Production and Turnover of Viruses and Dissolved DNA Pools at Station ALOHA:
Potential Effects on Bacteria and Roles in the Phosphorus Cycle

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWA'I IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

OCEANOGRAPHY

MAY 2003

By
Jennifer R. Brum

Thesis Committee:
David Karl, Chairperson
Grieg Steward
Roger Fujioka
Acknowledgments

The research described in this thesis was conducted with support from a National Science Foundation grant for the Hawaii Ocean Time-series program. There are many people who contributed their expertise, energy, and time in helping me with this research. Their efforts were invaluable and the goals of this project would not have been met without their support and contributions.

I want to thank David Bird for allowing me to visit him in Montreal and teaching me how to enumerate viruses. Roger Fujioka provided bacteriophage cultures for my research and assisted me in filling out all of the paperwork to obtain them. During the course of this research, I participated in many scientific cruises in the Hawaii Ocean Time-series program and I would like to thank the crews of the research vessels Ka'Imikai-O-Kanaloa, Wecoma, and Kilo Moana for their assistance in helping me achieve my goals for every cruise. I received an enormous amount of support, both scientific and emotional, from the scientists with whom I regularly go to sea including Mark Valenciano, Shimi Rii, Dan Fitzgerald, Cecelia Sheridan, Fernando Santiago-Mandujano, and members of the Karl lab.

I have been extremely fortunate to have worked with many great people in the Karl lab over the last few years. These people have taught me what they know, helped me in my research, and made it fun to work in the lab and at sea. These members of the Karl lab are: John Dore who initially showed me how to do research; Ursula Magaard, Louis Tupas, and Dale Hebel who were there to show me the way when I first became part of the lab; Chris Carrillo and Matt Church who imparted to me all of their experience
as graduate students; Tom Gregory, Tara Clemente, Anne Gasc, Karin Björkman, Paul Morris, and Dan Sadler who helped me get things done in the lab and at sea; Lance Fujieki who is my computer guru; and Lisa Lum who assisted me in ordering the things I needed to do my research. These few blurbs about the members of the Karl lab cannot do justice to how much I am indebted to them for the support they have given me. They have made my tenure in this lab both enjoyable and successful.

I wish to extend my deepest thanks to my committee members. Roger Fujioka gave me great feedback and a different perspective on my research. Grieg Steward allowed me to interrupt him and seek his advice on practically a daily basis. He helped me on nights and weekends with running viral DNA samples. None of this research would be possible without his insight, infinite patience, and dedication. Finally, being Dave Karl’s student has been an honor. Dave possesses an unparalleled knowledge and understanding of marine microbial ecology. Over the last few years, I have been fortunate in receiving his direction and advice which has truly helped my development as a scientist. I will forever be grateful for the knowledge he has imparted upon me and continue to be inspired by his energy and tenacity with which he approaches science.
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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 35S-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
unique in that it is the common information molecule of all living organisms and possesses a C:N:P ratio of approximately 10:4:1, making it a potentially rich nitrogen and phosphorus source for marine microorganisms. In the marine environment, DNA is present in particulate forms, within living organisms and as non-living organic matter, and in dissolved forms as extracellular DNA.

Dissolved DNA (D-DNA) is operationally defined as DNA that passes through a 0.2 µm-pore-size filter (DeFlaun et al. 1986). It has a spatial distribution pattern in the ocean similar to the total dissolved organic matter (DOM) pool including decreasing concentrations with distance from shore and with depth in the water column (DeFlaun et al. 1987). D-DNA has a size distribution of 0.12 to 35.2 kbp which is skewed towards lower molecular weight forms in more oligotrophic areas (DeFlaun et al. 1987).

The operationally defined D-DNA pool is comprised of three major categories including soluble (free) DNA, DNA within viruses, and an as yet uncharacterized form of bound DNA (Jiang and Paul 1995). Ultramicrobacteria (bacteria which pass through a 0.2 µm filter) are considered as part of the bound D-DNA pool but have been found to exist at such low concentrations that they do not comprise a large portion of this pool (Paul et al. 1991).

These three major pools of D-DNA have different properties which makes it desirable from an ecological perspective to quantify them individually. Free D-DNA can be hydrolyzed by nucleases whereas bound D-DNA, including DNA within viruses, is resistant to nuclease attack (Jiang and Paul 1995). This ability to be readily hydrolyzed could result in free D-DNA being more actively cycled than the other major D-DNA
pools because microorganisms will be able to break it down and take up the hydrolysis products (Weinbauer et al. 1993; Siuda and Chróst 2000). In addition to being resistant to nuclease attack, marine viruses infect hosts, use the host’s resources to replicate, and are then released into the dissolved pool again, usually killing the host and liberating the cell contents to the DOM pool. Thus, viruses are different from the other major pools of D-DNA in that they are not just passively cycled in the environment. Since the major D-DNA pools have such different properties, it is hypothesized that the study of each individual pool will be more ecologically meaningful than considering only the bulk D-DNA pool.

Previously, D-DNA has been quantified primarily using an ethanol precipitation method followed by Hoechst 33258 staining of DNA (DeFlaun et al. 1986) or with the CTAB precipitation method followed by reaction of DNA with 3,5-diaminobenzoic acid (Karl and Bailiff 1989). While both of these methods can be used to measure D-DNA, they have certain disadvantages including lengthy processing time on the order of days and the requirement of large volumes of water (>1L) for determinations in oligotrophic environments. In response to these disadvantages, a novel method has been developed in this study which allows quantification of D-DNA from marine environments requiring only 13.5 ml of sample and three hours of processing time.

This new method involves the concentration of >10 kDa compounds from 0.22 μm prefiltred seawater using a centrifugal filtration unit, followed by the quantification of DNA with the fluorescent stain SYBR Green I (Molecular Probes) that targets double-stranded DNA. In addition to low sample volume requirements and reduced processing time.
time, this method uses few reagents, offers more precise knowledge of the lower molecular weight limit of DNA being quantified, and allows for easy sample collection in the field. Furthermore, this novel method can be combined with deoxyribonuclease pretreatment and virus enumerations to quantify all three of the major D-DNA pools. The development and evaluation of this method will be discussed in the following section.

**Methods description**

A method was developed for the quantification of operationally defined dissolved DNA in seawater. The goals for the development of this method were that it should be relatively fast, easy to perform, and have a low detection limit suitable for use in oligotrophic seawaters with excellent precision and accuracy. Briefly, this method includes addition of tetraysodium ethylenediamine tetraacetic acid (EDTA) to 0.22 μm-filtered seawater, concentration of >10 kDa material with a centrifugal filtration unit, and fluorometric quantification of double-stranded DNA with the fluorescent stain SYBR Green I. $^{35}$S-labeled DNA is used as an internal standard for each sample to assess the percentage of D-DNA recovered. This method can be combined with DNase pretreatment to quantify enzymatically hydrolyzable D-DNA by difference. The following is a detailed description of the new method which is also shown in Figure 1.

Seawater is filtered through a 0.22 μm-pore-size Durapore membrane filter (Millipore) and 1 M EDTA is added to an aliquot of the filtrate to a final concentration of 100 mM. The addition of tetraysodium EDTA has three purposes: (1) it chelates divalent cations (Mg$^{2+}$, Mn$^{2+}$) that are cofactors for deoxyribonuclease, (2) it destabilizes viral
capsids thereby exposing viral DNA for analysis, and (3) it buffers the pH of the sample at 10.5 which reduces adsorption of DNA to containers during storage and analysis. The samples are then stored at 4 °C until analysis in the laboratory.

For laboratory analysis, 15 ml of sample is gently pipetted into a Centricon Plus-20 centrifugal filter device (Millipore). The sample is then centrifuged for 25 minutes at 1000 x g, reducing the sample volume to approximately 1 ml. The filtrate is then discarded and 10 ml of Tris-EDTA (TE) buffer (10 mM Tris-HCl, 1 mM disodium EDTA, pH 7.5) is added to the concentrated sample and centrifuged again for 25 minutes, reducing the sample volume to approximately 100-200 μl. This rinse is needed to exchange TE buffer for seawater since salts within seawater reduce the fluorescence of the SYBR Green I-DNA complex (Molecular Probes product literature). The concentrate is then recovered as directed by the manufacturer by adding 500 μl of TE buffer to the sample, inverting the retentate cup into the sample cup, and centrifuging for 3 minutes at 300 x g. The recovered sample is poured into a pre-weighed tube and the sample recovery process is repeated to ensure quantitative recovery of the concentrated material. The pooled sample is weighed to determine the volume of the concentrate and additional TE buffer is added to achieve a final sample volume of 1.75 ml.

In low light, 250 μl of 1.05x concentration of SYBR Green I (Molecular Probes) in TE buffer is then added to each sample. After 10 minutes of staining, the fluorescence of the sample is measured with a fluorescence spectrophotometer (Perkin-Elmer model LS-5; excitation, 497; emission, 520). An external standard curve is prepared by diluting
lambda phage double-stranded DNA (Molecular Probes) in the same volume and concentration of TE buffer and SYBR Green I as the samples.

Recovery for each sample is obtained using $^{35}$S-labeled DNA prepared by incorporation of $^{35}$S-dCTP (ICN Biomedicals) into double-stranded lambda phage DNA using a nick translation kit (Amersham Pharmacia Biotech). The labeled DNA obtained from nick translation is filtered using a 10 kDa Centricon unit and rinsed twice with TE buffer to remove the unincorporated $^{35}$S-dCTP. The recovered $^{35}$S-labeled DNA is then stored frozen until use. This $^{35}$S-labeled DNA is added to the initial sample at a final activity of approximately $6 \times 10^{-10}$ Ci ml$^{-1}$ after the addition of EDTA. The radioactivity of the recovered sample is measured by adding 1 ml of the final sample to 10 ml of Aquasol-2 liquid scintillation cocktail and counting with a liquid scintillation counter (Packard Tri-Carb 4640). The radioactivity of the final sample is compared to the radioactivity added to the sample initially. Since half of the final sample is counted, half of the volume of $^{35}$S-labeled DNA added to 15 ml of the initial sample is used for this comparison. To ensure that the samples are analyzed similarly, the $^{35}$S-labeled DNA used for comparison is added to the same volume and concentration of SYBR Green I and TE buffer as the samples. This procedure allows for the calculation of percent recovery of DNA throughout sample storage and analysis. The amount of $^{35}$S-labeled DNA added to samples varies with labeling efficiency of the nick translation step and with radioactive decay of $^{35}$S. This DNA is added at trace amounts and does not alter the fluorescence of samples.
Blanks are prepared exactly as samples using 10 kDa-filtered seawater. The concentration of DNA in each sample is calculated by subtracting the fluorescence of the blank from the fluorescence of the sample and dividing by the slope of the regression equation from the external standard curve. After corrections for volume, the DNA concentration is divided by the fraction of recovered $^{35}$S-labeled DNA to result in the final concentration of DNA per volume of seawater.

This method can also be used to quantify the concentration of dissolved DNA that is able to be hydrolyzed by the enzyme deoxyribonuclease (DNase). DNase (type IV, bovine pancreas, Sigma-Aldrich) is added to the 0.22 μm-filtered sample at a final concentration of 80 units ml$^{-1}$ and incubated at 23±2 °C for 30 minutes. After incubation, tetrasodium EDTA is added and the sample is processed as described above. Blanks for DNase-treated samples are prepared as described for non-DNase-treated samples except that DNase is added to the 10 kDa filtrate. The concentration of DNA that is enzymatically hydrolyzable (termed ehD-DNA) is calculated by subtracting the DNA concentration of DNase-treated samples from the DNA concentration of non-DNase-treated samples, with propagation of errors.

Methods development

Double-stranded DNA stain

Several steps of the proposed dissolved DNA quantification method were optimized in laboratory experiments using seawater collected from Station ALOHA prior to field application. First, the fluorescent nucleic acid stains PicoGreen, SYBR Gold, and
SYBR Green I (Molecular Probes) were compared using standard curves of double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), and RNA prepared in TE buffer. These stains were evaluated on the basis of their blank fluorescence, molar fluorescence yield (slope of fluorescence vs. nucleic acid concentration), and reaction specificity (e.g., relative reaction with ssDNA and RNA; Table 1), as well as the ability to use the stain to quantify viruses with epifluorescence microscopy. SYBR Gold was rejected because it reacted with ssDNA and had a larger blank than the other two stains. PicoGreen did not react with ssDNA or RNA, but was rejected because it required much more stain than SYBR Green I and had not been used in conjunction with epifluorescence microscopy. SYBR Green I had the highest molar fluorescence yield with dsDNA, displayed minimal activity towards ssDNA and RNA, had a low fluorescence blank, and has previously been used to enumerate viruses (Noble and Fuhrman 1998). The concentration of SYBR Green I was then varied from 0.5x to 0.125x in an effort to optimize the molar fluorescence yield with dsDNA and minimize the blank (Table 1). A final concentration of 0.125x was chosen because it had the lowest fluorescence blank. SYBR Green I was also evaluated to determine if it stained ssDNA and RNA viruses. Cultures of the ssDNA phage φX174 and the RNA phage MS-2 were stained with SYBR Green I and observed using epifluorescence microscopy. No stained phages were observed in the samples.
Centrifugal filter device

A 10 kDa centrifugal filter device was then chosen based on recovery of lambda phage DNA. Centricon Plus-20 centrifugal filter devices were chosen because they had a percent recovery six times higher than Ultrafree-15 centrifugal concentrators (Millipore), and also had much better replication between samples (typically ±5%).

Form and concentration of EDTA

The form and concentration of EDTA used to control hydrolysis of DNA were evaluated in several experiments to achieve high recoveries of DNA after storage and during sample processing (Table 2). First, the recovery of $^{35}$S-labeled DNA in seawater samples using 50 mM disodium EDTA was compared to recovery using 50 mM tetrasodium EDTA with no storage after sample preparation. The recovery of $^{35}$S-labeled DNA was on average 15% higher in samples with tetrasodium EDTA with improved precision (±4% vs. ±7%). This is thought to be a result of pH-dependant control of adsorption of DNA to sample containers and the Centricon units.

Storage of samples through freezing or refrigeration with 50 mM tetrasodium EDTA was then evaluated. A precipitate formed in the frozen samples and $^{35}$S-labeled DNA recoveries in both of these storage methods were lower than desired. Finally, refrigeration of seawater samples in 100 mM EDTA was tested and resulted in an average recovery of 83% with a typical precision of ±1%.
Prefiltration

Three types of 0.2 and 0.22 μm-pore-size filters were then evaluated to determine which filter adsorbed the least amount of D-DNA and viruses. Seawater collected from Station ALOHA was filtered through cellulose acetate (Millipore GS), polycarbonate (Nuclepore), and polyvinylidene fluoride (Durapore Millipore) filters. D-DNA and virus concentrations were measured in four replicate samples from the filtrates of each filter type. Viruses were also quantified in the seawater prior to filtration. Durapore filters allowed the most D-DNA and all of the viruses in the unfiltered seawater to pass through, and were therefore chosen for use with this method (Figure 2). Paul et al. (1991) reported that as much as 87% of the virus population was retained by Durapore filters but that this adsorption of viruses decreased with distance from shore which is consistent with these observations for Station ALOHA seawater.

Deoxyribonuclease incubation

In order to measure ehD-DNA, the length of sample incubation with DNase required to hydrolyze exogenous $^{35}$S-labeled DNA was determined. $^{35}$S-labeled DNA was added to 0.22 μm-filtered seawater samples followed by DNase addition (final concentration of 80 units ml$^{-1}$). The samples were incubated at 23±2 °C for 0, 30, and 60 minutes, at which time EDTA was added to stop the reaction. A negative control without DNase was also prepared for comparison. The negative controls and DNase amended samples without incubation were not significantly different (p<0.01; Figure 3). This demonstrates that the addition of 100 mM EDTA is sufficient to stop DNase activity.
Samples incubated with DNase for 30 and 60 minutes both showed approximately 10% recovery of $^{35}$S-labeled DNA. The lack of total hydrolysis of $^{35}$S-labeled DNA may be due to adsorption of the labeled DNA to particles in the seawater sample which would protect the DNA from hydrolysis by DNase. Since there was no significant difference between the recovery of labeled DNA in the 30 and 60 minute incubations ($p<0.01$), an incubation time of 30 minutes was chosen for the ehD-DNA quantification method to reduce biological activity during incubations.

**Methods evaluation**

*Internal standard curves and detection limits*

After the centrifugal ultrafiltration method to measure D-DNA and ehD-DNA was established, it was evaluated through a series of laboratory experiments with seawater collected from Station ALOHA. An internal standard curve (also known as a standard addition) was created by addition of varying amounts of lambda phage DNA to equal volumes of filtered seawater followed by processing with the centrifugal ultrafiltration method, including addition of $^{35}$S-labeled DNA. The fluorescence of the processed samples increased linearly with the amount of DNA added and standard deviations ($n=3$) were always less than 3% of the mean (Figure 4a). The recovery of each sample was assessed by calculating the recovery of added lambda phage DNA as well as $^{35}$S-labeled DNA. These recoveries were consistent over the range of DNA added, averaging 74% ($\pm 5\%$). The unrecovered DNA was most likely adsorbed to the Centricon filter and/or lost during sample manipulation. Furthermore, the recovery of $^{35}$S-labeled DNA was in
agreement (±2%) with the recovery of added lambda phage DNA calculated from fluorescence. These results show that the method accurately measures standard dsDNA over a 10 fold range of DNA concentrations and that $^{35}\text{S}$-labeled DNA recovery correctly represents sample DNA recovery.

The detection limit of the centrifugal ultrafiltration method was evaluated empirically with a very low range internal standard curve (0 to 0.1 ng ml$^{-1}$) in 10 kDa-filtered seawater followed by processing with this method. The recovered DNA had a linear relationship with added DNA through the lowest concentration added (Figure 4b). These results show that D-DNA can be measured in seawater with concentration as low as 0.01 ng ml$^{-1}$ using only 13.5 ml of seawater.

An internal standard curve was also created for samples with DNase treatment. DNase was added to the sample after EDTA addition to prevent DNA hydrolysis. The fluorescence of the samples increased linearly with the amount of DNA added (Figure 5a). Recovery of added lambda phage DNA was 76% (±5%) and was within 3% of $^{35}\text{S}$-labeled DNA recovery. The blank for samples with DNase is higher than samples without DNase added which may affect the lower detection limit. This lower limit for samples with DNase was evaluated exactly as for samples without DNase addition. Again, DNase was added to the sample after EDTA addition. The recovered DNA had a linear relationship with added DNA through 0.2 ng ml$^{-1}$ but was indistinguishable from blank values below this (Figure 5b).
Quantification of DNA within viruses

To insure that DNase was not hydrolyzing DNA within viruses, seawater samples and a cultured T-4 phage added to 10 kDa-filtered seawater were treated with DNase. Viruses were enumerated by epifluorescence microscopy before and after DNase addition. DNase did not significantly change the number of viruses in seawater or T-4 phage added to filtered seawater (p<0.01).

The measurement of DNA within viruses with the centrifugal ultrafiltration method was evaluated. Cultured T-4 phage was added to 10 kDa-filtered seawater after treatment with DNase to hydrolyze bacterial DNA existing in the culture. The concentration of T-4 phages was quantified by epifluorescence microscopy and converted to DNA within phages. The calculated amount of DNA within phages in the sample was 1.03 ng ml\(^{-1}\) (±0.08) and the DNA measured by the Centricon method was 0.97 ng ml\(^{-1}\) (±0.07; p<0.001). This shows that DNA within viruses is accurately quantified by the Centricon method.

Sample storage

The effect of sample storage on the recovery of \(^{35}\)S-labeled DNA was determined over the course of 35 days. Samples were collected from 0.22 \(\mu\)m-filtered seawater and stored exactly as previously described for samples collected in the field. Four samples were analyzed immediately and four more samples were analyzed per day at 2, 5, 10, 15, and 35 days after storage at 4 °C. The recovery of \(^{35}\)S-labeled DNA started to decrease after 5 days of sample storage and the measured DNA corrected for \(^{35}\)S-labeled DNA
recovery increased by 0.1 ng ml\textsuperscript{-1} after 10 days (Figure 6). These results show that through 10 days of sample storage, recovery of \textsuperscript{35}S-labeled DNA accurately corrects for losses of natural DNA. After 15 days of sample storage, the correction for recovery of \textsuperscript{35}S-labeled DNA overestimates the loss of natural DNA by approximately 13%.

Conclusions

The developed centrifugal ultrafiltration method has been shown to be an accurate method to measure double-stranded D-DNA in seawater. This method is easy to use, requires only 13.5 ml of seawater per determination even in oligotrophic water, and has a high precision. In addition, this method can be coupled with DNase treatment and virus enumeration to quantify the three major pools of double-stranded D-DNA in seawater: free DNA, DNA within viruses, and uncharacterized bound DNA.
Chapter II

Concentration, Production, and Turnover of Viruses and Dissolved DNA Pools
at Station ALOHA

Abstract

A newly developed centrifugal ultrafiltration method for quantification of dissolved DNA (D-DNA) was used to study the composition and production of the D-DNA pool in a depth profile at Station ALOHA in the subtropical North Pacific Gyre. By using deoxyribonuclease (DNase) treatment, virus enumeration, and the measurement of viral DNA content in conjunction with the D-DNA quantification method, the three major pools of D-DNA (free DNA, DNA within viruses, and uncharacterized bound DNA) were quantified and production and turnover time were measured for each pool.

The D-DNA pool at Station ALOHA was composed of two major pools: 49-63% was within viruses and the remaining D-DNA was able to be hydrolyzed in vitro by exogenous DNase (termed ehD-DNA). The turnover time of ehD-DNA in the upper water column ranged from 0.97 to 6.2 h, 3 to 10 times faster than the turnover of DNA within viruses. The low concentration and fast turnover of ehD-DNA at Station ALOHA is hypothesized to be a result of rapid uptake of this phosphorus-rich molecule in a phosphorus-stressed ecosystem. Based on virus production rates, viruses were estimated to lyse 3.2-16.5% of the standing stock of bacteria at Station ALOHA per hour, resulting in the release of ehD-DNA which was estimated to be 11-35% of the total ehD-DNA production.
This research supports the hypothesis that individual pools of D-DNA are cycled at different rates and therefore studies of these individual pools will be more ecologically meaningful than investigations of the total D-DNA pool. In addition, viruses in open-ocean gyre systems may have large impacts on the microbial community there, including viral-induced mortality and subsequent release of cellular contents to the DOM pool.

Introduction

Dissolved deoxyribonucleic acid (D-DNA) in aquatic environments is operationally defined as DNA that passes through a 0.2 μm-pore-size filter (DeFlaun et al. 1986). The three major pools of D-DNA are soluble (free) DNA, DNA within viruses, and an as yet uncharacterized form of bound DNA (Jiang and Paul 1995). The relative percentages of all three categories have been measured in the Gulf of Mexico resulting in 50% soluble D-DNA, 8-15% DNA within viruses, and 35-42% unidentified bound DNA (Jiang and Paul 1995). The percent of total D-DNA within viruses has been independently measured in several studies, usually resulting in a value less than 20% (Beebee 1991; Paul et al. 1991; Maruyama et al. 1993; Boehme et al. 1993; Weinbauer et al. 1993; Weinbauer et al. 1995; Jiang and Paul 1995).

D-DNA can be produced through protozoan grazing on bacteria (Turk et al. 1992; Ishii et al. 1998; Kawabata et al. 1998; Alonso et al. 2000), viral lysis (Weinbauer et al. 1993; Alonso et al. 2000), and potentially as exudates from growing bacterioplankton (Paul et al. 1987; Paul et al. 1990b). The D-DNA can then be taken up as intact polynucleotides by marine bacteria via transformation (DeFlaun et al. 1987; Frischer et al. 1987).
1990; Jeffrey et al. 1990; Paul et al. 1991; Frischer et al. 1994), or used by bacterioplankton as a nucleotide or nutrient source (Jørgensen et al. 1993; Jørgensen et al. 1994; Jørgensen and Jacobsen 1996; Siuda and Güde 1996; Paul et al. 1988; Finkel and Kolter 2001). Turnover time for the D-DNA pool, mediated by these production and uptake mechanisms, has been measured to be as rapid as 6.5 h (Paul et al. 1987).

However, due to the probable differential turnover times of the three major D-DNA pools because of their different production and uptake mechanisms, estimation of bulk D-DNA pool dynamics is of questionable ecological value. For example, it has been hypothesized that D-DNA which can be hydrolyzed by enzymes, including soluble or free DNA, will be more labile and thus have faster turnover times than bound D-DNA including DNA within viruses, which is resistant to enzymatic hydrolysis (Weinbauer et al. 1993; Siuda and Chróst 2000). Therefore, measurements of production, uptake, and turnover times of D-DNA which consider D-DNA as a bulk pool will be over- or underestimates depending on the method used, and will therefore not reflect the actual cycling of any of the individual pools.

In addition to being part of the D-DNA pool, marine viruses have been shown to affect microbial loop dynamics, bloom termination, and host community diversity (reviewed by Wommack and Colwell 2000). Viruses can affect microbial loop dynamics by infecting and lysing cells, resulting in cell death and the release of cell contents to the DOM pool. Virus-induced mortality of marine bacteria has been found to be similar in magnitude to protozoan grazing (reviewed by Wommack and Colwell 2000). This results in a large portion of microbial production in the oceans being cycled through the ‘viral
shunt', the transfer of cell contents to the DOM pool via viral lysis (Wilhelm and Suttle 1999), instead of being transferred to higher trophic levels through grazing.

The role of D-DNA as a phosphorus source has been considered as well as the effect of ambient phosphorus concentrations on the cycling of D-DNA and the production of viruses. The variability in turnover rates of D-DNA has been suggested to be a result of phosphorus limitation, with more rapid turnover in phosphorus-limited areas (Turk et al. 1992). This implies that D-DNA may have a nutritional role in the phosphorus cycle which may increase in importance with increasing phosphorus limitation. The production of viruses is thought to be affected by phosphorus concentration as well, with low concentrations acting as the "trigger" to switch from lysis to lysogeny, presumably due to the phosphorus requirement for the production of viral DNA (Bratbak et al. 1993; Wilson et al. 1996; Wilson and Mann 1997; Wilson et al. 1998; Jacquet et al. 2002).

The newly developed centrifugal ultrafiltration method for quantification of D-DNA (Chapter I) was employed in a study of the composition and production of the D-DNA pool at Station ALOHA in the subtropical North Pacific Gyre. By using DNase treatment, virus enumeration, and the measurement of viral DNA content in conjunction with the D-DNA quantification method, the three major pools of D-DNA were quantified and production and turnover time were measured for each pool. This is the first comprehensive study of the dynamics of these individual pools, and the first study of D-DNA cycling in an oceanic gyre system. The results of this study are discussed in the context of the impact of viruses on the microbial community as well as cycling of D-DNA in a phosphorus-stressed system.
Materials and Methods

Study location and goals

A study of viruses and dissolved DNA was conducted in December 2002 at Station ALOHA located in the subtropical North Pacific Gyre (22° 45' N, 158° W). This is in an ultraoligotrophic area that has an extensive 13 year set of biogeochemical data generated through the Hawaii Ocean Time-series (HOT) program. D-DNA has been quantified near this site (Karl and Bailiff 1989) and viral abundance has also been measured at Station ALOHA (Maranger and Bird 1995; Culley and Welschmeyer 2002). The goals of this study were to quantify the concentration, production, and turnover time of the major pools of D-DNA and investigate the ecological impact of viruses and D-DNA in an ultraoligotrophic open-ocean system.

Sampling

Seawater samples were collected with 12 L SIO polyvinylchloride sampling bottles attached to a rosette equipped with a Seabird CTD. Depth profiles of D-DNA, ehD-DNA, bacteria, viruses, dissolved organic phosphorus (DOP), and orthophosphate (Pi) were measured at eight depths extending from the surface to 500 m. Incubation experiments were conducted to measure production of viruses, D-DNA, and ehD-DNA at six depths in the profile. These incubation experiments were also conducted with phosphate addition at two depths.
D-DNA pools

D-DNA and ehD-DNA were measured using the previously described centrifugal ultrafiltration method (Chapter I). Samples were analyzed within ten days of collection.

Bacterial abundance

Samples of seawater were preserved with 1% paraformaldehyde (final concentration) and stored in liquid nitrogen until analysis. Bacterial abundance was quantified with a flow cytometer (Coulter EPICS 753, Monger and Landry 1993).

Virus abundance

Seawater samples were filtered through 0.22 μm Durapore filters, preserved with 2% 0.02 μm-filtered glutaraldehyde (final concentration) and stored at 4 °C. Viruses were enumerated within 5 days of sample collection using the method of Noble and Fuhrman (1998). Sample aliquots were filtered onto 0.02 μm Anodisc filters, stained with SYBR Green I, and viruses were enumerated using a Nikon Eclipse E600 epifluorescence microscope.

Viral genome fingerprinting

Genomic fingerprinting of viral assemblages with pulsed field gel electrophoresis (PFGE) was used to obtain a conversion factor for the amount of DNA per virus (Steward 2001). After filtration through 0.22 μm filters, approximately 8 L of seawater from each depth was concentrated using a vortex flow filtration system (Benchmark) equipped with
a 30 kDa filter (Membrex) followed by further concentration with 10 kDa Centricon units. PFGE was carried out using a CHEF-DR II pulsed field gel electrophoresis system (Bio-Rad) run for 18 h with pulses ramping from 1 to 15 s. DNA molecular weight markers (Midrange I and lambda ladder; New England BioLabs) and mass standards (high DNA mass ladder; Life Technologies) were run on the same gel as samples. The gel was stained with SYBR Green I and the average viral genome size for each depth was calculated after analysis of the gel with gel analysis software (Alpha Innotech).

Dissolved inorganic and organic phosphorus

Total dissolved phosphorus (TDP) and orthophosphate (Pi) were quantified in duplicate or triplicate samples at each depth using the MAGIC method including persulfate oxidation for TDP analysis (Karl and Björkman 2001). Dissolved organic phosphorus (DOP) was calculated by subtracting Pi from TDP measurements.

Virus and D-DNA production

Production of viruses, D-DNA, and ehD-DNA were measured at six depths using a dilution technique (Wilhelm et al. 2002). This method was originally described for virus production only, but preliminary experiments showed that D-DNA production could be measured by this technique as well. Seawater from each depth was collected the day before the incubation experiments were carried out and filtered through a 30 kDa filter using vortex flow filtration. Laboratory experiments showed that this filtration removes all viruses and over 90% of the D-DNA. Seawater samples (2 L) were gently vacuum-
filtered through 73 mm diameter, 0.22 μm-pore-size Durapore filters (Millipore). As the sample was filtering, particles on the filter were resuspended by pumping sample onto it using a sterile transfer pipet. As the residual volume began to decrease to below 300 ml, 30 kDa-filtered seawater collected the previous day from that depth was added to increase the volume to 500 ml. This addition of 30 kDa-filtered seawater was repeated three times in order to dilute viruses and D-DNA in the sample while retaining bacteria. The retained seawater was then diluted with the 30 kDa filtrate to a final volume of 2 L. Three aliquots of 500 ml of this prepared seawater were placed in 500 ml acid-rinsed polycarbonate bottles (Nuclepore) and incubated in the dark at in situ temperatures in ship-board incubators. Subsamples were collected every 3 hours for 12 hours to quantify bacteria, viruses, D-DNA and ehD-DNA as previously described.

Production rates of viruses, D-DNA, and ehD-DNA for individual incubations were calculated as the slope of the initial linear increase of each measured parameter versus time using first-order linear regression analysis. Because virus production is proportional to bacterial host abundance, the calculated production rates were then corrected for losses of bacteria during preparation of the incubation samples. Virus concentrations were only determined from the 0.22 μm filtrate, but prior experiments (Chapter I) show that all of the extracellular viruses at Station ALOHA pass through this filter. Therefore, the measured production of viruses in the 0.22 μm filtrate is also discussed as the total production of viruses.
Phosphate addition experiments

Incubation experiments were performed with samples from 5 and 75 m exactly as described above with addition of KH₂PO₄ to a final concentration of 100 nM PO₄³⁻. At these depths, 4 L of sample was processed and diluted to a final volume of 4 L so that all replicates for incubations with and without phosphate addition were from the same prepared seawater.

Results

D-DNA pool concentrations

Dissolved DNA was approximately 1.2 ng ml⁻¹ in the upper 100 m of the water column, declined significantly between 100 and 150 m, and continued to decrease with depth to 0.2 ng ml⁻¹ at 500 m (Figure 7, circles). Total D-DNA-P ranged from 1.5 to 4.1% of the DOP for all depths sampled. The concentration of the D-DNA that was hydrolyzable by DNase (ehD-DNA) had a similar profile to total D-DNA except that it was reduced by approximately 60% (Figure 7, squares). Virus concentrations were also highest in the upper 100 m with a slight maximum at 75 m (Figure 8).

Viral fingerprinting was used to obtain a conversion factor for the average amount of DNA per viral genome at each depth. The size distributions of viral genomes were bimodal and revealed a slight broadening of the size distribution in deeper samples (Figure 9). Despite small differences in the overall shape of the distributions, the average size of a viral genome remained fairly constant at all depths sampled. The average mass of DNA per viral genome calculated from average genome sizes ranged from 62.5 to 69.8...
attograms of DNA per virus (Table 3). These calculated values were then used to convert the concentration of viruses at each depth to concentrations of D-DNA within viruses.

The concentration of D-DNA within viruses (Figure 7, triangles) was not significantly different from the concentration of D-DNA that was not able to be hydrolyzed by DNase (t-test; p values greater than 0.3 at each depth). Since viruses are not hydrolyzed by DNase (Chapter I), it was concluded that the portion of D-DNA that could not be hydrolyzed by DNase consisted primarily of viruses. Therefore, at Station ALOHA there are two major pools of D-DNA: ehD-DNA and DNA within viruses. The percent of D-DNA that was within viruses at each depth ranged from 49 to 63% (Table 4).

**Virus and D-DNA production**

Preparations of seawater for incubation experiments resulted in the initial dilution of the concentrations of bacteria, viruses, and D-DNA by an average of 20, 7, and 16%, respectively, relative to the whole water sample (Table 5). Due to sampling error, production experiments were not conducted for 100 m samples. Treatment of all samples collected in incubations with DNase resulted in DNA measurements that were not significantly different from blank values. Therefore, ehD-DNA concentrations were calculated by subtracting the DNA within viruses from total D-DNA for each time point sampled. Increases in ehD-DNA and viruses were nearly linear for the first three time points (Figure 10). The ehD-DNA concentration in the 75 m incubations had already started to decrease by the third time point so the production rate was calculated as the
slope between the first two time points. Because the ehD-DNA may have already begun to be taken up by the second time point, the calculated production rate is probably a lower constraint on the gross ehD-DNA production at this depth. Due to only small fluctuations of ehD-DNA and virus concentrations, reliable production rates could not be determined for ehD-DNA in the 150 m and 200 m incubations, or for virus production in the 200 m incubation. Bacterial concentrations in the incubations did not change appreciably with time (data not shown).

Virus production rates were similar for the 5, 25, and 45 m incubations (4.0 - 6.0x10^6 ml^-1 h^-1) and increased to approximately twice this rate in the 75 m incubations (Table 6). There was no measurable production of viruses in 150 m incubations. Production of ehD-DNA was also similar at 5, 25, and 45 m (0.1 - 0.15 ng ml^-1 h^-1) but was approximately four times higher at 75 m (Table 6). Turnover times of viruses and ehD-DNA were calculated assuming a steady state for the concentrations of these pools at each depth. Turnover times of viruses ranged from 9.6 to 24 h and turnover times of ehD-DNA ranged from 0.97 to 6.2 h in the upper 75 m (Table 6).

In order to compare the production rates and turnover times of the two pools of D-DNA at Station ALOHA, virus production rates and turnover times were converted to viral DNA using the conversion factor for DNA per virus obtained for each depth. This resulted in production rates of DNA in viruses that were 3 to 6 times lower and turnover times which were 3 to 10 times longer than for ehD-DNA (Table 6).
Phosphate addition experiments

The addition of KH₂PO₄ to incubations from 5 and 75 m increased the concentration of Pi from 18 nM to 118 nM and 13 nM to 113 nM respectively. The concentration of viruses in the 5 m incubation increased significantly by the first time point then began to decline (Figure 11). The production of viruses was calculated from the slope between the first two time points and therefore may be an underestimate. In contrast, the concentration of viruses in the 75 m incubation continued to increase throughout the sampling period. There was no detectable increase in ehD-DNA in either of these incubations.

A comparison of the incubations with and without phosphate addition at both depths showed that the addition of phosphate resulted in no accumulation of ehD-DNA in the incubation samples, which suggests that production was halted (Figure 12). The effect of phosphate addition on virus production produced mixed results. Virus production increased by a factor of 2.5 in the 5 m incubations with the addition of phosphate, and decreased by 30% in the 75 m incubations (Figure 12). No appreciable change in bacterial concentration in the incubations was observed (data not shown).

Discussion

D-DNA pools

The concentration and shape of the depth profile of total D-DNA measured in this study is in agreement with the D-DNA profile measured by Karl and Bailiff (1989) near this station using the CTAB method. The average mass of DNA per viral genome
measured for Station ALOHA is slightly greater than the average of 55 attograms of DNA per virus from five marine environments reported by Steward et al. (2000). The difference is a result of a greater percentage of viral genomes in the 55-65 kb range than the 30-40 kb range at Station ALOHA, which is the opposite of the relationship between these two ranges found by Steward et al. The viral DNA content measured in this study is lower than the value determined for a range of known bacteriophage isolates (99 attograms DNA per phage; Freifelder 1987) which has been used by other marine researchers (refer to Table 7). This reflects the need to measure viral genome sizes in the sampling area in order to obtain accurate conversion factors for the amount of DNA per virus.

Using the conversion factor obtained from each depth at Station ALOHA resulted in 49.4-62.8% of the total D-DNA existing within viruses. The remaining D-DNA was able to be hydrolyzed by DNase (ehD-DNA). Jiang and Paul (1995) described three major pools of D-DNA in the Gulf of Mexico: soluble DNA (equivalent to ehD-DNA measured in this study), DNA within viruses, and an unidentified bound form of DNA which could not be hydrolyzed by DNase. This third pool was not found at Station ALOHA, although it may be present at low concentrations that were not able to be quantified due to the error in comparing viral DNA and D-DNA that could not be hydrolyzed by DNase. This uncharacterized bound D-DNA may be a characteristic of more eutrophic areas such as the coastal stations investigated by Jiang and Paul.

This percentage of D-DNA within viruses at Station ALOHA is significantly higher than the percentage obtained in most other studies (Table 7). A high percentage of
D-DNA within viruses was found by Beebee (1991) but the speed used in ultracentrifugation was later found to pellet high molecular weight soluble DNA as well as viruses (Jiang and Paul 1995). Maruyama et al. (1993) also found a high percentage of D-DNA within viruses but this was based on the assumption that all D-DNA that cannot be hydrolyzed by DNase is within viruses. This was refuted by Jiang and Paul (1995) who found that a portion of the non-DNase-digestible DNA at a coastal location was a bound form not contained within viruses. Therefore, the high values found for the percentage of D-DNA within viruses by Beebee and Maruyama et al. are most likely overestimates. Weinbauer et al. (1995) did find high percentages of D-DNA within viruses during the summer and autumn periods of their time-series measurements at coastal stations in the Northern Adriatic Sea, showing that the percentage of D-DNA within viruses can be dynamic. The remaining measurements in Table 7 suggest that DNA within viruses is not a large component of the D-DNA pool. However, these studies reflect a lack of data from oceanic gyre systems which comprise most of the area of the world ocean. Even those studies that report measurements from offshore oligotrophic stations in the Gulf of Mexico (Paul et al 1991; Jiang and Paul 1995) are conducted in areas where the D-DNA concentration is an order of magnitude greater than at Station ALOHA.

The comparatively high percentage of D-DNA that is within viruses found in this study is most likely due to the relative turnover rates of the two major D-DNA pools. eD-DNA was produced at a rate 3 to 6 times greater than D-DNA within viruses, resulting in turnover times that were 3 to 10 times faster. This much more rapid turnover
of ehD-DNA may be a result of the nature of ultraoligotrophic gyre systems. Virus and D-DNA production has not previously been measured at the same location so a direct comparison to other environments cannot be made. However, comparisons of these rates individually can be used to infer general relationships among different trophic environments.

Cycling of major D-DNA pools: comparison to other marine environments

The only other direct measurement of D-DNA production was made by Paul et al. (1987) in the Gulf of Mexico. They employed the incorporation of $[^3]H$-thymidine into bacteria and measured the subsequent release of this labeled DNA into the D-DNA pool. This method only measures D-DNA production by actively growing bacterioplankton but is still several orders of magnitude higher than the production of ehD-DNA at Station ALOHA (Table 8). The turnover time calculated from these production rates was much longer than those measured at Station ALOHA owing to the much higher concentrations of D-DNA in the Gulf of Mexico. Unfortunately, turnover times were calculated using total D-DNA concentrations. It was later found that only approximately 50% of the D-DNA in that area is soluble DNA (Jiang and Paul 1995). Since the turnover of soluble DNA is most likely much faster than bound forms of DNA, the turnover times calculated by Paul et al. (1987) are probably longer than turnover times for the ehD-DNA pool.

Despite this, a relationship between turnover time of D-DNA pools and trophic status can be inferred. The turnover of D-DNA was faster in oligotrophic offshore environments in the Gulf of Mexico than the eutrophic coastal environments. If this trend
can be extended to Station ALOHA, there emerges a relationship between lower D-DNA concentrations and faster turnover times with increasingly oligotrophic conditions.

Weinbauer et al. (1993) speculated that low D-DNA concentrations may be due to increased utilization of D-DNA in phosphorus-limited systems. Since Station ALOHA is considered to be a phosphorus-stressed system, this would explain the higher turnover rates of D-DNA there. A D-DNA turnover time of 6.5 h in an estuary was measured by Paul et al. (1987). However, this was done by measuring uptake of labeled DNA which is highly dependent on the size of DNA added (Jørgensen and Jacobsen 1996) and therefore may or may not reflect actual turnover times of D-DNA at that location.

Virus production rates at Station ALOHA were within the range of rates measured for other locations (Table 9). A decrease in virus production and an increase in turnover time with distance offshore was noted in several studies (Steward et al. 1992; Steward et al. 1996; Noble and Fuhrman 2000). However, virus turnover times at Station ALOHA are within the range reported for nearshore stations (Noble and Fuhrman 2000) and are even faster than some reported coastal values (Steward et al. 1992).

Therefore, while ehD-DNA turnover rates are dramatically faster at Station ALOHA, virus turnover rates remain within the range observed for nearshore and coastal stations. This has direct implications for the relative percent of D-DNA that is within viruses. The faster turnover rates of ehD-DNA at Station ALOHA imply a greater utilization of ehD-DNA most likely owing to the ultraoligotrophic status of this station. Therefore, ehD-DNA concentrations will be kept low by preferential uptake of this phosphorus-rich, energetically expensive-to-synthesize molecule. DNA within viruses is
not accessible to DNase and therefore cannot be used by bacteria as a source of nutrients or nucleotides without prior degradation of the viral capsid. The combination of these turnover times for the individual D-DNA pools would serve to increase the percentage of D-DNA within viruses relative to most other areas where this has been measured. Thus, the conclusion that DNA within viruses is not a large fraction of the D-DNA pool, which has been reached by multiple investigators, may not be true for much of the ocean since the vast oligotrophic oceanic gyres have not been taken into account.

Virus and ehD-DNA production and turnover

The production of viruses and ehD-DNA at Station ALOHA decreased dramatically to undetectable levels below 75 m. This has also been noted below 10 m in offshore waters of the Southern California Bight (Steward et al. 1992) and below 100 m in the Bering and Chukchi Seas (Steward et al. 1996). This undetectable production would explain the sharp decrease in concentrations of viruses and D-DNA below 100 m. Bacteria are currently thought to be the major host to viruses and the main source of D-DNA in marine environments. The reasons for this will be discussed later, but the depth profile of bacterial concentration at Station ALOHA is similar to the virus and D-DNA depth profiles (Figure 13). Bacterial concentrations are uniform in the upper 75 m of the water column where viruses and D-DNA are produced and dramatically decrease below this depth. This is only circumstantial evidence, since phytoplankton concentrations may also affect this profile, but it appears that bacteria could be the main source and sink for
viruses and D-DNA at Station ALOHA. A study including quantification of visibly infected bacteria may be able to resolve this.

The production of virus and ehD-DNA in the upper ocean at Station ALOHA was relatively similar for all depths except for samples collected from 75 m. Virus production rates at 75 m were two times greater than the three depths measured above 75 m, and ehD-DNA production rates were four times greater. There is no increase in the concentration of viruses or ehD-DNA at this depth, resulting in turnover times that greatly exceed those measured above 75 m. These lines of evidence suggest a greater activity of production and uptake or destruction of viruses and ehD-DNA at 75 m. No direct conclusion can be reached about the reason for this high activity with the current data set. Using past estimates of bacterial production at or near Station ALOHA (Jones et al. 1996; Church unpublished data), there does not appear to be an increase in bacterial production at 75 m. Since this depth represented a major portion of the virus and ehD-DNA production, it bears further research to determine if this feature is replicated over temporal and spatial scales in the North Pacific Gyre and to understand the causes of its existence.

It seems logical to assume that the three major pools of D-DNA described by Jiang and Paul (1995) will have different turnover rates based on their relative lability and different production and uptake mechanisms. Unless cells take up D-DNA via transformation, it must be hydrolyzed by cell-associated or extracellular enzymes before being transported into the cell (Paul et al. 1988). It is therefore reasonable to assume that ehD-DNA will have a faster turnover time than bound forms of D-DNA which are not
readily hydrolyzed by enzymes. In contrast, DNA within viruses will be turned over as a function of the ability of viruses to infect hosts and replicate. Therefore, as suggested by Siuda and Chróst (2000) and Weinbauer et al. (1993), measurements of the turnover of the entire D-DNA pool will not accurately reflect the ecological roles of D-DNA. This study supports these hypotheses by demonstrating that eH-DNA turns over 3 to 10 times faster than DNA within viruses. Given that eH-DNA is less than half of the total D-DNA pool at Station ALOHA, a calculation of turnover times using the total D-DNA pool would have underestimated turnover by more than a factor of 2.

Phosphate addition experiments

The results of the phosphate addition experiments are difficult to interpret. The goal of these experiments was to see if the addition of phosphate to the phosphorus-stressed microbial community at Station ALOHA would cause any changes in the production of viruses or eH-DNA. Multiple studies have found that phosphate can stimulate virus production possibly by acting as the “trigger” for viruses to switch from a lysogenic to a lytic pathway (Bratbak et al. 1993; Wilson et al. 1996; Wilson and Mann 1997; Wilson et al. 1998; Jacquet et al. 2002). This may be a result of the requirement for phosphorus in the production of viral DNA.

The addition of phosphate to the 5 m incubation in this study caused an increase in virus production by a factor of 2.5 with no noticeable change in bacterial abundance. This would suggest that virus production at 5 m is limited in part by low phosphate concentrations. The mechanism causing this increase in production is uncertain. Higher
concentrations of phosphate can cause an increase in burst size per lysed cell (Wilson et al. 1996; Jacquet et al. 2002; Clasen and Elser submitted) which would explain the increase in virus production without a significant decrease in bacteria. A study including TEM could help to determine if phosphate causes an increase in burst size or the frequency of visibly infected cells in these incubations.

In contrast to the 5 m incubation, virus production was decreased by phosphate addition to the 75 m incubation. An explanation for this may involve proximity of this depth to deeper waters with much higher phosphate concentrations (Figure 14). However, if virus production was not limited by phosphate, there would be an expectation of no change in virus production in this incubation. Further study including more than two depths investigated is required to understand if phosphate addition affects virus production as a function of depth and phosphorus concentration.

A very surprising result of these experiments was the complete arrest of any measurable ehD-DNA production. The expected impact of phosphate addition on ehD-DNA production would be no change or a slight increase in production as a result of cell DNA released due to lysis by viruses. There is no precedent for an experiment of this type regarding DNA production and no conclusions can be drawn at this time, although it definitely bears further study.

Impact of viruses at Station ALOHA

The virus-mediated bacterial mortality at Station ALOHA was estimated from virus production. It was assumed that bacteria were the only source of viruses in this
area. This assumption is certainly not true since viruses that infect phytoplankton have been identified, but it is reasonable to assume that bacteria are the main source of viruses. This assumption has been inferred from studies that have found positive correlations between bacterial and viral concentrations (Cochlan et al. 1993; Steward et al. 1996; Culley and Welschmeyer 2002). The study by Culley and Welschmeyer (2002) was a transect from California to Station ALOHA and concluded that prokaryotes, not phytoplankton, were probably the major hosts of viruses in this area. A range of burst sizes (15-28) determined for an oligotrophic oceanic environment by Weinbauer and Suttle (1999) was divided into the virus production to calculate average bacterial mortality due to virus infection and replication. Because these calculations are highly dependent on burst size which can be spatially variable, the effect of viruses on bacteria at Station ALOHA can only be roughly estimated. Independent measurements of burst size from this region would improve accuracy in determining the impact of viruses at Station ALOHA.

The percent of bacteria lysed between 5 and 75 m at Station ALOHA was estimated to range from 3.2 to 16.5% of the standing stock per hour (Table 10). Using estimates of bacterial production from a station northeast of Station ALOHA (Jones et al. 1996), virus-mediated mortality of bacteria ranged from 52-134% of gross bacteria production above 75 m and 366-687% at 75 m (Table 10). These estimates would be improved with bacterial production measurements at the time of sampling and determination of burst sizes for this location. However, it appears that viruses exert considerable control over the bacterial population at Station ALOHA.
In addition to their impact on bacterial mortality, the role of viruses in the recycling of nutrients and other materials in the microbial loop has been studied (reviewed by Fuhrman 1992; Bratbak et al. 1994; Fuhrman 1999; Wilhelm and Suttle 1999). Lysis of bacteria by viruses results in a release of cell constituents which can then be utilized by other organisms, primarily bacteria. D-DNA produced from lysis of bacteria in this study was calculated by multiplying the rate of bacterial lysis by a DNA content per bacterium of 1.6 fg as determined by Fuhrman and Azam (1982) for an offshore station. This calculated rate ranged from 11 to 35% of the ehD-DNA production (Table 10) which is slightly lower than the estimates indirectly inferred from the Northern Adriatic Sea (Weinbauer et al. 1995). Thus, the release of D-DNA through viral lysis of bacteria is a significant portion of ehD-DNA production at Station ALOHA but viral lysis is probably not the major mechanism of ehD-DNA production.

Sources of ehD-DNA at Station ALOHA

In addition to viral lysis, D-DNA can be produced from predation on bacteria (Turk et al. 1992; Ishii et al. 1998; Kawabata et al. 1998; Alonso et al. 2000) and as exudates by growing bacterioplankton (Paul et al. 1987; Paul et al. 1990b). Although \textit{rbcL}, a large subunit of RUBISCO, has been found in the D-DNA pool of a reservoir (Paul et al. 1990a), it has not yet been demonstrated that growing phytoplankton produce D-DNA (Paul et al. 1987; Paul et al. 1990a). Therefore, in an attempt to identify the major mechanism resulting in the remaining 65-89% of ehD-DNA production at Station ALOHA, particulate DNA (P-DNA) production was estimated based on gross bacterial
production and phosphorus uptake. The reason for these calculations is that for D-DNA to be produced, it must first be synthesized within cells and then released. A lower constraint on the P-DNA production can be calculated from bacterial production measured near Station ALOHA (Jones et al. 1996) and assuming a DNA content per bacteria of 1.6 fg (Fuhrman and Azam 1982). This estimate assumes that bacteria are only producing enough P-DNA to replicate and that no D-DNA is being produced through exudation. An upper constraint on the production of P-DNA can be estimated using net phosphorus uptake measurements at Station ALOHA (Björkman and Karl in press). These measurements were converted to gross phosphorus uptake by addition of 20% of these values, which represents DOP recycling during incubations to obtain these measurements (Björkman personal communication). This assumes that all phosphorus that is taken up by the cell is used to produce P-DNA which is composed of approximately 9.7% phosphorus.

These estimates of gross P-DNA production can then be compared to gross ehD-DNA production (Table 11). P-DNA production estimated from bacterial production was much lower than the production of ehD-DNA. In contrast, P-DNA production estimated from phosphorus uptake was very close to, and at some depths less than, ehD-DNA production. Together, this suggests that bacteria are producing much more DNA than is required to replicate and a large fraction of phosphorus uptake is being used to produce DNA. If ehD-DNA was primarily being produced by predation on bacteria, then its production should be closer to or less than P-DNA production estimated from bacterial production. Therefore, the majority of ehD-DNA is probably being produced through
exudation by growing bacterioplankton or autolysis of non-replicating bacteria. It should be noted that these conclusions are based solely on considerations of bacterial DNA. Dead or senescent phytoplankton could be a source of D-DNA (Paul et al. 1990a) but this source is unable to be quantified at this time.

**ehD-DNA as a phosphorus source**

As alluded to previously, fast turnover of ehD-DNA at Station ALOHA is an indication of a high rate of recycling of this molecule within the microbial loop. Once D-DNA is produced, it can be hydrolyzed by cell-associated or extracellular nucleases (Paul et al. 1988). Assuming a 50% G+C content, DNA has a C:N:P ratio of approximately 10:4:1 which makes it a rich source of phosphorus. This would suggest that while D-DNA is a very small portion of the DOP pool, 1.5-4.1% in this study, it has the potential to be a large source of phosphorus for microorganisms, especially considering its rapid turnover.

Previous research indicates that D-DNA is taken up primarily by bacteria (Turk et al. 1992; Siuda and Güde 1996; Siuda et al. 1998; Paul and David 1989), although it can be hydrolyzed by cell-associated or extracellular nucleases prior to uptake. D-DNA as a phosphorus source has been studied through several experimental approaches. In a stratified lake, Matsui et al. (2001) observed phosphate release from the extracellular breakdown of plasmid DNA, concluding that DNA is part of a phosphate regeneration mechanism. Paul et al. (1988) suggested that hydrolysis products of D-DNA taken up by bacteria were primarily salvaged into nucleic acids. D-DNA uptake has also been linked
to the phosphorus status of bacterioplankton in the marine environment. Turk et al. (1992) demonstrated that labeled DNA added to incubations in the phosphorus-limited Northern Adriatic Sea was degraded much more rapidly than when it was added to the nitrogen-limited Southern California Bight incubations. This labeled DNA was mainly transferred to the <3 μm size fraction indicating that it was supporting a portion of the bacterial phosphorus demand. Jørgensen and Jacobsen (1996) demonstrated that prior to nutrient addition, D-DNA was taken up as 46% of the bacterial phosphorus requirement in mesocosms, and after phosphate was added, D-DNA uptake was reduced to 9% of the bacterial phosphorus requirements. Together, these studies suggest that in phosphorus-limited systems, D-DNA can be exploited as a source of phosphorus alone and/or a source of nucleotides which require phosphorus for the cell to synthesize. The high turnover of ehD-DNA at Station ALOHA can therefore be hypothesized to be a response of bacteria to quickly recycle this phosphorus-rich compound.

Over the last decade, there has been a decrease in the soluble reactive phosphorus (SRP) pool at Station ALOHA (Karl et al. 2001 a, b). As discussed by Björkman and Karl (in press), this implies that microorganisms at Station ALOHA must intensify recycling of phosphorus compounds or exploit other phosphorus sources. The measured production of ehD-DNA in the upper 75 m at Station ALOHA contains enough phosphorus to support the biologically available phosphorus (BAP) demand (Table 12) measured by Björkman and Karl (in press). This, coupled with fast turnover rates, suggests that ehD-DNA could be a significant phosphorus and/or nucleotide source for the microbial community in the phosphorus-stressed subtropical North Pacific Gyre.
Conclusions

The D-DNA pool at Station ALOHA is composed of two major pools: 49-63% within viruses and the remaining D-DNA as free DNA, able to be hydrolyzed in vitro by exogenous DNase. The reason for the relatively high percent of D-DNA within viruses is the very different turnover times for each of these D-DNA pools. The fast turnover of ehD-DNA (0.97-6.2 h) is hypothesized to be a result of rapid uptake of this phosphorus-rich molecule by bacteria in a phosphorus-stressed ecosystem. In fact, based on ehD-DNA production, the phosphorus demand of microorganisms at Station ALOHA may be able to be met by the uptake of ehD-DNA.

Viruses at Station ALOHA can have a large impact on the microbial community. The measured production of viruses shows that viruses can lyse 3.2-16.5% of the standing stock of bacteria per hour in the upper 75 m. This estimated rate of bacterial lysis translates into an estimated production of ehD-DNA which is 11-35% of the total ehD-DNA production. This suggests that the release of other cell contents via viral lysis may have an important role in cycling of DOM at Station ALOHA. In addition, this production of viruses may be limited by phosphate concentrations in the surface ocean.

Together, this information suggests that viruses and D-DNA have important roles within the microbial loop in the subtropical North Pacific Gyre which need to be investigated further. Future studies regarding viruses should include identification of their hosts, further investigation of the role of phosphate in virus production, evaluation of the role of viruses in nutrient cycling, time-series analysis of the effects of viruses on host community dynamics, and the role of viruses during blooms at Station ALOHA. The
use of eh-DNA as a phosphorus source should also be investigated including
identification and quantification of individual sources and sinks for this molecule.
Table 1. Regression data from standard curves of fluorescent nucleic acid stains. Concentration of nucleic acids ranged from 0 to 3.5 ng ml\(^{-1}\).

<table>
<thead>
<tr>
<th>Stain</th>
<th>Final Concentration of Stain</th>
<th>Blank Fluorescence</th>
<th>Nucleic Acid</th>
<th>Slope (fluorescence / ng ml(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>PicoGreen 1x</td>
<td>1x</td>
<td>0.2</td>
<td>dsDNA</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ssDNA</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RNA</td>
<td>0.04</td>
</tr>
<tr>
<td>SYBR Gold 1x</td>
<td>1x</td>
<td>1.1</td>
<td>dsDNA</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ssDNA</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RNA</td>
<td>0.05</td>
</tr>
<tr>
<td>SYBR Green I 1x</td>
<td>1x</td>
<td>0.4</td>
<td>dsDNA</td>
<td>1.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ssDNA</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RNA</td>
<td>0.005</td>
</tr>
<tr>
<td>SYBR Green I 0.5x</td>
<td>0.2</td>
<td></td>
<td>dsDNA</td>
<td>1.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ssDNA</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RNA</td>
<td>0.005</td>
</tr>
<tr>
<td>SYBR Green I 0.125x</td>
<td>0.1</td>
<td></td>
<td>dsDNA</td>
<td>1.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ssDNA</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RNA</td>
<td>0.004</td>
</tr>
</tbody>
</table>
Table 2. Recovery of $^{35}$S-labeled DNA from seawater samples using the centrifugal ultrafiltration method with different EDTA concentrations and storage methods. Frozen and refrigerated samples were stored for less than 1 day. Standard deviations are given in parentheses (n=4).

<table>
<thead>
<tr>
<th>EDTA Used</th>
<th>Final Concentration</th>
<th>Storage Method</th>
<th>Average Percent Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>disodium EDTA</td>
<td>20 mM</td>
<td>analyzed immediately</td>
<td>71% (±7)</td>
</tr>
<tr>
<td>tetrasodium EDTA</td>
<td>50 mM</td>
<td>analyzed immediately</td>
<td>86% (±4)</td>
</tr>
<tr>
<td>tetrasodium EDTA</td>
<td>50 mM</td>
<td>frozen</td>
<td>61% (±2)</td>
</tr>
<tr>
<td>tetrasodium EDTA</td>
<td>50 mM</td>
<td>refrigerated</td>
<td>45% (±2)</td>
</tr>
<tr>
<td>tetrasodium EDTA</td>
<td>100 mM</td>
<td>refrigerated</td>
<td>83% (±1)</td>
</tr>
</tbody>
</table>
Table 3. Average mass of DNA per viral genome at each sampled depth calculated from average genome sizes. Calculations assume a 50% G+C content of double-stranded DNA (0.1076 attograms per kbp).

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Average DNA Content Per Viral Genome (attograms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>66.9</td>
</tr>
<tr>
<td>25</td>
<td>69.8</td>
</tr>
<tr>
<td>45</td>
<td>67.7</td>
</tr>
<tr>
<td>75</td>
<td>65.0</td>
</tr>
<tr>
<td>100</td>
<td>69.7</td>
</tr>
<tr>
<td>150</td>
<td>65.6</td>
</tr>
<tr>
<td>200</td>
<td>65.7</td>
</tr>
<tr>
<td>500</td>
<td>62.5</td>
</tr>
</tbody>
</table>
Table 4. Percent of total D-DNA that is within viruses for each depth sampled. Average concentrations of DNA within viruses (n=3) were divided into average total D-DNA concentrations (n=4) for each depth. Standard deviations were propagated throughout the calculation and are given in parentheses.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Percent of D-DNA Within Viruses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>52.5 (±3.4)</td>
</tr>
<tr>
<td>25</td>
<td>56.6 (±2.5)</td>
</tr>
<tr>
<td>45</td>
<td>49.8 (±7.9)</td>
</tr>
<tr>
<td>75</td>
<td>62.8 (±6.4)</td>
</tr>
<tr>
<td>100</td>
<td>51.1 (±3.2)</td>
</tr>
<tr>
<td>150</td>
<td>54.4 (±4.7)</td>
</tr>
<tr>
<td>200</td>
<td>49.4 (±12.8)</td>
</tr>
<tr>
<td>500</td>
<td>60.1 (±4.5)</td>
</tr>
</tbody>
</table>
Table 5. Percent of the natural concentration of bacteria, viruses, and D-DNA present within incubations at the start of the incubation period.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Bacteria (%)</th>
<th>Viruses (%)</th>
<th>D-DNA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>21</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>25</td>
<td>21</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>45</td>
<td>21</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>75</td>
<td>15</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>100</td>
<td>20</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>200</td>
<td>20</td>
<td>11</td>
<td>19</td>
</tr>
</tbody>
</table>
Table 6. Estimated production rates and turnover times of viruses, ehD-DNA (D-DNA able to be hydrolyzed by DNase), and DNA within viruses. Standard deviations of the mean are given in parentheses.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Viruses</th>
<th>ehD-DNA</th>
<th>DNA Within Viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Production (x 10^5 ml^-1 h^-1)</td>
<td>Turnover Time (h)</td>
<td>Production (ng ml^-1 h^-1)</td>
</tr>
<tr>
<td>5</td>
<td>4.0 (±1.6)</td>
<td>24 (±9.7)</td>
<td>0.15 (±0.06)</td>
</tr>
<tr>
<td>25</td>
<td>6.0 (±1.1)</td>
<td>16 (±3.0)</td>
<td>0.13 (±0.02)</td>
</tr>
<tr>
<td>45</td>
<td>4.3 (±0.5)</td>
<td>22 (±4.1)</td>
<td>0.10 (±0.01)</td>
</tr>
<tr>
<td>75</td>
<td>11 (±1.1)</td>
<td>9.6 (±1.2)</td>
<td>0.41 (±0.12)</td>
</tr>
<tr>
<td>150</td>
<td>1.2 (±1.2)^t</td>
<td>-</td>
<td>nd</td>
</tr>
<tr>
<td>200</td>
<td>nd</td>
<td>-</td>
<td>nd</td>
</tr>
</tbody>
</table>

^t not significantly different from zero
nd not able to be determined due to small changes in concentration during incubations
- calculations not applied where rates were not significantly different from zero or not able to be detected
Table 7. Measurements of the percentage of D-DNA contained within viruses in marine environments. The range of measurements is shown in parentheses where applicable.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Method Used</th>
<th>Study Location</th>
<th>Percentage of D-DNA Within Viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beebee (1991)</td>
<td>ultracentrifugation (DNA in pellet is from viruses)</td>
<td>coastal southeast England</td>
<td>85 ± 10%</td>
</tr>
<tr>
<td>Paul et al. (1991)</td>
<td>virus concentration converted to DNA¹</td>
<td>Gulf of Mexico (coastal and offshore stations)</td>
<td>3.7 ± 3.8% (0.9 - 12.3%)</td>
</tr>
<tr>
<td>Maruyama et al. (1993)</td>
<td>deoxyribonuclease digestion (DNA not digested is within viruses)</td>
<td>Tokyo Bay</td>
<td>90%</td>
</tr>
<tr>
<td>Boehme et al. (1993)</td>
<td>virus concentration converted to DNA¹</td>
<td>Gulf of Mexico (coastal and offshore stations)</td>
<td>4 ± 5.8%</td>
</tr>
<tr>
<td>Weinbauer et al. (1993)</td>
<td>virus concentration converted to DNA²</td>
<td>Northern Adriatic Sea (time series of multiple stations)</td>
<td>17.1 ± 19.6% (0.7 - 88.3%)</td>
</tr>
<tr>
<td>Weinbauer et al. (1995)</td>
<td>virus concentration converted to DNA²</td>
<td>Northern Adriatic Sea (time series of coastal stations)</td>
<td>18.3 ± 26.2% (0.1 - 96.1%)</td>
</tr>
<tr>
<td>Jiang and Paul (1995)</td>
<td>virus concentration converted to DNA¹</td>
<td>Gulf of Mexico (coastal and offshore stations)</td>
<td>10.5 ± 7.8% (2.2 - 21.8%)</td>
</tr>
<tr>
<td>This Study</td>
<td>virus concentration converted to DNA³</td>
<td>subtropical North Pacific Ocean (Station ALOHA)</td>
<td>54.6 ± 4.9% (49.4 - 62.8%)</td>
</tr>
</tbody>
</table>

¹ conversion factor of 0.09 fg DNA per virus (Freifelder 1987)
² conversion factor of 0.099 fg DNA per virus (Reanney and Ackerman 1982)
³ conversion factor of DNA per virus determined by viral fingerprinting for each depth sampled (65 - 70 attograms DNA per virus)
Table 8. Direct estimates of D-DNA production and turnover time in the marine environment. Averages are reported with ranges given in parentheses.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Method Used</th>
<th>Study Location</th>
<th>D-DNA Production (ng ml$^{-1}$ h$^{-1}$)</th>
<th>Turnover Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paul et al. (1987)</td>
<td>TdR labeled DNA release*</td>
<td>Gulf of Mexico coastal</td>
<td>8 x 10$^3$</td>
<td>2064 (545 - 3504)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>offshore</td>
<td>9.29 x 10$^2$</td>
<td>775</td>
</tr>
<tr>
<td>This Study</td>
<td>dilution technique (production of DNA able to be hydrolyzed by DNase)</td>
<td>subtropical North Pacific Ocean (Station ALOHA) &lt;100m</td>
<td>0.2 (0.10 - 0.41)</td>
<td>3.7 (0.97 - 6.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;100m</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* method only measures D-DNA production from growing bacteria
- no production detected
Table 9. Direct estimates of virus production and turnover time in natural marine environments. Results are averages with ranges given in parentheses.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Method Used</th>
<th>Study Location</th>
<th>Virus Production (mL⁻¹ h⁻¹)</th>
<th>Virus Turnover Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steward et al. (1992)</td>
<td>³²Pi incorporation into DNA</td>
<td>Southern California Bight coastal surface</td>
<td>3.7 x 10⁶*&lt;br&gt;(0 - 9.6 x 10⁶)</td>
<td>79.2†&lt;br&gt;(64.8 - 93.6)†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nearshore &lt;10m</td>
<td>4.7 x 10⁵&lt;br&gt;(5.0 - 10.0 x 10⁵)</td>
<td>18.6&lt;br&gt;(13.7 - 23.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>offshore &lt;10m</td>
<td>2.1 x 10⁴*&lt;br&gt;(0 - 5.8 x 10⁴)</td>
<td>467†&lt;br&gt;(214 - 720)†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;10m</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>offshor e &lt;10m</td>
<td>4.7 x 10⁴</td>
<td>18.6</td>
</tr>
<tr>
<td>Steward et al. (1996)</td>
<td>FVIC²</td>
<td>Bering and Chukchi Seas 0-100m</td>
<td>1 x 10⁵&lt;br&gt;(0.2 - 5.8 x 10⁵)</td>
<td>(9.12 - 235)</td>
</tr>
<tr>
<td></td>
<td>TdR incorporation into DNA</td>
<td>&gt;100m</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0-100m</td>
<td>0.75 x 10⁵&lt;br&gt;(0.21 - 1.8 x 10⁵)</td>
<td>(28.8 - 360)</td>
</tr>
<tr>
<td>Noble and Fuhrman (2000)</td>
<td>FLV³ tracer method</td>
<td>nearshore and offshore California 0-60m</td>
<td>4.8 x 10⁵&lt;br&gt;(1.2 - 11.7 x 10⁵)</td>
<td>33&lt;br&gt;(16 - 53)</td>
</tr>
<tr>
<td>Wilhelm et al. (2002)</td>
<td>dilution technique</td>
<td>coastal British Columbia surface</td>
<td>3.9 x 10⁷&lt;br&gt;(1.4 - 9.7 x 10⁷)</td>
<td>2.8&lt;br&gt;(0.9 - 5.1)</td>
</tr>
<tr>
<td>This Study</td>
<td>dilution technique</td>
<td>subtropical North Pacific Ocean (Station ALOHA) &lt;100m</td>
<td>6.3 x 10⁵&lt;br&gt;(4.0 - 11 x 10⁵)</td>
<td>18&lt;br&gt;(9.6 - 24)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;100m</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* measurements that were not significantly different from zero are included as zero production
- no production detected or rates not significantly different from zero
† calculated from virus production estimates which were greater than zero
‡ reported production estimates corrected for loss of bacteria in preparation of incubations
§ frequency of visibly infected cells
$ fluorescently labeled viruses
Table 10. Impact of viral lysis on mortality of bacteria and the subsequent release of bacterial DNA to the D-DNA pool. Calculations are based on virus production with a burst size range for oligotrophic oceanic environments of 15-28 (Weinbauer and Suttle 1999). Percent of bacterial production lysed by viruses is calculated from bacterial production estimates by Jones et al. (1996) for a station northeast of Station ALOHA. DNA produced due to lysis is based on a DNA content of 1.6 fg DNA per bacterium for an offshore station (Fuhrman and Azam 1982).

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Bacteria Lysed ($\times 10^4 \text{ ml}^{-1} \text{ h}^{-1}$)</th>
<th>Percent Bacteria Lysed ($% \text{ h}^{-1}$)</th>
<th>Percent of Bacterial Production Lysed (%)</th>
<th>D-DNA Produced From Lysis (ng ml$^{-1}$ hr$^{-1}$)</th>
<th>Percent D-DNA Production Produced Through Lysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.4 - 2.6</td>
<td>3.2 - 5.9</td>
<td>52 - 97</td>
<td>0.019 - 0.042</td>
<td>11 - 26</td>
</tr>
<tr>
<td>25</td>
<td>2.1 - 4.0</td>
<td>4.7 - 8.8</td>
<td>71 - 134</td>
<td>0.034 - 0.063</td>
<td>19 - 35</td>
</tr>
<tr>
<td>45</td>
<td>1.5 - 2.9</td>
<td>3.4 - 6.4</td>
<td>70 - 134</td>
<td>0.025 - 0.046</td>
<td>18 - 34</td>
</tr>
<tr>
<td>75</td>
<td>3.9 - 7.3</td>
<td>8.8 - 16.5</td>
<td>366 - 687</td>
<td>0.062 - 0.12</td>
<td>13 - 24</td>
</tr>
</tbody>
</table>
Table 11. Estimates of gross particulate DNA production compared to D-DNA production. Estimates based on gross bacterial production are calculated from bacterial production at 26°N 155°W (Jones et al. 1996) assuming that bacteria do not produce excess DNA. Estimates based on phosphorus uptake are calculated from uptake of BAP at Station ALOHA (Björkman and Karl in press) assuming that all phosphorus taken up by cells is used to make DNA. Calculations use 1.6 fg DNA per bacterium as in Table 10 and a 9.7% phosphorus content of DNA. Standard deviations are given in parentheses.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>ehD-DNA Production (ng DNA ml⁻¹ d⁻¹)</th>
<th>Particulate DNA Production Estimates Based On Bacterial Production (ng DNA ml⁻¹ d⁻¹)</th>
<th>Particulate DNA Production Estimates Based On Phosphorus Uptake (ng DNA ml⁻¹ d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3.6 (±1.5)</td>
<td>1.0</td>
<td>2.1 (±0.2)</td>
</tr>
<tr>
<td>25</td>
<td>3.1 (±0.5)</td>
<td>1.1</td>
<td>2.8 (±0.4)</td>
</tr>
<tr>
<td>45</td>
<td>2.4 (±0.3)</td>
<td>0.83</td>
<td>3.1 (±0.4)</td>
</tr>
<tr>
<td>75</td>
<td>9.8 (±3.0)</td>
<td>0.41</td>
<td>2.3 (±0.5)</td>
</tr>
</tbody>
</table>
Table 12. Production of phosphorus within ehD-DNA compared to the biologically available phosphorus (BAP) uptake (Björkman and Karl in press) for the upper water column at Station ALOHA. DNA is assumed to have a 9.7% phosphorus content.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>ehD-DNA Production (ng ml(^{-1}) h(^{-1}))</th>
<th>ehD-DNA-P Production (nM P d(^{-1}))</th>
<th>BAP Uptake (nM P d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.15</td>
<td>11.3</td>
<td>6.6</td>
</tr>
<tr>
<td>25</td>
<td>0.13</td>
<td>9.8</td>
<td>8.8</td>
</tr>
<tr>
<td>45</td>
<td>0.10</td>
<td>7.5</td>
<td>9.6</td>
</tr>
<tr>
<td>75</td>
<td>0.41</td>
<td>30.8</td>
<td>7.3</td>
</tr>
</tbody>
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Appendix B: Figures

filter seawater through 0.22 μm Durapore filter
↓
add 1 M tetrasodium EDTA
(final concentration 100 mM)
↓
add 35S-labeled DNA
(final specific activity 6x10^{10} Ci ml^{-1})
↓
store at 4 °C until analysis
↓
add 15 ml of sample to Centricon
↓
centrifuge
(1000 x g, 25 min)
↓
add 10 ml TE buffer
↓
centrifuge
(1000 x g, 25 min)
↓
add 500 μl TE buffer and recover concentrate
↓
repeat concentrate recovery step
↓
increase concentrate volume to 1.75 ml with TE buffer
↓
add 250 μl 1.05x SYBR Green I
↓
stain for 10 min
↓
measure fluorescence of sample
(excitation 497, emission 520)
↓
combine 1 ml of sample with 10 ml of LSC cocktail
↓
count radioactivity to determine percent D-DNA standard recovery

Figure 1. Flow diagram of developed method for measuring dissolved DNA in seawater.
Figure 2. Comparison of 0.2 and 0.22 μm-pore-size filter types used for prefiltration in the centrifugal ultrafiltration method. Filters were evaluated for the amount of D-DNA allowed to pass through (black bars) and the percent of viruses in whole water that were recovered from the 0.2 and 0.22 μm filtrate (grey bars).
Figure 3. Recovery of $^{35}$S-labeled DNA with and without DNase addition. Times given refer to the time elapsed until EDTA was added after DNase addition to the sample.
Figure 4. Internal standard curve of lambda phage DNA added to filtered seawater (A) and determination of the detection limit of the centrifugal ultrafiltration method (B). Error bars represent one standard deviation of the mean. The $r^2$ values are >0.99.
Figure 5. Internal standard curve of lambda phage DNA added to filtered seawater with DNase (A) and determination of the detection limit of the centrifugal ultrafiltration method with DNase addition (B). Error bars represent one standard deviation of the mean. The $r^2$ value for the regression line (A) is $>0.99$. 
Figure 6. Effect of storage time on recovery of $^{35}$S-labeled DNA (circles) and D-DNA concentration corrected for $^{35}$S-labeled DNA recovery (triangles) in seawater samples.
Figure 7. Depth profiles of total D-DNA, ehD-DNA, and D-DNA within viruses. Error bars represent one standard deviation of the mean of n=4 (total D-DNA, ehD-DNA) and n=3 (D-DNA within viruses).
Figure 8. Depth profile of virus concentration enumerated with epifluorescence microscopy. Error bars represent one standard deviation of the mean for triplicate samples.
Figure 10. Changes in the concentrations of ehD-DNA and viruses in incubations from each depth. Values at zero hours are means and standards deviations of measurements from the initial prepared incubation sample (n=3). Symbols represent individual replicate incubations.
Figure 10. (Continued) Changes in the concentrations of ehD-DNA and viruses in incubations from each depth. Values at zero hours are means and standard deviations of measurements from the initial prepared incubation sample (n=3). Symbols represent individual replicate incubations.
Figure 11. Changes in the concentrations of ehD-DNA and viruses in phosphate addition incubations from each depth. Values at zero hours are means and standards deviations of measurements from the initial prepared incubation sample. Symbols represent individual replicate incubations.
Figure 12. Comparison of virus and ehD-DNA production rates in incubations with, and without phosphate addition. Error bars represent standard deviations of the mean of triplicate samples. No ehD-DNA production was able to be detected in phosphate addition incubations.
Figure 13. Depth profile of bacteria concentrations determined by flow cytometry. Error bars represent one standard deviation of the mean for duplicate samples.
Figure 14. Depth profile of Pi concentration determined by the MAGIC method (Karl and Björkman 2001).
References


Björkman KM (personal communication)


Church M (unpublished data)


