Final report
Project Name: A new HAB invader in the Inland Bays: Initial isolation and characterization of a low-salinity raphidophyte species unknown to science
Major PIs: Drs David A. Hutchins and Kathy Coyne (College of Marine Studies, UD)
Graduate Student: Elif Demir (College of Marine Studies, UD)

Accomplishments
In the past year, we have successfully isolated the "New Raphidophyte" and currently have the culture in our laboratory. Our efforts in identifying this new species can be summarized as:
-Microscopy analysis
-Molecular and phylogenetic analysis
-Environmental characterization (including bottom-up and top-down controls).

Microscopy analysis: We completed light microscopy, scanning electron microscopy, transmission electron microscopy and confocal microscopy analysis of the new species along with *Heterosigma akashiwo* for comparison purposes. These analyses have resulted in a collection of high quality images on the cellular structure of this alga that is being used in the manuscript in preparation. Our microscopy results indicate that there is a definite difference between *Heterosigma* and the new species in average cell size (length and width), chloroplast shape and numbers per cell, mitochondria inner tubular structure and the size of the fibrous body located in the cell.

Molecular and phylogenetic analysis: We have completed analysis of the complete 18S (rDNA) sequence of this species, and have compared it to all the other raphidophyceae sequences available on Genbank (both marine/brackish and fresh water). Additionally, we have started collaborating with Dr. Gertrud Cronberg (Lund University, Sweden) to obtain *Gonyostomum* sp. DNA to add to our phylogenetic analysis since the DNA sequence has not been deposited to Genbank previously. Our phylogenetic tree includes all the marine/brackish water and fresh water genera of raphidophytes which has not been reported in any publication previously as well. Our results from phylogenetic analysis indicate that the new species is a marine/brackish water raphidophyte that is located between *Chattonella* and *Heterosigma*, and as far removed from *Heterosigma* as much as *Heterosigma* is removed from *Chattonella*. Therefore, within the current description of the group Raphidophyceae, the new species belongs to a novel genus. As proposed in our project, we also designed species-specific primer set and a probe to use in our Quantitative Real Time PCR (QPCR) analyses of environmental samples.

Environmental characterization: Since the first observation of this new alga was made in a low salinity environment, in the summer of 2005 we sampled Miller Pond, Cedar Creek Millpond, Blair Pond, Griffith Lake, Heaven Lake, Waples Mill Pond and Red Mill Pond, which are fresh water ponds along with Love Creek. Our results indicate that the new raphidophte species was present in Love Creek, however was not observed in any of the fresh water locations. We also performed microzooplankton grazing experiments (top-down control -Love Creek), salinity transect of Love Creek (0 – 9 ppt) and Sassafrass Landing (16 – 18 ppt) and weekly sampling of Love Creek, Bill's Canal and Russells

Canal (2005 and 2006). We have compiled an extensive DNA sample collection from various locations (such as Delaware Bay salinity transect, Delaware Inland Bays). We have also collected salinity, temperature and dissolved nutrient data and the analyses are underway to establish a relationship with the occurrence of this new species with any of these environmental factors. Winter of 2006, we performed a salinity/growth tolerance experiment on the culture of the new alga that's been isolated from DIB. Our results indicate that this species can grow in salinities between 5 and 17 ppt and tolerate salinities as low as 3 ppt for at least one generation. Additionally we have collected pigment samples from our cultures and got them analyzed along with *Heterosigma* cultures, confirming that this new species is in fact a marine/brackish water raphidophyte.

Overall, we have compiled enough evidence that this new species belongs to a new genus within raphidophyceae. Our first paper describing the new species/genus as Viridita minima has been submitted to Journal of Phycology. We are currently working up results of laboratory experiments to establish growth requirements (nutrients) and analyzing data from our environmental collections which will be included in our second paper that will come out of this project. We have also raised additional funding through Delaware Epscor, ECOHAB and MERHAB. Results of the species description part of this project were presented in the 12th International Conference on Harmful Algae, 4-8 September 2006 in Copenhagen, Denmark, and at the STAC meeting on October 27, 2006.

Publications

Demir, E, Hutchins, D.A., Czymmeck, K.J., and Coyne, K.J. (In review). Description of *Viridita minima* (gen. et sp. nov.), a new raphidophyte from Delaware's Inland Bays. *Journal of Phycology*

Presentations

Demir, E., Coyne, K.J., Czymmek, K. and Hutchins, D.A. Description of a novel raphidophyte species and genus from Delaware's Inland Bays, USA. Poster presented by E. Demir.