LOCO: Characterization of Phytoplankton in Thin Optical Layers

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LONG-TERM GOALS

Our long-term goal is to understand the ecology of phytoplankton inhabiting coastal shelves, upwelling areas, fjords and banks. We are especially interested in ways in which species-specific properties, including colony size and shape (diatoms) and motility (dinoflagellates) interact with physical mixing processes to regulate spatio-temporal distribution patterns. We wish to understand these processes in sufficient detail to be able to predict bloom dynamics, size structure, and the impact of species-specific characteristics of the phytoplankton on ocean optics.

OBJECTIVES

Our goals within the LOCO DRI program were (1) to thoroughly characterize the phytoplankton community within thin layers and compare it to that outside of layers, (2) to increase our understanding of the importance of species-specific characteristics of the plankton to both ecology and ocean optics, and (3) to expand our understanding of the role that biological-physical processes play in thin layer dynamics.

APPROACH

Under previous ONR funding (N000149610247, N000140210247), we have demonstrated that interactions between physical processes at multiple time and space scales, and the species-specific properties of diatoms and dinoflagellates (*e.g.* size, shape, behavior etc.) are important factors contributing to phytoplankton distribution, bloom dynamics, particle size structure and optical characteristics in the ocean. In order to continue this work within the LOCO framework, we have (1) adapted our earlier protocols for use in the open waters of Monterey Bay (*i.e.* exposed, coastal locations), and (2) developed methodologies that will allow us to collect new kinds of data, so that we can begin to investigate our 'next generation' of questions. In August/September of 2005, and in July 2006, we employed our refined protocols during the LOCO field experiments in Monterey Bay, California. Our primary effort was carried out in close collaboration with Donaghay, Sullivan,

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Holliday and Hanson. We are fortunate to have also had the opportunity to collaborate with many other PIs in the LOCO program.

WORK COMPLETED

During the course of this grant, our efforts were divided between the LOCO field program, and preliminary evaluation of our CytoSense scanning flow cytometer – http://www.cytobuoy.com/

LOCO: Our sample set from the LOCO experiments includes over 300 preserved whole water samples collected from inside and outside thin layers and surface slicks, about 30 offshore samples from *R/V New Horizon* (2005), live counts (2005) of the fragile dinoflagellate *Akashiwo sanguinea* (which does not reliably preserve), over 100 filter samples for epifluorescence-based image analysis of picoplankton, over 20 hours of videotaped record of microscopic examination of live phytoplankton, and about 70 CytoSense samples (2006). Analysis of samples has been prioritized to address specific questions, and to interact with other members of the LOCO team. Two manuscripts have been published in a special issue of Continental Shelf Research entitled *The Ecology and Oceanography of Thin Plankton Layers*, two additional manuscripts have been submitted by my students, and several others are in preparation. This is a large data set, which will remain invaluable for many years to come.

CytoSense/CytoSub Evaluation: We continue to develop methodologies for using this exciting instrument to further the long-term goals of our research program. To date, we have:

- Used CytoSense in bench top mode to quantify many different types (e.g. sizes, shapes, pigments, cell coverings) of phytoplankton in both natural field samples, and cultures.
- Deployed CytoSense *in situ*, and obtained *in situ* profiling data.
- Operated at multiple gain settings, in order to characterize different size classes of particles
- Improved data evaluation capabilities.
- Evaluated our ability to match CytoSense data to species-specific, microscopy-based observations made from discrete water column samples, as well as to *in situ* optical data.
- Conducted a preliminary evaluation of the potential to use CytoSense with fluorochrome dyes, in order to address an entirely new set of questions.

RESULTS

LOCO: During this program, Monterey Bay was home to an extraordinarily diverse community of phytoplankton and other protists comprising diatoms, photosynthetic, heterotrophic, mixotrophic and parasitic dinoflagellates, coccolithophorids, ciliates, cryptomonads, silicoflagellates, acantharians, cyanobacteria and other taxonomic groups (Figures 1 and 2). Bioluminescent dinoflagellates, and Harmful Algal Bloom (HAB) taxa were common. Although many of the same taxa were present in both 2005 and 2006, the dynamics of each year were quite different, and were correlated to hydrographic patterns. In 2005, the dinoflagellate *Akashiwo sanguinea* formed intense thin layers near the pycnocline at night (Figure 3), and migrated to near surface waters at dawn. This population was superimposed on a diverse background of other planktonic organisms, some of which also underwent vertical migration, and others (e.g. diatoms), which were non-motile. The healthiest diatoms were found in low concentrations at depth, near the sediment/water interface.

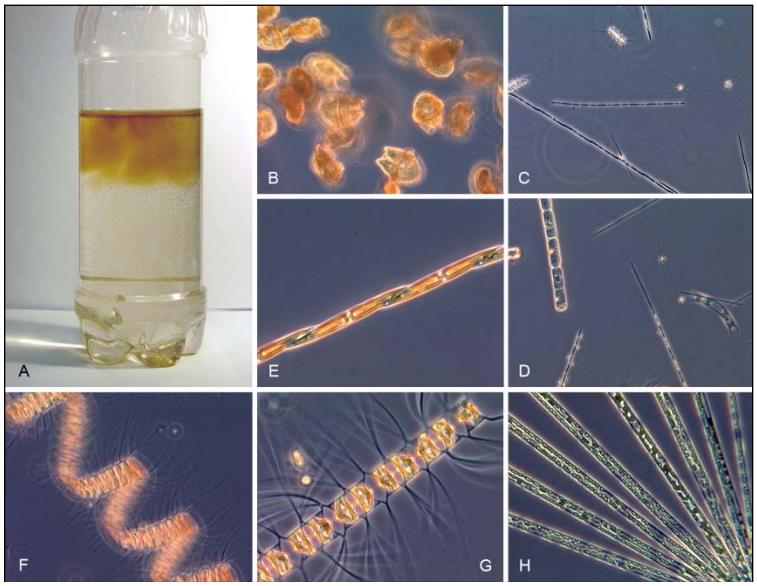


Figure 1. Representative phytoplankton from the 2005 experiment. (A) bucket sample from a nearly monospecific surface slick of Akashiwo sanguinea. (B-H) phase contrast photomicrographs, (B) cells in a single drop from a highly concentrated region of the bottle in A. (C-D) midwater diatoms in poor physiological condition. (E-H) healthy diatoms from just above the sediment/water interface, (E) Pseudo-nitzschia, (F) Chaetoceros debilis, (G) Chaetoceros protuberans, (H) Lioloma pacifica.

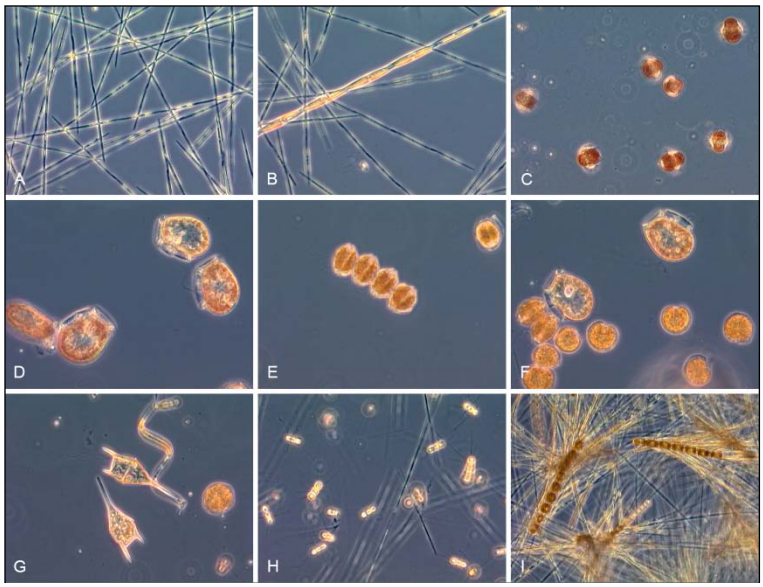


Figure 2. Photomicrographs of representative phytoplankton from the 2006 experiment. (A-B) Pseudo-nitzschia spp. from Regime I. (C) Myrionecta rubra, especially common during Regimes I and II. (D-F) taxa from the thin layer at ~ 3m during Regime II, (D) Dinophysis fortii, (E) four cell chain of Alexandrium catenella, (F) unicellular Alexandrium cf. catenella and Dinophysis fortii, (G) Ceratium lineatum and Proboscia sp. from the thin layer at ~ 5 m during Regime II. (H-I) taxa from Regime III, (H) Chaetoceros cf. perpusillus and Pseudo-nitzschia sp. from the upper water column, (I) Chaetoceros concavicornis from a layer at depth.

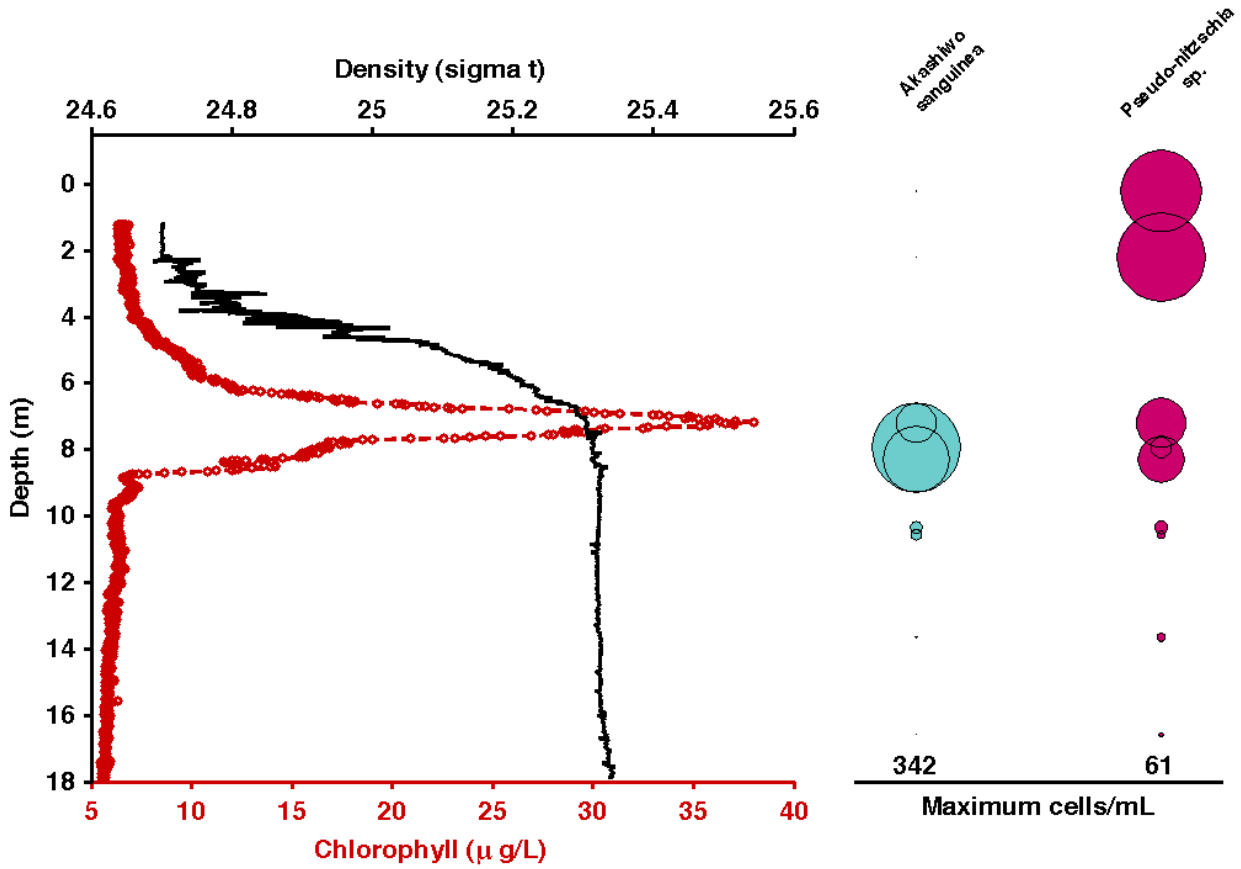


Figure 3. Vertical distribution of selected thin layer-forming, and non-layer-forming taxa in relation to density and chlorophyll on 26 August 2005 at 23:14 h. For each taxon, circle size is proportional to cell concentration normalized by the maximum observed concentration in this profile. Numbers under circles indicate the maximum observed concentrations (cells ml^{-1}) for each taxon. When this nighttime profile was taken, an intense chlorophyll thin layer was present at ~ 7 m depth. Nearly all of the Akashiwo sanguinea cells were located here, with concentrations as high as 342 cells ml^{-1}, whereas other taxa such as Pseudo-nitzschia sp. were located elsewhere in the water column.

In 2006, hydrography varied between periods of vertical mixing and periods of increased stratification and enhanced biological productivity, and could be divided into three successive phases, or regimes. Phytoplankton were very patchy in spatial distribution, and communities varied in floristic composition. As a result of patchy horizontal patterns, advection of water masses through the array resulted in dramatic shifts in community composition. Each hydrographic regime contained its own phytoplankton community and pattern(s) of vertical structure. A representative 2006 profile (Regime II) is depicted in Figure 4 (for examples from Regimes I and III, please see Rines *et al.* 2010).

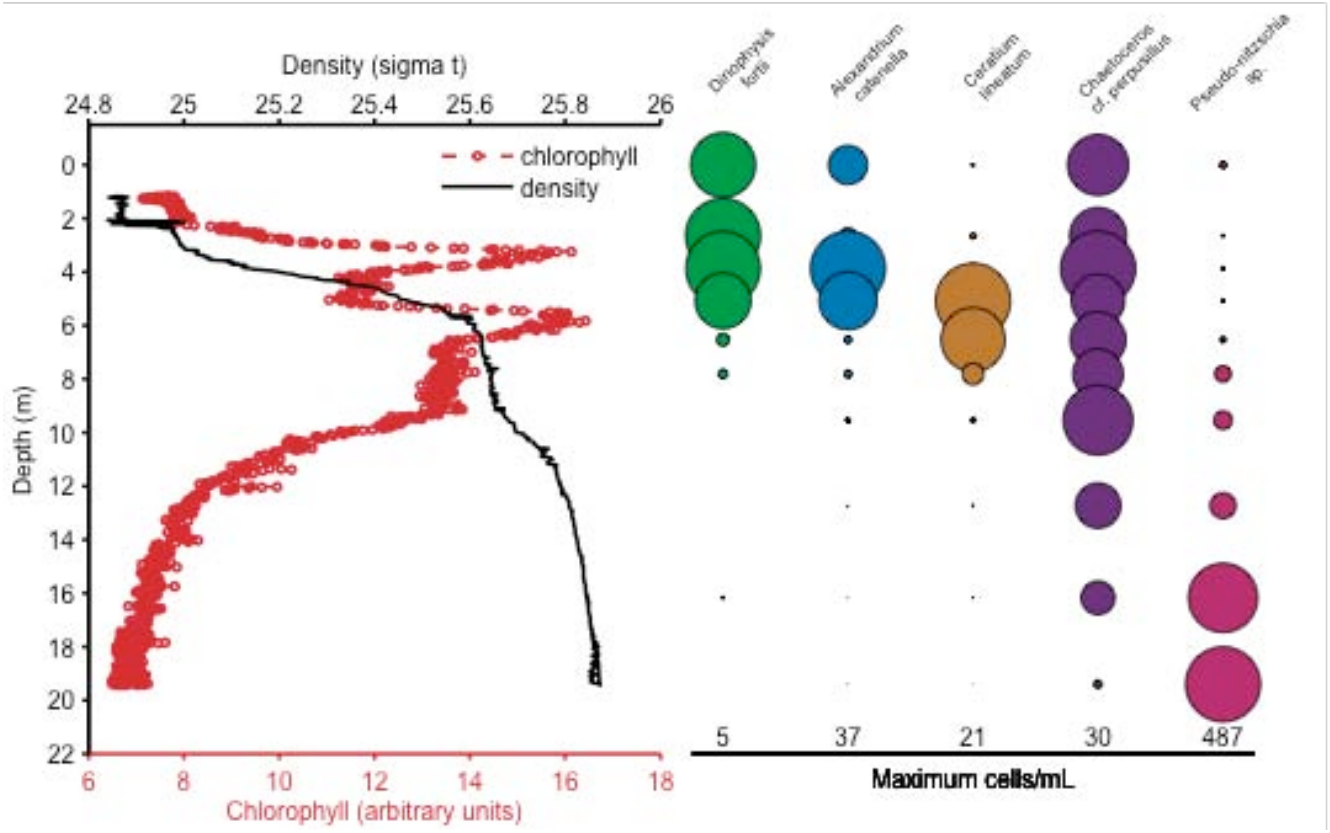


Figure 4. Vertical distribution of selected taxa from inside, and outside of thin layers in relation to density and chlorophyll on 19 July 2006 (Regime II). For each taxon, circle size is proportional to cell concentration normalized by the maximum observed concentration in this profile. Numbers under circles indicate the maximum observed concentrations (cells ml^{-1}) for each taxon. Chlorophyll profiles reveal thin layers at about 3 and 5 meters. The vertical distribution of 5 different species of phytoplankton shows distinct, species-specific patterns of distribution. Some taxa are restricted to thin layers, whereas others are independent of these structures. Chlorophyll is not necessarily a good predictor of cell abundance.

Our extensive LOCO data set, coupled with those of our colleagues, allowed us to address many of our originally proposed questions. We found that:

- Thin layers can be composed of an enhanced concentration of the total integrated phytoplankton community, or can be dominated by a particular taxon, or size/shape class.
- Spatially extensive layers can be taxonomically uniform.
- Many different taxa and size classes of phytoplankton are capable of forming layers.
- Both motile, and non-motile phytoplankton can form layers.

CytoSense Evaluation: Our CytoSense scanning flow cytometer (Figure 5) was specifically designed to study the size, shape, physiological and optical properties of phytoplankton colonies and individual cells within the colonies, but it can also be used for the more traditional analyses of small, single cells.

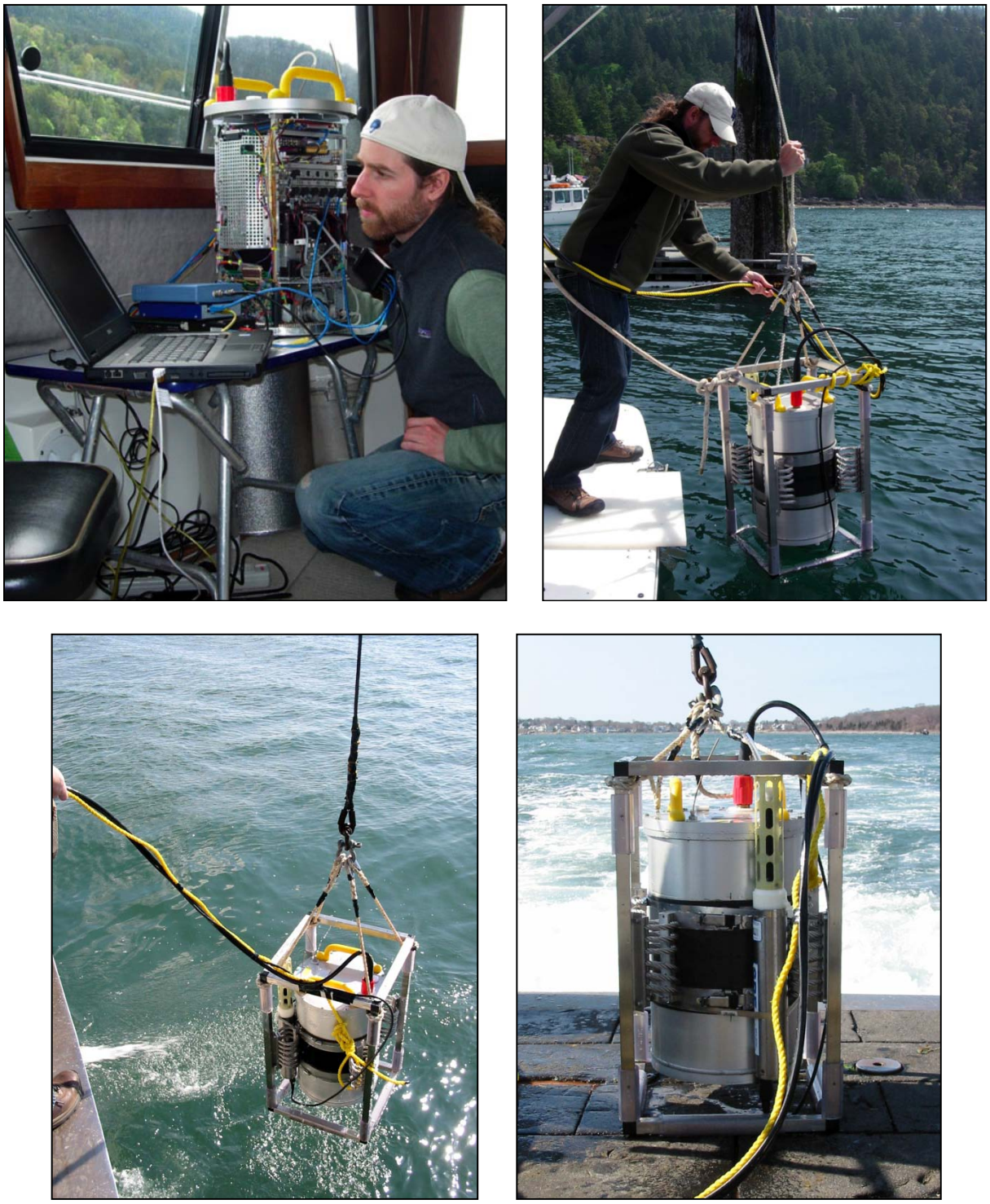


Figure 5. Clockwise from top left, discrete water samples are run through the instrument on deck, CytoSense is deployed from the dock for testing prior to successful experimental deployment in situ, CytoSense sits on deck in its underwater housing, and lastly, lowered into the water for a profile.

It operates in both bench-top, and *in situ* mode. It streams near-real-time, multi-channel data on the size and optical properties of each particle as it flows past the sensors, creating a detailed scan of thevariations in complexity of each parameter at 0.5 µm resolution over the length of the particle. Our

instrument contains a blue (488nm) laser, and sensors to measure forward scatter, side scatter, red, orange, yellow and green fluorescence, and curvature. Data from each sample can be summarized as scatter plots (Figure 6). Each dot represents a discrete particle, and the full pulse profile can be called up for each one (*e.g.* Figures 7, 8). This instrument is ideally suited for our continuing investigations of the species-specific interactions between cell/colony size and morphology of the larger phytoplankton (*e.g.* diatoms and dinoflagellates), small-scale turbulence, and optics!

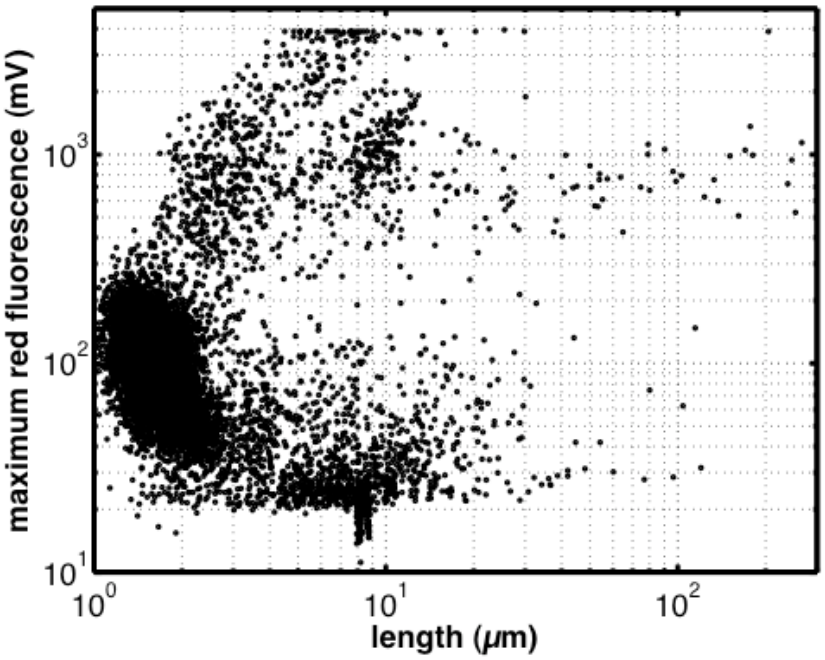


Figure 6. A typical scatter plot of CytoSense field data. This graph summarizes the particles quantified and is plotted as maximum fluorescence in the red channel vs. particle length. Clusters of similar organisms are obvious, but most interesting to us are the relatively rare, large outliers in the upper right quadrant. These are beautiful, large, chain forming diatoms! We can learn a lot about them by examining each pulse profile, however, we would be able to make a far more precise match of CytoSense data to the organisms by adding the new 'Image-in-flow' module to our instrument, which will provide photomicrographs of user-targeted particles based on their pulse profiles.

Many of the morphological and cytological features that can be seen in the microscope can be quantified in CytoSense's pulse profiles. Figures 7 and 8 provide examples of diatoms characterized by markedly different morphology. Now that we have demonstrated that CytoSense can be used to collect quantitative data in both laboratory and field settings, we can pursue research on the impact of turbulence on chain forming diatoms, and on the optical properties of individual particles. We are currently using this exciting instrument to increase our understanding of the species-specific biology of the organisms, and their impact on the IOPs of the water column.

In 2009 and 2010, CytoSense was extensively used in conjunction with three separate, but closely related field experiments in East Sound, WA. We employed the instrument both on deck, and *in situ* (Figure 5). The scientific results of this project are reported upon in the 2009 and 2010 ONR Annual Reports of Donaghay, Rines & Sullivan. We were thrilled that CytoSense proved so effective at collecting data, that we were able to do far more than allowed for in our original experimental design!

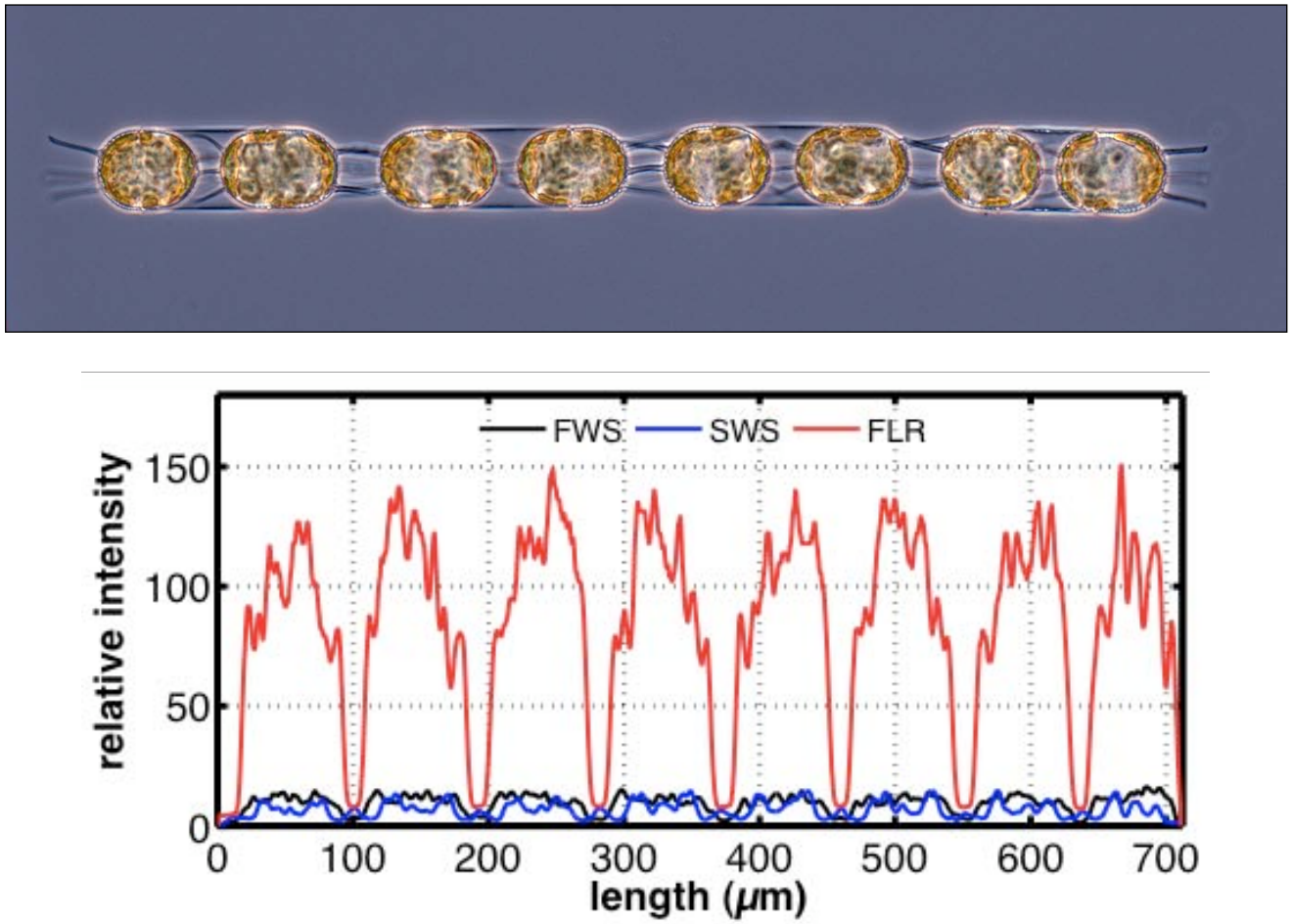
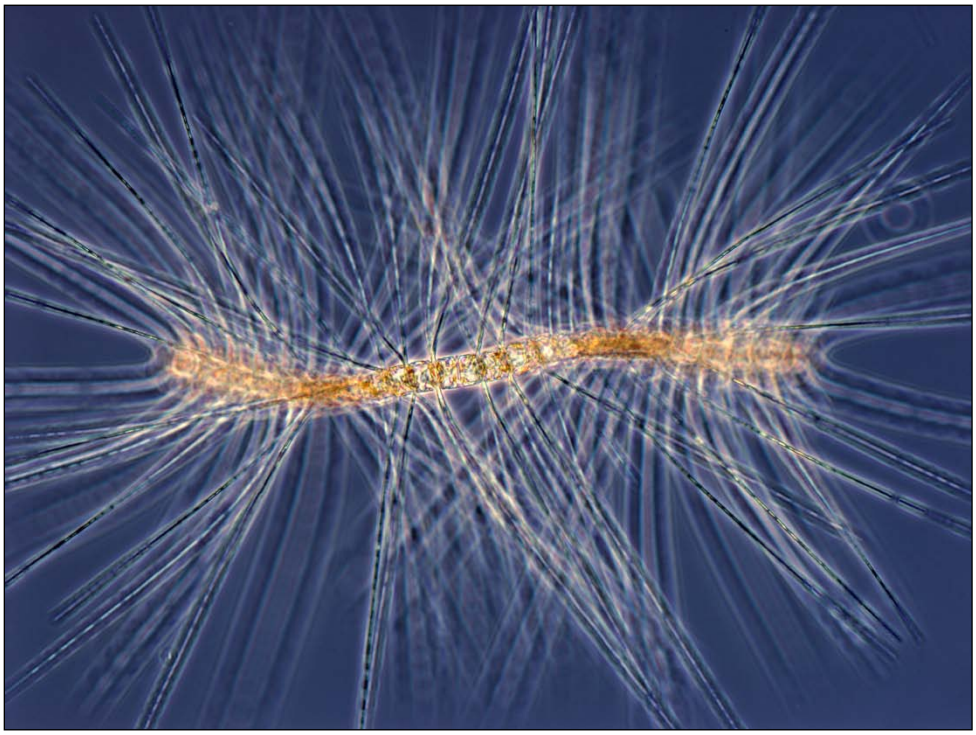


Figure 7. Stephanopyxis turris (cultured material). The fairly simple morphology of this diatom produces a repetitive signal. Chloroplasts are tightly packed in each cell, resulting in discrete pulses of strong red fluorescence. There are 8 cells in this chain. Above, a phase contrast photomicrograph. Below, a CytoSense pulse profile from a similar colony depicting changes in forward scatter, side scatter and red fluorescence over the length of the particle.



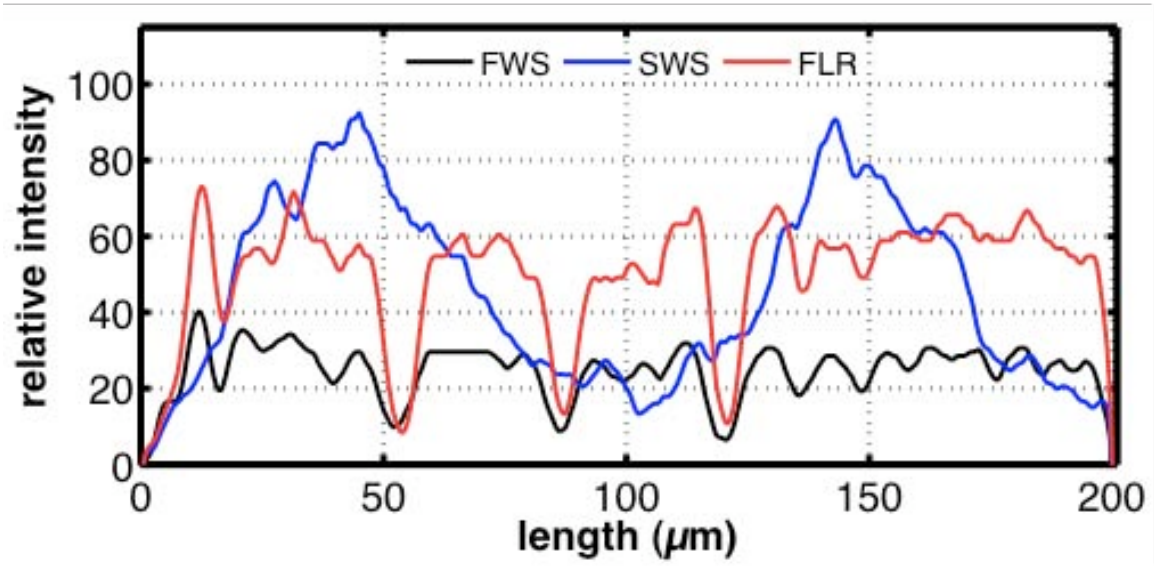


Figure 8. Chaetoceros densus (cultured material). This diatom is characterized by siliceous spines, or setae, which project in 360° around the colony axis and produce strong side scatter. Torsion along the colony axis adds to morphological complexity. Chloroplasts migrate in and out of the setae in response to light levels, and alter the spatial distribution of pigment. Colonies can reach ~ 1 mm in length. Above, a phase contrast micrograph. Below, a CytoSense pulse profile from a similar colony depicting changes in forward scatter, side scatter and red fluorescence over the length of the particle.

We now turn our attention to the possibilities of using CytoSense in an entirely different way, by incorporating fluorochrome dyes for the study of intracellular and colonial structures and physiological processes. These compounds can be used with both epifluorescence microscopy, and flow cytometry. One example is SYBR Green, which readily penetrates living cells, and binds with nucleic acids. The DNA-SYBR complex absorbs blue light and emits green light, and thus is compatible with CytoSense's optical systems. This technique can be used to address questions related to phytoplankton vitality, cell cycles and ploidal levels. This data is relevant to elucidating the developmental mechanisms that regulate colony morphology and size, which ultimately impact both particle, and *in situ* optics. Figure 9 depicts SYBR stained material visualized with microscopy, and Figure 10 demonstrates that CytoSense can quantify SYBR fluorescence, and thus will be invaluable in this kind of research.

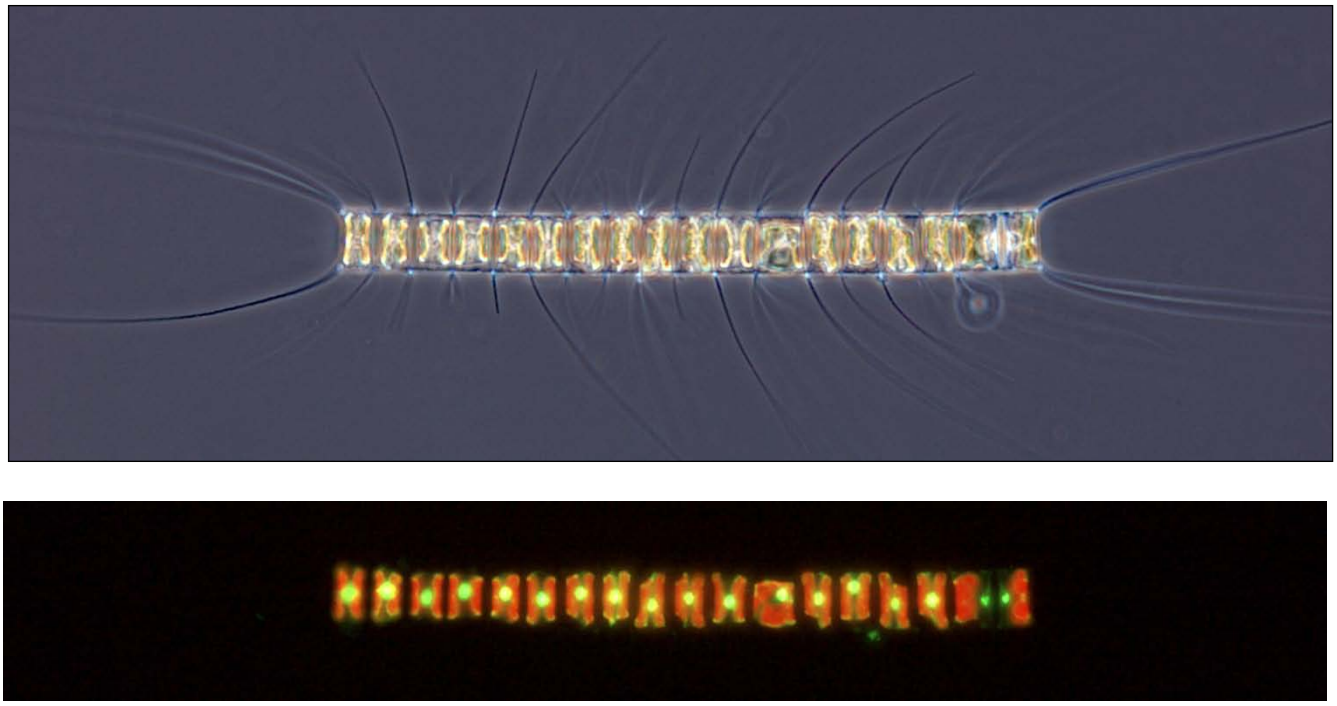


Figure 9. Light micrographs of a colony of Chaetoceros vanheurckii, isolated from East Sound, WA. This material has been stained with the fluorochrome dye SYBR green. Top, colony viewed with phase contrast microscopy. Note the siliceous spines, or setae. The cell at the right end of the chain is in the process of mitotic division. Bottom, the same colony viewed with epifluorescence microscopy. Each cell's two chloroplasts glow red. The SYBR green stained nuclei are yellow/green. Note the recently divided nuclei are smaller than the others in the chain, indicative of their different cell cycle phase.

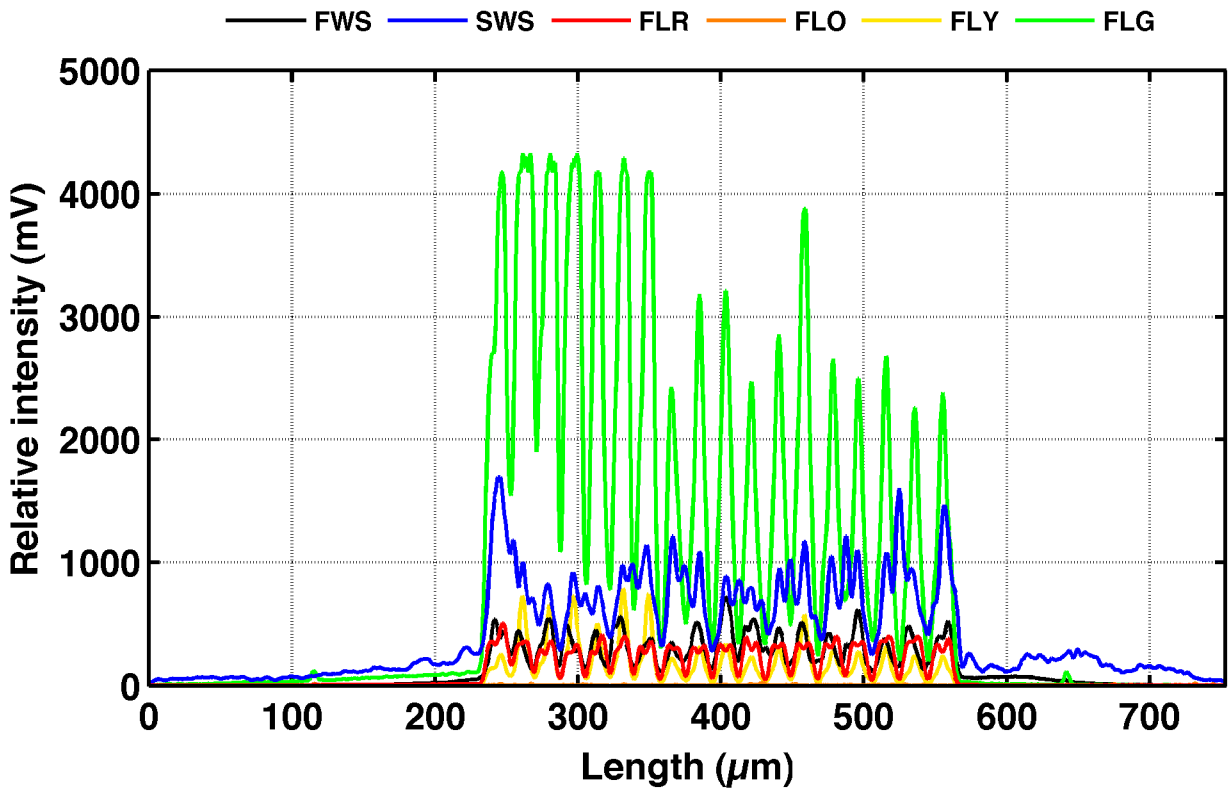


Figure 10. A CytoSense pulse profile of a SYBR Green stained Chaetoceros vanheurkii colony. This is the same sample, but a different colony than the one depicted in Figure 9. The blue trace shows the large amount of side scatter generated by the siliceous setae (spines). Red depicts chlorophyll fluorescence. The very strong green channel signal is the SYBR stained nuclear material. In this example, the left half of the colony has generated almost twice the signal as the right half, suggesting that the cell cycle phases of the two ends of the colony are offset.

IMPACT/APPLICATIONS

Thin Layers of phytoplankton are important features of the coastal ocean. However, they don't exist in isolation – they are a component of the biological and hydrographic dynamics of the entire water column, and must be studied as such. Thin layers may simply contain an enhanced concentration of the phytoplankton community found throughout the water column, but frequently they contain a unique flora, with layers at different depths dominated by different taxa. Patterns exist at multiple scales. In addition to species-specific differences, we have demonstrated that *groups* of organisms (*e.g.* diatoms, dinoflagellates and picoplankton) can exhibit separate patterns of vertical distribution, thus different processes must regulate their dynamics. These relationships are not static: layers of motile organisms may migrate in and out of other structures. Thus, there may be many simultaneously occurring and kaleidoscopically interacting patterns, operating on multiple spatio-temporal scales.

Once characterized, the implications of these features of the water column can be explored. Thin layers are of interest for multiple reasons, including their potential impact on both the oceanographic environment, and Navy sensor systems. For example, high-biomass thin layers affect the inherent

optical properties (IOPs) of the water column (Sullivan *et al.* 2005, Sullivan *et al.* 2010), and because they offer dense concentrations of food potentially attractive to zooplankton and planktivorous fish (acoustical scatterers), they also impact ocean acoustics (Holiday *et al.* 2003, Holliday & Stanton 2005, Holliday *et al.* 2010). But we also found that several low-abundance taxa appeared to be restricted to narrow depth intervals in the water column. These include some Harmful Algal Bloom (HAB) taxa, which can be toxic at incredibly low concentrations. Even at low densities, they may constitute functional thin layers, with large ecological impact even if their biomass is too low to dominate an optically defined thin layer. Moreover, life within a thin layer may facilitate finding food, a mate, or other concentration-dependent resources. Therefore, from the perspective of the organism, thin layers can be viewed as *critical scale* phenomena, which may be essential to their ecological success.

Species-specific properties of phytoplankton such as size, shape, biomineralization, pigment composition and toxin production are known to play important ecological and oceanographic roles. However, the classical 'form and function' questions remain largely unanswered (Sournia 1982), and to my mind are amongst the most fascinating in biological oceanography. Our CytoSense flow cytometer gives us a new, innovative tool with which to pursue the significance of particle variability with respect to biological/ecological questions, and also from the perspective of impact of species-specific properties of the phytoplankton on ocean optics. This instrument does not replace a microscope – its tremendous power lies in generating data to link IOPs to the highest quality, detailed microscopic images that we can obtain of the organisms themselves. We believe that CytoSense can quantify the optical properties of plankton is such a detailed way that it will both revolutionize our studies of phytoplankton ecology, and provide data critical to linking microscope-based studies of the species-specific properties of phytoplankton to the *in situ* inherent optical properties (IOPs) measured by our team.

Our species-specific characterization of phytoplankton in thin optical layers and throughout the water column of Monterey Bay during the LOCO experiments has revealed more complexity of structure than we had imagined possible for open coastal systems, and has allowed us to challenge the theoretical framework within which we study phytoplankton ecology. Further advances will come with the next generation of technological innovation – smart samplers, which can be mounted on the ORCAS profilers, and programmed to autonomously collect water samples when sensor data meets specific criteria. Integration of fluorochromes and molecular technologies will allow us to study specific organisms, even those that are rare. With *in situ* CytoSense scanning flow cytometry, we can probe the properties of individual cells and colonies within an interdisciplinary oceanographic framework. Moreover, such a system can operate around the clock in all weather, and circumvent the difficulties of trying to target a micro-scale feature from a moving ship platform, in a sea of internal waves. We predict that the future will bring discovery of even more fascinating ecological complexity!

RELATED PROJECTS

During 2009 and 2010 field work in East Sound, Washington, we extensively utilized our CytoSense flow cytometer to pursue the goals of our current ONR projects: *In situ* quantification of the impact of episodic enhanced turbulent events on large phytoplankton (Donaghay, Rines & Sullivan), and *In situ* validation of the source of thin layers detected by NOAA airborne fish lidar (Donaghay, Sullivan, Rines & Churnside). We also collaborated closely with Dr. Alan Weidemann (NRL, Stennis), who was conducting a related project as part of this effort.

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Rines, J., McFarland, M., Donaghay, P., Sullivan, J. (2010) Thin layers and species-specific characterization of the phytoplankton community in Monterey Bay, California USA. *Special Issue*: The Ecology and Oceanography of Thin Plankton Layers, Continental Shelf Research 30: 66-80, refereed. http://doi:10.1016/j.csr.2009.11.001
Note: This paper was listed as one of the journal's 10 most downloaded publications!

Sullivan. J.M., Donaghay, P.L., **Rines, J.E.B.** Coastal thin layer dynamics: consequences to biology and optics. *Special Issue*: The Ecology and Oceanography of Thin Plankton Layers, Continental Shelf Research 30: 50-65, refereed.

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