Characterization of the Microcystis Bloom and Its Nitrogen Supply in San Francisco Estuary Using Stable Isotopes

Estuaries and Coasts
Impact Factor 2.535 | Peer-Reviewed | 2014

P. W. Lehman, C. Kendall, M. A. Guerin, M. B. Young, S. R. Silva, G. L. Boyer, S.J. The

"Summertime blooms of the toxic cyanobacteria Microcystis aeruginosa (Microcycstis) have occurred regularly in San Francisco Estuary (SFE) since 1999…. A suite of particulate and dissolved organic and inorganic stable isotopes were needed to determine the source of the nutrients and cells that initiate and sustain the toxic cyanobacteria bloom…."

METHODS & DISCUSSION "Microcystis samples for determination of cell abundance were stained and preserved with Lugol's solution. The volume of Microcystis colonies within each sample was computed from the area of the colonies and an assumption of a spherical volume using a FlowCam digital imaging flow cytometer made by Fluid Imaging Technologies. Cell abundance estimates based on FlowCam measurements were closely correlated with those determined by microscopic analyses (r=0.88, p<0.01). In order to more easily measure the diameter of the colonies, the samples were size fractionated into 12-35, 36-300 and >300 µm diameter sub-samples using sieves, diluted to a maximum of 200 cells ml-1 and read at a magnification of either 4X or 2X."

"The surface seston during the Microcystis bloom was primarily composed of cyanobacteria and phytoplankton."

FlowCam as a tool for studying small (80-1000µm) metazooplankton communities

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Baptiste Le Bourg, Véronique Cornet-Barthaux, Marc Pagano, Jean Blanchot

Le Bourg, et al. compared counting methods with the FlowCam and a stereomicroscope to evaluate FlowCam's effectiveness in enumerating eight metazooplankton communities sized 80-1000µm.

METHODS & RESULTS "Each sample was first analyzed using FlowCam and then using microscopy. Both analyses were carried out by the same person."

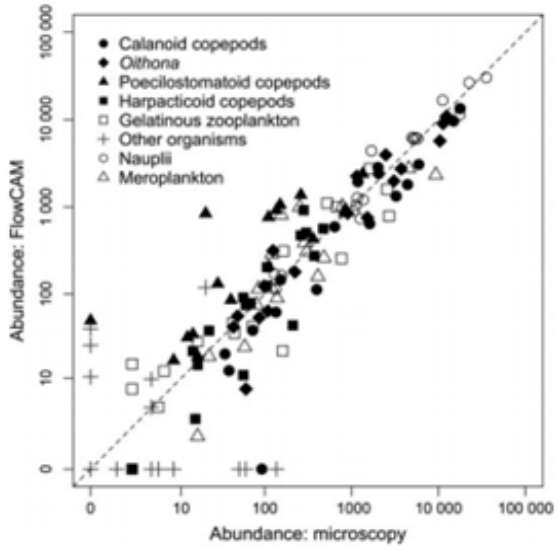

Fig. 2. Comparison of estimated abundances (number of individuals in samples) using a stereomicroscope (x-axis) and FlowCAM (y-axis) for each group. The dashed line shows the 1:1 line.

DISCUSSION "Our results indicate that the FlowCam system is suitable for studying zooplankton communities as it produced similar results to those obtained by microscopy for nearly all the groups of organisms considered."

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Future Climate Scenarios for a Coastal Productive Planktonic Food Web Resulting in Microplankton Phenology Changes and Decreased Trophic Transfer

PLOS ONE – 2014
Impact Factor 3.234 | Peer-Reviewed

Albert Calbet, Andrey F. Sazhin2, Jens C. Nejstgaard, Stella A. Berger, Zachary S. Tait, Lorena Olmos, Despoina Sousoni, Stamatina Isari, Rodrigo A. Martınez, Jean-Marie Bouquet, Eric M. Thompson, Ulf Ba°mstedt, Hans H. Jakobsen

Calbet, et al. "studied the effects of future climate change scenarios on plankton communities… using a mesocosm approach.... Future predicted warming conditions should lower the overall food web transfer efficiency of matter and energy from the primary producers to higher trophic levels." The research predicts decreased phytoplankton bloom periods, potentially impacting "top consumers such as fish if intermediate metazoan grazers such as copepods "miss" the bloom".

METHODS Calbet, et al. explored "the affects of different global change scenarios on the trophic efficiency of a coastal food web by analyzing the numerical, biomass (e.g., ratio heterotrophs/ autotrophs), and stoichiometric responses of the planktonic protist community. We simulated possible future synergistic climate conditions by manipulating nutrients, temperature, and pH. The contemplated scenarios were i) unaltered communities, ii) eutrophication, iii) eutrophication combined with acidification, and iv) eutrophication combined with acidification and warming. This was conducted in a mesocosm experiment with enclosed natural plankton in outdoor tanks.

"Sample preservation may result in considerable loss of larger delicate protists. Therefore, we also analyzed samples of untreated live plankton from both replicates of each mesocosm using two black and white FlowCam II instruments. The FlowCams were run in autoimage-mode, using 4X magnification to analyze particles ranging between 15 and 1000 µm, focusing on ciliates and the dominant athecate dinoflagellate Gyrodinium spirale. The samples were kept in dim light at 12° C until analyzed within 4 hours after sampling."

DISCUSSION "Mesocosms are a powerful experimental tool to address plankton food-web responses to climactic change…. Another common criticism of mesocosm experiments is the lack of replication. Here, we use duplicated factorial treatments that allow for statistical analysis of the effects. Given the large amount of samples acquired during the experiment, and the negative effects of long-term preservation on these sorts of samples, we opted by a fast analysis (within the day of collection) of only one replicate per treatment. We are, nevertheless, confident regarding the accuracy of these data because they matched the patterns obtained by chl a analysis and by automated particle counters (FlowCam). The latter measurements were performed on both replicates of each mesocosm and showed consistent responses among duplicates."

Feasibility Study for Early Warning Systems for Algae-induced Tastes & Odors

American Water Works Association, Water Research Foundation
Impact Factor 0.52 | 2009

Phillip Tarrant, Tyler Sawyer, Rebecca Mestek and Susanne Neuer

Tarrant et al. explored the relationship between cyanobacteria abundance and levels of MIB and geosmin, nuisance compounds associated with taste and odor events in public water supplies. The study also compared semi-automated particle analysis with a FlowCam® and microscopic analysis to help agencies and utilities evaluate which "technologies may contribute to simplifying and speeding up the process of monitoring water supplies".

METHODS & RESULTS "Cyanobacteria filaments were quantified using two methods: 1) epifluorescence microscopy of cells filtered onto membrane filders and 2) automated particle counting of water samples using a FlowCam® particle analyzer."

- Epifluorescence Microscopy: 60-75 minutes/sample
- FlowCam Semi-Automated Particle Analysis: 10-30 minutes/sample
- FlowCam requires an initial time investment to create digital filter libraries (Basic filter 20 hours, complex filter 40 hours)

"The counts of filamentous cyanobacteria produced by microscopic examination and those produced by the FlowCam were in good agreement. While some variation existed between sample estimates, this difference was not significant (t-test, DF=23, p = 0.89)."

DISCUSSION "Agencies choosing to use microscopy may expect to invest more time in sample processing on a sample by sample basis…. Taxonomic identification of species requires technicians to have received the necessary training or education.

Organizations choosing to adopt the automated technologies now available may be able to process samples with less experienced personnel. Also, the FlowCam… can be left unattended for short periods of time. This would allow personnel to conduct other tasks in parallel with sample processing. The use of this technology, however, requires an upfront investment in the development of "filters" capable of isolating target organisms. While these filters are reasonably effective in identifying filamentous particles, they cannot easily isolate curved or spiral cyanobacteria filaments. Also, it is unlikely in our view that filters could operate at a species level, although this is not necessarily an issue as so many filamentous cyanobacteria species are known MIB producers. However, all images processed by the particle analyzer are retained and can be further examined by the operator if taxonomic classification is required."

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Automated measurement of diatom size

Limnology and Oceanography: Methods
Impact Factor 2.254 | Peer-Reviewed | 2012

Sarah A. Spaulding, David H. Jewson, Rebecca J. Bixby, Harry Nelson, Diane M. McKnight

Spaulding et al. compared the FlowCam to a light microscope to examine census and morphological characteristics of diatom populations. Size analysis of diatoms through microscopy is uncommon due to equipment and time limitations. The FlowCam introduces efficient measure of diatom size, allowing scientists to better understand diatom community dynamics. Spaulding et al. developed "a methodological approach for size analysis of diatom populations by determining the effectiveness of different instrument-operating conditions and highlighted aspects and limitations of the method…. The increased speed of data acquisition through use of imaging flow cytometers like the FlowCam is an essential step for advancing studies of diatom populations."

DISCUSSION "To quantify the role of the sex clock in natural systems, it is essential to know the age-size structure of populations…. Asymmetric specimens require special consideration. Feret measurements are appropriate for symmetric cells, while geodesic measurements (not tested in this study) reportedly measure asymmetric objects more accurately. For example, feret measurements worked well to estimate apical lengths of A. minutissimum and D. geminata but introduced errors for the curved values of H. baicalensis.

The shape of the edge of a diatom species, therefore, is important to consider in imaging dimensions of diatom cells.

Time of post-processing [with a FlowCam] can be minimized by selection of samples with the taxon of interest dominant in the assemblage, or a taxon that is particularly distinct in size or shape compared with other species in the assemblage."

Use of the FlowCAM® for semi-automated recognition and enumeration of red tide cells (Karenia brevis) in natural plankton samples

Harmful Algae
Impact Factor 3.874 | Peer-Reviewed | 2006

Edward J. Buskey, Cammie J. Hyatt

Buskey and Hyatt examined the FlowCam's ability "to improve routine monitoring protocols for HAB species by automatically recording information on size and fluorescence per cell…."

OBJECTIVE Buskey and Hyatt "characterized the size and fluorescence characteristics of seven different clones of K. brevis and on of K. mikimotoi… under exponential, stationary, and declining growth phases to examine the range of characteristics that might be expected for K. brevis under natural conditions."

RESULTS "The ability of the image recognition software to correctly identify target cells was found to be limited in this study. A low match threshold must be used (e.g. 40%) in order for 80% or more of the target cells to be recognized…. However, use of the image recognition software can still help

to streamline the screening of natural samples for rare HAB species. A low threshold setting should correctly identify >80% of the target cells, and should on average eliminate more than half of the non-target cell images that need to be screened by the operator."

"Typical time to collect 200 images of chlorophyll-bearing cells of similar size to K. brevis in coastal waters is about 15 min. To count and make size measurements of 200 cells with inverted microscopy, and enter them into a computerized database would take 5-10 times longer, and no information on cell fluorescence would be collected."

A mesocale study of phytoplankton assemblages around the South Shetland Islands (Antarctica)

Polar Bio
Impact Factor 1.586 | Peer-Reviewed | 2012

Cristina Garcia-Munoz, Luis M. Lubian, Carlos M. Garcia, Angeles Marrero-Diaz, Pablo Sangra, Maria Vernet

Garcia-Munoz et al. described phytoplankton assemblages "using flow cytometry, FlowCam and HPLC/CHEMTAX pigment analysis.

INTRODUCTION Nanophytoplankton (2-20 µm) was predominant throughout the study area, which was dominated by small diatoms…. While assemblage studies have been performed, 'this resolution for a physical-biological coupled sampling has not been reported previously in this region and has allowed [Garcia-Munoz et al.] to test the distribution of the different phytoplankton groups in relation to the mesoscale physical features."

DISCUSSION Flow cytometry (FCM) "does not resolve scarcer and larger phytoplankton, especially microplankton (>20 µm ESD). The technique used to cover the microplankton size range was the Flow Cytometer and Microscope (FlowCam®), which combines the capabilities of FCM, microscopy and image analysis."

"Our results highlight that for an accurate estimation of cell size distribution needed in ecological studies, it is necessary to combine different analytical methods. We combined FCM and FlowCam to cover the whole size spectra from pico- to microphytoplankton…. FlowCam results indicated that the well-mixed waters of the AS [Arctic Shelf] were dominated by microplanktonic diatoms, with chain-forming Thalassiosira sp. being the main taxon."

"The photosynthetic dinoflagellates actually represent an evolutionarily diverse collection of different chloroplast types. FlowCam analyses revealed a widespread presence of dinoflagellates throughout the study area…. No taxonomical approximation could be made to classify them into autotrophs and heterotrophs (or even mixotrophs), which implies a possible overestimation of the haptophytes 8-CHEMTAX group, being type-2 dinoflagellates masked within this group."

Low-Energy Input Continuous Flow Rapid Pre-Concentration of Microalgae through Electro-Coagulation Flocculation

Chemical Engineering Journal
Impact Factor 4.321 | Peer-Reviewed | 2016

Teodora Rutar Shuman, Gregory Mason, Daniel Reeve, Alexander Schacht, Ann Goodrich, Katerine Napan, Jason Quinn

Shuman et al. quantified microalgae cell viability by examining fluorescence using Sytox Green Nucleic Acid Stain and a FlowCam.

INTRODUCTION Microalgae cells are small (typically 1-10 µm) and grow in low concentration. These biological parameters raise operational costs for algae producers by increasing the input of energy and water, as well as harvest time. Teodora et al. studied alternatives to current systems by "rapidly [processing] algae in a continuous flow process and [pre-concentrating] them in a separate collection vessel…. The effect of the ECF process on cell viability was examined using optical data collected using a Fluid Imaging Technologies FlowCam® to quantify fluorescence from Sytox Green Nucleic Acid Stain (Invitrogen, Molecular Probes). Sytox Green penetrates cells with compromised plasma membranes and does not cross the membranes of live cells."

DISCUSSION "The ECF Efficiency is seen to rapidly increase in the first 30 min after treated saltwater is mixed with algae suspension…. Most of the settling occurs in the first 5 min. This is a desirable result for commercial applications of a continuous flow apparatus…. Once the treated saltwater is mixed into the algae solution, algae flocs begin to form, seen with FlowCam and the hydroxides collect flocs and sweep them to the bottom in fairly short time."

"The data show that "increased input voltage and slower flow rate reduce algal cell viability and that samples do not tend to recover over time. At higher flow rates and lower voltages, the number of viable cells increases over time along with an observed increase in OD. The increase in OD is attributed to regrowth and not biological contamination as no other cells were observed on FlowCam® photos."