Investigation of Toxic Raphidophyte Population Dynamics Using Molecular and Physiological Tools

Sara M. Hardy, Kathryn J. Cooney, S. Craig Cary, Martina Dotoli, Yaohang Zheng, Edward Wherrett, Kevin J. Portune and David A. Hutchins
University of Delaware Graduate College of Marine Studies, 700 Fletchertown Rd., Lewes, DE 19958

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 notorious 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 Raphidophyte 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 led to devastating effects on marine and coastal ecosystems. ECOHAB was created to help understand and stop this trend. ECOHAB is in part funded by EPA.

Laboratory Work
- Molecular algal samples have been collected.
- Genes have been isolated.
- Qualitative parameters are used to understand environmental preferences of different Raphidophyte species.
- Nutrients
- Laboratory cultures of Chattonella are used in this investigation.

Field Work
- Environmental water and sediment samples are collected for molecular analysis.
- Environmental and climatic factors are measured and correlated to presence of Chattonella species.

Molecular Methods
Denaturing Gradient Gel Electrophoresis (DGGE)
DGGE is a 'fingerprint' of total phytoplankton diversity and is used to assess changes in community structure. Each lane in the gel represents a different sample. The bands in each lane express different species.

PCR-Fluorescent Fragment Detection (PCR-FFD)
PCR-FFD is used to evaluate Raphidophytes population dynamics. The PCR primer amplifies all Raphidophytes species, so that any of the 4 or more local species of Raphidophytes, in any combination, will be detectable. For this reason, labeled PCR products are separated on an ABI Prism S1000 Genetic Analyzer and the fluorescein 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 Raphidophytes present in both environmental samples and laboratory experiments using labeled cultures.

Quantitative Real-Time PCR (QRT-PCR)
QRT-PCR is used to measure the abundance of individual elements of Raphidophytes in environmental samples and laboratory cultures. In contrast to PCR-FFD, which only amplifies global phytoplankton, PCR-FFD provides a accurate count of each species. This is especially useful in field situations when relationships between environmental variables and cysts are not prevalent.

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 our research far beyond the local Delaware area.

Acknowledgements
This research is funded by EPA-ECOHAB. We are grateful to Bill Davis for photography of environmental bloom sample and field sites. Photos of Raphidophyte years taken from the Maryland Department of Natural Resources webpage.

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