Investigation of Toxic Raphidophyte Population Dynamics Using Molecular and Physiological Tools

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Healthy Communities and Ecosystems

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Introduction:

Harmful algal blooms are phytoplankton blooms that are detrimental to humans or the ecosystem. The incidence of these blooms has increased over the past 30 years. Raphidophytes are among the most noxious red tide phytoplankton. In 2000, an abrupt and unprecedented bloom of the toxic Raphidophyte *Chattonella verruculosa* in the Delaware Inland Bays (DIB) caused massive mortality of marine life. Extensive monitoring revealed the presence of Raphidophytes throughout the bays, and blooms have occurred several times since their discovery. Initially thought to consist of unialgal blooms of *Chattonella*, it has since become clear that Raphidophyte blooms in the bays are instead made up of a consortium of four Raphidophyte species. The effects of environmental and physical factors, as well as biotic interactions, on the dominance and succession of mixed Raphidophyte blooms are currently unknown. This investigation addresses fundamental questions of Raphidophyte physiology and population dynamics.

Objectives:

- To gain a better understanding of the effects of environmental perturbations and grazing pressure on Raphidophyte community dynamics;
- To identify environmental factors that stimulate the growth of Raphidophytes relative to other algal species;
- To investigate the potential of Raphidophyte cyst distributions as an indicator of seasonal bloom "hot spots".

Approach:

- Raphidophyte assemblages at key sites in the DIB are routinely monitored in collaboration with Delaware's Dept. of Natural Resources and Environmental Control (DNREC) and the Volunteer Phytoplankton Monitoring Group.
- The relative abundances of Raphidophytes are monitored using sensitive molecular techniques
- Using laboratory cultures of unialgal Raphidophytes, environmental parameters such as nutrients, light, temperature and salinity are then correlated to Raphidophyte population dynamics.
- Sediment samples are also collected to determine the distribution and relative abundance of Raphidophyte cyst (resting stage) populations.

What is ECOHAB?

Ecology and Oceanography of Harmful Algal Blooms

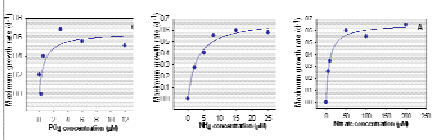
The increasing trend of harmful algal blooms has had devastating effects on people and economics. ECOHAB was created to help understand and stop this trend. ECOHAB is in part funded by EPA.

Laboratory Work

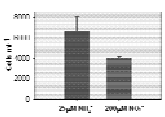
- Isolate algal samples from natural blooms
- Grow cultures individually
- Measure growth parameters to understand environmental preferences of different Raphidophyte species

Nutrients

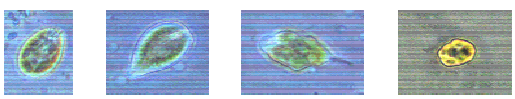
Laboratory cultures of *Chattonella subsalsa* were used in this investigation.



The growth rates of cultured *Chattonella subsalsa* with varying nitrate (A), ammonium (B) and phosphate (C) concentrations

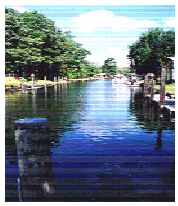


The *Chattonella subsalsa* growth in response to different inorganic nitrogen sources



Field Work

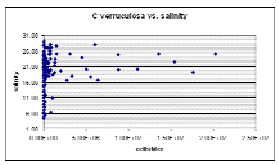
- Environmental water and sediment samples are collected for molecular analyses
- Physical and chemical factors are measured and correlated to presence of Raphidophyte species.

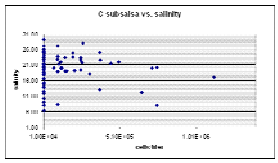


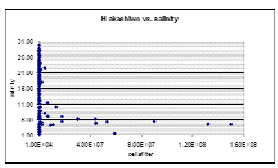
Example of dead-end canal where *Chattonella subsalsa* frequently blooms.

Salinity

Appearance of three Raphidophyte species compared with salinity in environmental samples.







Molecular Methods

Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE is a "fingerprint" of total phytoplankton diversity and is used to assess changes in community composition. Each lane on the gel represents a different sample. The bands in each lane represent different species.

Figure at right: DGGE analysis of total phytoplankton community at a single site over 4 weeks in 2003. Left panel is from surface water, right panel is from subsurface water.

PCR-Fluorescent Fragment Detection (PCR-FFD)

PCR-FFD is used to evaluate Raphidophyte population dynamics. The PCR primers amplify all Raphidophyte species, so that any of the 4 (or more) local species of Raphidophytes, in any combination, will be detected. For this method, fluorescently-labeled PCR products are separated on an ABI Prism 310 Genetic Analyzer and the fragment length and abundance of each PCR product is evaluated using GeneScan analysis software. This method is being used to track changes in relative abundances of Raphidophyte populations in both environmental samples and laboratory experiments using mixed cultures.

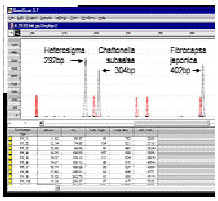
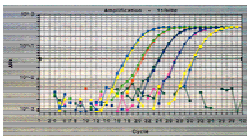
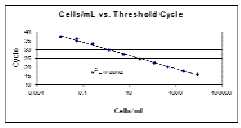


Image of PCR-FFD analysis of three Raphidophyte species.

Quantitative Real-Time PCR (QRT-PCR)

QRT-PCR is used to measure the abundance of individual species of Raphidophytes in environmental samples and laboratory cultures. In contrast to PCR-FFD, which only gives relative abundances, QRT-PCR provides an accurate count of each species. This is especially useful at low densities when microscopic cell counts are not possible.





QRT-PCR analysis of spiked samples of Chattonella subsalsa demonstrating sensitivity and wide dynamic range of detection.

IMPACT OF INVESTIGATION

❖A detailed picture of how interacting environmental factors, such as **nutrients, light, and salinity** together affect the growth and biomass of our local Raphidophyte community is essential in order to build a truly predictive understanding of bloom formation.

❖Information on biological factors such as **grazing and cyst distributions** will add to our emerging picture of bloom dynamics.

❖The development of state-of-the-art **molecular methods** for Raphidophyte detection and enumeration will **benefit algal research** far beyond the local Delaware area

Acknowledgements

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