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Final Report: **Sea Grant Award R/CZ-145**

Title: Assessing Environmental Control of Domoic Acid Production by the Planktonic Diatom *Pseudo-nitzschia australis* in California Waters

Principle Investigators: Mary Silver, David Garrison, and Ron Tjeerdema

Narrative Summary

This Sea Grant project focused on domoic acid production by the toxin-producing diatoms (*Pseudo-nitzschia australis and P. multisieries)* in coastal California waters. There were two major aspects of our work: 1) the development of a laboratory HPLC assay for measuring domoic acid and the tracking of DA in a field mortality event using the HPLC assay, and 2) developing continuous culture facilities and examining the nutrient physiology of *Pseudo-nitzschia* species.

1. Development of a Laboratory HPLC assay for Domoic Acid and Field Studies of a Domoic Acid Event

UV HPLC. We found the basic method of Quilliam et al. 1991, to be quite suitable after applying the modification of Hatfield et al. (1994). Basically, DA is extracted via homogenization, aqueous extraction and filtration through a strong anion exchange column. Twenty ul of sample is injected and analyzed using an isocratic elution profile on a Hewlett-Packard 1090 HPLC equipped with a diode array detector set at 280 nm and a flow rate of -.3 ml/min. The instrument detection limit is 0.5 um domoic acid/ml. This method can be used for animal tissues such as those of anchovies.

FMOC. This method is used to determine trace amounts of DA and our method, used for phytoplankton material is essentially similar to the published one (Pocklington et al, 1990) with slight modifications. The method involves pre-column derivitization of DA in cell and seawater samples with 9-fluorenylmethylchloroformate reagent to form the FMOC-derivative. The reaction occurs in 45 seconds at pH 7.2-7.6. Using gradient elution, 20 ul sample injection, and dihydrokainic acid as an internal standard, the detection limit is 1 ng/l. The fluorescence detector used in the HPLC analysis is highly sensitive and enable the detection of much lower levels of DA than can be measured by the HPLC-UV method.

(Text References: Quilliam, M. A., M. Xie, and W. R. Hardstaff. 1991. Nat. Res. Counc. Canad. Techn. Rep 64:No. 33002; Hatfield, C. L., J. C. Wekell, E. J. Gauglitz Jr., H. J. Barnett. Nat. Toxins 2:206; Pocklington, R, J. E. Milley, S. S. Bates, C. J. Bird, A. S. W. Defreitas, M. A. Quilliam. 1990. Int. J. Environ. Analyt. Chem 38:351)

The availability of the HPLC assay for domoic acid allowed us to measure domoic acid in a dramatic mortality event that occurred in May 1998 in Monterey Bay. At the time, nearly 100 sea lions (*Zalophus californianus*) were found seizuring or ill on coastal beaches and many died. They appeared to have been poisoned as a result of eating anchovies (*Engraulis mordax*) that had consumed *Pseudo-nitzschia australis* during an unusual spring bloom of that toxic species. Sea Grant trainee Kathi Lefebvre undertook the analysis of animal samples from the event. Measurements at UCSC of DA in anchovy tissues and sea lion feces were made using HPLC-UV. The highest DA concentrations in anchovies occurred in the viscera, where levels reached over 220 ug/g tissue. HPLC values for DA in sea lion feces ranged from 136-137 ug DA/g. Confirmation of the presence of *P. australis* in sea lion fecal material and anchovy stomach contents was made by scanning electron microscopy, which revealed the diagnostic frustules of this diatom and confirmed the *Pseudo-nitzschia* source of the toxin. These results were published (Lefebvre et al., 1999) and widely publicized in the media.

2. Continuous Culture Facilities and Nutrient Dynamics Experiments.

With support from Sea Grant, a continuous culture facility was developed at the Institute of Marine Sciences at the University of California. The experimental apparatus was established in a walk-in cold room capable of holding constant temperatures with in the range of approximately 12-18 °C. A bank of eight high-intensity fluorescent lamps provide light levels up to 125 µmol photons m^{-2} sec^{-1} to allow experiments at light saturation for surface phytoplankton. Illumination can be reduced by using neutral density filters. Flow of media to the culture vessels is controlled by two peristaltic pumps (Watson Marlow multichannel cartridge pump and Masterflex L/S Modular Pump). Experimental vessels are 1000 ml polycarbonate cylinders. The present design allows us to follow growth dynamics in four replicate culture vessels. The entire continuous culture apparatus is fabricated without glass components so that silicate-limited experiments can be done. Protocols have been developed for performing standardized experiments under silicate, nitrate, and phosphate-limited conditions. In addition the cultures protocols have been developed to isolate and maintain *Pseudo-nitzschia* clones. This facility is now being used extensively as part of an EPA and NOAA supported studies of *Pseudo-nitzschia.*

Physiological studies were performed by a Sea Grant Trainee, Wendy Enright Storms. Preliminary findings were reported at national and international meetings and the final work was submitted in partial fulfillment of a M.A. degree at the University of California at San Diego (see publication section).

Wendy's work focused on examining domoic acid production by *Pseudo-nitzschia multiseries* under four different media combinations. Because this work was concurrent with the development of the continuous culture apparatus, much of the work was done using batch cultures. These experiments, however, provided valuable information is subsequent continuous culture work.

Results of this experimental work indicated that *P. multiseries* produced domoic acid in greater amounts that *P. australis.* These results are at variance with many field measurements of domoic acid content of these two species. *P. australis* is a much larger cell than *P. multiseries,* so higher per cell accumulation of domoic acid is expected (and has been observed) by *P. australis.* These results apparently reflect the difficulty of maintaining healthy cultures of *P. australis* for extended periods. In this work, and as also observed by other workers, *P. australis* loses viability after only a short time in culture. The ability to isolate and maintain healthy P. australis cultures for experimental work continue to be a major impediment to studies of domoic acid by this important species in California waters.

Because of the difficulty working with *P. australis,* most of the comparative work with different macronutrient regimes was completed using *P. multiseries.* Domoic acid was producted by this species under silicate, nitrogen, and phosphate limited culture conditions. Silicate limited conditions resulted in most domoic acid production. Production of domoic acid under nitrogen limitation has not previous been found for this species. Domoic acid production does not appear to be limited to periods of stationary growth (as most previous reports suggest), but a consistent relationship of domoic acid production with growth rates could not be established. Most domoic acid was found in the particulate fraction (e.g. within cells of *Pseudo-nitzschia*), except near the end of experiments when significant amounts of domoic acid were found in the dissolved fractions. This finding was attributed to release of domoic acid by senescent or dying cells.

Publications.

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Storms, W.E., D.L. Garrison, and P.J.S. Franks. 2000. Growth and domoic acid production by *Pseudo-nitzschia multiseries* and *P. Australis* under nitrate, silicate, and phosphate limited conditions (abstract). International Conference on Harmful Algal Blooms, Ninety Conference Tasmania 2000, 5-10 February, 2000. Hobart, Tasmania.

Storms, Wendy Enright, 2000. Growth and Domoic Acid in Two Species of a Toxic Diatom. MS Thesis, Scripps Institution of Oceanography, University of California, San Diego. 104 p.