USING A FLOWCAM TO FACILITATE HAB CELL COUNTS: COMPARING CELL ENUMERATION METHODS

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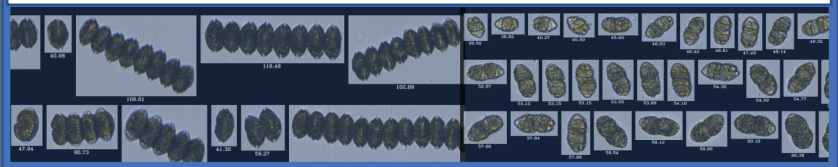
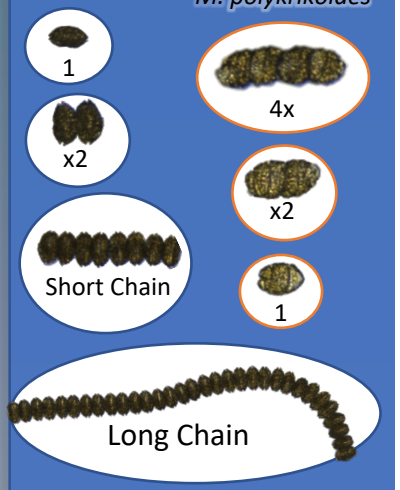
Introduction

Coastal environments, aquatic and human health can be devastated by harmful algal blooms (HAB), thus efficient monitoring of HAB species is critical. Two HAB species in the lower Chesapeake Bay, *Alexandrium monilatum* and *Margalefidinium polykrikoides* form cell chains in bloom conditions and the individual cells are tedious to count with traditional microscopy methods. To improve HAB monitoring efficiency, we used a FlowCam in addition to a traditional microscopy method (Sedgewick Rafter Counting Chamber) and quantitative PCR (qPCR) during the 2020 *A. monilatum* and the termination of the *M. polykrikoides* blooms in the York River, VA.

FlowCam Cell Enumeration Methodology

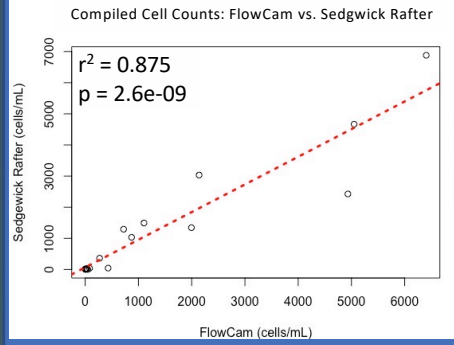


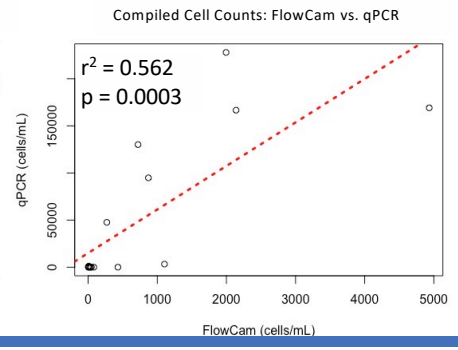


Using VisualSpreadsheet:

1. Build image libraries of different species from processed samples.
2. Create classifications of cell images from libraries based on species and length of cell chain. Classifications created for this study are pictured on the left.
3. Apply classifications as a filter on processed samples.
4. Extract data from into an EXCEL spreadsheet.
5. Compute cell density (µm^2) by finding the average area of a single cell for each class
6. Calculate cell concentration by dividing cell density by the product of the mean cell area for the sample multiplied by particles per milliliter.
*((mean cell area * particles/mL)/average cell density)*

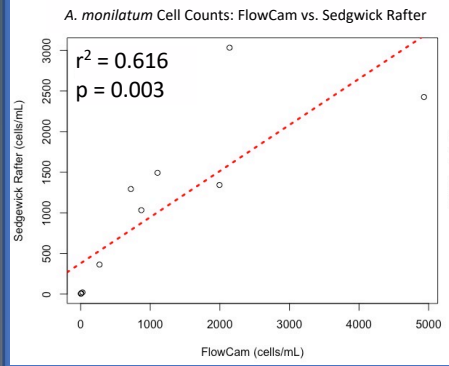
Cell Enumeration Methods Comparison Results

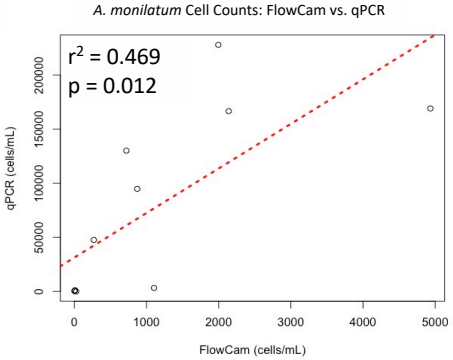




Linear regression comparison of FlowCam and Sedgwick Rafter cell counts, and FlowCam and qPCR cell counts. Compiled results include *A. monilatum* and *M. polykrikoides* cell count data. Correlations between FlowCam vs. Sedgewick Rafter and FlowCam vs. qPCR counts are statistically significant. FlowCam, Sedgwick Rafter, and qPCR cell counts were comparable for *M. polykrikoides*, though there were not enough data points to determine significance of correlations.

A. monilatum Results





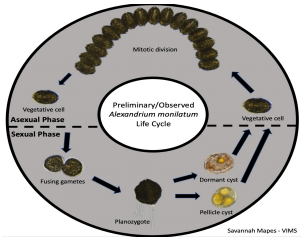
Significant correlations between FlowCam vs. Sedgewick Rafter and FlowCam vs. qPCR were observed. Raw data (not present) of FlowCam and Sedgewick Rafter cell counts were consistently comparable, while qPCR cell counts for *A. monilatum* were consistently much higher (x10^2) than either FlowCam or Sedgewick Rafter counts.

Discussion

The FlowCam is an efficient alternative to manual cell counts, providing more data (particle images and parameters) in less time. Results of all three cell counting methods are comparable for *M. polykrikoides.* With *A. monilatum*, the Sedgewick Rafter and FlowCam method are approximate, but the qPCR method greatly overestimates. It is possible that this is due to using a single clonal culture as the standard for qPCR. rDNA variability among strains is observed for other *Alexandrium* species[1,2]. In addition, multiple life stages with different ploidy levels are observed in field samples. Additional clonal cultures are being established to obtain additional qPCR curves.

Next Steps

- Create virtual libraries of other common and/or threatening phytoplankton found in the lower Chesapeake Bay to further enhance the HAB monitoring program
- Solve discrepancy between visual and FlowCam cell counts vs. qPCR method for *A. monilatum*
  - rDNA copy variations among strains?
  - Ploidy variation among life cycle stages
- Combine FlowCam data collected from the 2020 *A. monilatum* bloom with lab analyses (microscopy, flow cytometry, growth experiments) of *A. monilatum* cultures to determine its complete life cycle and ploidy in all life stages.



Acknowledgements

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References

[1] Galluzzi et al. 2010 *Analysis of rRNA gene content in* A. catenella *and* A. taylori….
[2] Murray et al., 2019 *Evaluation of sxtA and rDNA qPCR assays in* A. catenella…