Production and Turnover of Viruses and Dissolved DNA Pools at Station ALOHA:

Potential Effects on Bacteria and Roles in the Phosphorus Cycle

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Chapter I

Development of a Novel Method for Dissolved DNA Quantification

Abstract

A novel method was developed for the quantification of dissolved DNA (D-DNA) in seawater. This method includes addition of tetrasodium EDTA to 0.22 μm-filtered seawater, concentration of >10kDa material in the filtrate with a Centricon centrifugal ultrafiltration unit, and quantification of the concentrated D-DNA with the fluorescent DNA stain SYBR Green I. This method requires only 13.5 ml of seawater per sample even in ultraoligotrophic environments and samples can be analyzed in less than 3 hours. The recovery of D-DNA with this method is 75-85% and can be determined for each sample by measuring recovery of ^{35}S-labeled DNA added at trace amounts. This method can be used to quantify D-DNA concentrations as low as 0.01 ng ml^{-1} with high precision (standard deviation <5% of the mean). Deoxyribonuclease (DNase) treatment of samples and virus enumeration can be used in conjunction with this method to determine the three major pools of D-DNA: free or soluble DNA (the fraction hydrolyzable by DNase), DNA within viruses, and uncharacterized bound DNA.

Introduction

The dissolved organic phosphorus (DOP) pool in the ocean is complex, with only approximately 50% of it identifiable by current analytical methods (reviewed by Karl and Björkman 2002). Of the recognizable DOP compounds, deoxyribonucleic acid (DNA) is