

A STUDY OF DISSOLVED DEOXYRIBONUCLEIC ACID
IN THE OCEAN:
METHOD DEVELOPMENT AND FIELD APPLICATION

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN OCEANOGRAPHY

By

Megan Diane Bailiff

Thesis Committee

David M. Karl, Chairman
Christopher D. Winn
Francis J. Sansone

ABSTRACT

Often considered a relatively minor component of seawater, dissolved organic matter (DOM), measured in terms of carbon, has come to be recognized as an important link between the various biological, chemical and geological processes in the ocean. Although there has been rather extensive research on the origin and utilization of low molecular weight DOM, little such work has been carried out on dissolved macromolecules. A component of this high molecular weight DOM is the molecule of heredity, deoxyribonucleic acid (DNA). It has been hypothesized that this polymeric compound may be important in the cycling of high molecular weight DOM.

The cycling of organic matter is effected by the activity of bacteria and other microorganisms due to their small size, abundance and rapid potential growth rates. One way to assess the impact that the microbial food web has on carbon flow in the aquatic ecosystem is to monitor the release and cycling of the dissolved nucleic acid, DNA. This study was designed primarily to develop a sensitive and convenient method for measuring dissolved DNA (D-DNA) in seawater. The secondary task was to evaluate the method in the field and to test several ecological hypotheses concerning the production of D-DNA in the ocean, hence, the potential impact of the microbial community on the cycling

of DOM.

Fulfilling the primary task, a method has been developed for measuring D-DNA in seawater. It is unique in its simplicity and can detect dissolved DNA concentrations as low as 100 ng DNA in seawater. The method employs cetyltrimethylammonium bromide (CTAB) as a precipitating agent for dissolved nucleic acids and the DNA specific dye diaminobenzoic acid (DABA) for the quantitative analysis of the D-DNA in a sample. In summary, soluble nucleic acids are precipitated as CTA-salts, filtered onto combusted Whatman GF/F filters and analyzed spectrofluorometrically using DABA.

The CTAB/DABA method was initially tested in Kaneohe Bay, Oahu, Hawaii where the D-DNA concentrations measured using the CTAB/DABA method ($10-16 \mu\text{g DNA l}^{-1}$) were found to be similar to previous measurements in a variety of aquatic habitats. Subsequently an extensive examination of the distribution of D-DNA was conducted in the neritic Southern ocean in conjunction with the RACER (Research on Antarctic Coastal Ecosystem Rates) program from December 1986 - March 1987, a period which encompasses the massive spring bloom observed in that region.

I had predicted that early in the Antarctic summer D-DNA concentration would be low, and that there would be a time dependent and significant increase in concentration of D-DNA during and following the spring bloom. However, an

antethitical situation was observed; In the beginning of the sampling season there was a measurable surface concentration of D-DNA throughout the study area ($2-20 \mu\text{g l}^{-1}$), then in the weeks and months that followed D-DNA declined to below the detection limit of the CTAB/DABA method at most stations by February. A detailed protocol for the CTAB/DABA method and examination of the results obtained are presented in the following study.