

## **PHYTOPLANKTON COMMUNITY REGULATION ON THE NEW ENGLAND SHELF: INSIGHTS FROM AUTOMATED SUBMERSIBLE FLOW CYTOMETRY**

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### **ABSTRACT**

Phytoplankton communities in temperate continental shelf systems typically exhibit dramatic seasonal variations. We are taking advantage of the Martha's Vineyard Coastal Observatory (MVCO), a cabled facility on the New England inner shelf, to better understand the physical and biological processes that interact to produce this variability. Our approach depends on high resolution (~hourly) multi-year time series of taxonomically resolved phytoplankton acquired with FlowCytobot and Imaging FlowCytobot, custom-built automated submersible flow cytometers optimized for measurement of picoplankton and microplankton, respectively. These observational capabilities enable new approaches to understanding classical problems in plankton ecology. Annually at MVCO, chlorophyll concentrations are highest during bloom events in fall and winter, which Imaging FlowCytobot measurements show are dominated by microplankton, especially large (often chain-forming) diatoms. As water temperatures warm in spring, the community shifts to one dominated by pico- and small nanophytoplankton. Notably, this transition is associated not only with a decline in abundance of large diatoms, but also with a dramatic increase (100- to 1000-fold for picoplankton) in small cells. From FlowCytobot-based growth rate estimates, we show that the seasonal picoplankton variability is linked to effects of temperature and light limitation. On-going work is focused on exploring the hypothesis that fall and winter diatom blooms are linked to processes that control the availability of macronutrients.

### **INTRODUCTION**

Temperate continental shelf ecosystems have long been known to exhibit strong seasonal and interannual variability in phytoplankton biomass and community structure. These systems are highly productive and play important roles in the regional and global cycling of carbon and other elements but, especially for the inner shelf, the combination of physical and biological processes that regulate them are not well understood. The availability of new optical sampling technologies and cabled observatory infrastructure provides an unprecedented means to acquire high resolution time series of long duration. These time series can provide insights into natural variability and its sources.

### **STUDY SITE AND APPROACH**

We are taking advantage of the Martha's Vineyard Coastal Observatory<sup>1</sup> (MVCO) for its cabled power and communications capabilities and its location in an environment that is a model for wide temperate zone shelves (Fig 1). The site is located in a near shore area exposed to the open shelf off the eastern seaboard of the United States. This is a region characterized by dramatic physical and chemical

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<sup>1</sup> <http://www.whoi.edu/mvco>

changes over the annual cycle (e.g., Fig. 2) and these changes are expected to impact the phytoplankton community over a range of scales.

MVCO is a research facility operated by the Woods Hole Oceanographic Institution and located approximately 3 km from the southern shore of the island of Martha's Vineyard. MVCO includes a meteorological mast at the beach, an undersea node at a depth of 12-m, and an offshore tower, which spans the water column and extends approximately 20 m into the atmosphere, at a water depth of 15 m. Each of these locations is connected to a shore laboratory by cables that supply power and two-way communications for core instruments and user-supplied instruments. Core measurements include wind velocity, air temperature, solar radiation, wave height, temperature, salinity and water currents. These observations are publicly available in near real time<sup>1</sup>. Additional optical (chlorophyll and CDOM fluorescence, backscattering, reflectance) and chemical (oxygen concentration) measurements are also routinely made.

The cabled power and bandwidth at MVCO permit continuous use of automated submersible flow cytometers that we have developed specifically for in depth study of phytoplankton communities (Olson et al. 2003; Sosik et al. 2003; Olson and Sosik 2007; Sosik and Olson 2007). We deploy two instruments side-by-side at the offshore tower: FlowCytobot, optimized for analysis for pico- and small nanophytoplankton (~1-10  $\mu\text{m}$  cells), and Imaging FlowCytobot, optimized for analysis of large nano- and micro plankton including chain-forming diatoms (~5-200  $\mu\text{m}$ ) (Fig. 3). Detailed descriptions of these instruments appear elsewhere (Olson et al. 2003; Olson and Sosik 2007). In brief, they each characterize individual cells (or particles in general) according to their light scattering and fluorescence properties as the cells flow through a focused laser beam. Imaging FlowCytobot has higher sample volumes necessary to quantify rarer large cells and includes addition of video technology to capture correlated images of the organisms for identification. In both FlowCytobot and Imaging FlowCytobot, programmable syringe pump/distribution valve systems allows us to inject antifouling and cleaning agents, as well as internal standards (fluorescent microspheres) that enable instrument performance to be evaluated during extended operation. Experience to date suggests daily automated antifouling measures (which take only a few minutes to execute) are sufficient to prevent bio-fouling from affecting deployment durations, which now routinely reach 6 months (at which time reagents and certain mechanical components must be replaced).

FlowCytobot observations provide abundance and cell size information for *Synechococcus* and a mixed assemblage of 2-10  $\mu\text{m}$  eukaryotes; furthermore for *Synechococcus* we can estimate population growth rates (independent of grazing and other losses) from diel changes in cell size distributions (Sosik et al. 2003). When combined with image processing and automated classification techniques (utilizing a supervised machine learning algorithm), Imaging FlowCytobot observations provide abundance and size information for a wide range of taxonomic groups of microplankton, with most identifications possible to at least the genus level (Sosik and Olson 2007).

## PHYTOPLANKTON TIME SERIES

Historical observations in waters near Woods Hole (MA) document strong seasonality and point to fall and late winter-early spring as periods of transition in the phytoplankton community. Blooms of large-celled species and chain-forming diatoms have been more commonly reported in fall and winter than at other times of year (e.g., Lillick 1937; Riley 1947; Glibert et al. 1985). Our observations in recent years (2003-2008) show that chlorophyll concentrations are typically lowest in late spring and early

summer and consistently higher throughout the fall and winter (Fig. 4). Filter fractionation, while not a definitive method, suggests that bloom levels in fall and winter are related to an increase in the presence of cells larger than 10  $\mu\text{m}$  (Fig. 4).

Multi-year time series are now available from both FlowCytobot (since 2003) and Imaging FlowCytobot (since 2006) enabling us to explore these patterns in much greater detail, and in relation to changes in environmental conditions. With the two instruments deployed at the same time, it is possible to characterize the phytoplankton community from pico- through microplankton, with many cell types resolved to genus level and at temporal resolution from hours to days.

Picophytoplankton are numerically dominated by *Synechococcus*, which exhibit a distinct annual cycle with low abundance in late winter, a 2-3 month spring increase, and then a slow decline beginning in late fall continuing through to the next winter (Fig. 5). In this system, larger size classes are dominated by diatoms, which exhibit a completely different seasonal pattern; their biomass is highest throughout the fall and winter and relatively low in summer when small cells are most abundant (Fig. 5). Thus the high chlorophyll conditions of late fall and winter are periods associated not only with increased biomass of microphytoplankton, but also a decline in picophytoplankton.

To quantitatively compare the contributions of different cell size classes to the phytoplankton community at MVCO, we have used the flow cytometry and imaging results to construct phytoplankton carbon budgets. For small cells ( $< 10 \mu\text{m}$  measured by FlowCytobot), we estimate volume of each cell from light scattering (Olson et al. 2003), and for all others we use automated image processing to determine cell dimensions (Sosik and Olson 2007) and then calculate volume. Carbon content of each cell is then estimated from published relationships with cell volume, with a different factor applied for large diatoms (identified by automated classification) according to Menden-Deuer and Lessard (2000).

The resulting carbon budgets (summing contributions of each cell) show high levels of interannual variability at high frequencies, but also support some generalizations about the annual cycle. Microplankton consistently dominate in winter,  $< 10 \mu\text{m}$  cells dominate in summer, and all size classes can contribute substantively to events in fall (Fig. 6). Nanophytoplankton contribute more to total carbon than picoplankton during summer and they can also be as important as microplankton in other seasons (Fig. 7). Considering all size classes, the annual cycle for total phytoplankton carbon shows remarkably little variation (Fig. 7), especially in light of the high seasonality in chlorophyll concentration (Fig. 4). This result is consistent with  $<10 \mu\text{m}$  cells that dominate in summer having high carbon-to-chlorophyll ratios compared to wintertime diatom blooms.

## **SOURCES OF SEASONAL VARIABILITY**

High temporal resolution flow cytometric analysis of picophytoplankton provides the opportunity to derive more than abundance estimates and to begin to explore the role of environmental factors in regulating natural populations. For *Synechococcus* in particular, we have developed an approach to estimate daily population growth rates, independent of grazing or other losses. The rates are determined from observed diel changes in cell size distributions (Sosik et al. 2003). When this approach is applied to an annual cycle, the results reveal strong seasonal variability, with very low growth rates in wintertime associated with low water temperatures (Fig. 8). Lack of growth at cold temperatures has been previously documented for *Synechococcus* (Moore et al. 1995) and is evident in

these natural populations, though new results from MVCO isolates show they are more cold tolerant (lower minimum temperature) than previously studied open ocean ecotypes. Growth rates in the natural populations are similar to those found in controlled laboratory studies during the period January-June (Fig. 8), suggesting that temperature limitation controls most of the physiological variability. In the second half of the year, however, growth rates are often below those predicted from water temperature alone. This is likely associated with the added impact of lower light levels during fall and early winter, both due to lower incident radiation (Fig. 2) and wintertime conditions leading to vertical mixing. We find no evidence that nutrient limitation is a substantial factor affecting picoplankton dynamics at MVCO.

Wintertime conditions that result in light and temperature limitation of picophytoplankton are apparently favorable for diatom blooms. Presumably consistently low nutrient conditions (associated with high light and stratification) in summer explain the lack of large diatoms during that period. During fall and winter, Imaging FlowCytobot observations reveal a series of bloom events with different taxonomic structure suggesting that nutrients supply may be periodic but persist throughout both seasons (as opposed to being concentrated during transitions such as the fall breakdown in stratification). We are currently investigating conditions that may be responsible for unsteady, but persistent supply of macronutrients to the inner shelf during fall and winter (Fig. 2). MVCO observations allow us to consider dynamics related to wind and wave events as well as tidal processes and along and cross-shelf advection.

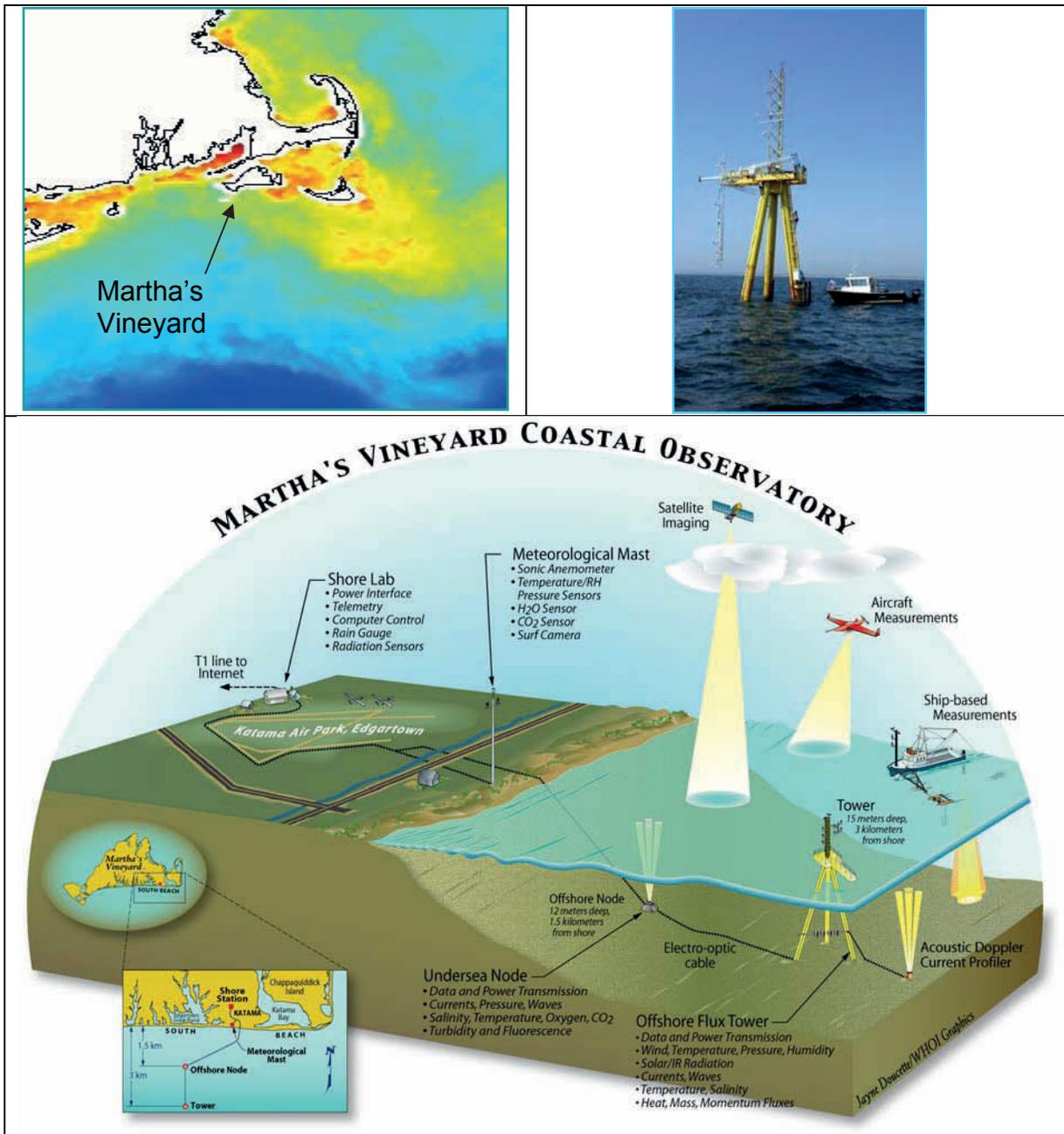
## ACKNOWLEDGMENTS

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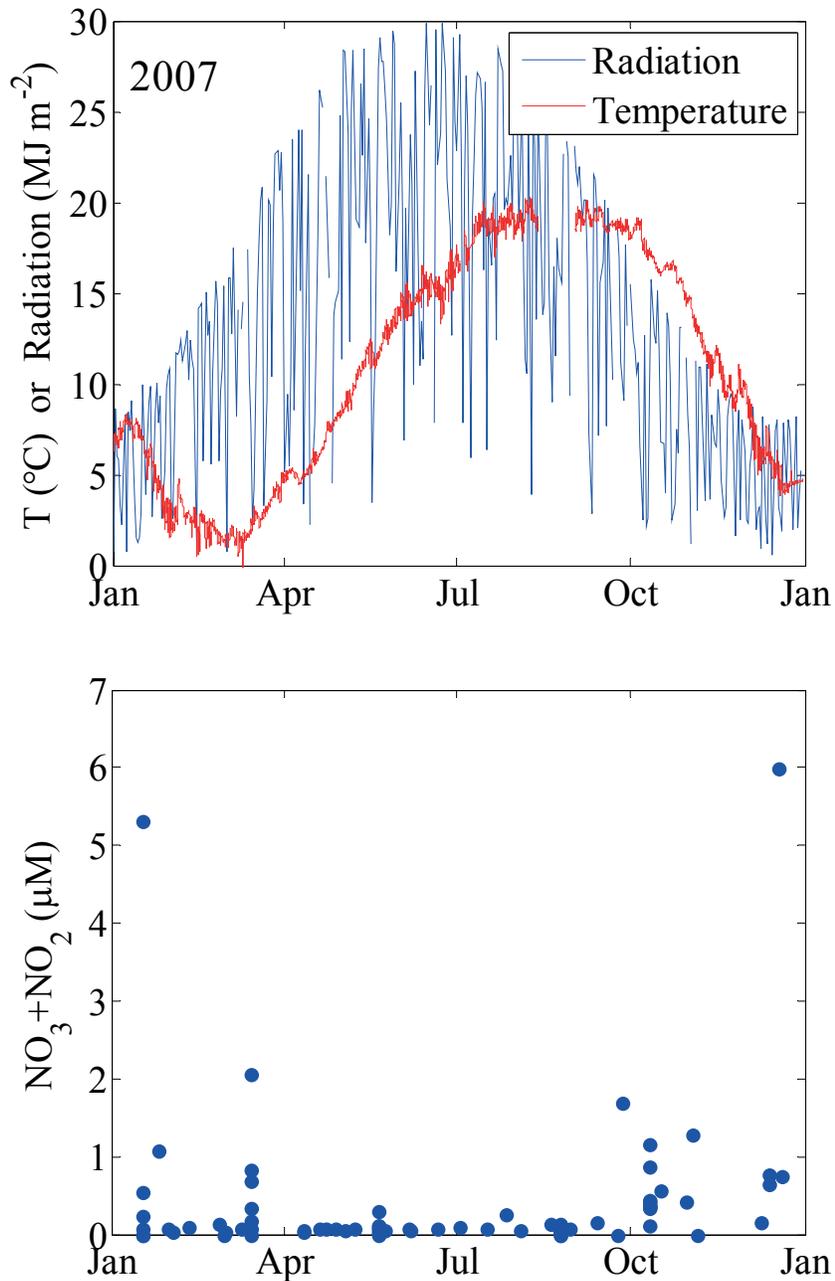
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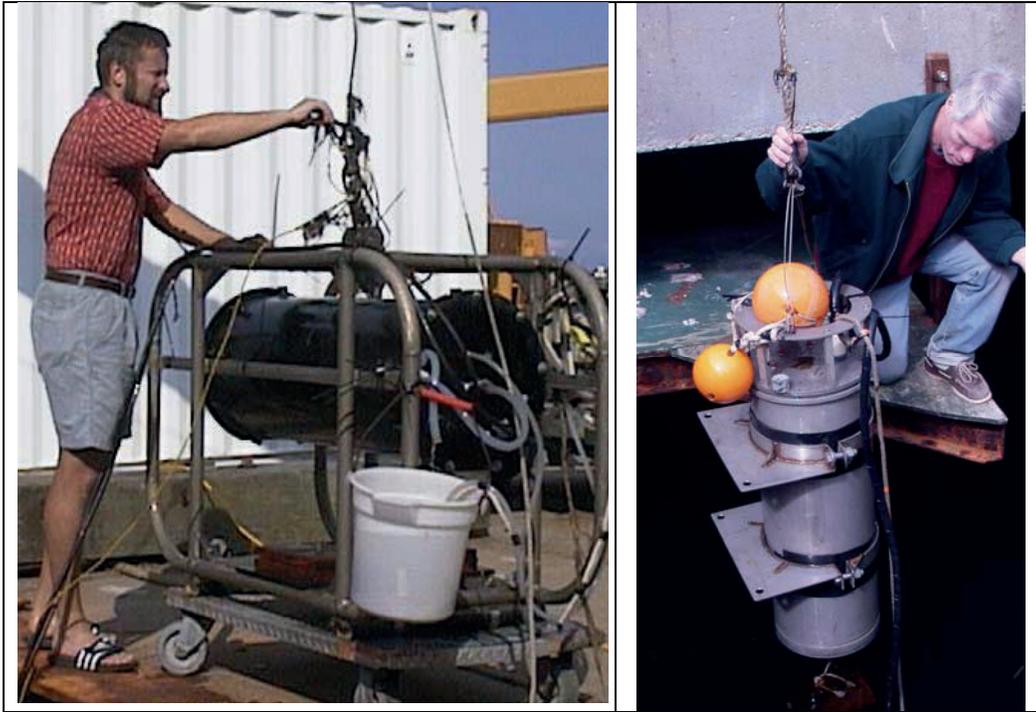
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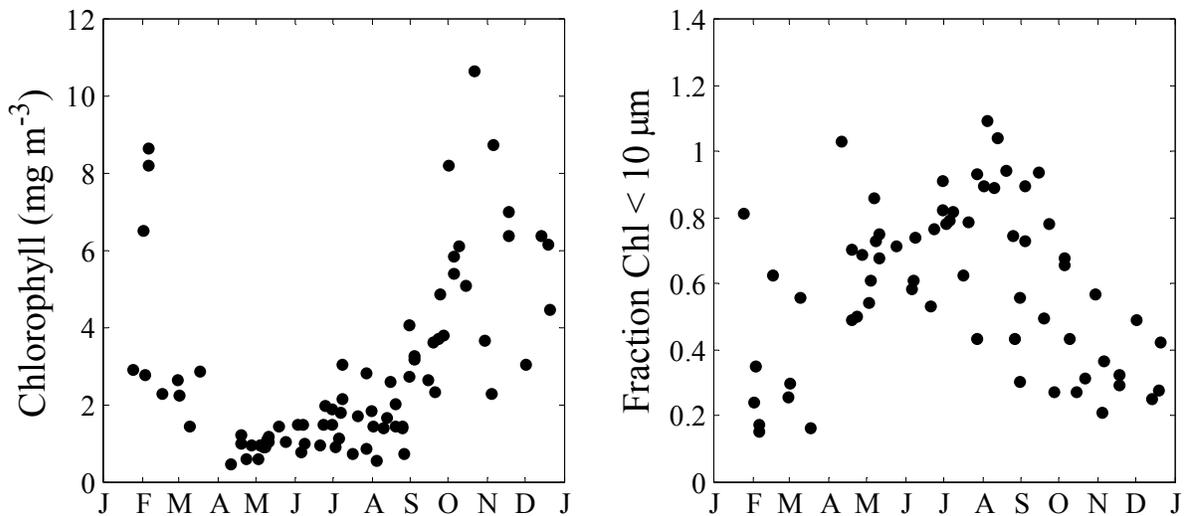
**Figure 1.** *The Martha's Vineyard Coastal Observatory (MVCO), operated by the Woods Hole Oceanographic Institution, is a cabled research facility located approximately 3 km from the southern shore of Martha's Vineyard. Upper left: SeaWiFS chlorophyll image showing the region around the island of Martha's Vineyard, Massachusetts. Upper right: Photograph of the offshore tower structure at MVCO where FlowCytobot and Imaging FlowCytobot are deployed at 4 m below the mean water level. Lower center: Schematic representation of MVCO showing the shore laboratory on the island and cable runs that connect the meteorological mast, undersea node, and offshore tower.*



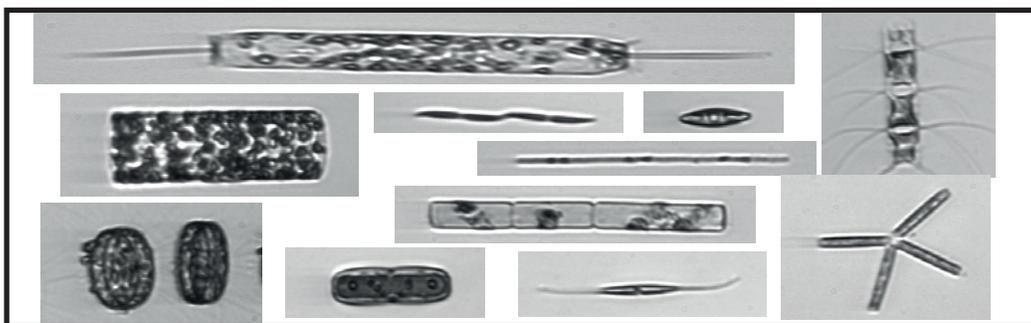
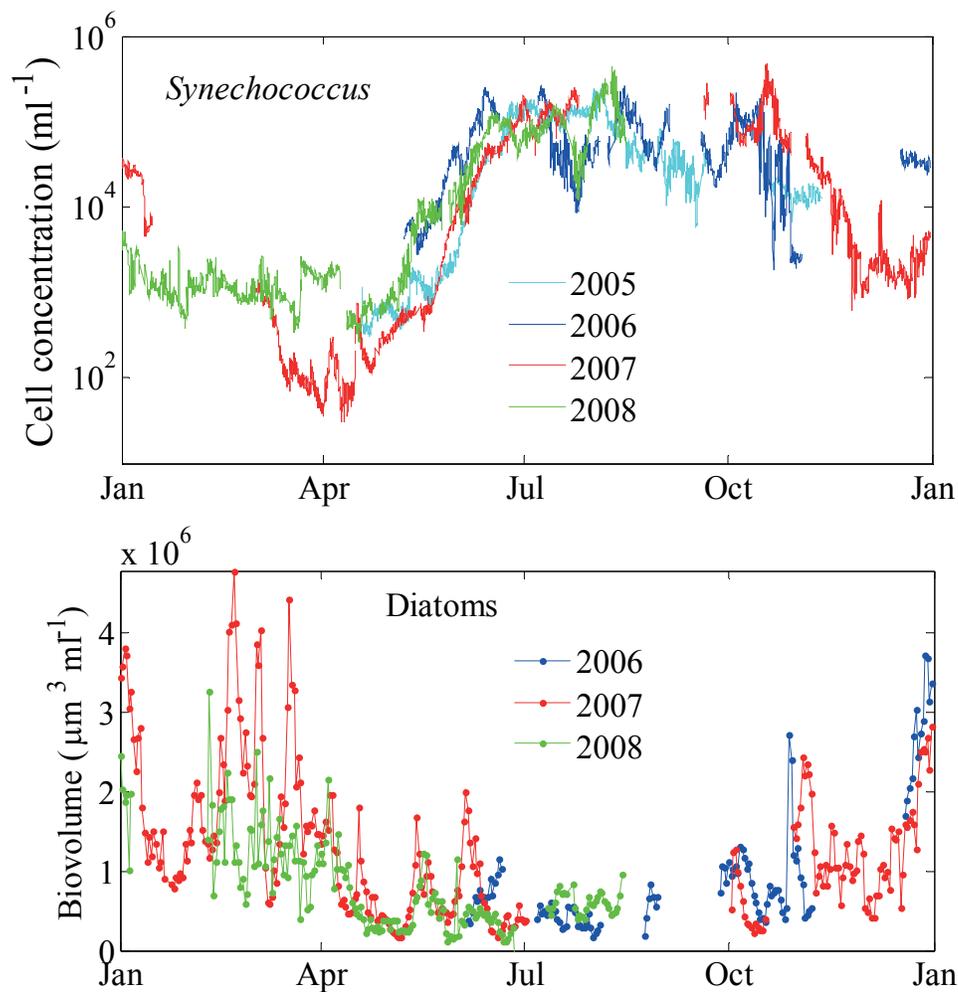
**Figure 2. Annual cycle of water temperature and incident radiation at MVCO during 2007 (top), showing season increases in spring and decreases in fall with temperature lagging behind incident radiation in both seasons. Nitrate plus nitrite concentrations at MVCO (bottom) are at or near detection limits during spring and summer, and intermittently higher in fall and winter when diatom blooms are prevalent.**



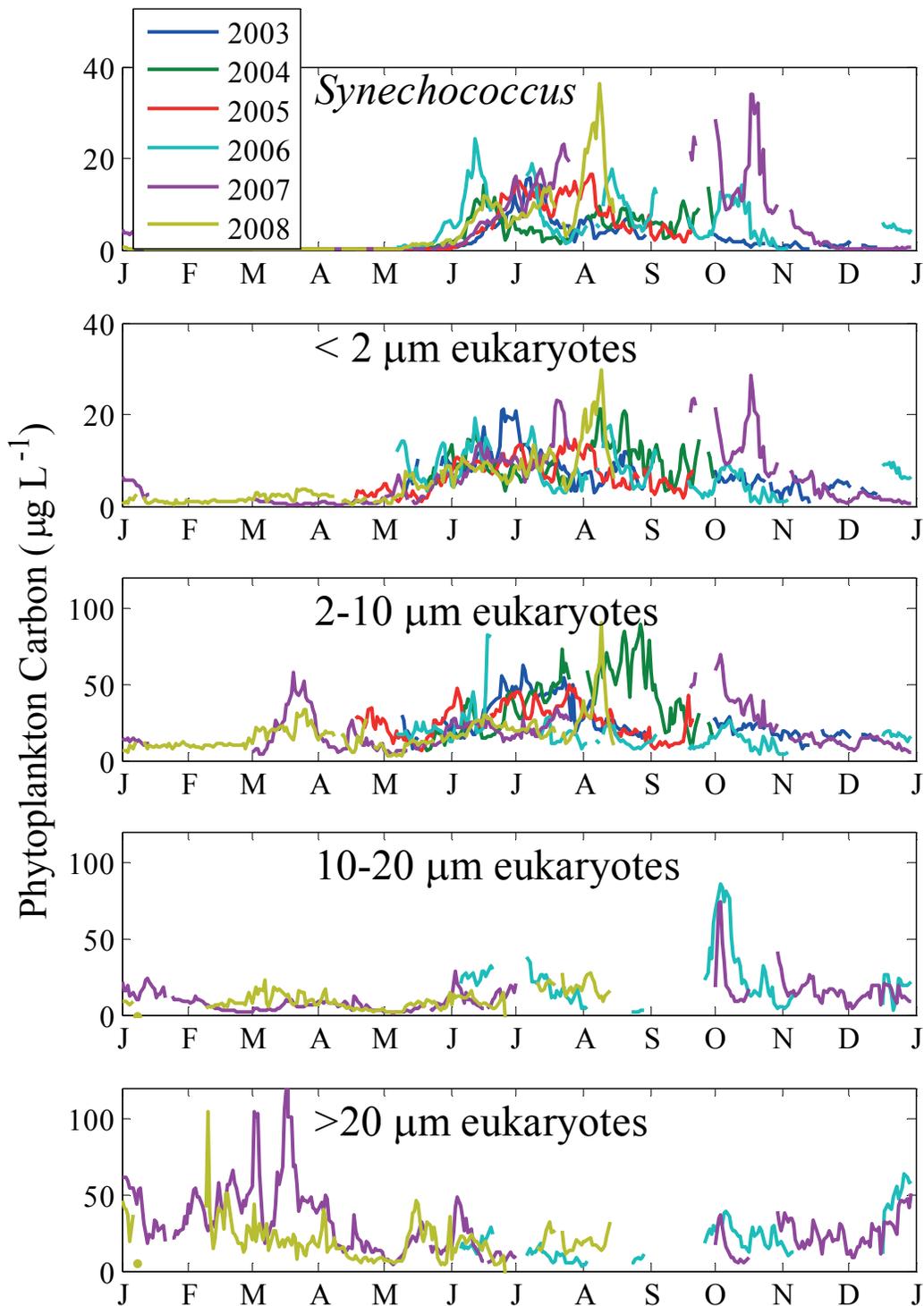
**Figure 3.** *FlowCytobot (left) and Imaging FlowCytobot (right) ready for underwater deployment in their pressure housings and mounting frames.*



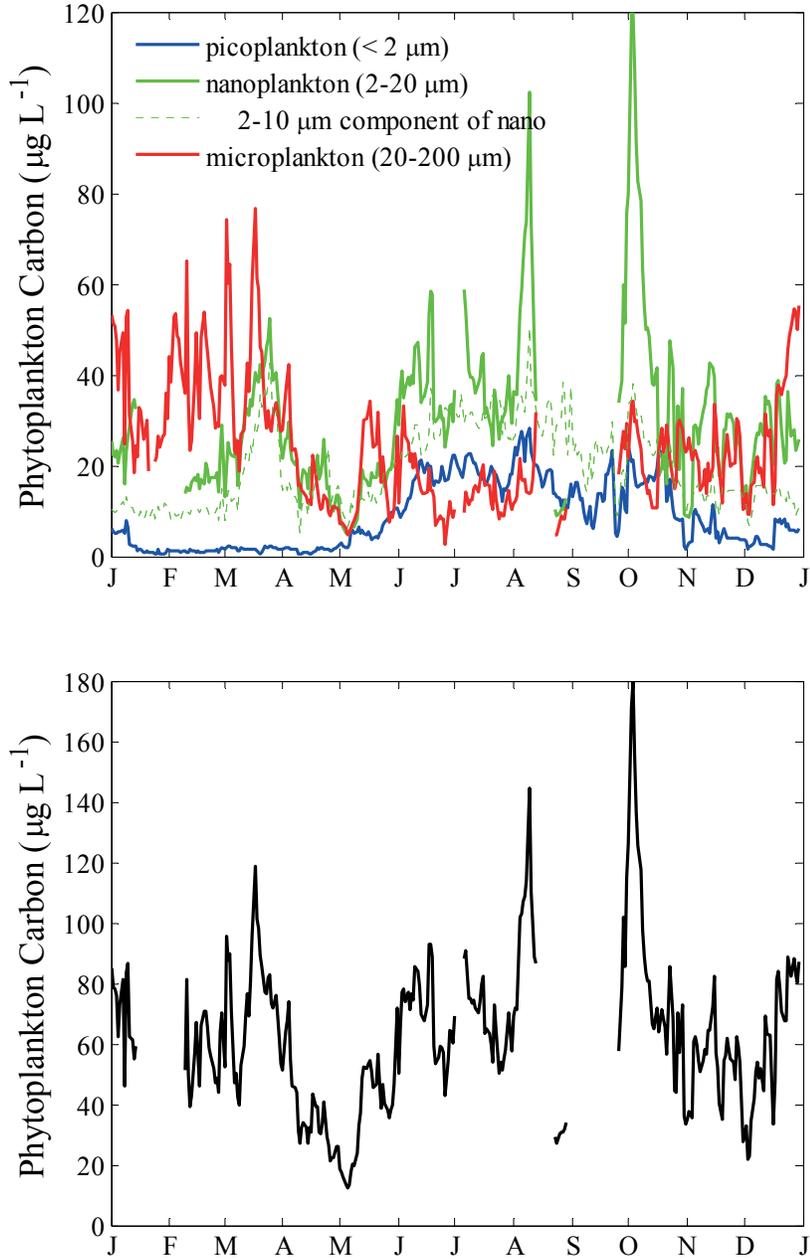
**Figure 4.** *Annual variability in chlorophyll concentration (from fluorometric analysis of discrete samples) at MVCO (left panel) showing highest concentration occurring in fall and winter. Filter-based size fractionation (right panel) reveals that during the high chlorophyll periods only 20-40% of the pigment is in cells that pass through 10 μm mesh, while in summer the < 10 μm fraction often exceeds 70%.*



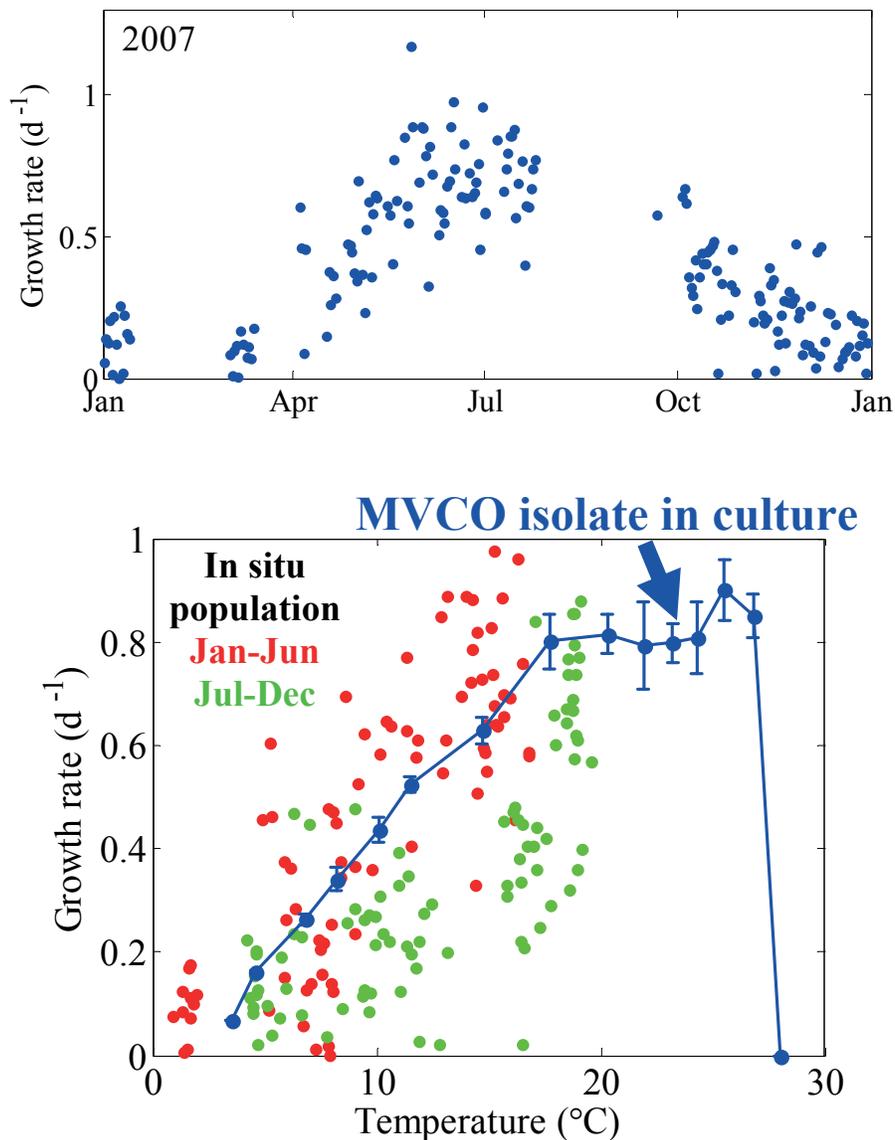
**Figure 5.** FlowCytobot observations at MVCO emphasize a distinct annual cycle in *Synechococcus* abundance, with a notable spring increase (top). There is some interannual variability in timing of this spring bloom, but a remarkably consistent rate of increase each year. In contrast, diatoms (which dominate the microplankton at MVCO) tend to bloom on event-scales in fall and winter (middle). Note, the diatoms time series is shown as integrated biovolume as an index for total biomass that accounts for the large differences in cell size among species in this group. Example images (collected by Imaging FlowCytobot) of typical diatom genera at MVCO are shown at bottom.



**Figure 6. Multi-year size-resolved phytoplankton carbon budgets derived from analysis of ~1 billion individual particles analyzed by submersible flow cytometry at MVCO. Interannual variability is high, but microplankton consistently dominate in winter, < 10 µm cells dominate in summer, and all sizes can contribute substantially to events in fall. Note the different y-axis scale on the picoplankton panels.**



**Figure 7.** Annual composite size-resolved phytoplankton carbon budgets derived from the time series in Figure 6 (upper). The sum of all size classes (lower) exhibits much less seasonal variation than observed for chlorophyll concentration (Fig. 4) suggesting that small cells important in summertime have higher carbon-to-chlorophyll ratios.



**Figure 8.** Daily growth rates for the *Synechococcus* population at MVCO, derived from diel changes in cell size distributions measured by FlowCytobot during 2007. Growth rates increase in the spring, remain relatively high all summer and then decline again in fall (upper). Water temperature explains most of the variability in growth rate in the first half of the year, while in the fall rates are lower than expected on the basis of temperature alone (lower), most likely associated with light limitation.