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Phytoplankton Excretion Revisited: Healthy Cells May Not do It,
But How Many Cells Are Healthy?

CONTINUATION PROGRESS REPORT

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Dr. A.M. Wood

Dept. of Biology
University of Oregon
Eugene, Oregon 97403

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ABSTRACT

The primary purpose of the proposed research is to develop molecular tools for determining the health of marine phytoplankton on an individual cell basis. Since the definition of "healthy" in phytoplankton cells is elusive, we propose to develop markers for several different metabolic processes indicative of physiological state: photosynthetic activity, esterase activity, membrane permeability, and mitochondrial activity. One underlying motivation is to develop methods which will allow us to evaluate the hypothesis that, while healthy cells release very little DOC, many phytoplankton communities are comprised of "unhealthy" or physiologically stressed cells which release a large proportion of total photosynthate directly into the pool of labile DOC. This is proposed to be especially true in continental shelf and coastal environments where zones of productivity are patchy and phytoplankton populations adapted to one regime can be easily transported into waters which differ in salinity, nutrient supply, and/or turbidity.

The significance of the work, however, extends beyond this immediate goal since there are presently relatively few methods which allow us to estimate the physiological state of phytoplankton cells. When we evaluate population sizes of phytoplankton in the water column or examine fecal pellets, particulate aggregates, or other material, we generally work in ignorance of the activity of the cells except as the average cell-specific activity is estimated from bulk measurements. This approach effectively hides any differences in the relative contribution of different taxa or individuals to overall productivity even though most flux processes are sensitive to physiological and taxonomically determined differences among members of the community.

OBJECTIVES

The primary purpose of the research in progress is to develop molecular tools for determining the health of marine phytoplankton on an individual cell basis. Since the definition of "healthy" in phytoplankton cells is elusive, we are developing markers for several different metabolic processes indicative of physiological state: photosynthetic activity, esterase activity, membrane permeability, and mitochondrial activity. The ultimate objective is to develop a set of complementary probes which can be used simultaneously to provide a multi-parameter description of cell physiological state. One underlying motivation is to develop methods which will allow us to evaluate the hypothesis that, while healthy cells release very little dissolved organic carbon (DOC), many phytoplankton communities are comprised of "unhealthy" or physiologically stressed cells which release a large proportion of total photosynthate directly into the pool of labile DOC. This is proposed to be especially true in continental shelf environments where zones of productivity are patchy and phytoplankton populations adapted to one regime can be easily transported into waters which differ in salinity, nutrient supply, and/or turbidity.

BRIEF SUMMARY OF ACHIEVEMENTS TO DATE

So far, things are going well. Nile Red, a fluorescent dye for neutral lipids appears to provide useful information on the growth state of bloom-forming diatoms (Figure 1) and we are now linking the probe work to measurement of DOC production. A one-week research trip to Port Aransas to do DOC measurements in Ron Benner's Lab is planned for March, and we plan to continue this collaboration during Year 3.

We participated in a July cruise off the Cape Hatteras region. We sampled stations arranged on two hydrographic grids (Fig. 2) with the intent of examining spatial heterogeneity in water mass distribution, chlorophyll concentration, and phytoplankton community structure. The results from this cruise are extremely exciting. first, we observed that, on the shelf, under these summer stratified conditions, the bottom was euphotic and that nearly all the chlorophyll in the water column was in the benthic boundary layer (Fig. 3). Data from the shelf break clearly indicate off-shelf transport of the chlorophyll-enriched water mass (Fig. 4).

RESEARCH PLAN FOR YEAR 3

With the additional goal of submitting some of our results for publication (See Appendix 1 and above), our goals for Year 3 remain much as they were in Year 2. The one additional research objective is to develop a method for preserving samples

for staining with intracellular probes. At present all our staining protocols involve use of live material.

Specific Aims for Year 3:

The first goal of the project is to develop protocols for estimation of individual cell health, defined as a multiparameter character. We plan to continue to evaluate new probes and develop a quantitative way of expressing the character.

The second goal of the research is to characterize the metabolic state of exponentially growing, fully-acclimated, nutrient replete cultures of bloom-forming diatoms using the newly developed probe protocols.

The third goal is to examine the physiological response of bloom-forming diatoms to perturbations associated with increased excretion of photosynthate using the protocols for measurement of cell health. We are particularly interested in evaluating the amount and composition of the DOC released by phytoplankton in different growth stages.

The fourth goal of the project is to apply the measures of cell health to natural populations. We are working on techniques for the analysis of preserved samples, and will have samples collected for us on the spring, 1994, OMP cruises off Cape Hatteras. We continue to use Coos Bay estuary as a site for methods development, and are planning to conduct a small experiment in summer, 1994, to look at the physiological response of benthic diatoms that are entrained into the water column by tidal mixing.

PUBLICATIONS DURING PROJECT PERIOD :

Wood, A.M. and T. Leatham. 1992. The species concept in phytoplankton ecology. *J. Phycol.* 28:723-29.

Wood, A.M., H. Rai, J. Garnier, T. Kairesalo, S. Gresens, E. Orive, and B. Ravail. 1993. Practical approaches to algal excretion. *Marine Microbial Food Webs*, 6:21-38. (Appendix II)

Abstracts Submitted to ASLO/PSA, June, 1994 (Appendix I)

Figure 1. Exponentially growing (A) and late log phase (B) specimens of *Skeletonema costatum*, a common bloom-forming diatom, stained with Nile Red. Nile Red is a stain which makes neutral lipids fluoresce yellow; in the specimens the red fluorescence is from chlorophyll. Note the relative decrease in chlorophyll:lipid fluorescence in the older culture.

A

B

Figure 2. Map of study area from July, 1993, Cape Hatteras cruise. Stations were arranged in two hydrographic grid patterns. Stations 3-18 were selected to represent a mid-shelf environment in which station depth was relatively invariant $33 \pm 3\text{m}$ and stations 21-40 were selected to examine variation in water mass structure, phytoplankton community composition and chlorophyll concentration across the shelf-break

Figure 3. Chlorophyll concentration along two west-to-east transects in the mid-shelf grid; stations 3-18 (A) and stations 5-16 (B). Dashed red line indicates approximate depth of the bottom contour.

Figure 4. Chlorophyll concentration along a west-to-east transect in the cross-shelf grid; data are for stations 38-23. (A) data interpolated for the upper 50 meters (sampled at 1m intervals at each station); (B) data interpolated for the upper 450 meters (or to within 5 meters of the bottom, whichever was more shallow). Below 50 meters, samples were collected at 5 meter intervals at each station. Dashed red line indicates approximate depth of the bottom contour.

Abstracts from research resulting from DE-FG06-92 ER 61417
Submitted to ASLO/PSA Joint meeting, June 12-16, 1994

1. A. M. Wood, N.D. Sherry, and B. Flesher. Dept. of Biology, Univ. of Oregon, Eugene, Oregon, 97403. MOLECULAR PROBES AND PHYTOPLANKTON PHYSIOLOGY.

The notion of "cell health" has long been associated with consideration of extracellular release in phytoplankton. Do healthy cells do it? A central question, however, is, "What is a healthy cell?". Fluorogenic enzyme substrates, radiolabelling techniques, and fluorescent stains for neutral lipids and membrane permeability can be used to assess the "health" of individual cells and bulk populations as a multi-parameter character. Changes in this character, which can be quantified in vector form, were monitored through exponential growth and into stationary phase in batch cultures of *Skeletonema costatum* and correlated with rates of extracellular release. A similar approach was used to evaluate changes in the physiological condition of an oceanic phytoplankton community transported into a major estuarine system.

2. Sherry, N. D., and A. M. Wood, University of Oregon, Eugene, OR, 97403, U.S.A., A. J. Huyer, Oregon State University, Corvallis, OR, 97331, U.S.A., SUMMER CHLOROPHYLL AND WATER MASS DISTRIBUTION ON THE CONTINENTAL SHELF OFF CAPE HATTERAS

Vertical profiles of fluorescence, transmittance, conductivity, temperature, and pressure were determined at 37 stations off Cape Hatteras, North Carolina, U.S.A. in July, 1993. A 16 station mid-shelf grid was sampled along with a 21 station grid composed of four transects across the shelf break. Discrete water samples were collected at regular intervals to determine the correlation between *in-situ* fluorescence and chlorophyll concentration as determined by standard methods (Parsons *et al.* 1984). Secchi disk measurements, chlorophyll concentration, and turbidity estimates all indicate $> 1\%$ light penetration to the sediment surface at all mid-shelf stations suggesting a potential for significant benthic primary production on the shelf. Chlorophyll concentrations at the mid-shelf stations ranged from < 0.15 ppb in the surface mixed layer to greater than 8 ppb in the benthic boundary layer.

Abstracts from research resulting from DE-FG06-92 ER 61417
Submitted to ASLO/PSA Joint meeting, June 12-16, 1994

3. Gondek, S.M., N.D. Sherry, and A. M. Wood, Dept. of Biology, Univ. of Oregon, Eugene, Oregon, 97403, PHYTOPLANKTON COMMUNITIES OFF THE OREGON COAST.

Utermohl's settling technique was used to enumerate large ($>10\ \mu\text{m}$) phytoplankton in whole water samples collected off the Oregon coast in August, 1992. At each of the thirteen stations, samples were collected at a minimum of five depths, including the surface mixed layer, the pycnocline, and the deep chlorophyll maximum. All stations were less than 400 km offshore, and only two stations were inshore of the 100 fathom depth contour. Throughout the sampling region, the community of large phytoplankton was dominated by diatoms, particularly *Rhizosolenia*, *Eucampia*, and *Chaetocerus* spp., but total biomass decreased dramatically along the onshore-to-offshelf transect. *Nitzschia* spp., common at the most nearshore stations, may be a source of domoic acid in razor clams on the Oregon coast since these molluscs live in the sands of high energy exposed beaches.

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