## APOPTOSIS AND CELL LYSIS RATES DURING A DIATOM BLOOM IN THE NORTH ADRIATIC SEA

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## Abstract

During the late winter diatom bloom in the Northern Adriatic Sea in 2002, the proportion of apoptotic cells were estimated using the TUNEL assay, and cell lysis rates using the FDA method. Apoptotic cells were more abundant (up to 85%) at the peak phase of the bloom, when also cell lysis rates increased (up to  $0.8 d^{-1}$ ), indicating that phytoplankton was experiencing physiological stress conditions. The occurrence of apoptotic cells may indicate an endogenous control of diatom abundances when nutrients are close to depletion, possibly triggered by chemical signaling within the population.

Keywords: diatoms, bloom, apoptosis, fronts, Adriatic

In February-March 2002, six stations were sampled on a weekly basis to estimate phytoplankton cell concentrations along a coast-tooffshore transect in correspondence to the Po river delta. Phytoplankton concentrations were estimated using flow cytometry, microscopy, HPLC-pigment analysis, and remote sensing (SeaWifs). Percentages of apoptotic cells were estimated using the TUNEL assay, while cell lysis rates were estimated using the FDA method.

Pigment indicators (Dt/Dt+Dd) suggested a light-induced stress on phytoplankton trapped within the front separating Po river freshwater from offshore marine waters.

The evolution of total phytoplankton concentrations was evident only at the three most offshore stations, situated on the external side of an haline front, caused by the freshwater river outflow. Cell concentrations ranged from 500 cell ml<sup>-1</sup> before the bloom to  $10^5$  cell ml<sup>-1</sup> at its peak (20 March). The phytoplankton bloomwas mainly composed of diatoms (up to 90% of total counts), with *Skeletonema costatum* dominating the diatoms at the peak phase of the bloom.

At the frontal area (station 4) high percentages of Diatoxanthin and Diadinoxanthin were observed, indicating the occurrence of light-induced stress within the phytoplankton population trapped within the haline front.

Within the *S. costatum* population, apoptotic cells ranged from 30 to 85% of total cells at all stations, and at station 4 their evolution strongly correlated with *S. costatum* cell numbers (Fig. 1). The peak in apoptotic cells corresponded to a peak in cell lysis rates (from 0.1 to 0.8 d<sup>-1</sup>).

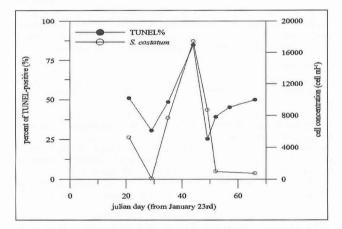


Fig. 1. *S. costatum* cell concentrations (cell ml<sup>-1</sup>, closed symbols) and percentages of TUNEL-positive cells (open symbols) in the northern Adriatic Sea in February-March 2002.

The high percentage of apoptotic cells may indicate an endogenously-controlled process of initiation of lysis of phytoplankton cells.

Apoptosis is the phenotype of Programmed Cell Death, an active mechanism of cell disruption activated by cells as a response to stress or changes in environmental factors.

The presence of apoptosis in unicellular organisms may represent an evolutionary relic, but also support the hypothesis that it is a widespread and crucial feature of the eukaryote world, best suited for adaptation to different environments, and, as in our case, as a reaction to unfavorable conditions.

The late winter diatom bloom is a recurrent feature in the North Adriatic Sea, starting in February and persisting in a large area for more than two months. With respect to other coastal areas, this bloom is characterized by the dominance of Skeletonema costatum, which is later on replaced by Chaetoceros species, during the following spring bloom. No detailed information is available on the dynamics of this bloom, which is peculiar for its duration and almost monospecificity. Miralto et al. (1) have shown that the North Adriatic late winter diatom bloom has a strong effect on copepod reproduction and therefore recruitment, strongly impacting the whole food web. This effect has been attributed to the production of unsaturated aldehydes by diatom species. These molecules appear to be involved in a defence mechanism activated after cell membrane disruption, as during grazing (2). It is reasonable to expect that aldehydes are also released in the seawater following cell lysis, a common process at sea, controlled by exogenous (bacteria, viral attack, nutrient limitation), or endogenous (senescence, automortality) factors (3; 4). Algal aldehydes have been shown to be toxic to other phytoplankton and to induce apoptosis in Copepods and sea urchin embryos (5). Possibly, the realease of such compounds via natural lysis of cells during the bloom may have determined apoptosis of cells during the late winter bloom of 2002. Apoptosis may therefore be regarded as a general mechanism acting at sea to control population growth, in order to optimize resources utilization at the end of the bloom.

## References

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