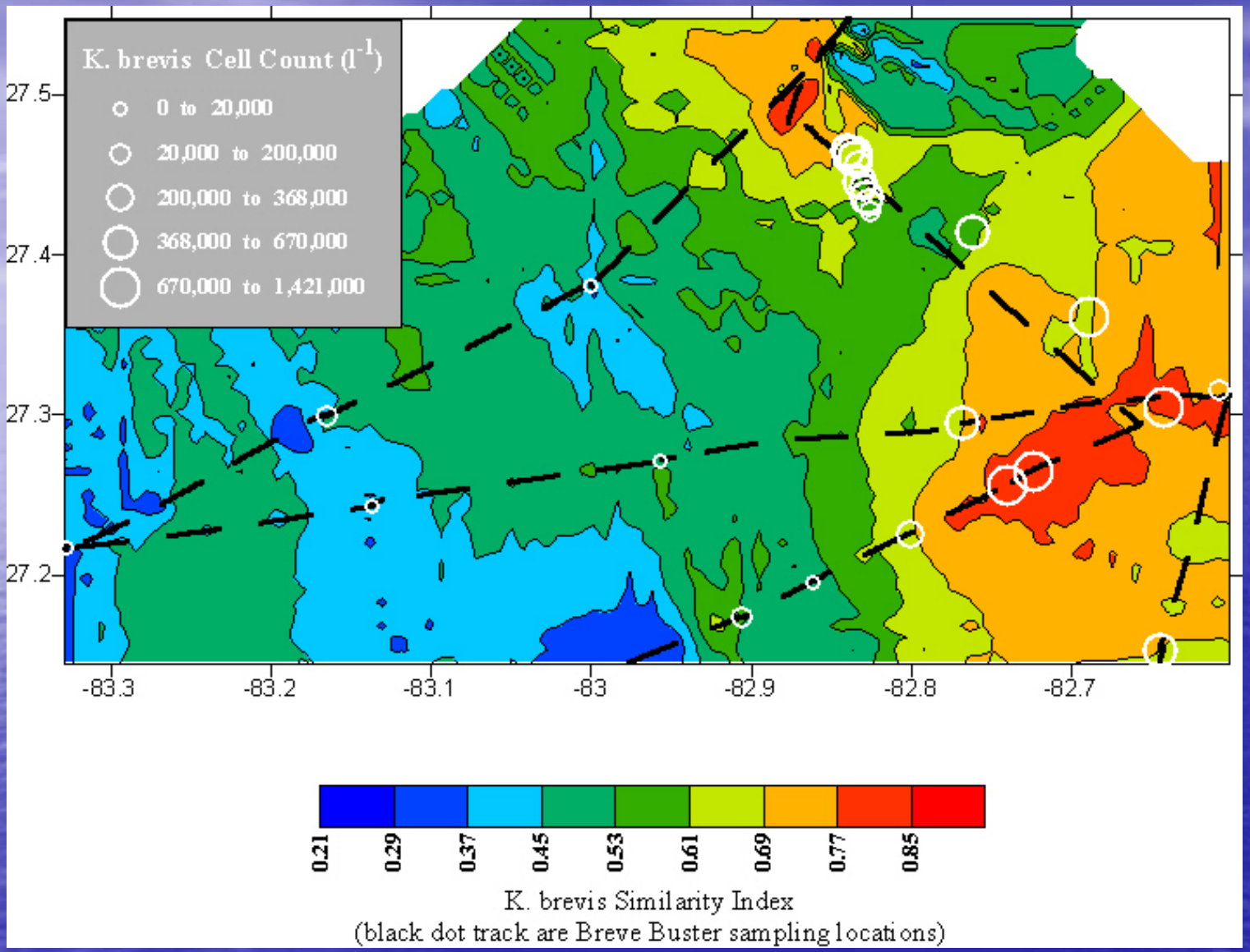


Fig. 8. Similarity index map during a recent cruise to the Eastern Gulf of Mexico. Broken line are the ship tracks and circle denote locations where samples for individual counts of G. breve where collected. Size of circle is proportional to the number of individuals.

3. Techniques to derive taxonomic information from remote sensing

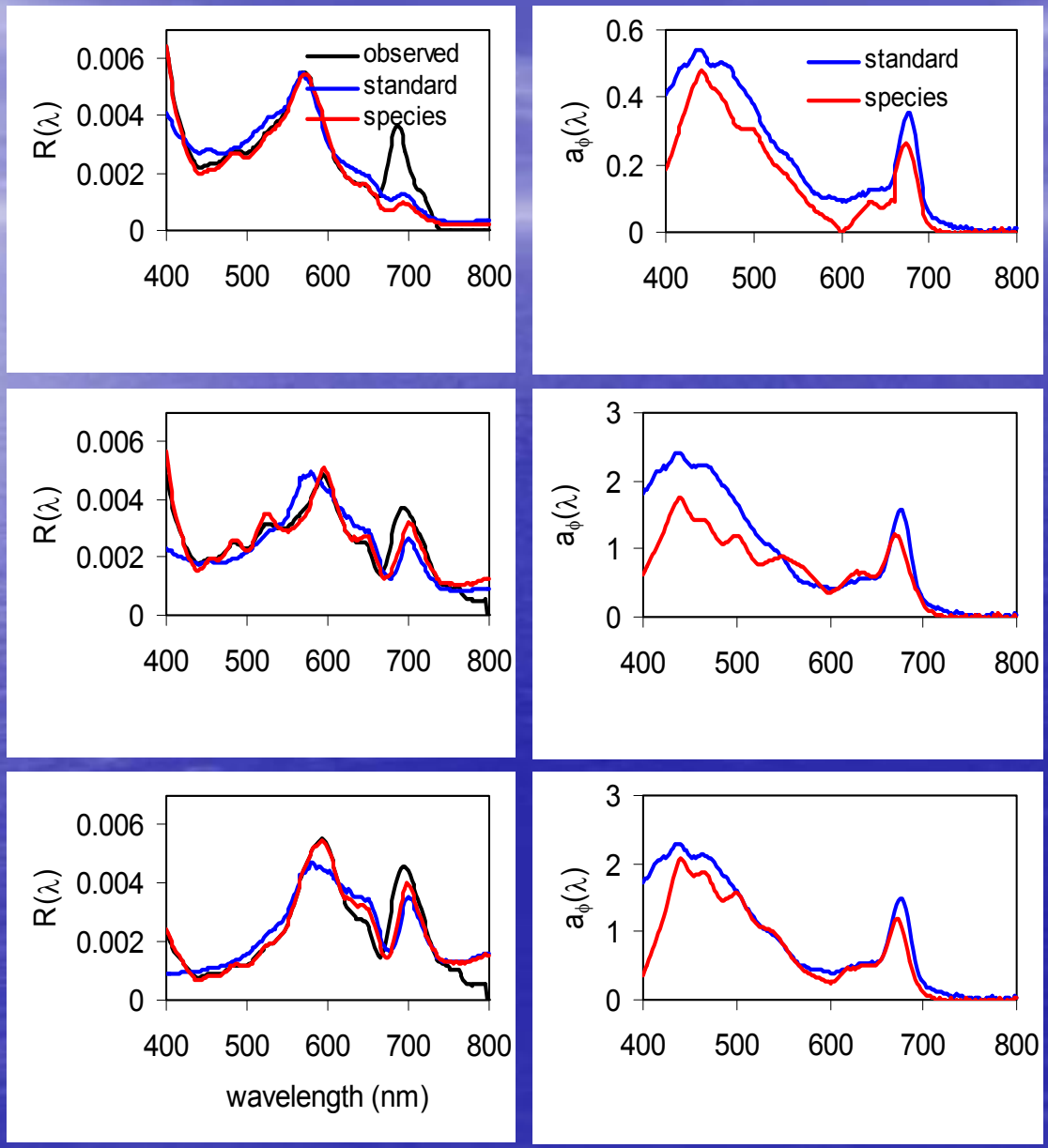


Fig. 9. Reflectance can be expressed as a function of the backscattering to absorption ratio, which are in turn parameterized by linear combinations of optically active components: $R = (bbw + bbp)/(aw + af + aCPM + aCDM)$ where subscripts w, p, f, CPM, and CDM are water, particles, phytoplankton, colored particulate and dissolved materials, respectively. By assuming spectral shapes for each component (eigenfunctions), the magnitude (eigenvalues) can be estimated by non-linear regression. A single phytoplankton eigenfunction (standard model) does not provide species information while a set of six species-specific phytoplankton eigenfunctions (Fig. 10) not only provides a better model fit but provides an estimate of species composition. Data from inshore of the Benguela upwelling front during the onset and development of a red tide during which species composition varied significantly from day to day (Fig. 11) (Roesler, Etheridge, and Pitcher).

Species specific eigenfunctions:

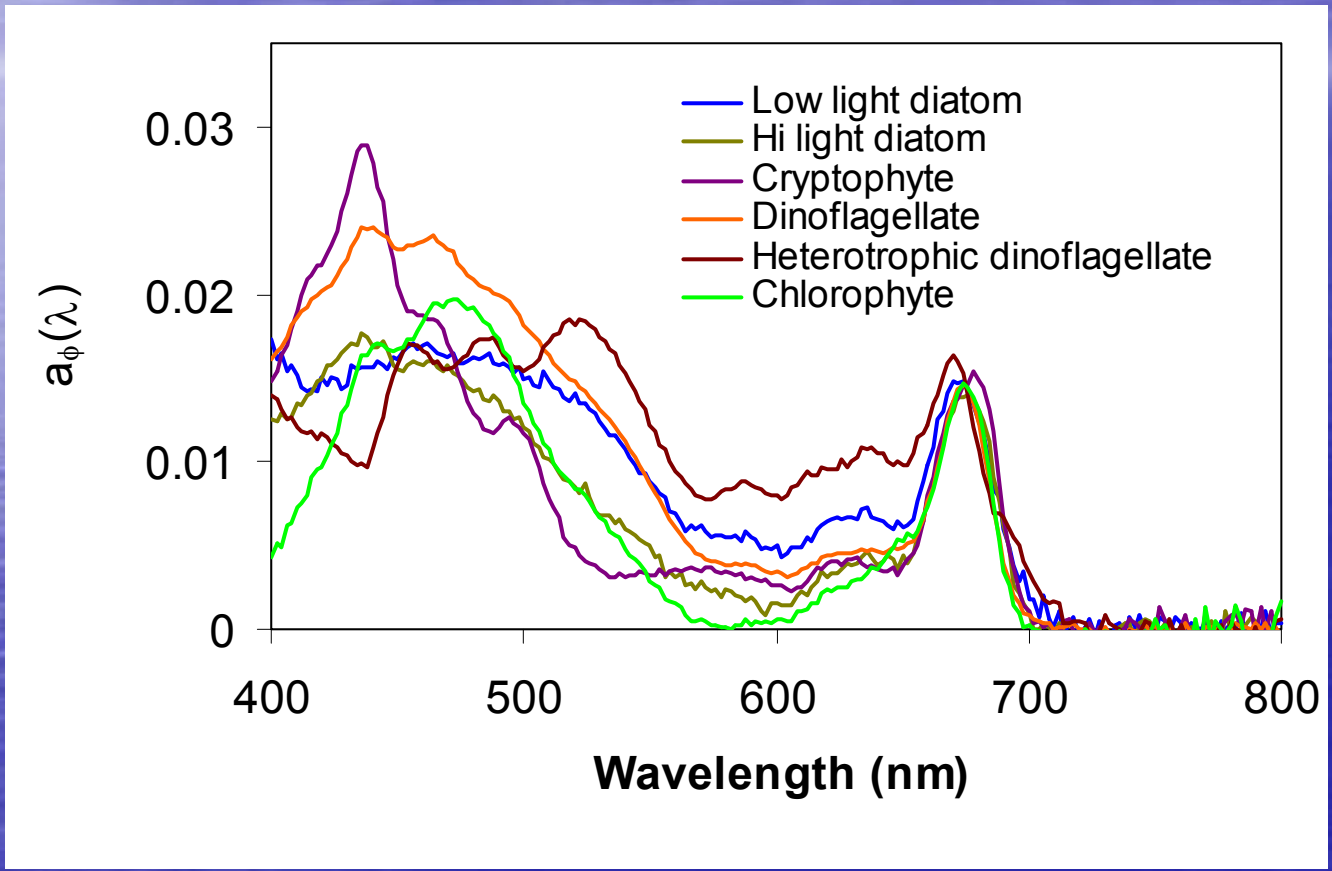


Fig. 10 Spectral dependence of the six phytoplankton absorption eigenfunctions used in the species-dependent reflectance inversion model in Fig. 9. Spectral differences are due primarily to pigment composition and secondarily to relative pigment concentrations.

Derived Species Composition:

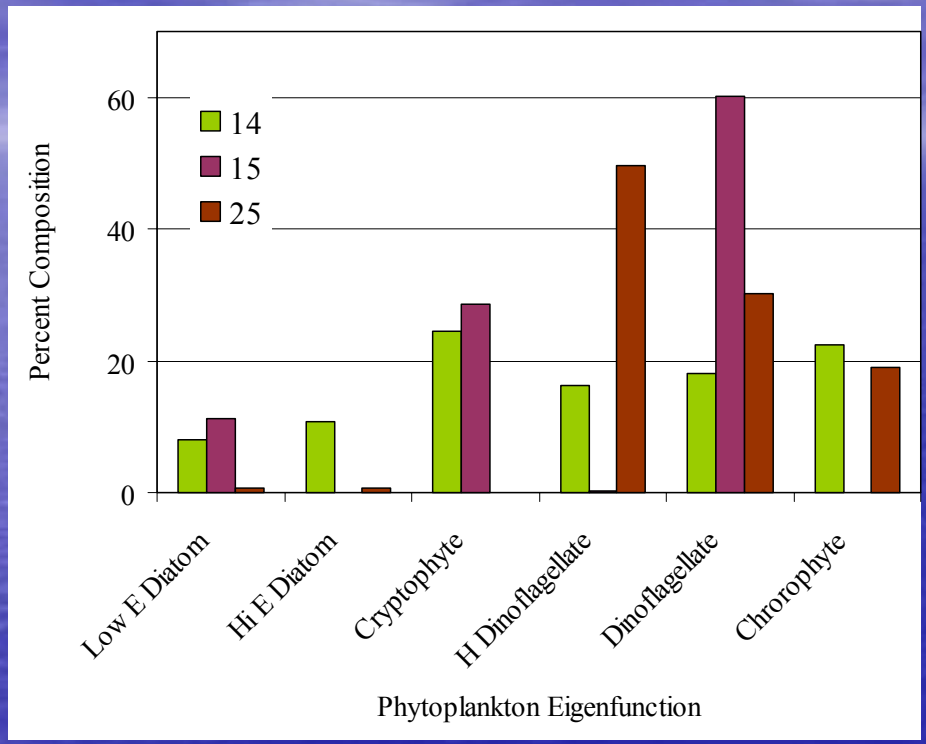


Fig. 11. Species composition derived from the inversion model in Fig. 9 for three dates in March 2001. The observed species composition for the three dates evolved from one mixed with significant diatom contributions (14); to dominance by a small *Gymnodinium* species and large *Mesodinium rubrum*, which contain a symbiotic cryptophyte (15); to a community dominated by the heterotrophic dinoflagellate *Zygobikodinium* sp., *Dinophysis* sp., responsible for diuretic shellfish poisoning, and a <5 μm chlorophyte (25). These observed results are consistent with the model derived results. The color of the bars approximates the observed water color. (Roesler, Etheridge, and Pitcher).

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Methods based on the optical properties of single cells:

THE FUTURE (based on present trends):

I. Bench top optical methods are being packaged for in-situ analysis on moorings, hydrocasts, and AUV (e.g. flow-cytometry).

II. Molecular techniques are combined with optical tags to provide genetic taxonomic information.

Methods based on the optical properties of bulk cells:

THE FUTURE (based on present trends):

I. Bench top optical methods are being packaged for in-situ analysis on moorings, hydrocasts and AUV (e.g. absorption spectroscopy).
II. Routine inversions of hyper-spectral remote sensing.