Title
A Mechanism Coupling Sewage Effluent to the Dark Survival of the Toxic Diatom Pseudo-nitzschia sp. and Subsequent Initiation of Toxic Blooms by Coastal Upwelling

Author
Menqelt, Claudia

Publication Date
2005-01-28

Final Progress Report
California Coastal Environmental Quality Initiative Fellowship
University of California Marine Council

Project Reference Number 02T CEQI 08 0094

Attachments: - Copy Manuscript "Dark Survival and Light Recovery of *Pseudo-nitzschia multiseries*"
- Copy Manuscript "UVA Enhancement of Carbon Fixation and Elevated Resilience to UVA Inhibition for the Genus *Pseudo-nitzschia* May Provide Competitive Advantage in High UV Surface Waters"

Claudia Mengelt

A Mechanism Coupling Sewage Effluent to the Dark Survival of the Toxic Diatom *Pseudo-nitzschia sp.* and Subsequent Initiation of Toxic Blooms by Coastal Upwelling

Proposed Research:

The goal of this project was to examine a potential link between subsurface enrichment of organic nutrients due to sewage effluent and enhanced dark survival of toxigenic diatoms of the genus *Pseudo-nitzschia*. The aim was to test the hypothesis that organic nutrient addition increases the survival and viability of a *Pseudo-nitzschia sp.* population in the dark thus leading to an increased seed bank of viable *Pseudo-nitzschia sp.* cells near the seafloor dominating the diatom community during a subsequent upwelling event and ensuing diatom bloom. The following research priorities were identified to examine above-mentioned hypothesis:

I. *Pseudo-nitzschia sp* survival and viability in the dark near the bottom is enhanced by the addition of sewage effluent due to its heterotrophic capability.

II. *Pseudo-nitzschia sp* is capable of adapting to an extreme light shift, such as associated with an upwelling event from very dim light near the bottom to high light in the surface mixed layer.

Project Results:

<u>Hypothesis I.</u> ***Pseudo-nitzschia sp*** **survival and viability in the dark near the bottom is enhanced by the addition of sewage effluent due to its heterotrophic capability.**

In order to address this hypothesis, I first investigated the ability of the culture *Pseudo-nitzschia multiseries* to survive long periods in the dark and subsequently recover in the light. The organic substrates, glutamate and glutamine, were added to the culture in order to test for enhanced dark survival (up to 10 weeks), and to examine its ability for light recovery after these extended periods in the dark. Growth rates following dark survival were enhanced for cultures surviving under heterotrophic conditions. However, duration of dark survival was not enhanced with glutamate and glutamine additions, possibly due to bacterial contamination or due to the rapid disappearance of the organic substrate. Our experiments confirmed the ability of *P. multiseries* to survive six weeks in the dark without forming a morphologically distinct resting stage regardless of the availability of organic substrate. Cultures in both treatments were able to grow photosynthetically after extended periods in the dark, confirming the hypothesis that upwelling of seed populations from below the euphotic zone could initiate a new bloom. Further details are available in the draft manuscript attached to this report that summarizes the approach and results for this experiment.

Additional experiments were conducted with antibiotics to examine effects of glutamate and glutamine on short-term light growth both in the presence and absence of bacteria. Results from these experiments confirm that glutamate enhances growth of *P. multiseries* in the light. These results suggest that the increase in cell numbers during the first week in the organic treatment in the dark was indeed the consequence of growth stimulated by glutamate and glutamine.

Two follow-up experiments were initiated to investigate the role of glutamate and glutamine in the dark in the absence of bacteria, however during one experiment fungal contamination was discovered and resulted in termination of the experiment. During the second bacterial contamination persisted despite attempts to minimize it using sterile techniques and antibiotics. Due to these difficulties with maintaining bacteria-free cultures a sewage addition experiment was deemed not feasible.

<u>Hypothesis II.</u> ***Pseudo-nitzschia sp.*** **is capable of adapting to an extreme light shift, such as associated with an upwelling event from very dim light near the bottom to high light in the surface mixed layer.**

In order to address this hypothesis, the organism's ability to adapt to high-light was examined and the following approach was undertaken:

a) **Results from field experiments designed to study the UV sensitivity of diverse algal communities were analyzed and compared to the UV sensitivity of *Pseudo-nitzschia australis* dominated communities**. The comparison allows us to determine the ability of *P. australis* to adapt to high-light/UV exposure *in situ* relative to other phytoplankton. Based on these studies we concluded that *P. australis* photosynthesis is well adapted for a high light environment and may have a competitive advantage over other algal communities in surface waters where UVA and PAR penetrates but UVB is attenuated. These findings are described in detail in the manuscript "UVA Enhancement of Carbon Fixation and Elevated Resilience to UVA Inhibition for the Genus *Pseudo-nitzschia* May Provide Competitive Advantage in High UV Surface Waters." (see attachment), which has been submitted to the journal Marine Ecology Progress Series and is currently in review.

b) **Field samples of *P. australis* dominated bloom communities were incubated to test for the UV sensitivity of light harvesting pigments, cellular growth and cellular domoic acid concentration.** Results from these experiments allow us to determine mechanisms of high light adaptation and sensitivity of important light harvesting functions of photosynthetic pigments. Samples under varying light treatments were analyzed to observe changes in absorption spectra resulting from differential UV exposure. Analysis of these results is in progress and expected to be completed by spring and a copy of any manuscript published will be submitted.

c) **A culture of *P. multiseries* grown under low light was incubated in high-light, and high-light + UV to test for the high-light/UV sensitivity of light harvesting pigments, photosynthetic yield, cellular growth, cDOM production and cellular domoic acid concentration.** Findings are currently being analyzed and prepared for publication and indicate that the organism's growth ability is not reduced by increasing the light levels to 30% surface light or by adding UVA irradiance. However, the photosynthesis and growth is inhibited by the addition of UVB irradiance, which is common for phytoplankton growth. Changes in light harvesting occur during the afternoon hours and appear to be induced by UVA. These results are expected to be prepared for publication by spring or summer of 2005 and a copy of any manuscript published will be submitted.

Related Projects:

The funding for this provided unanticipated opportunities to expand on this project and foster undergraduate research and collaborations. An undergraduate senior honors thesis in Environmental Science addressed the question of organic nutrient enhancement of photosynthetic growth. The undergraduate thesis project has allowed the student to compete successfully for several campus-wide undergraduate research awards. Based on the undergraduates academic and scientific credentials she was also selected to present this research at the U.C. Day in Sacramento (2003) as a representative of UCSB undergraduate researchers. In addition, funding provided by this project allowed our research laboratory to establish collaborations with Mary Silver, who has been monitoring the occurrence of *Pseudo-nitzschia sp.* and associated toxins in the food chain in Monterey Bay. Within a year of this project the collaboration yielded a multi-campus proposal (PIs: M. Silver, E Venrick, B. Prézelin) to work on a coastal wide effort to monitor for the occurrence of harmful algal blooms, including *Pseudo-nitzschia sp*., which was generously awarded with two years of funding by the CEQI. In the context of this second project I developed the outreach component of our multi-campus collaboration, focused on harmful algal blooms and toxin transfer in the marine food chain. Several lesson plans were developed to meet 6-8 grade science standards, which will be available to the public online at www.habtrac.com.

Conclusions

The funding for this project allowed for the discovery of unusual UV adaptive characteristic of *P. australis* (Mengelt et al in review), experimental determination of length of dark survival of *P. multiseries* (Mengelt & Prézelin, in prep), and experimental determination of high light adaptation in *P. australis* and *P. multiseries.* While not initially part of the project proposal, funding for this project also allowed for the mentoring of an undergraduate senior honors thesis and for the initiation of a multi-campus project to monitor for harmful algal species along the California Coast and development of an outreach component for 6 - 8 grade science curriculum.

Recommendations

The literature provides evidence that *Pseudo-nitzschia* species in experimental setup can grow better on organic substrate and that under certain conditions sewage effluent will result in *P. multiseries* dominating the phytoplankton community (Hillebrand & Sommer 1996; Pan & Subba Rao 1997). Our results do not support the hypothesis that organic nutrients increase the period of dark survival or the viability of a seed population in the dark. Hence the role organic nutrients play in supplementing growth and predominance of *P. australis* remains unclear along our coast, especially considering the relative high oceanic flushing rates in the Santa Barbara Channel. However, around the world the occurrence of harmful algal blooms is on the rise and in many instances has been linked to increased coastal population and/or nutrient pollution (Smayda, 1990). It is therefore of utmost importance to monitor continuously for the occurrence of toxic or harmful algae and reassess the eutrophication status of our coastal oceans on a regular basis. For that purpose I feel that nutrient loading from runoff, sewage effluent, but also from groundwater flux needs to be carefully monitored and emphasis should not only be placed on inorganic but also organic algal nutrients.

Publication Record

Results from testing the first hypothesis have been presented at the 10th International Harmful Algal Bloom conference in St. Petersburg (Mengelt & Prézelin, poster), at the California & the World Ocean Conference in Santa Barbara (Mengelt & Prézelin, talk), and at the U.C. Day in Sacramento 2003 (Hernandez et al, poster). Conference proceedings have been published for the 10th International Harmful Algal Bloom conference in St. Petersburg and the California & the World Ocean Conference in Santa Barbara. These results are also summarized in a draft manuscript attached to this report (see attachments). Results from testing hypothesis II have been presented at the Ocean Science Conference in Hawaii (Mengelt & Prézelin, poster). A manuscript summarizing findings from Hypothesis II a) has been submitted for publication, is in review and a current draft is attached to this report (Mengelt & Prézelin, submitted).

Reference:

Hillebrand, H., and Sommer, U. (1996). "Nitrogenous nutrition of the potentially toxic diatom *Pseudonitzchia pungens f. multiseries* Hasle." *Journal of Plankton Research*, 18(2): 295-301.

Mengelt, C. and B.B Prézelin, submitted. “UVA Enhancement of Carbon Fixation and Elevated Resilience to UVA Inhibition for the Genus *Pseudo-nitzschia* May Provide Competitive Advantage in High UV Surface Waters.” *Marine Ecology Progress Series.*

Pan, Y., Subba Rao, D.V. (1997). "Impacts of Domestic Sewage Effluent on Phytoplankton from Bedford Basin, Eastern Canada." *Marine Pollution Bulletin*, 32(12): 1001-1005.

Smayda, T. J. (1990). “Novel and nuisance phytoplankton blooms in the sea: Evidence for a global epidemic.” *Toxic Marine Phytoplankton.* E. Graneli [ed.], Elsevier Science Publishing Co., Inc.: 29-40.