# METAL CYCLING THROUGH PLANKTON COMMUNITIES: A SINGLE-CELL APPROACH USING SYNCHROTRON-BASED X-RAY FLUORESCENCE

Benjamin S. Twining†, Stephen B. Baines, and Nicholas S. Fisher*

Marine Sciences Research Center, State University of New York, Stony Brook, NY 11794-5000 USA - * nfisher@notes.cc.sunysb.edu

†Present address: Yale School of Forestry and Environmental Studies,
Environmental Science Center, PO Box 208105, New Haven, CT 06520

## Abstract

We have applied a synchrotron-based x-ray fluorescence microprobe to analyze the concentrations and cytological distributions of trace elements in autotrophic and heterotrophic protists from remote waters. Using this approach it is now possible to discern different elemental stoichiometries exhibited by different types of co-occurring protists. This technique, combined with other geochemical measurements, should enable important new advances in our understanding of the marine biogeochemistry of trace elements.

*Keywords: x-ray fluorescence, microprobe, microbial loop, trace metals*

## Introduction

The elemental composition of marine protists is of great interest to oceanographers. The elemental stoichiometries of plankton simultaneously reflect the nutrient ratios of the aquatic environment and control the input of recycled elements through remineralization of plankton (1). While most attention has been focused on the C, N, and P content of plankton, several early studies determined the trace metal composition of plankton as well (2, 3). More recent evidence that trace metal nutrients such as iron can limit primary production in both open-ocean and coastal environments (4, 5) has spurred researchers to further study the trace metal contents of plankton.

Bioactive trace metals such as Mn, Fe, Co, Ni, Cu, and Zn are typically measured in plankton by first concentrating cells onto filter membranes and then digesting the filters and analyzing the resulting solution with atomic absorption spectrometry or inductively-coupled plasma mass spectrometry. This approach requires concentrating large amounts of similarly sized cellular and abiotic material on membranes of various pore-size. Thus co-occurring plankton with overlapping size ranges cannot be separated, and the potentially contaminating influence of suspended abiotic particles cannot be eliminated.

We have developed a new approach to the analysis of trace elements in marine protists that utilizes the unique sensitivity of synchrotron x-ray radiation to measure trace metals in individual nanoplankton cells. Further, the synchrotron x-ray fluorescence (SXRF) technique produces a two-dimensional map of the metals in each cell, providing additional information on the co-localization of elements. Here we present an example of the information that can be collected using this technique for cells collected from the Southern Ocean during a recent mesoscale iron enrichment experiment (SOFeX).

## Materials and methods

Complete descriptions of the sample preparation and analysis protocols are presented elsewhere (6). Briefly, cells were collected with trace-metal 'clean' techniques from the Southern Ocean, before and after Fe fertilization, and immediately centrifuged onto gold electron microscopy grids following fixation with Chelexed glutaraldehyde. The mounted cells were briefly rinsed with Milli-Q deionized water and then dried in a Class 100 laminar flow hood. Light and epifluorescence micrographs of the dried cells were taken on-board the ship, and the cells stored in a plastic dessicator until analysis. SXRF analyses were performed at the 2-ID-E beamline of the Advanced Photon Source, Argonne National Laboratory, Argonne, IL. Each cell was raster scanned across a highly focused x-ray spot, and the excited x-ray fluorescence spectra were recorded at each pixel with a 3-element germanium detector. Spectra were averaged over the cell, corrected for fluorescence from nearby background regions, and the peak areas modeled. Peak areas were converted to element concentration with NIST thin-film standards. Trace element contents were normalized to cellular P, which was measured directly with SXRF.

## Results and discussion

SXRF enables the identification of different trace metal compositions of co-occurring protists cells. Table 1 presents an example of SXRF data collected from the Southern Ocean, which are compared to data from bulk analyses of collected plankton from other waters. There are notable differences in the metal contents of diatoms and flagellated cells that cannot be detected with bulk analyses. Flagellated cells were significantly more enriched in P and diatoms more enriched in Mn, Ni, and Zn. Iron fertilization resulted in sharp increases in cellular concentrations of Mn, Ni, and Zn and smaller increases in P. Generally, P, Fe, and Zn were found distributed within cells and Si in the frustules of diatoms. Adsorbed Fe, localized in high concentrations attached to some cells in a way that doesn't correspond to any cellular feature, can be identified and removed from the elemental analysis with SXRF. Explanations for varying elemental stoichiometries in different co-occurring taxa remain to be discovered. Stoichiometric variations among different taxa suggest that the concept of a constant Redfield-type elemental ratio may not extend to trace metals. Application of SXRF analyses to protists in other waters, such as P-limited waters of the eastern Mediterranean, may reveal different stoichiometric relationships and may help explain the distribution of plankton in those waters.

**Table 1. Elemental composition (ratios normalized to cellular P, mmol mol$^{-1}$) of natural plankton assemblages as measured with bulk analysis and SXRF.**

| | Martin & Knauer (2) | Collier & Edmond (3) | Cullen et al. (10) | This study | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *Low Fe* | | | *High Fe* | | |
| | | | | Diatom | A flag | H flag | Diatom | A flag | H flag |
| *Elements normalized to P* | | | | | | | | | |
| Mn | 0.39 | 0.34 | 1.7 | 0.42 | 0.16 | 0.14 | 0.28 | 0.22 | 0.17 |
| Fe | 5.2 | 4.6 | -- | 0.71 | 0.54 | 0.63 | 1.9 | 1.9 | 0.94 |
| Ni | 0.21 | 0.86 | -- | 1.2 | 0.16 | 0.22 | 0.73 | 0.21 | 0.20 |
| Zn | 0.84 | 3.0 | 11.1 | 8.1 | 1.4 | 2.1 | 6.2 | 1.8 | 2.6 |

Shown are geometric mean stoichiometries for three cell types (diatoms, autotrophic flagellated cells—A flag, heterotrophic flagellated cells—H flag) collected from either low Fe (unenriched) or high Fe (enriched) stations. The Martin and Knauer (2) and Collier and Edmond (3) data are shown as selected by Bruland *et al.* (7). The SXRF Fe data are from Twining *et al.* (8, 9).

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