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Millimeter scale profiles of chlorophyll fluorescence: Deciphering the microscale spatial structure of phytoplankton

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ABSTRACT

Marine food webs and biogeochemical cycles are driven by interactions between individual phytoplankton and other micro-organisms embedded within turbulent flows. Understanding the causes and ecological consequences of these interactions requires measurement of the spatial distribution of organisms across sub-meter scales relevant to their activities. However, estimates of many microscale processes (e.g., encounter rates, competition) are implicitly based on a random distribution of plankton despite increasing evidence of patchy distributions of turbulence and phytoplankton at the oceans microscale. Further complicating our understanding of microscale phytoplankton ecology, recent studies have suggested that the high levels of fluorescence variability measured at sub-centimeter scales may be due to the detection of separate, large phytoplankton particles (i.e. large cells, chains and aggregates) rather than 'patches' of increased cell abundances. By comparing coincident fluorescence estimates measured with millimeter (μL) and centimeter (mL) scale resolution, we show that estimates of phytoplankton biomass made at centimeter scales are consistent with averaging discrete variations in fluorescence measured at millimeter scales and that a critical scale exists where measures of fluorescence variability transitions from representing an individual to a patch. Application of nearest neighbor analysis to the discrete fluorescence patterns showed deviations from complete spatial randomness towards clustering across scales of millimeters to tens of centimeters. The strength of the deviation from random increased significantly in regions of elevated phytoplankton concentrations. No relationship was observed between fluorescent particle concentrations or nearest neighbor distances with the rate of dissipation of turbulent kinetic energy. Our results provide empirical evidence that the scale at which phytoplankton distributions are estimated by chlorophyll fluorescence may be critical to the interpretation of microscale phytoplankton spatial patterns and that biological processes may strongly influence the microscale structure and ecology of marine systems.

1. Introduction

A microscopic inspection of seawater reveals that plankton and many of the resources for which they compete are distributed discretely in a dynamic seascape of organic matter (Azam and Malfatti, 2007). For example, phytoplankton occur as individual cells, chains or aggregates, all of which are competing for nutrient molecules as well as providing food for grazers and loci for bacteria and viruses. Embedded within turbulent flows, interactions among phytoplankton, other organisms and biogenic particles drive plankton trophodynamics and biogeochemical cycling with cumulative influences for ocean systems predicted across multiple scales (Azam and Worden, 2004). Advancing our understanding of the influence of individual interactions between organisms on marine systems therefore requires the measurement of phytoplankton distributions (as well as other microscopic organisms) and turbulence across the nanometer to centimeter scales (microscale) relevant to their activities (Azam and Malfatti, 2007).

Phytoplankton play a central role in plankton ecology and are strongly influenced by turbulence across its entire size spectra (Kiørboe, 1993; Martin, 2003; Jumars et al., 2009). At the smallest dissipative scales of turbulence, eddies of the order of millimeters interact intimately with phytoplankton influencing many fundamental ecological processes such as behavior (Dower et al., 1997), nutrient uptake (Karp-Boss et al., 1996), plankton trophodynamics (Rothschild and Osborn, 1988) and organism growth (Peters et al., 2006). However, since microscale turbulent motions have typically been regarded to be a homogenizing factor, the modeling of microscale planktonic processes such as encounter rates (Rothschild and Osborn, 1988), coagulation (Jackson, 1990),

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nutrient competition (Siegel, 1998) and ecosystem metabolism (Rothschild, 1992) have implicitly assumed a random (Poisson) spatial distribution of particles. Progress in our understanding of the microscale structure of turbulence at the scale of phytoplankters (Jumars et al., 2009) now reveals that vortical motions may lead to aggregation (Crimaldi et al., 2006). Similarly, biological processes such as reproduction have the potential for causing non-random, aggregative distribution patterns (Young et al., 2001). As technological advances allow for the measurement of phytoplankton distributions with greater resolution, deciphering the in situ spatial patterns observed over a range of natural systems is fundamental to developing our knowledge of the mechanisms underpinning the microscale spatial structure and ecology of marine ecosystems (Levin, 1992).

The microscale distribution of phytoplankton has recently been studied with a variety of high-resolution instruments including water sampling devices (Waters et al., 2003), microstructure profiling fluorometers (Desiderio et al., 1993; Wolk et al., 2001; Doubell et al., 2006, 2009) as well as fluorescence imaging systems (Franks and Jaffe, 2001; Prairie et al., 2010) and holography (Katz et al., 1999; Davis et al., 2005; Malkiel et al., 2006). Various fluorescence based measurement systems have all shown increasing levels of fluorescent intermittency with a reduction in sample volume size (Desiderio et al., 1993; Franks and Jaffe, 2001; Doubell et al., 2006, 2009) attributable to the preferential detection of larger individual cells, chains and aggregates (Franks, 2005). Coincident measurements of the spatial distribution of phytoplankton and turbulence has remained a technically challenging endeavor limited to microstructure profilers that contain adjacent fluorescence and shear sensors (Wolk et al., 2001, 2002; Mitchell et al., 2008; Doubell et al., 2009). Moreover, efforts to understand the mechanisms driving the high levels of observed spatial variability using conventional (Gaussian) models such as spectral analysis (Denman and Gargett, 1995) appear to be limited by the discrete nature of phytoplankton distributions when resolved at these small scales (Franks, 2005; Doubell et al., 2009). These findings suggest that for any given system, a critical scale exist such that measures of chlorophyll fluorescence (used as an indicator of phytoplankton biomass) transition from representing a continuum (i.e. Gaussian process) to representing a discrete variable (i.e. point process). Identifying this scale is central to the interpretation of microscale phytoplankton patchiness.

In this context, the first part of this paper identifies the length scale at which the measurement of phytoplankton fluorescence transitions from a bulk property to discrete phytoplankton particles by investigating the probability density function (PDF) of the fluorescence signal measured over different sample volume sizes. Second, we evaluate the spatial structure of separate fluorescent particles through the application of point-process statistics (Diggle, 1983; Rothschild, 1992). Specifically, the statistical distribution of nearest neighbor distances (*NND*) is compared to those drawn from a random (Poisson) distribution in order to identify the microscale spatial structure of phytoplankton. Finally, spatial and temporal shifts in particle concentrations and *NND* are compared with changes in the background particle concentrations and turbulence conditions to understand how the spatial distribution of fluorescent particles fit with the patterns and processes observed at larger scales.

2. Methods

2.1. Field site and sampling

Tokyo Bay is a semi-enclosed bay with an estuarine circulation which is intensified during summer due to increased freshwater input into the bay and increased thermal stratification (Nakayama et al., 2005). At the narrow mouth (6 km wide, 50 m depth) outflowing freshwater overlies denser in-flowing seawater creating strong stratification. The vertical structure of the water column is periodically modulated by tidal mixing which restricts seawater exchange in the bay (Yanagi et al., 2003). As a consequence of the limited exchange and increased eutrophication of the inner bay region, frequent bloom events including harmful algal blooms typically occur throughout the spring, summer and autumn periods (Han and Furuya, 2000).

We sampled at the entrance of Tokyo Bay (35°16′26″N, 139°43′14″E) using the Tokyo University of Marine Science and Technology *RTV Seiyo Maru* on May 18 and 19, 2009. Profiling was conducted every 15 min over a 16 h period using a TurboMAP-L microstructure profiler (Doubell et al., 2009). TurboMAP-L provided undisturbed measures of turbulent shear ($\partial u/\partial z$, $\partial v/\partial z$), *in vivo* chlorophyll-a fluorescence, turbidity as well as standard hydrographic parameters (conductivity, temperature and depth). TurboMAP-L sensors are sampled at a rate between 64 and 512 Hz for different channels. Typical free-fall descent rates were about 0.65 m s^{-1}.

TurboMAP-L contains two fluorescence sensors for the measurement of microscale phytoplankton distributions. The sensors are separated by a distance of 12 cm and both sensors sample at 256 Hz. Fluorescence concentrations measured with the light-emitting-diode (LED) fluorescence/turbidity sensor are averaged over a sample volume of approximately 4 mL and have a spatial resolution of approximately 2 cm (Wolk et al., 2001, 2006). The LASER sensor, with a sample volume of 32 μL can resolve spatial features as small as 1 mm and at typical descent rates gives independent measures of the fluorescence field approximately every 2–3 mm (Doubell et al., 2009). Both fluorescence sensors are calibrated so that the normally arbitrary units used to measure chlorophyll-a fluorescence are approximately equivalent to μg L^{-1} (Wolk et al., 2001, 2002; Doubell et al., 2009).

Intense spikes in fluorescence measured by the TurboMAP-L LASER fluorescence sensor have been shown to be consistent with the measurement of separate particles with a mean size of 1 mm in Tokyo Bay (Doubell et al., 2009). Additional comparisons of the TurboMAP-L LASER profiles with fluorescence images obtained using a planer laser imaging system (Prairie et al., 2011) have shown that the high fluorescence concentration of spikes measured by TurboMAP-L are driven by the preferential detection of large individual (>20 μm) fluorescent particles (Franks, 2005). The composition of these particles is varied and includes individual large cells, chains and aggregates (Franks and Jaffe, 2008; Prairie et al., 2010, 2011). Since identification of the composition of fluorescence peaks was not possible in this study we simply refer to these peaks as fluorescent particles.

Supplementary measures of phytoplankton community composition, species abundance and biomass were made by water sampling and standard CTD and fluorescence profiling using a Seapoint system. Near surface and bottom water samples were taken at 10 and 40 m depths and 1 L samples for phytoplankton identification and enumeration were fixed with buffered formalin (1% final concentration). Water samples were settled using the Utermöhl technique and counted under an inverted microscope.

2.2. Fluorescence PDF fitting

Fluorescence measures from the LED sensor were first low-pass filtered at 50 Hz to suppress sensor noise whilst retaining the spatial scales resolved by the sensor (Wolk et al., 2006; Sato and Yamazaki, 2008). No correction was required for fluorescence measured by the LASER sensor (Doubell et al., 2009). The fluorescence signal for each sensor was then divided into 4 m