Impact of Turbulence And Growth Rate on the Scattering Signatures OF Marine Phytoplankton

William M. Balch
Bigelow Laboratory for Ocean Sciences
P.O. Box 475, McKown Point
W. Boothbay Harbor, ME 04575
Phone (207) 633-9600 Fax (207) 633-9641 INTERNET bbalch@bigelow.org
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LONG-TERM GOALS

The long-range goal of my ONR-sponsored research has been to use bio-optical techniques to understand the distributions of phytoplankton, in space and time in the sea. An important control in this effort is to examine physical factors which affect particle size and shape (which would change their optical volume scattering function). Turbulent shear is one such process which can have profound effects on the shape of large cells, and the length of algal chains and is the focus of my work in year 1. In year 2, I focused on a method for identifying particles in the sea using their depolarization scattering properties. Phytoplankton species contain unique arrays of organelles and sub cellular particles which depolarize light to varying degrees. I am using the angular dependence of depolarization to aid in identification of phytoplankton species (and other particles).

OBJECTIVES

The objectives of this work are to:

Year 1

- Examine the role of micro-scale turbulence in the rate of aggregate formation in filtered sea water, and its subsequent impact on the bulk volume scattering function.

-Examine the role of micro-scale turbulence in affecting the volume scattering functions of a range of phytoplankton species. Turbulence will be varied from those typical of quiescent mid-water environments, highly tidally-mixed environments and extremely high turbulence levels found in a flow-through fluorometer or beam transmissometer.

Year 2

-Measure the volume scattering function of various phytoplankton species and inorganic particulate material, either with vertical polarization (same as the incident laser beam), or after the plane has been rotated 90º to the horizontal. The objective is to verify the conservative nature of the ratio of these two volume scattering functions (β_h/β_v) versus solid angle within an algal taxa (such that they could be used for identification along with other traditional optical indicators such as fluorescence and/or absorbance).

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APPROACH

Turbulence generation

To generate quantified micro-scale turbulence, an oscillating grid approach has been used, in which a grid is reciprocated through a cylindrical optical cuvette at a precise rate using a computer-driven stepping motor. Calculation of the energy dissipation rate (ε; Watts kg^{-1}) imparted to the sample requires measurement of the stress to push the grid through the water (S= Newton's m^{-2}; measured using a precision balance and knowing the area of the bottom of the cuvette through which the stress is conveyed), the volume of fluid which passes through the grid (V_{grid}; m^{-3}) and T_{stroke}, the time for the grid to move from the top of the stroke to the bottom (1/2 of a complete cycle). We must also correct for any Archimedes effect from the grid shaft which moves in and out of the fluid. Energy dissipation is calculated as:

$$\varepsilon = [(S \times V_{grid} / T_{stroke}] / (V_{cuvette} \times \rho)$$

where $V_{cuvette}$ is the cuvette volume ($V_{cuvette}$; m^3) and ρ is density ($kg\ m^{-3}$). When all of the fluid moves by the grid in one stroke, then the above equation simplifies to:

$$\varepsilon = (S \times \rho) / T_{stroke}$$

With knowledge of the kinematic viscosity (γ; $m^2\ s^{-1}$), the equivalent turbulent shear (G; s^{-1}) is calculated as:

$$G = (\varepsilon / \gamma)^{1/2}$$

Finally, the Kolmogorov eddy length scale (η ; m) is calculated as:

$$\eta = (\gamma^3/\varepsilon)^{1/4}$$

Given quantitative energy dissipation, volume scattering of the samples are monitored using a laser light scattering photometer. The approach to the control experiments is to quantify any changes in volume scattering as filter-sterilized sea water is examined in a sterile cuvette. For the experimental samples, cultures are carefully introduced into the cuvette, and short-term (several minutes) measurements are made at a given energy dissipation level. The process is repeated several times, with and without turbulence to verify the values. Microscopic enumeration is used to estimate cell concentration and chain length.

Polarization Experiments

Cells and/or inorganic particles are suspended in 0.2µm filtered sea water and placed into our Wyatt light-scattering photometer. Half of the 14 volume scattering detectors are covered with a vertically polarizing filter. The other half are covered with a horizontally-polarizing filter. The incident light is vertically-polarized output of an Argon Ion laser (512 nm). The instrument is absolutely calibrated with a solid scattering standard supplied with the instrument. We also routinely perform checks of the calibration using ultra-filtered distilled water (HPLC grade water pre-filtered through a 0.02µm anodisc

filter). Suspended samples of cells or inorganic particles are inserted into the instrument and horizontally-polarized and vertically-polarized volume scattering measured in 10 replicate scans (10 s of scanning, at a detector scan rate of 400hz). The contribution of water volume scattering (horizontally or vertically polarized) was subtracted from all sample volume scattering, in order to estimate the particulate volume scattering. The Beardsley-Zaneveld (1969) function was then fit to the vertically-polarized results, and used to predict vertically-polarized volume scattering at the same angles used for horizontally-polarized volume scattering. The ratio of horizontally-polarized, particulate volume scattering to vertically-polarized, particulate volume scattering ($\beta_{\theta H}/\beta_{\theta V}$) was calculated at 7 common angles.

WORK COMPLETED

Turbulence Experiments

More control experiments were performed this year which involved adding 0.2μm pore-size filtered sea water to a pre-sterilized, ultra-clean cuvette. This water was continuously circulated through a 0.2μm pore-size filter cartridge while the cuvette was sitting in a Wyatt Dawn laser light scattering photometer. When the volume scattering function and backscattering values stabilized at values seen in pure sea water, filtration was stopped, and the volume scattering function was monitored over time either with no turbulence imparted to the sea water, or with the grid oscillating. As aggregates increased in size, the change in volume scattering was measured.

Species of phytoplankton were grown in batch culture in K media (with Si added for diatoms). They were carefully transferred to the optical cuvette, and measurements first were made on the stability of their volume scattering function in quiescent water. Then shear was imparted by oscillating the grid and again watching the volume scattering function. Care was taken to minimize shear when sampling cells for microscopy. More species were examined in year 2 to examine for any turbulence effects on cellular volume scattering. In particular, we focused on large algal species that formed chains. We also performed experiments in which we examined aggregate formation in a sterile environment. Finally, using several algal species, we concentrated on the time scales of optical changes caused by turbulence. The reason for this was that changes over hours of treatment were often permanent (chain breakage due to shear) while changes over minutes appeared to be reversible (chain stretching from short-term shear, chain alignment when sinking in quiescent conditions, etc.). Microscope counts were used to verify changes in chain length between treatment and controls.

Polarization Experiments

Depolarization scattering "signatures" of 21 particle types (14 species of phytoplankton and 7 types of inorganic particles found in case II waters) were performed. Phytoplankton species were examined during logarithmic growth phase. The $\beta_{\theta H}/\beta_{\theta V}$ of each particle type was examined at variable dilution to insure optimal signal:noise ratio in the scattering measurements. The contribution of water was subtracted from all data, in order to examine the polarization ratios of the particulate material.

RESULTS

Turbulence Experiments

We performed additional control experiments during year 2 of this grant and once again observed submicron aggregation/polymerization occurring, which affected particle scattering. These experiments were in sterile media. Filter sterilized sea water increased its particulate backscattering when shear was imparted to the media. If this water is filtered again, the process will repeat itself. But note, cells do not pass the filtration process, so the aggregation likely represents the production of biologically active, sub-micron particles . As part of these experiments, we have established the time constants of the process.

In terms of the species volume scattering functions, relatively minor changes were observed as the equivalent turbulent shear was increased from quiescent conditions to ~2 s^{-1}. Such results even apply for large diatoms such as *Ditylum brightwellii*, dinoflagellates such *Gymnodinium catenatum*, all the way down to small diatoms such as *Skeletonema costatum*.. Remaining species measurements have been completed during year 2, and our conclusion still holds, the volume scattering function of phytoplankton appears to be relatively stable over a range of turbulence levels. Short term changes are evident in some chain-forming species, as shear causes chain distortion, or randomization of chain orientation.

Polarization Experiments

Depolarization volume scattering signatures ($\beta_{\theta H}/\beta_{\theta V}$ vs. angle) are significantly lower in the forward angle (35.5o) for phytoplankton than for suspended minerals (with one exception, the mineral Benitoite ($BaTi(SiO_3)_3$). Depolarization signatures at higher angles did not show the differences between minerogenic particles and organic matter. Sometimes the difference in this ratio is as great as a factor of 2. It also should be noted that opal, $CaCO_3$ and clay sediments, which dominate the sea floor, all showed significantly higher $\beta_{\theta H}/\beta_{\theta V}$ ratios in the forward angle than marine phytoplankton, and at concentrations as low as 1PPM. While phytoplankton species identification does not appear to be possible using depolarization signatures, the forward angle $\beta_{\theta H}/\beta_{\theta V}$ data allows almost unambiguous separation of organic and inorganic contributions. Experiments will begin shortly with mixtures of phytoplankton and sediments, to test the discrimination of case I and II waters using this optical analysis.

IMPACT/APPLICATIONS

The first impact of these results is based on the control experiments in which variability on the order of 2-3X was observed in the backscattering of 0.2μm-filter-sterilized sea water. This variability likely represents the aggregation of biologically-active, sub-micron particles. Since dissolved and particulate scattering are usually calculated by difference between an unfiltered and 0.2μm-filtered samples, then if the scattering of dissolved blanks vary by 2-3X, this will translate to associated differences in particle scattering blanks. These results also have consequence to understanding reflectance in the sea because reflectance is a function of absorption and backscattering (Gordon et al., 1988). Higher backscattering attributed to a dissolved "blank" will mean that the backscattering (and reflectance) attributed to particulate material will be similarly reduced.

The other impact of this work is that if we can assume that volume scattering functions of particulate matter are stable under variable turbulence in the sea, then scattering changes can be attributed to processes of particle production, destruction, advection, upwelling and sinking, not *in situ* changes associated with transient conditions of micro-scale turbulence. That is, turbulence measurements over

short time scales apparently are not required to interpret optical data. The secondary factor of cell sinking is obviously closely tied to turbulence via particle suspension, however, which can obviously affect the inherent optical properties of the water column.

The depolarization signature results allow the detection mineral "contamination" in sea water, which signals the transition between case I and case II waters. Long a major hurdle in optical oceanography, discrimination between case I and II waters requires concurrent changes in pigment algorithms, and model coefficients. Thus, easy detection of suspended sediments by using forward angle $\beta_{\theta H}/\beta_{\theta V}$ ratios will allow better application of optical algorithms.

TRANSITIONS

The project is now in the last phase of the depolarization experiments in which we will examine more phytoplankton species, and mineral types.

RELATED PROJECTS

Collaborative relationships are maintained with Dr. Ken Voss and Dr. Howard Gordon, both ONR-funded investigators at the University of Miami Dept. of Physics. Discussions with Dr. Alan Brandt (Applied Physics Laboratory, Johns Hopkins University) were very helpful in the experimental design as well as the interpretation of the oscillating grid results.

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