The 2008 North Atlantic Bloom Experiment

Abundance and Biomass Determination of heterotrophic and autotrophic bacteria, phototrophic and heterotrophic nanoplankton, & microplankton - including instrument calibration

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1. Flow Cytometry

All samples were analyzed with a Becton Dickinson FACScan flow cytometer on board the ship (KN-193-3). The volume of sample analyzed was determined by a "time" method. The flow rate of the instrument was monitored twice a day using volume standards. These were tubes containing DI water that had been weighed and sealed ashore and stored refrigerated. Approximately twice a day a volume standard tube was opened and run on the instrument for about 5 min at high flow rate. Tubes were then resealed, stored in the refrigerator, and weighed upon return to the lab. From these results it was determined that the flow rate was constant over the first 2 days of the cruise, then declined over the middle portion of the cruise, and then was higher again and constant for the last 2 days. The trend was fit to a linear regression and the resulting flow rate was used to calculate cell abundances for samples run on those days, while a constant rate was used for the first and last periods. The high flow rate averaged 51.0 µL min^{-1} (2.4 sd, 4.6% cv, range: 46.8 – 55.7), over the whole cruise.

1.1 Heterotrophic bacteria

Samples for heterotrophic bacteria were preserved with 10% paraformaldehyde (0.5% final), 100 uL of preserved sample was stained with 10 µL PicoGreen (Invitrogen - 1:10 dilution in DIW) for 24 h, diluted with 900 µL of filtered seawater (FSW) and analyzed via flow cytometry (Veldhuis et al. 1997).

1.2 Phototrophic Pico- and Nanoplankton

Live samples were run undiluted at high flow rate as per standard flow cytometric procedures (Marie et al. 2005).

1.3 Heterotrophic nanoplankton

Live samples were stained with LysoTracker Green (Invitrogen). A 1mM stock of LysoTracker Green was diluted 1:10 in 0.2 µm-filtered seawater added to live seawater samples (75 nM final concentration) and incubated at in-situ temperatures for 10 minutes and enumerated via flow cytometry (Rose et al. 2004).

1.4 Cell size determination

Mean cell sizes of heterotrophic and phototrophic nanoplankton were determined from flow cytometric forward light scatter (FSC). The relationship between FSC and size was determined using a set of standard microbeads (1, 2, 2.5, 4, 6, 10, 15 and 20 µm). These microbeads were run at the beginning and end of the cruise and at sea when any adjustment was made to the flow cytometer. These results were used to create calibration curves relating forward scatter to "bead diameter" (µm) - see Figure 1. After the cruise, in the laboratory, a set of 14 cultures ranging in size from 2 to 30 µm (see Table 1) were analyzed along with the microbeads on both a Coulter Counter (Beckman Coulter Multisizer) and on the BD FACScan to determine mean forward light scatter. Cultures of cells too small for the Coulter Counter (*Synechococcus* and *Micromonas*) were measured by image analysis using a Zeiss epifluorescence microscope and a Diagnostic Research Instruments color camera. Bead and Cell size vs. forward light scatter calibration curves were determined (Figure 2) and these results were used to establish a standard curve relating "bead diameter" to "cell diameter" (µm) using forward light scatter - see Figure 3. Cell diameters from the cruise were determined using the bead to cell standard curve relationship in Figure 3, all heterotrophic and phototrophic nanoplankton biovolumes (μm^3) were then calculated and used to determine biomass in carbon units.

Table 1. Cultures used for size calibration of forward light scatter from the BD FACScan and Coulter Counter. CCMP=Culture Center for Marine Phytoplankton.

Species Name	**Stain ID – if available**	**Approximate Size (microns)**
Alexandrium fundyense	CB-501	30
Pycnococcus provasolii	CCMP 1203	2-4
Brachiomonas		10x18
Dunaliella tertiolecta	CCMP-1302	8-13
Isochrysis galbana	CCMP 1324	4-8
Emiliana huxleyi		3-5
Thalassiosira pseudonana	CCMP 1335	4-6
Amphidinium carterae	CCMP 1314	12-18
Pleurochrysis carterae	CCMP 645	8-12
Thalassiosira weissflogii	TW	14-18
Phaeocystis globosa	CCMP 2754	5-7
Synechococcus sp. (cyanin)	8c-1k	1-2
Micromonoas pusilla	CCMP 494	1-2
Rhodomonas salina	3C / CCMP1319	9-15

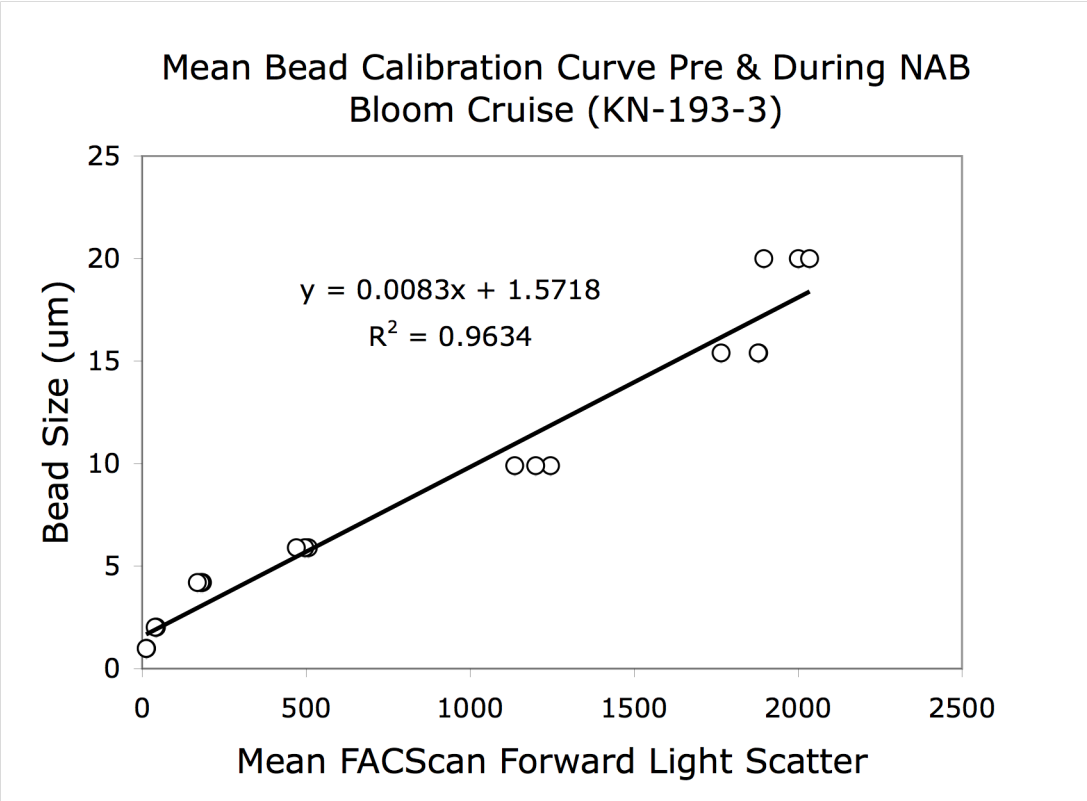

Figure 1. Relationship between Bead Size and Mean Forward Light Scatter (FSC) just prior and during the KN-193-3 2008 North Atlantic Bloom Research Cruise.

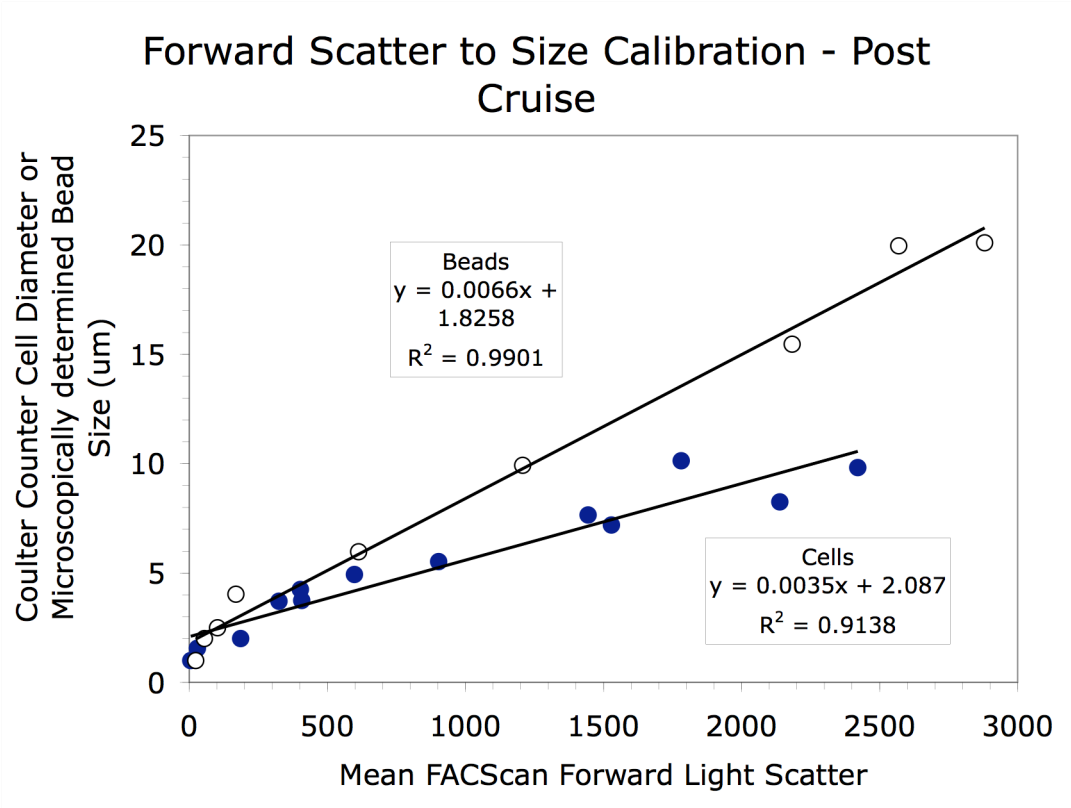

Figure 2. Bead and culture cell calibration curves relating bead or cell diameter to mean forward light scatter (FSC) – determined in the laboratory (after cruise).

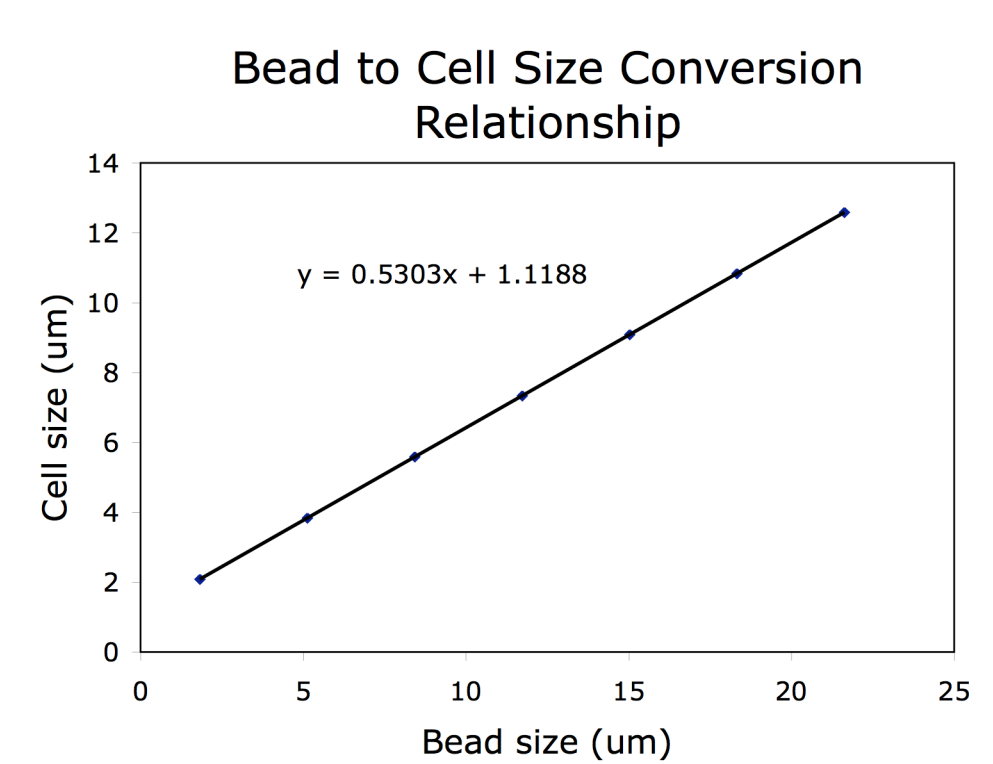

Figure 3. Standard Curve relating Bead Size to Cell Size.

2. Biomass estimation

2.1 Flow Cytometric Samples

Established carbon density factors from the literature were used to convert cell biovolumes to biomass in C units. For heterotrophic bacteria we used a cell carbon density of 20 fgC cell^{-1} (Lee and Fuhrman 1987). For *Synechococcus* and phototrophic and heterotrophic nanoplankton we used the biovolume/biomass relationship of Verity et al. (1992). We assumed the *Synechococcus* had a diameter of 1 µm, yielding a value of 248 fgC cell^{-1} (compare to the average measured value of 250 fgC cell^{-1} measured by Kana and Glibert (1987)).

2.2 Microplankton

Live samples for microplankton analysis were run on the FlowCAM (Fluid Imaging Technologies) imaging-in-flow system (Sieracki et al. 1998). The instrument was equipped with a 4X objective and a 300 µm flow cell, and was operated in fluorescence trigger mode at a flow rate of about 2.5 mL min^{-1}. Typically between 50 -100 mL of sample was analyzed from each depth, followed by a rinse of filtered seawater. Some of the microplankton FlowCAM runs were sub-classified using Visual Spreadsheet software (Fluid Imaging Technologies) by visual identification into 17 different classes. Four major sub-groups were identified, diatoms, dinoflagellates (autotrophic and mixotrophic), ciliates, and other microplankton. From the images within each class, biovolume estimates were determined using the SVW (Sieracki, Viles and Webb) method (Sieracki et al 1989) which applies a modified integration-by slice to estimate the volume of aquatic cells from 2d images. SVW scans along a rotated (and filled) boundary for each x position. The rotation of the boundary is required to present the long dimension of the

particle parallel to the abscissa. Finding the boundary points of a particle proved tricky. ImageJ's native particle tracing assigns the boundary to the crack between pixels. This is different than that described by the original work, and between our desire to replicate SVW and the rounding issue we decided to abandon ImageJ's built-in capabilities. We used Connected Component Labeling algorithm by Chang, Chen and Lu (CCL, 2004) described and implemented in Burger and Berge (2009).

From Menden-Deuer et al. (2000) a set of functions relating carbon content to cell biovolume for protists was utilized. These functions account for the fact that larger cells have lower carbon density. Diatoms, in particular, have a distinct function with less carbon per biovolume than ciliates and dinoflagellates. In cases of cells and colonies that were not spherically symmetric (e.g. *Thallasiosira*, and *Pseudonitzschia*) the SVW algorithm was not appropriate and gave exaggerated biovolumes. For these image types an algorithm was developed that used colony perimeter and skeletonization to yield better estimates the biovolume.

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