Concentration, production and turnover of viruses and dissolved DNA pools at Stn ALOHA, North Pacific Subtropical Gyre

Jennifer R. Brum*

School of Ocean and Earth Science and Technology, University of Hawaii, 1000 Pope Road, Honolulu, Hawaii 96822, USA

ABSTRACT: The concentrations, production rates and turnover times of the components of the dissolved DNA (D-DNA) pool, including viruses, were investigated in a depth profile at Stn ALOHA in the North Pacific Subtropical Gyre. A recently developed centrifugal concentration method was used to quantify the 3 major components of the D-DNA pool: free or enzymatically-hydrolyzable D-DNA (ehD-DNA), D-DNA within viruses and uncharacterized bound D-DNA. The production rates of each of these components of the D-DNA pool were estimated using a dilution technique and the turnover times of each component were calculated. Concentrations of total D-DNA and ehD-DNA were approximately 1.2 and 0.6 ng ml^{-1}, respectively, throughout the mixed layer (upper 100 m), and decreased with increasing depth to 0.2 and 0.06 ng ml^{-1} at 500 m, resulting in ehD-DNA constituting 27 to 51% of the total D-DNA pool. Concentrations of viruses ranged from 0.9 to 1.0×10^7 ml^{-1} within the mixed layer and also decreased with increasing depth to 0.2×10^7 ml^{-1} at 500 m. The average mass of DNA per viral genome was estimated at each depth with viral genome fingerprinting, and ranged from 62.5 to 69.8 ag DNA per virus. Multiplying the concentration of viruses by the average mass of DNA per virus at each depth revealed the concentration of D-DNA within viruses, which ranged from 0.13 to 0.68 ng ml^{-1} and constituted 49 to 63% of the total D-DNA pool. There was no measurable concentration of uncharacterized bound D-DNA in the depth profile. The production rates of ehD-DNA and D-DNA within viruses ranged from 0.10 to 0.41 and 0.03 to 0.07 ng ml^{-1} h^{-1}, respectively, within the mixed layer, but no production of D-DNA could be measured below the mixed layer. The calculated turnover times of ehD-DNA ranged from 0.97 to 6.2 h within the mixed layer and were 3 to 10 times shorter than the turnover times of D-DNA within viruses, which ranged from 9.6 to 24 h. In addition, the amount of phosphorus that was calculated to be produced within the ehD-DNA was able to support the biologically available phosphorus (BAP) demand, based on previously reported measurements of BAP uptake at Stn ALOHA. Using the measured virus production rates, viruses were estimated to lyse 3.2 to 16.5% of the standing stock of bacteria at Stn ALOHA h^{-1}, resulting in the release of ehD-DNA, which was estimated as 11 to 35% of the total ehD-DNA production. This research supports the hypothesis that individual components of the D-DNA pool are cycled at different rates and shows that 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 dissolved organic matter pool.

KEY WORDS: Dissolved DNA · Virus · Stn ALOHA · North Pacific Subtropical Gyre

INTRODUCTION

Dissolved deoxyribonucleic acid (D-DNA) in aquatic environments is operationally defined as DNA that passes through a 0.2 or 0.22 µm pore-size filter (DeFlaun et al. 1986, Karl & Bailiff 1989). The 3 major components of the D-DNA pool are free or enzymatically-hydrolyzable D-DNA (ehD-DNA; Siuda & Chróst 2000), D-DNA within viruses, and an as-yet-uncharacterized form of bound D-DNA (Jiang & Paul 1995). The

percent of total D-DNA of all 3 components measured in the Gulf of Mexico was 50% free D-DNA, 8 to 15% D-DNA within viruses, and 35 to 42% unidentified bound D-DNA (Jiang & Paul 1995). The percent of total D-DNA within viruses has been independently measured in many studies, usually resulting in an average of <20% (Paul et al. 1991, Boehme et al. 1993, Weinbauer et al. 1993, 1995, Jiang & 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 bacteria (Paul et al. 1987, 1990). The D-DNA can then be taken up as intact polynucleotides by marine bacteria during transformation (DeFlaun et al. 1987, Frischer et al. 1990, 1994, Jeffrey et al. 1990, Paul et al. 1991), or used by bacteria as a nucleotide or nutrient source (Paul et al. 1988, Turk et al. 1992, Jørgensen et al. 1993, 1994, Jørgensen & Jacobsen 1996, Siuda & Güde 1996, Finkel & Kolter 2001). Turnover times for the D-DNA pool, mediated by these production and uptake mechanisms, have been calculated to be 22.7 to 146 d in the Gulf of Mexico (Paul et al. 1987). However, estimates of bulk D-DNA pool dynamics are of limited ecological value because the individual D-DNA components are probably turning over at different rates due to their differing bioavailability and production and uptake mechanisms. For example, it has been hypothesized that ehD-DNA will be more labile and thus have faster turnover rates than bound D-DNA (including DNA within viruses), because the latter is resistant to enzymatic hydrolysis (Weinbauer et al. 1993, Siuda & Chróst 2000). Therefore, measurements of production and turnover rates of the individual D-DNA pool components will result in more realistic conclusions regarding the roles of D-DNA in aquatic environments.

In addition to being part of the D-DNA pool, aquatic viruses have been shown to affect microbial loop dynamics, bloom termination, and host-community diversity (Wommack & 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 dissolved organic matter (DOM) pool. Virus-induced mortality of marine bacteria has been found to be similar in magnitude to that due to protozoan grazing (Fuhrman & Noble 1995). 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, instead of being transferred to higher trophic levels through grazing (Wilhelm & Suttle 1999).

The recently developed centrifugal concentration method for quantification of D-DNA (Brum et al. 2004) was employed in a study of the composition and pro-

duction of the D-DNA pool at Stn ALOHA in the North Pacific Subtropical Gyre. By using DNase treatment, virus enumeration and the measurement of viral DNA content in conjunction with the D-DNA quantification method, the 3 major components of D-DNA were quantified and the production rates and turnover times were measured for each component. This is the first comprehensive study of the dynamics of these individual pools, and the first study of D-DNA cycling in an open-ocean gyre system. The results 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 (Björkman & Karl 2003) marine ecosystem.

MATERIALS AND METHODS

Study location and sampling. All samples were collected from December 13 to 15, 2002, at Stn ALOHA (22° 45′ N, 158° W) in the North Pacific Subtropical Gyre. This is an oligotrophic site that has an extensive 15 yr set of biogeochemical data generated through the Hawaii Ocean Time-series (HOT) program. Seawater samples were collected with 12 l SIO polyvinyl-chloride sampling bottles attached to a rosette equipped with a Seabird CTD. The concentrations of total D-DNA, ehD-DNA, uncharacterized bound D-DNA, viruses and bacteria as well as the average amount of DNA per virus were measured at 5, 25, 45, 75, 100, 150, 200 and 500 m. The production rates of total D-DNA, ehD-DNA, uncharacterized bound D-DNA and viruses were measured at 5, 25, 45, 75, 150, and 200 m in this profile.

D-DNA concentrations. The concentrations of total D-DNA, ehD-DNA, D-DNA within viruses and uncharacterized bound D-DNA were each measured in 4 replicate samples per depth using the centrifugal concentration method exactly as previously described (Brum et al. 2004). By this method, tetrasodium ethylenediamine tetraacetic acid (tetrasodium EDTA) was added to seawater that was vacuum-filtered through 0.22 μm pore-size PVDF (polyvinylidene difluoride) filters (Millipore) and stored at 4°C until analysis. Replicate samples were incubated for 30 min at 25°C with DNase (Sigma-Aldrich No. D5025) prior to addition of tetrasodium EDTA to stop DNase activity. Within 10 d of sample collection, a Centricon concentration unit (Millipore) was used to concentrate material greater than 10 kDa from 15 ml of sample. After the concentrate had been rinsed with Tris-EDTA buffer, DNA in the concentrate was quantified with SYBR Green I (Molecular Probes), a fluorescent double-stranded DNA stain, using a fluorescence spectrophotometer (Perkin-Elmer LS-5). ^{35}S-labeled DNA was

initially added to samples at tracer concentrations (final concentration 1300 dpm ml^{-1}) to estimate recovery efficiency of each sample, and ^{35}S radioactivity was measured with a liquid scintillation counter (Packard Tri-Carb 4640).

D-DNA within viruses was calculated by multiplying the concentration of viruses in the 0.22 µm filtrate by the average mass of DNA per virus determined by viral genome fingerprinting (Steward et al. 2000, Steward 2001) at each depth sampled. The concentration of ehD-DNA was calculated by subtracting the concentration of D-DNA that was not hydrolyzed by DNase from the concentration of total D-DNA. The concentration of uncharacterized bound D-DNA was calculated by subtracting the concentration of D-DNA within viruses from the concentration of D-DNA that could not be hydrolyzed by DNase. The final error values for D-DNA component concentrations were determined by propagation of errors throughout all calculations (Skoog et al. 2000).

Viral and bacterial concentrations. Concentrations of viruses were measured using epifluorescence microscopy (Noble & Fuhrman 1998). Samples were filtered through 0.22 µm pore-size PVDF filters (Millipore), preserved with 0.02 µm filtered glutaraldehyde (final concentration 2%), and stored at 4°C. Within 5 d of sample collection, triplicate sample aliquots (1 ml) were filtered onto 0.02 µm pore-size Anodisc (Whatman) filters and stained with SYBR Green I; viruses were enumerated using a Nikon Eclipse E600 epifluorescence microscope. Bacterial concentrations were determined from duplicate seawater samples (2 ml) preserved with paraformaldehyde (final concentration 1%) which were stored at −80°C until analysis with a flow cytometer (Coulter EPICS 753; Monger & Landry 1993). The pre-filtration of virus enumeration samples through a 0.22 µm pore-size filter was required so that virus and D-DNA concentrations could be determined from the same sample. However, previous experiments had shown that all of the extracellular viruses at Stn ALOHA passed through this filter (Brum et al. 2004). Therefore, the quantified concentrations of viruses are reported and discussed as the total concentration of viruses.

Viral genome fingerprinting. Pulsed field gel electrophoresis (PFGE) was used to estimate the average DNA content per virus at each depth sampled (Steward et al. 2000, Steward 2001). After filtration through 0.22 µm pore-size PVDF filters (Millipore), 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 (Millipore). PFGE was carried out with these samples using a CHEF-DR II pulsed field gel elec-

trophoresis 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 Bio-Labs) and mass standards (high DNA mass ladder; Invitrogen) were run on the same gel as samples. The gel was stained with SYBR Green I and the bands in each lane of the gel were analyzed with gel analysis software (Alpha Innotech). The average viral genome size for each depth was then calculated by dividing the sum of the abundance-weighted molecular weights for each band by the sum of the genome copy numbers for each band, in each lane. These average viral genome sizes were then used to calculate the average mass of DNA per viral genome assuming that the viral DNA was composed of equal amounts of adenine, thymine, guanine and cytosine.

Virus and D-DNA production. Production of viruses, total D-DNA, ehD-DNA, and uncharacterized bound D-DNA were measured using a dilution technique (Wilhelm et al. 2002). This method was originally described for virus production, but preliminary experiments had shown that D-DNA production could be measured by this technique as well, using the same principles. Seawater was collected from each depth the day before the incubation experiments, filtered through a 30 kDa filter using a vortex flow filtration system (Benchmark), and stored at 4°C for use as a diluent. Preliminary experiments had shown that this filtration removes all viruses and over 90% of the D-DNA in seawater collected from Stn ALOHA. On the subsequent day, seawater samples (2 l) from the same depths were vacuum-filtered through 73 mm-diameter, 0.22 µm pore-size PVDF filters (Millipore). As each sample was filtering, a sterile transfer pipette was used to repeatedly direct jets of sample across the filter surface to help maintain particles in suspension. 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 3 times in order to dilute viruses and D-DNA and allow them to pass through the filter while retaining bacteria. The retained seawater (approximate volume 200 ml) was then diluted with the 30 kDa filtrate to a final volume of 2 l, resulting in a target dilution of viruses and D-DNA to approximately 10 to 20% of their initial concentrations. Of this prepared seawater, 3 aliquots of 500 ml each 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 from each replicate every 3 h for 12 h to quantify the concentration of bacteria, viruses, total D-DNA, ehD-DNA, and uncharacterized bound D-DNA as previously described.

Production rates of ehD-DNA and viruses 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 (Minitab). 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 (Wilhelm et al. 2002). Again, virus concentrations were only determined from the 0.22 µm filtrate, but this filtration has been shown not to exclude extracellular viruses at Stn ALOHA (Brum et al. 2004), so the measured production of viruses in the 0.22 µm filtrate is also reported as the total production of viruses. Turnover times for each component of the D-DNA pool were calculated by dividing their concentrations by their production rates at each depth, assuming that their concentrations remained at steady state.

The production rates of ehD-DNA and viruses were then used to ascertain the potential for ehD-DNA as a phosphorus source, the rate of bacterial lysis by viruses, and the rate of D-DNA production as a result of viral lysis. The production rates of phosphorus within ehD-DNA were calculated by multiplying the estimated ehD-DNA production rates by the phosphorus content of DNA, which was assumed to be 9.7%. Virus-induced bacterial mortality was estimated by dividing a range of burst sizes (15 to 28 viruses cell^{-1}) determined for bacteria in an oligotrophic oceanic environment (Weinbauer & Suttle 1999) by the estimated virus production rates to obtain the rate of bacterial lysis at Stn ALOHA. The rate of D-DNA production from this viral-induced lysis was calculated by multiplying the rate of bacterial lysis by a DNA content per bacterium of 1.6 fg, as determined at an offshore station by Fuhrman & Azam (1982). These calculations assumed that all virus production is the result of bacterial lysis and that all the bacterial DNA is released to the D-DNA pool.

RESULTS

D-DNA concentrations

The concentration of total D-DNA was approximately 1.2 ng ml^{-1} throughout the mixed layer (upper 100 m), declined significantly between 100 and 150 m, and continued to decrease with increasing depth to 0.2 ng ml^{-1} at 500 m (Fig. 1). The concentration of ehD-DNA had a depth profile similar to total D-DNA, but ranged from 0.06 to 0.66 ng ml^{-1} (Fig. 1), constituting 27 to 51% of the total D-DNA pool. Concentrations of viruses and bacteria were also highest in the mixed layer, ranging from 3.5 to 4.5 × 10^5 ml^{-1} and 9.0 to 10.4 × 10^6 ml^{-1}, respectively, and declined sharply below 100 m
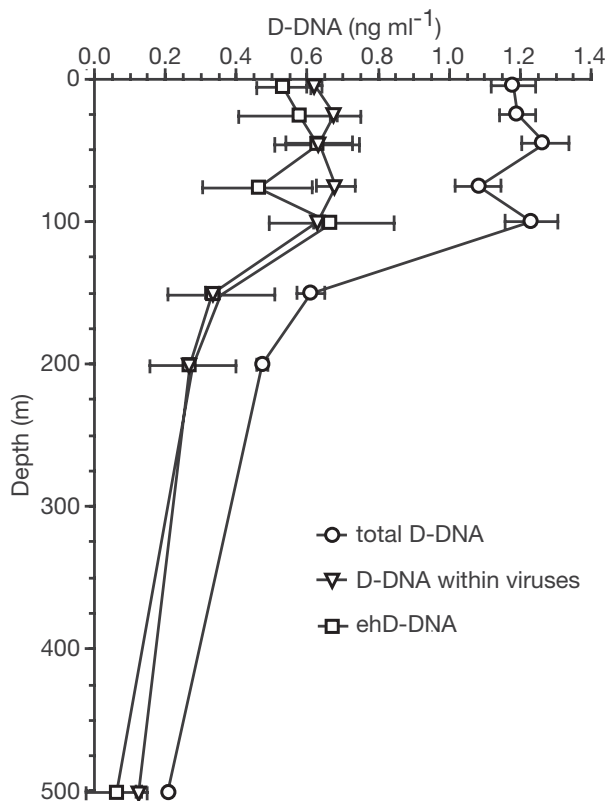

Fig. 1. Depth profiles of the concentrations of total D-DNA, ehD-DNA and D-DNA within viruses at Stn ALOHA. Error bars represent ±1 SD of the mean (n = 4)

to 0.7 × 10^5 ml^{-1} and 2.0 × 10^6 ml^{-1}, respectively, at 500 m (Fig. 2). Average viral genome sizes ranged from 58 to 65 kb over all depths sampled and the average mass of DNA per viral genome calculated from the genome sizes ranged from 62.5 to 69.8 ag DNA virus^{-1} (Fig. 3). These calculated values were then used to convert the concentration of viruses at each depth to concentrations of D-DNA within viruses, which ranged from 0.13 to 0.68 ng ml^{-1} (Fig. 1), constituting 49 to 63% of the total D-DNA concentration. The concentration of D-DNA within viruses was not significantly different from the concentration of D-DNA that was not hydrolyzable by DNase (Student's *t*-tests, p > 0.3 for every depth) resulting in no measurable concentration of uncharacterized bound D-DNA at any depth sampled. Therefore, at Stn ALOHA, there were 2 major components of the D-DNA pool: ehD-DNA and D-DNA within viruses.

ehD-DNA and virus production

Preparations of seawater for incubation experiments diluted bacteria, viruses and total D-DNA, resulting in average initial concentrations of 20, 7 and 16%, respectively, relative to whole water samples. Because
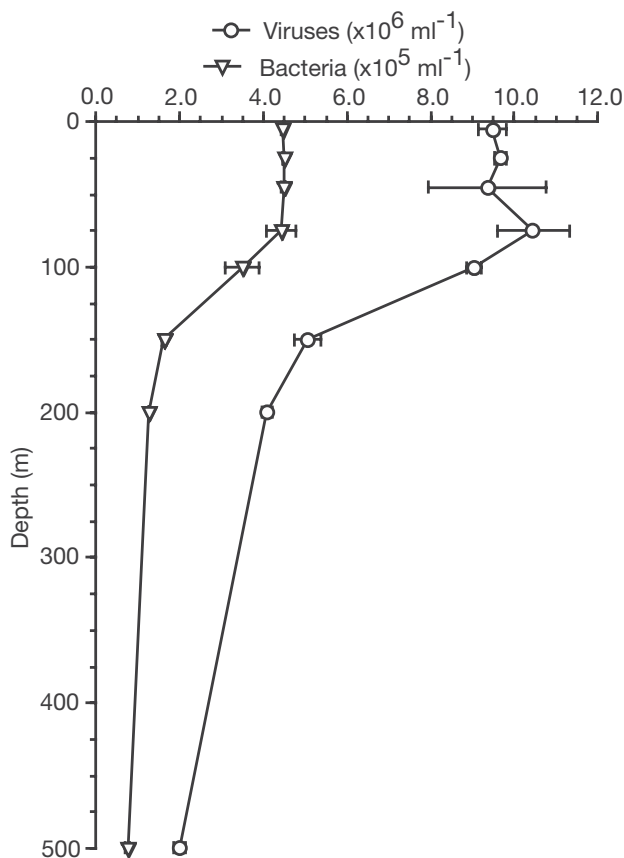
Fig. 2. Depth profiles of the concentrations of viruses and bacteria at Stn ALOHA. Error bars represent ±1 SD of the mean of triplicate samples for viruses and duplicate samples for bacteria

of low virus concentrations in the incubations, treatment of all samples with DNase resulted in D-DNA measurements that were not significantly different from blank values. Therefore, ehD-DNA concentrations were calculated by subtracting the D-DNA within viruses from total D-DNA for each time point sampled. Increases in ehD-DNA and virus concentrations were nearly linear for the first 3 to 4 time points (Fig. 4). The ehD-DNA concentration in the 75 m incubations had stopped increasing by the third time point (Fig. 4), so the production rate was calculated as the slope between the first 2 time points of the incubations. Because significant uptake of ehD-DNA may have already begun by the second time point, the calculated production rate is a minimum estimate of gross ehD-DNA production at 75 m. Because of only small fluctuations of ehD-DNA and virus concentrations, reliable production rates could not be determined in the 150 and 200 m incubations. Bacterial concentrations in the incubations did not change appreciably with time.

Virus production rates were similar for the 5, 25 and 45 m incubations (4.0 to 6.0 × 10^5 ml^{-1} h^{-1}) and

increased to approximately twice this rate in the 75 m incubations (Table 1). Using the calculated average DNA content per virion, these values translated into production rates of 0.03 to 0.07 ng ml^{-1} h^{-1} for D-DNA within viruses in the mixed layer (Table 1). Production of ehD-DNA was also similar at 5, 25, and 45 m (0.1 to 0.15 ng ml^{-1} h^{-1}) but was approximately 4 times higher at 75 m (Table 1). Turnover times of D-DNA within viruses ranged from 9.6 to 24 h and turnover times of ehD-DNA ranged from 0.97 to 6.2 h in the mixed layer (Table 1). Thus, production rates of D-DNA within viruses were 3 to 6 times lower and turnover times were 3 to 10 times longer than for ehD-DNA.

The production rates of phosphorus within ehD-DNA were calculated to range from 7.5 to 30.8 nM P d^{-1} in the mixed layer (Table 2). These rates ranged from 78 to 422% of the rates of BAP uptake previously measured at the same depths at Station ALOHA (Björkman & Karl 2003). The rate at which bacteria were lysed by viruses ranged from 1.4 × 10^4 bacteria ml^{-1} h^{-1} at 5 m to 7.3 × 10^4 bacteria ml^{-1} h^{-1} at 75 m, resulting in 3.2 to 16.5% of the standing stock of bacteria lysed per hour in the mixed layer at Stn ALOHA (Table 3). This bacterial lysis was calculated to result in the production of 0.019 to 0.12 ng DNA ml^{-1} h^{-1} as a result of bacterial DNA being released from the viral lysis of cells (Table 3). This calculated rate of D-DNA production from viral lysis of bacteria constituted 11 to 35% of the measured production of ehD-DNA in the mixed layer at Stn ALOHA (Table 3).

DISCUSSION

Components of D-DNA pool

The total D-DNA depth profile in this study is in agreement with the D-DNA profile measured near this site using the CTAB (cetyltrimethylammonium bromide) method (Karl & Bailiff 1989). The D-DNA pool was composed of an average of 48% ehD-DNA, which is similar to results for free D-DNA in the Gulf of Mexico (Jiang & Paul 1995). However, the uncharacterized bound D-DNA described in the latter report was not detected at Stn ALOHA and may be a characteristic of more eutrophic areas.

The average percentage of total D-DNA within viruses at Stn ALOHA was 54.6% which is higher than the average percentages reported for other environments, which range from 3.7% in the Gulf of Mexico (Paul et al. 1991) to 18.3% in the Northern Adriatic Sea (Weinbauer et al. 1995). Previous studies have reported that DNA within viruses constitutes an average of 85% (Beebee 1991) and 90% (Maruyama et al. 1993) of the total D-DNA pool, but the methods employed in those studies
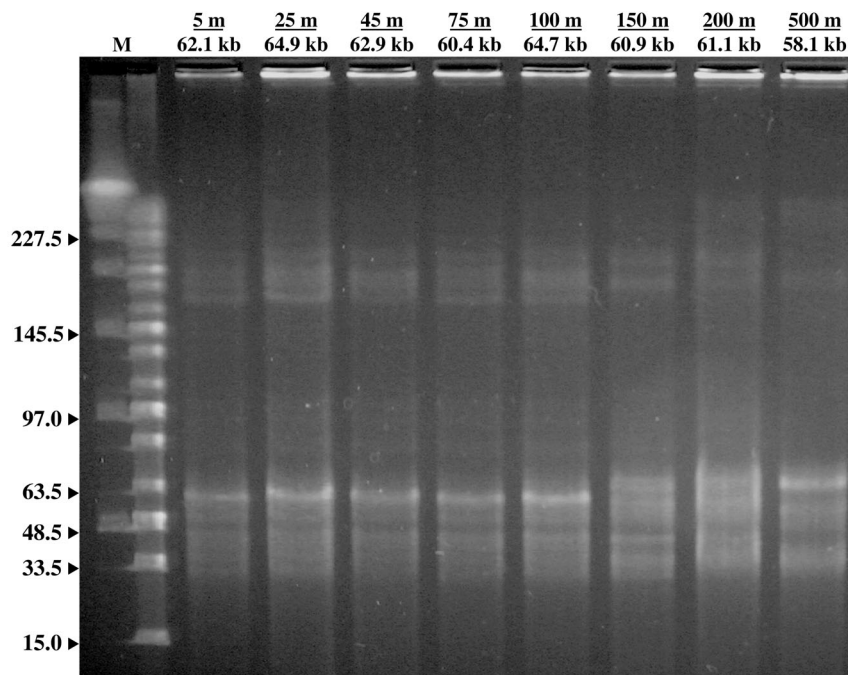
Fig. 3. Pulsed field gel electrophoresis (PFGE) banding patterns for viral concentrates from 8 depths at Stn ALOHA. The average length of DNA per viral genome is shown below the sampling depths at the top of the gel lanes. The marker lane (M) contains a 15 to 300 kb ladder and a lambda ladder. Sizes (kb) of selected marker bands are indicated on the left

rates were 4 times greater and virus production rates 2 times greater than at the other depths sampled in the mixed layer. There was no increase in the concentration of ehD-DNA or viruses at this depth, resulting in turnover times that were much shorter than those measured at other depths in the mixed layer. This suggests that there was greater uptake or degradation of ehD-DNA and viruses at 75 m as well as higher production rates. The cause of faster cycling rates at 75 m is unknown, but could potentially be caused by greater bacterial activity or a large viral lysis event.

The production rates of ehD-DNA and viruses were undetectable below the mixed layer and coincided with the decrease in the concentration of bacteria, which are thought to be the major producers of viruses (Wommack & Colwell 2000) and perhaps D-DNA (Paul et al. 1987, 1990). Nearly undetectable rates of virus production below 100 m were also reported for the Bering and Chukchi Seas, where it was also found that concentrations of bacteria declined below this depth (Steward et al. 1996). This undetectable production is the most likely cause of the sharp decrease in ehD-DNA and virus concentrations below the mixed layer at Stn ALOHA.

The production of ehD-DNA was 3 to 6 times faster than the production of D-DNA within viruses, resulting in turnover times of ehD-DNA that were 3 to 10 times shorter than turnover times of D-DNA within viruses. It seems logical that the 3 major components of the D-DNA pool described by Jiang & Paul (1995) would turn over at different rates, based on their relative lability and different production and uptake mechanisms. Unless cells take up D-DNA during 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 that ehD-DNA will be turned over more rapidly than bound forms of D-DNA that are not readily hydrolyzed by DNase. In contrast, D-DNA within viruses will be turned over primarily as a function of the ability of viruses to infect their hosts and replicate. Therefore, as previously suggested (Weinbauer et al. 1993, Siuda & Chróst 2000), measurements of the turnover of individual components of the D-DNA pool will more accurately reflect the ecological roles of D-DNA. This study supports these hypotheses by demonstrating that ehD-DNA turns over much faster than D-DNA within viruses at Stn ALOHA.

were later shown to overestimate the percentage of D-DNA within viruses by including high molecular weight D-DNA and uncharacterized bound D-DNA, respectively (Jiang & Paul 1995). Seasonally high percentages of total D-DNA within viruses of up to 96.1% were found in the phosphorus-limited Northern Adriatic Sea (Weinbauer et al. 1993, 1995), but the remaining measurements in these studies (Table 4) suggest that D-DNA contained within viruses is not, overall, a large component of the D-DNA pool. However, none of the previous studies sampled open-ocean gyre systems, which comprise most of the world ocean. In this study, the faster turnover of ehD-DNA compared to D-DNA within viruses, coupled with the lack of uncharacterized bound D-DNA, may have served to increase the percentage of total D-DNA within viruses at Station ALOHA relative to other locations. It is not known whether the high percentage of D-DNA within viruses found in this study is typical of oligotrophic, open-ocean gyre environments.

Production and turnover of ehD-DNA and viruses

The production of ehD-DNA and viruses were relatively uniform throughout the mixed layer at Stn ALOHA, except at 75 m where ehD-DNA production

ehD-DNA as a phosphorus or nucleotide source

It has been speculated that low D-DNA concentrations may result from increased utilization of D-DNA in phosphorus-limited systems (Weinbauer et al. 1993). Direct measurements of D-DNA production have been made in the Gulf of Mexico (Paul et al. 1987), but these measurements only included D-DNA produced by actively growing bacteria and were much lower than the production rates measured at Stn ALOHA, which include D-DNA produced from all sources. However, Paul et al. (1987) did find lower concentrations and shorter turnover times of the D-DNA pool at an oligotrophic offshore environment than at eutrophic coastal environments. Turnover times of ehD-DNA at Stn ALOHA were even shorter than those reported for the offshore station in the Gulf of Mexico. This suggests that there may be a relationship between lower D-DNA concentrations and shorter turnover times with increasingly oligotrophic conditions.

DNA is rich in phosphorus, with a molecular C:N:P ratio of approximately 10:4:1, and is the major information molecule of all living organisms. Considering its rapid turnover, ehD-DNA can potentially be a large source of nucleotides or phosphorus for microorganisms in the mixed layer at Stn ALOHA. Previous research suggests that D-DNA is taken up primarily by bacteria (Paul & David 1989, Turk et al. 1992, Siuda & Güde 1996, Siuda et al. 1998) and that the hydrolysis products of D-DNA are mostly salvaged into nucleic acids within the cell (Paul et al. 1988). It has also been found that orphophosphate is generated from 5′-nucleotides by bacterial cell-surface 5′-nucleotidase (Ammerman & Azam 1985) and that extracellular breakdown of plasmid DNA results in the release of phosphorus (Matsui et al. 2001), indicating that D-DNA is part of a phosphorus regeneration mechanism. D-DNA uptake has also been linked to the phosphorus status of bacteria in the marine environment. D-DNA has been shown to be taken up more rapidly when added to phosphorus-limited samples than when added to nitrogen-limited (Turk et al. 1992) or phosphate-amended (Jørgensen & Jacobsen 1996) samples. These studies suggest that in phosphorus-limited or phosphorus-stressed systems such as Stn ALOHA, D-DNA can be exploited as a source of phosphorus and/or as a source of nucleotides which require phosphorus for the cell to synthesize.

Over the last decade, there has been a decrease in the soluble reactive phosphorus (SRP) pool at Stn ALOHA (Karl et al. 2001a,b). As discussed by Björkman & Karl (2003), this implies that microorganisms at Stn ALOHA must intensify recycling of phosphorus compounds or exploit other phosphorus sources. The measured production of ehD-DNA in the mixed layer at Stn ALOHA contains enough phosphorus to support
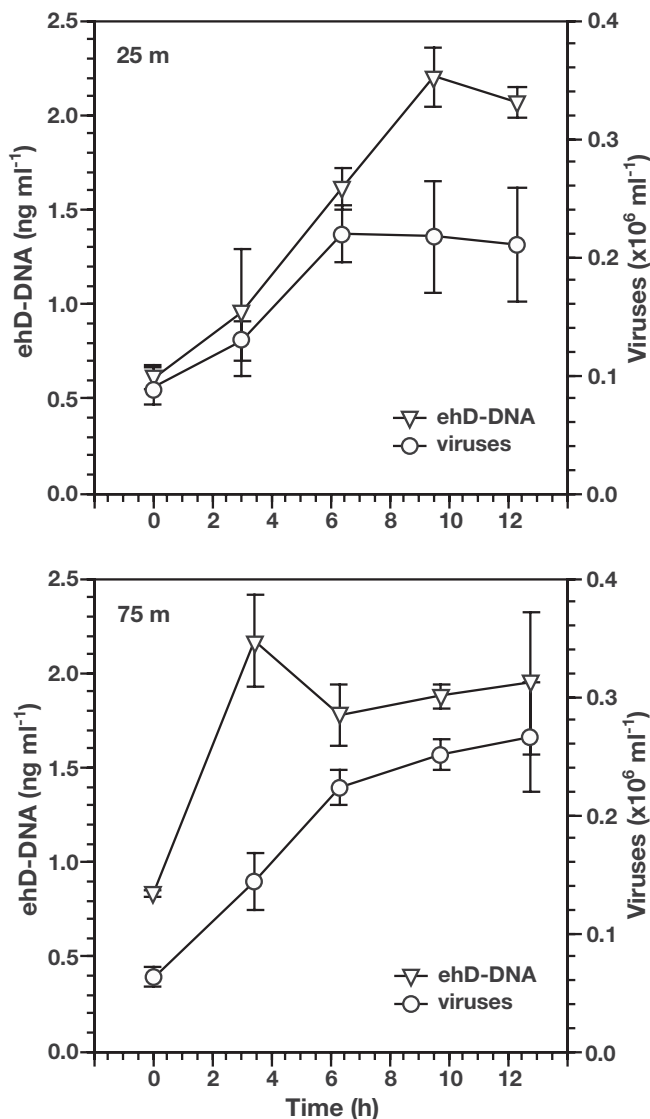

Fig. 4. Examples from 25 and 75 m of the change in concentrations of ehD-DNA and viruses over time in the incubations used to measure production rates. Error bars represent ±1 SD of the mean (n = 3)

the BAP demand previously measured there (Björkman & Karl 2003). Thus, it is feasible that ehD-DNA could be a significant phosphorus and/or nucleotide source for the microbial community in the phosphorus-stressed North Pacific Subtropical Gyre.

Impact of viruses at Stn ALOHA

The virus-mediated bacterial mortality at Stn ALOHA was estimated from virus production, assuming that bacteria were the only source of viruses at this station. This assumption is certainly not true, since viruses that infect phytoplankton have been

Table 1. Estimated production rates and turnover times of viruses, D-DNA within viruses and ehD-DNA. Data are means (±1 SD) of triplicate samples. Data from 150 and 200 m not shown as production rates at these depths were below detection limit

Depth (m)	Viruses Production ($\times 10^5$ ml^{-1} h^{-1})	D-DNA within viruses		ehD-DNA	
		Production (ng ml^{-1} h^{-1})	Turnover time (h)	Production (ng ml^{-1} h^{-1})	Turnover time (h)
5	4.0 (± 1.6)	0.03 (± 0.01)	24 (± 9.5)	0.15 (± 0.06)	3.7 (± 1.5)
25	6.0 (± 1.1)	0.04 (± 0.01)	16 (± 3.0)	0.13 (± 0.02)	4.0 (± 0.8)
45	4.3 (± 0.5)	0.03 (± 0.003)	22 (± 4.1)	0.10 (± 0.01)	6.2 (± 1.2)
75	11 (± 1.1)	0.07 (± 0.007)	9.6 (± 1.2)	0.41 (± 0.12)	0.97 (± 0.34)

identified; but positive correlations between bacteria and virus concentrations have been reported in the North Pacific Subtropical Gyre (Culley & Welschmeyer 2002), adding to the evidence that bacteria may be the major hosts of viruses (Wommack & Colwell 2000). The percent of bacteria lysed in the mixed layer at Stn ALOHA was estimated to range from 3.2 to 16.5% of the standing stock of bacteria per hour. Because of the relatively slow growth of bacteria at Station ALOHA (Jones et al. 1996) the high percent of bacteria lysed at 75 m is almost certainly not sustainable, but it may be indicative of a large viral lysis event or a short-lived increase in bacterial activity fueled by nutrient input from below the mixed layer. A time series of virus production measurements at Stn ALOHA would improve accuracy in determining the impact of viruses at this site. However, it appears that viruses can exert considerable control over the bacterial population at Stn ALOHA.

As a result of viral lysis, the cell contents of the lysed bacteria are released to the dissolved pool, where they may be utilized by other organisms, particularly bacteria (Fuhrman 1992, 1999, Bratbak et al. 1994, Wilhelm & Suttle 1999). The rate of D-DNA production from viral-induced lysis of bacteria ranged from 11 to 35% of the ehD-DNA production, which is slightly lower than 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 Stn ALOHA, but viral lysis is probably not the major mechanism of its production. The remaining ehD-DNA is most likely produced through grazing, exudation by growing bacteria, or autolysis of bacteria.

Methodological considerations

The estimates of ehD-DNA and virus production in this study relied on measuring the rates at which they were produced from bacteria recovered in the incubation samples, and multiplying these rates to reflect production from the entire bacterial population. Improvements in recovery of bacteria for these incubations will undoubtedly

Table 2. Production of phosphorus within ehD-DNA compared to biologically available phosphorus (BAP) uptake measured previously (Björkman & Karl 2003) in the mixed layer at Stn ALOHA. D-DNA is assumed to have 9.7% phosphorus content. Data are means (±1 SD where applicable)

Depth (m)	ehD-DNA production (nM P d^{-1})	BAP uptake (nM P d^{-1})
5	11.3 (± 4.5)	6.6
25	9.8 (± 1.5)	8.8
45	7.5 (± 0.75)	9.6
75	30.8 (± 9.0)	7.3

Table 3. Impact of viral lysis on mortality of bacteria and subsequent release of bacterial DNA to D-DNA pool. Data based on virus production, with burst size range for oligotrophic oceanic environments of 15 to 28 (Wainbauer & Suttle 1999). D-DNA produced due to lysis based on DNA content of 1.6 fg DNA per bacterium for an offshore station (Fuhrman & Azam 1982)

Depth (m)	Bacteria lysed ($\times 10^4$ ml^{-1} h^{-1})	(% h^{-1})	D-DNA from viral lysis (ng ml^{-1} h^{-1})	ehD-DNA from viral lysis (%)
5	1.4–2.6	3.2–5.9	0.019–0.042	11–26
25	2.1–4.0	4.7–8.8	0.034–0.063	19–35
45	1.5–2.9	3.4–6.4	0.025–0.046	18–34
75	3.9–7.3	8.8–16.5	0.062–0.12	13–24

Table 4. Percentage of D-DNA within viruses in marine environments. Data are means ±SD; range of measurements is shown in parentheses where applicable, n: no. of samples

Location	%	n	Source
Gulf of Mexico			
Coastal and offshore stations	3.7 ± 3.8 (0.9–12.3)	5	Paul et al. (1991)
Coastal and offshore stations	4 ± 5.8	29	Boehme et al. (1993)
Coastal and offshore stations	12.9 ± 5.8 (6.6–21.8)	5	Jiang & Paul (1995)
Northern Adriatic Sea			
Time-series of coastal and offshore stations	17.1 ± 19.6 (0.7–88.3)	35	Weinbauer et al. (1993)
Time-series of coastal stations	18.3 ± 26.2 (0.1–96.1)	24	Weinbauer et al. (1995)
North Pacific Subtropical Gyre			
Stn ALOHA	54.6 ± 4.9 (49.4–62.8)	8	This study

increase the accuracy of these production measurements.

Since this study was conducted, other reports have shown that the concentration of viruses in a sample will decrease with time when stored with 2% formaldehyde or 0.5% glutaraldehyde at 4°C (Brussaard 2004, Wen et al. 2004). These reports did not include data for storage of viruses in 2% glutaraldehyde, the concentration used in this study, but a discussion of the possible effects of storage on the results presented in this study is warranted. The production rates of viruses would not be considerably affected by loss of viruses during storage because the samples for virus enumeration were all filtered on the same day, resulting in a similar decrease in virus concentration (if any) for all samples, which would not affect the calculated rates of increase in virus concentrations in the incubations. Any loss of viruses in stored samples would have the most direct effect on the concentrations of viruses reported in the depth profile, and thus also affect calculations of virus turnover times. However, an underestimation of virus concentrations by approximately 40 to 50% as a result of storage for 5 d (Wen et al. 2004) would mean that D-DNA within viruses was approximately 100% of the total D-DNA pool at Stn ALOHA. This result would be highly unlikely, especially since an average of 45% of the D-DNA pool was hydrolyzable by DNase, which does not hydrolyze DNA within viruses (Brum et al. 2004). Furthermore, a major factor in the decrease of enumerated bacteria in preserved samples is protease activity (Gundersen et al. 1996), which would presumably also affect viruses in preserved samples. The majority of ectoenzyme activity in aquatic environments is associated with microbial cells (Chróst 1990), which were removed from the virus enumeration samples in this study by 0.22 µm filtration. Brussard (2004) and Wen et al. (2004) also conducted their studies of virus preservation with samples from coastal environments which would have higher concentrations of microbes, and therefore higher protease activity to reduce virus counts, than samples collected from an oligotrophic open-ocean environment such as Stn ALOHA. Finally, the concentrations of viruses in the depth profile of this study were slightly higher than the concentrations reported for Stn ALOHA by Culley & Welschmeyer (2002), who also used 2% glutaraldehyde as a fixative but filtered their samples at sea with no stated storage time. It is therefore unlikely that the virus concentrations reported in this study were significantly affected by sample storage conditions, but further investigation into the effects of 2% glutaraldehyde fixation of virus samples in oligotrophic environments is needed to confirm this.

CONCLUSIONS

This research has shown that measurements of concentration, production and turnover of the individual components of the D-DNA pool greatly increase the understanding of the ecological roles of D-DNA in the marine environment. ehD-DNA was shown to be quickly cycled in this open-ocean gyre system, potentially supporting bacterial phosphorus and/or nucleotide demand, and viruses were shown to lyse a significant portion of the bacterial population at this site. These are important roles within the microbial loop that should be investigated further to understand how ehD-DNA is utilized by bacteria and to what extent viruses affect individual microbial population dynamics and recycling of DOM in the open ocean.

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