

Top FlowCam® Studies for Phytoplankton and Zooplankton

High-resolution imaging particle analysis of freshwater cyanobacterial blooms

Limnol. Oceanogr.: Methods (2018)

doi:10.1002/lom3.10274

M.D. Graham, J. Cook, J. Graydon, K. Kinniburgh, H. Nelson, S. Pileci, R.D. Vinebrooke

INTRODUCTION “Here, we report on the development of a FlowCam-based method for providing high taxonomic resolution and accurate cell count data in a timely manner (e.g. 50 samples enumerated per week) to the Alberta Health Services cyanobacterial monitoring network of 50–60 lake beaches across the province ... We compared total cyanobacterial and finer taxonomic cell counts generated through the use of a FlowCam vs. a standard light microscopy method.”

FlowCam METHOD “All taxonomic analyses using both the FlowCam and light microscope were performed by the same taxonomist (M.D. Graham).” First, Graham performed an “initial [sample] inspection using standard light microscopy ... to qualitatively determine the general taxonomic composition [and maximum particle size to inform flow cell and objective selection] ... Then, samples were taxonomically enumerated [using] a modified Utermöhl technique ... [The FlowCam’s] 50-µm deep flow cell with the x20 objective maximize[d] taxonomic resolution [but] was only feasible [for] cells less than 50 µm in diameter [and] require[d] longer run times ... We did not dilute or size fractionate via sieving dense samples to avoid both time consuming additional processing of samples and potential introduction of handling error... [W]e occasionally observed cyanobacterial cells adhering to ... the flow cell [which we remedied using a Lugol’s solution] ... Thereafter, each Lugol’s preserved sample passed through a 50-µm deep flow cell at a flow rate of 0.02 mL min⁻¹ and analyzed using the x20 objective... Concordance between light microscopy- and FlowCam-based cyanobacterial cell counts was determined using correlation and correspondence analyses.”

RESULTS & CONCLUSIONS “In general, use of the FlowCam equipped with a x20 objective [and 50 µm flow cell] enabled taxonomic identification to the genus- and

often species-level, thereby equating approximately to light microscopy performed using a x63 objective...[The FlowCam with a x20 objective] also enabled detection of smaller picocyanobacteria (2–5 µm diameters)”.

“Total [FlowCam] cyanobacterial cell counts for live and preserved water samples ... showed significant positive correlation Lugol’s preserved samples tended to produce higher estimates of cyanobacterial abundance [relative to live samples]”.

The x20 FlowCam objective “resulted in cell density estimates ... four times higher than the ×10 objective ...

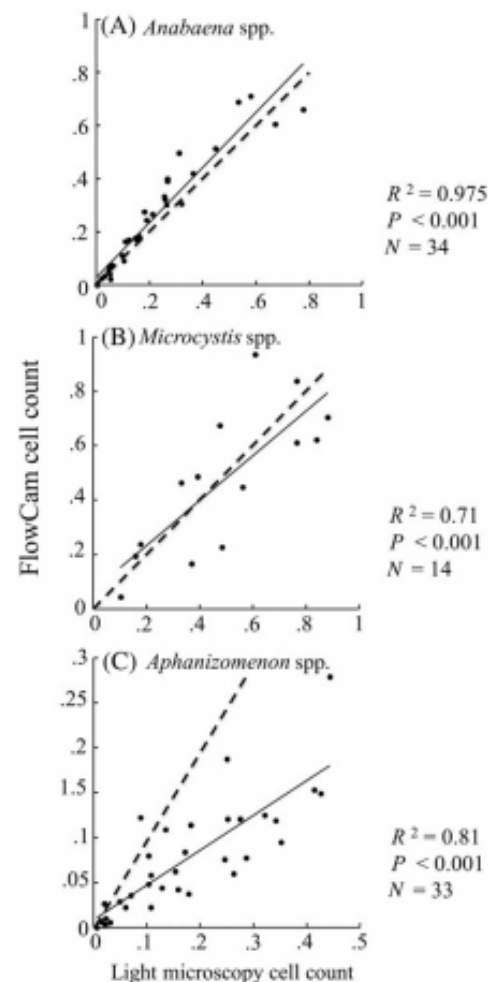


Fig. 5. Comparisons of total *Anabaena* spp. (A), *Microcystis* spp. (B), and *Aphanizomenon* spp. (C) densities (X 10⁵ cells/mL) obtained with light microscopy (x-axis) vs. the density obtained with FlowCam (y-axis). All samples were preserved with 5% Lugol’s solution. Solid line represents the best-fit line while the dashed line depicts the 1 : 1 relationship.

Better image resolution ... helped maximize both detection and taxonomical identification of cyanobacteria ... [H]igher total cell counts and greater species richness were mainly attributable to detection of ... smaller genera measuring less than 5 µm in cell diameter, such as *Cyanodictyon*, *Merismopedia*, and *Synechococcus*."

"Total cyanobacterial cell counts based on light microscopy vs. the FlowCam showed significant positive correlation ... [as did] total cell counts for major cyanobacterial taxa (*Anabaena* spp., *Microcystis* spp., and *Aphanizomenon* spp.) ... [and] *Microcystis*." By comparison, "total cell counts for *Aphanizomenon* were often underestimated [with the] FlowCam [Figure 5] ... [T]otal cyanobacterial and species-level cell counts performed using either FlowCam or light microscopy showed strong agreement, attesting to the reliability of cyanobacterial enumerations derived from the relatively less time-consuming digital flow cytometry approach."

"Unfortunately, accurate species-level identification ... using AutoClassification in VisualSpreadsheet ... remains a work in progress because of several challenging factors, including their relatively small cell sizes, polymorphism, and lack of taxonomically diagnostic sexual reproductive structures ... [I]nterference from multiple co-occurring cyanobacteria within a sample can also hamper detection and enumeration of certain taxa ... [W]e strongly believe that complementary use of light microscopy is essential to QA/QC assurance when using a FlowCam for ... enumeration of cyanobacterial cells within a water sample ... Nevertheless, ... FlowCam greatly relieves operator fatigue, ... facilitating turnaround times and greater throughput of sample enumerations [e.g. 50 samples per week vs. 15] ... Most importantly, our comparative investigation ... highlights that use of a FlowCam can facilitate intensive lake monitoring by providing the data to end users in an equally reliable, yet more timely manner."

Comparison of microscopy to a semiautomated method (FlowCam) for characterization of individual-, population-, and community-level measurements of zooplankton

Hydrobiologia (2019)

doi:10.1007/s10750-019-03980-w

T. Detmer, K. Broadway, C. Potter, S. Collins, J. Parkos, D. Wahl

INTRODUCTION "We evaluated the reliability of a semiautomated method by comparing it to traditional microscopy. Our goals were to (1) characterize detection rates and body sizes, (2) assess the accuracy of population and community analyses, and (3) determine processing efficiency in terms of processing time per sample."

FlowCam METHOD The researchers used the FlowCam VS-1 benchtop model and VisualSpreadsheet version 4.1.95 to "enumerate and measure body characteristics of individuals." Mesozooplankton runs used a 2x objective, FC1000 Field of View flow cell and ran at an imaging rate of 20 frames/sec and flow rate of 7.5 mL/min. Microzooplankton runs, by contrast, used a 4x objective, FC300 flow cell, and ran at an imaging rate of 20 frames/sec and flow rate of 2mL/min. Auto-Image mode was used to process both groups as this setting is recommended for both high density...and preserved samples..." Both groups were diluted and processed with a 12.5-ml internal syringe pump and a magnetic stir plate to ensure uniformity. "Once the sample was photographed, images were auto-identified by the VisualSpreadsheet software and categorized by taxonomic group...manual post-processing validation was included because sorting accuracy can vary between runs. Once images were correctly sorted, the total number of images per taxa was recorded and densities derived...[and] particles lengths were then estimated for each identified organism."

RESULTS & CONCLUSIONS "We found congruence between microscopy and FlowCam methods across several tests and levels of biological organization... The reliability of semi-automated methods requires validation before usage. After a thorough vetting, semiautomated methods can increase processing

efficiency of zooplankton samples by reducing processing times without sacrificing accuracy... Similar to previous studies, however, FlowCam length estimates for several zooplankton taxa were slightly greater than microscopy-based estimates... The present study provides evidence that there can be a reduction in processing effort associated with the use of the semi-automated approach. We found that the identification software was fairly accurate at identifying zooplankton correctly and it rarely misclassified zooplankton as non-zooplankton particles."

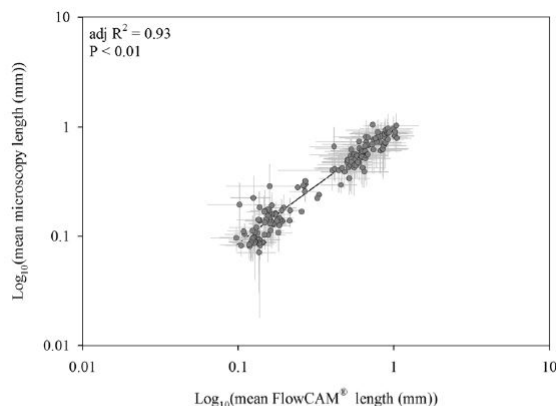


Fig. 3 The relationship between zooplankton length as determined by microscopy and FlowCAM® ($\log_{10}(\text{mean microscopy length}) = 0.02 + 0.95 \times \log_{10}(\text{mean FlowCAM}^\circ \text{ length})$)

Field Mark-Recapture of Calcein-Stained Larval Oysters (*Crassostrea virginica*) in a Freshwater-Dominated Estuary

Estuaries and Coasts (2019)

doi:10.1007/s12237-019-00582-6

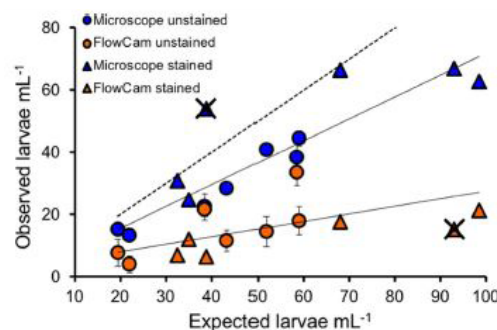
H. Gancel, R. Carmichael, K. Park, J. Krause, S. Rikard

INTRODUCTION "We conducted a large-scale field-based study to refine calcein marking and detection methods, in which we measured growth and survival of calcein-stained oyster larvae at different salinities common to oyster-growing areas and in different holding tank characteristics for pre-release salinity acclimation."

FlowCam METHOD "To quantify detection, stained larvae were visualized and counted with [a]... fluorescent microscope...and using a FlowCam® VS series...equipped with a blue laser (λ_{em} 488nm) and red/green filter (λ_{em} 525±15nm bandpass and long-pass

650 nm). Results were compared to determine if the microscope or FlowCam was better for detecting stained oyster larvae." FlowCam configuration included a field of view flow cell with a 2X objective in trigger mode (flow rate of 10 mL min⁻¹ with 13 frames per second) and Autolmage mode (8 mL min⁻¹ with 19 fps)... To detect stained and unstained larvae in our high particulate background, high volume field samples, where recapture was expected to be low, we opted to use the FlowCam VS series...because whole samples could be relatively quickly imaged."

RESULTS & CONCLUSIONS "Calcein has potential to be a useful marker to track larval movement at large scales needed for field-based studies, providing critical information to aid in selection of restoration sites and management of commercially important shellfish species... Although not perfect, the FlowCam was the most viable option for detection of large-volume, high-background field sampling."



Comparison of FlowCam and microscope biovolume measurements for a diverse freshwater phytoplankton community

Journal of Plankton Research (2019)

doi:10.1093/plankt/fbz056

A.R. Hrycik, A. Shambaugh, J.D. Stockwell

INTRODUCTION "FlowCam combines flow cytometry and imaging to rapidly enumerate, classify and measure particles. The instrument potentially increases processing speed of phytoplankton samples. FlowCam, however, requires extensive comparison to microscopy before incorporation into monitoring and research. Past studies have compared FlowCam and microscopy results for mostly marine rather than freshwater phytoplankton communities. We compared

phytoplankton biovolume, density and taxonomic classifications between FlowCam and microscopy for 113 samples from Lake Champlain, USA - a large freshwater system with diverse phytoplankton."

FlowCam METHOD The researchers preserved samples in 1% acid Lugol's solution or more, and first enumerated and measured phytoplankton using a microscope. "Additional aliquots from the same samples...were then analyzed using a Benchtop B3 Series FlowCam Model VS-IV... Samples were diluted [and] each sample was run at x4 and x10 objectives... Samples were filtered through 100- μ m mesh for the x10 objective and 300- μ m mesh for the x4 objective to prevent flow cell clogging." FlowCam data was processed by eliminating particles that were: <63 μ m, dead or detritus, and that touched the bottom or left sides of the field of view. The researchers found manual processing of FlowCam data was more reliable than using libraries to automate image processing, "especially for species that can take different colony shapes such as chain-forming diatoms and coccoid cyanobacteria." They employed the "sort" function in VisualSpreadsheet® to expedite the process. The authors estimated biovolume using two different methods (ABD and shape-specific) and used genus richness to compare diversity between FlowCam and microscope samples. They also chose ten random samples to test reproducibility of biovolume estimates.

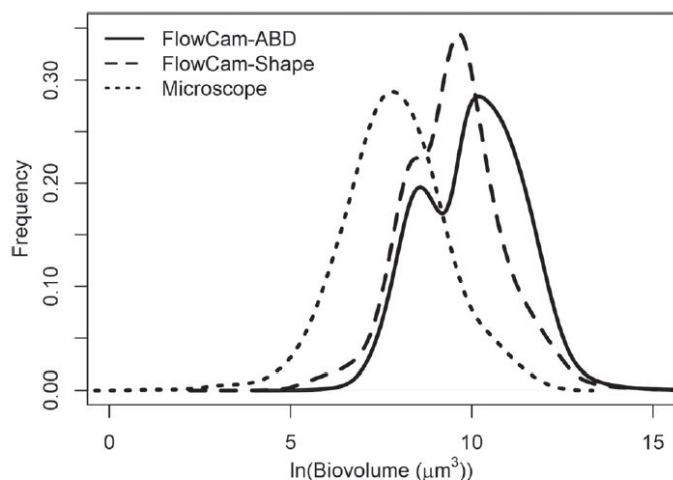


Fig. 8. Comparison of log_e-transformed biovolume distributions of individual particles (natural units) measured on the FlowCAM (both ABD and shape-specific methods) versus microscope. All particles counted and measured are included.

RESULTS & CONCLUSIONS "With careful calibration to the phytoplankton community in question, FlowCam can be a powerful tool for rapid phytoplankton processing and may be valuable for research programs limited by personnel or time... Although FlowCam and microscope biovolumes do not follow a one-to-one ratio, we found strong positive relationships between... the two methods for total sample biovolumes and for most group-specific biovolumes, excluding coccoid cyanobacteria and groups that were sparse in our samples. Close relationships between microscope and FlowCam biovolumes demonstrate that results can be calibrated from one method to the other if a similar comparison is completed for the system in question. Both methods were highly reproducible... [and] FlowCam has the added reproducibility benefit that classified images are automatically saved and can be revisited later. The main drawback to FlowCam is that it necessitates coarser taxonomic resolution than is typically available with microscopy."

A new method for acquiring images of meiobenthic images using the FlowCam

Elsevier (2018)

doi:10.1016/j.mex.2018.10.012

T. Kitahashi, H.K. Watanabe, M. Tsuchiya, H. Yamamoto, H. Yamamoto

INTRODUCTION "The purpose of this study was to develop a new method for investigating sediment-inhabiting meiobenthos using the Flow Cytometer and Microscope (FlowCam). Meiobenthos are...a useful indicator for assessing the effects of anthropogenic and natural disturbances in both shallow and deep ocean ecosystems. These small benthic invertebrates are traditionally investigated by individually counting and identifying specimens under a microscope, which is labor intensive and time consuming. However, FlowCam...has the potential to resolve these challenges."

FlowCam METHODS The authors utilized a portable FlowCam and presented three problems and corresponding solutions they encountered when using the instrument to investigate meiobenthos:

1) "Problem: Sediment particles block the narrow flow cell. Solution: Sieving and separation." The sediment samples were sieved through 1mm, 250µm and 63µm mesh to obtain particles "equivalent to the lower size of meiobenthos." They separated meiobenthic specimens from sediment and debris by centrifuging three times in colloidal silica.

2) "Problem: Syringe pump destroys meiobenthic specimens. Solution: Use an external pump." Instead of using the typical syringe pump, the researchers fitted their FlowCam with an external peristaltic pump to retrieve specimens intact so they could be reexamined under a microscope afterwards.

3) "Problem: Sorting the obtained pictures is time consuming. Solution: Stain meiobenthic specimens with Rose Bengal." The researchers used Rose Bengal to successfully discriminate organisms from inorganic particles using VisualSpreadsheet® to sort by red/green ratio and then successfully classified and counted organisms in each taxonomic group.

RESULTS & CONCLUSIONS "FlowCam successfully captured the images of the meiobenthic specimens in the samples, including metazoan meiobenthos and protists (mainly, foraminifera)... The FlowCam imaging efficiencies (i.e., the ratio of the numbers of individuals observed with FlowCam to microscopy) were $57\% \pm 19.6\%$ for *Nematoda*, and $34.6\% \pm 3.7\%$ for *Copepoda*, and there were significant linear relationships detected between the numbers of individuals observed with FlowCam and those with microscopy" ($r = 0.95$, $p < 0.05$ for all meiobenthos and *Nematoda*; $r = 1.00$, $p < 0.01$ for *Copepoda*).

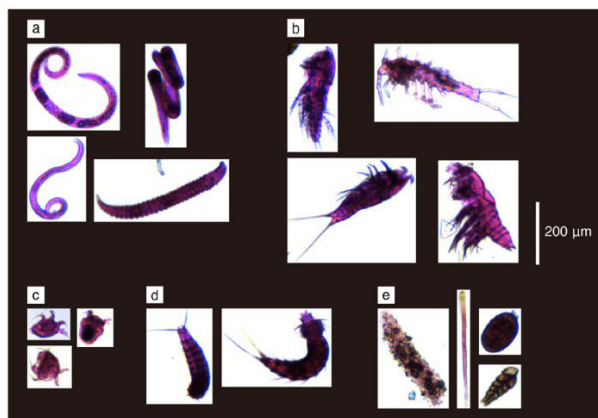


Figure 2. Meiobenthos images captured using FlowCAM. Labels indicate the following meiobenthic taxa: a. *Nematoda*; b. *Copepoda*; c. *Nauplius* larvae; d. *Kinorhyncha*; e. *Foraminifera*

Plankton abundance, biovolume, and normalized biovolume size spectra in the northern slope of the South China Sea in autumn 2014 and summer 2015

Deep-Sea Research Part II (2019)

doi:10.1016/j.dsr2.2019.07.006

W. Zhang, X. Sun, S. Zheng, M. Zhu, J. Liang, J. Du, C. Yang

INTRODUCTION "In the present study, phytoplankton and zooplankton in the continental slope of the South China Sea were analyzed in terms of plankton abundance, biovolume and NBSS [normalized biovolume size spectra] by using flow cytometry and microscopy (FlowCam) and ZooScan. The relationship between plankton distributions and mesoscale currents, as well as that between environment factors and NBSS slopes, were analyzed."

FlowCam METHOD "All samples were fixed in 5% formaldehyde and analyzed in the laboratory... The samples from the microplankton net were analyzed by FlowCam... Phytoplankton were counted and estimated using FlowCam in an autotrigger mode... Taxa information was obtained based on the South China Sea phytoplankton sample library of the FlowCam system... Before constructing the NBSS, all the organisms were picked out from the original FlowCam...pictures, and all the detrital particles were discarded. Then, the phytoplankton...information (including the classification information, plankton length and volume and other particle parameters) was exported from the FlowCam."

RESULTS & CONCLUSIONS "*Rhizosolenia* dominated the phytoplankton community in autumn. In summer, the dominant phytoplankton were *Chaetoceros* and *Thalassionema*... Significantly higher phytoplankton and zooplankton abundances were observed in summer than in autumn... Small plankton were the predominant group in the summer, which in turn resulted in steeper NBSS slopes than those observed during autumn."

[Plankton composition data on next page.]

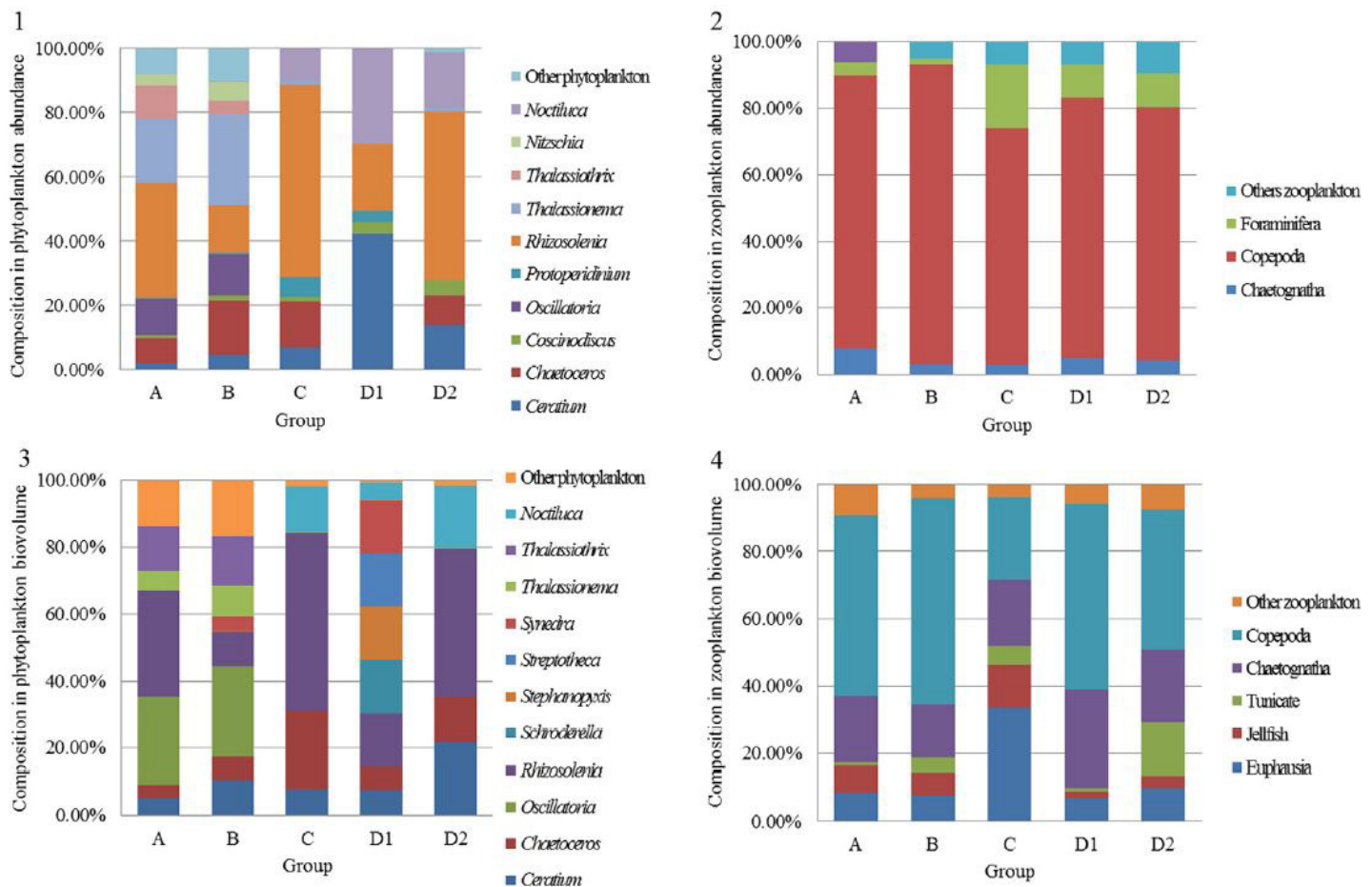


Fig. 4. Plankton composition of the 5 groups in the northern slope of the South China Sea.

Resource availability affects temporal variation of phytoplankton size structure in the Kuroshio east of Taiwan

Limnology and Oceanography (2019)

doi:10.1002/lno.11294

F. Lin, P. Ho, A. Sastri, C. Chen, G. Gong, S. Jan, C. Hsieh

INTRODUCTION "The size structure of phytoplankton assemblages has been a research focus since the 1960s and 1970s...because [they represent] nearly 50% of global primary production... The normalized biomass size spectrum (NBSS) slope has been used...to investigate how the size structure of a community changes in response to environmental variation..." due to factors such as resource supply and temperature. "The resource-size relationship and the temperature-size relationship have been widely investigated...[but] which relationship dominates in seas?... [We] investigated the size structure of the phytoplankton assemblage in the Kuroshio Current east of Taiwan... Our

aim is to examine how size spectral slopes of phytoplankton assemblages vary with environmental conditions in an oligotrophic sea."

FlowCam METHOD The researchers measured phytoplankton cell sizes using the FlowCam in autoimage mode with the 10X objective/100µm flow cell configuration for nanoplankton, and the 4X objective/300µm flow cell for microphytoplankton. All images were manually reviewed in VisualSpreadsheet® to remove detritus. Particles in the 3 - 5µm range were too small to identify visually, so all circular particles were assumed to be cells using VisualSpreadsheet's "Circle-Fit" function. "Cells in the 20-200µm range were classified into phytoplankton taxa...[and] images of phytoplankton [in] the 3-200µm ESD [equivalent spherical diameter] range were taken [and] converted to biovolume...to construct NBSS..." The researchers calculated biomass from biovolume via "taxon-specific equations for conversion."

RESULTS & CONCLUSIONS “While our results generally support the resource-size relationship, we found a clear exception during the cold season when resource availability is at its highest. We hypothesize that this difference occurs during the initial stage of resource enrichment after a long period of resource depletion in oligotrophic marine systems, when the small cells with low diffusive boundary layers take up resources more efficiently than the larger cells.”

Massive Blooms of *Chattonella subsalsa* Biecheler (*Raphidophyceae*) in Hypereutrophic, Tropical Estuary - Guanabara Bay, Brazil

Frontiers in Marine Science (2019)

doi:10.3389/fmars.2019.00085

T. Viana, G. Fistarol, M. Amario, R. Menezes, B. Carneiro, D. Chaves, P. Hargreaves, A. Silva-Lima, J. Valentin, D. Tenenbaum, E. Arruda, R. Paranhos, P. Salomon

INTRODUCTION “In this study we present the results of an intense, 4-year monitoring program in Guanabara Bay [a large, highly-eutrophicated bay on the southeast coast of Brazil] that unveiled the occurrence of massive blooms of the raphidophyte *Chattonella subsalsa* in this environment. The taxonomic identity, tolerance to salinity and toxic potential of *C. subsalsa* was assessed by means of cultures established from cells isolated from the bay.”

FlowCam METHOD The researchers obtained cell counts from live samples within 3h of sampling using the FlowCam. Samples were sieved through a 100µm nylon mesh to remove larger particles and prevent blockages, then run through the FlowCam in autoimage mode using a 90µm field-of-view flow cell and 10X objective at a rate of 0.1 mL min⁻¹ and 20 frames per second for 20 minutes. “The threshold for acquisition [was] set on an ESD (equivalent spherical diameter) value of 5µm. Classification of *C. subsalsa* cells was done with the VisualSpreadsheet®... based on a library of ca. five thousand *C. subsalsa* cells images selected from the Guanabara Bay database. A visual inspection was then performed by a human operator to correct for false positive and false negative errors by the software... A total of 1,776 sharp images from the FlowCam displaying the cells from the dorso-ventral view were selected for measurements of cell length and width.”

All samples with detectable numbers of *Chattonella* were also examined live on the day of sampling using inverted microscope with a digital camera.

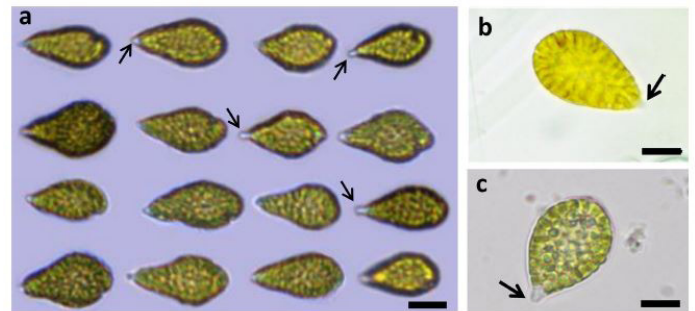


Figure 4. Live *C. subsalsa* cells from plankton samples collected in Guanabara Bay. (a) Live cells imaged by the FlowCam®; (b,c) Live cells observed in an inverted microscope at 40X magnification. The hyaline, tail-like protrusion at the posterior end of the cells typical of *C. subsalsa* can be seen (arrows). Scale: a: 10µm, b and c: 20µm.

RESULTS & CONCLUSIONS “Bloom episodes ($>0.1 \times 10^6$ cells L⁻¹) were observed in 37 samples, mostly in a shallow (<10 m) area with extremely high nutrient and organic matter loads (average total P = 19µM and total N = 344µM), intermediate salinity (average 24.5), and low water transparency (average Secchi depth = 0.54m) due to continental runoff. Blooms in this area reached up to 13.3×10^6 cells L⁻¹. *C. subsalsa* cell concentration was correlated with parameters linked to eutrophication of the bay... A set of 1,776 cells measured in the images from the FlowCam were $34.9 \pm 4.5\mu\text{m}$ (mean \pm SD) long and $20.1 \pm 3.1\mu\text{m}$ (mean \pm SD) wide... A hyaline, tail-like protrusion at the posterior end of the cells, one of the hallmarks of *C. subsalsa* Biecheler... was often observed both in the microscope and in the FlowCam images... Our four-year monitoring unveiled a consistent presence of *C. subsalsa* in the surface waters of Guanabara Bay, with recurrent blooming events at very high concentrations.”