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Viruses, bacterioplankton, and phytoplankton in the southeastern Gulf of Mexico: distribution and contribution to oceanic DNA pools

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ABSTRACT: Little is known concerning the factors which might control the distribution of viral abundance in oceanic environments and the relationship of viruses to the oceanic DNA pool. We have measured the distribution of viruses, bacterioplankton and phytoplankton in the subtropical southeastern Gulf of Mexico and related these parameters to the distribution of DNA (dissolved and particulate) in these waters. Viral direct counts were 4.6 to 27 $\times 10^6$ ml^{-1} in Tampa Bay (Florida, USA), 3.8 to 8.5 $\times 10^5$ ml^{-1} in all oceanic euphotic zone samples and 1.4 to 4.7 $\times 10^4$ ml^{-1} in deep (200 to 2500 m) waters, and were highly correlated with chlorophyll *a* concentrations (r = 0.97), particulate DNA (r = 0.96) and bacterial direct counts (BDC, r = 0.94). A vertical profile indicated a subsurface euphotic zone maximum in viral direct counts that corresponded with maxima for particulate and dissolved DNA, and picocyanobacterial direct counts. For all stations, the vertical distribution of viruses most closely followed the distribution of particulate DNA. Bacterioplankton made the largest contribution (> 50 %) to the particulate (> 0.2 µm) DNA pool while phytoplankton averaged 8 %. A predictive model for particulate DNA was determined to be: Particulate DNA (µg l^{-1}) = 4.94 $\times 10^{-9}$(BDC l^{-1}) + 2.31 [chlorophyll *a* (µg l^{-1})] + 2.77. DNA in viral particles was estimated to comprise only ca 4 % of the dissolved DNA pool. These results suggest that the distribution of viruses is tightly coupled to the distribution of microbial biomass in subtropical oceanic water column environments. Viruses may be instrumental in the production of dissolved DNA, but themselves are not a significant component of the dissolved DNA.

INTRODUCTION

Even though the existence of bacteriophages in the marine environment has been known for some time (Zobell 1946, Spencer 1955, Wiebe & Liston 1968) the quantitative importance of viruses in seawater has only come to light relatively recently (Sieburth et al. 1988, Bergh et al. 1989, Proctor & Fuhrman 1990, Suttle et al. 1990, 1991, Hara et al. 1991, Paul et al. 1991). These reports all indicate that viruses or virus-like particles (VLP) may be the most numerically dominant form of life in the oceans, with reports of concentrations from 10^3 to over 10^8 viruses ml^{-1}. Since these initial observations on viral abundance in diverse aquatic environments, recent reports have focused on the ecological significance of viruses and attempts have been made to quantitate their role in the 'microbial loop' (Heldal & Bratbak 1991, Bratbak et al. 1992, Gonzalez & Suttle 1993, Gonzalez et al. 1993). For example, as many as 70 % of the procaryotes in the oceans could be infected by phage, resulting in 100 % of the bacterial mortality (Proctor & Fuhrman 1990). Other estimates of the proportion of the bacterial population lysed by phage ranged from 24 to 576 % d^{-1} (Heldal & Bratbak 1991) and that carbon released from viral lysis of bacteria exceeded bacterial carbon production by a factor of 6

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(Bratbak et al. 1992). These estimates leave little room for bacterial mortality by other causes. Most of these observations were for coastal environments, where viral abundance and virus to bacterial ratios (VBR, Wommack et al. 1992) seem to be the largest.

As thought provoking as these estimations may be, there has not yet been a systematic study on viral abundance in offshore oceanic environments nor any attempt to relate viral abundance to other microbial biomass measurements in oceanic waters. In this paper we report on the abundance of viruses in surface and subsurface waters in a 10 station transect from Tampa Bay (Florida, USA) to the oligotrophic southeastern Gulf of Mexico. We relate viral abundance to bacterioplankton, phytoplankton abundance, dissolved and particulate DNA, and devise a model to explain the distribution of DNA in the oceans.

MATERIALS AND METHODS

Sampling sites. All samples were collected during a cruise aboard the RV 'Pelican', from June 14 to 26, 1992. The stations sampled appear in Fig. 1A and depths sampled appear in Fig. 1B. Subsurface samples were taken with 5 or 20 l Niskin bottles. The 5 l bottles were used in conjunction with a rosette sampler and Seabird SBE9 CTD equipped with a Sea Tech fluorometer. Surface samples (3 m) were either taken with this rosette sampler or by use of a Sears well pump connected to a 2 cm diameter hose. Two to three depths were sampled at each station except for Stn 10, where 5 depths were sampled in the euphotic zone and 3 in the deep aphotic zone. *In situ* fluorescence maxima were sampled at Stns 8 (85 m), 9 (118 m) & 10 (68 m). Salinity and temperature measurements were made with the CTD except for deep (>1000 m) samples, which were measured at the surface. Processing of samples was initiated immediately upon retrieval.

Viral direct counts. Microbial populations in water samples (5 to 100 l) were concentrated to 30 to 70 ml by vortex flow filtration using a Benchmark Rotary Biofiltration Device equipped with a 100 kDa, 400 cm^2 filter, and run in the recirculating mode (Paul et al. 1991, Jiang et al. 1992). Samples from 1000 and 2500 m at Stn 10 were concentrated to 750 ml using an Amicon DC-10 filtration device equipped with a spiral wound, 30 kDa filter. The 750 ml was further concentrated to 30 to 50 ml by use of a Benchmark VFF device as described above. The concentrated samples were fixed with 2% glutaraldehyde and stored at 4°C until counting by TEM as previously described (Paul et al. 1991).

Bacterial direct counts. Samples were fixed with 1.85% filtered formalin (final concentration) until counted. Subsamples were stained with filtered 10^{-5} M 4',6'diamidino-2-phenylindole (DAPI) as previously described (Paul 1982).

Autotrophic cell counts. Samples were filtered onto 0.2 µm Nuclepore filters that had been counterstained in 0.2% Irgalen black. The filters were placed cell-side up onto glass microscope slides and a drop of glycerol was added to the filter surface followed by a #1½ coverslip (Vernet et al. 1990). The slides were stored

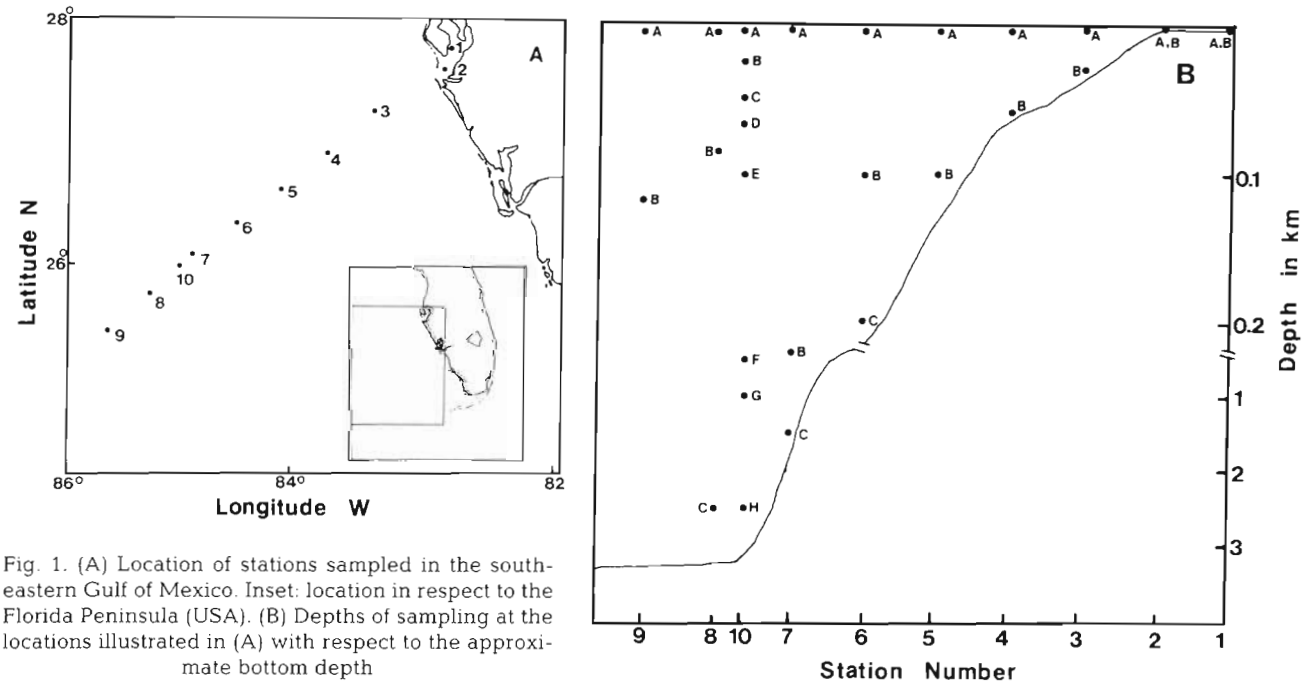

Fig. 1. (A) Location of stations sampled in the southeastern Gulf of Mexico. Inset: location in respect to the Florida Peninsula (USA). (B) Depths of sampling at the locations illustrated in (A) with respect to the approximate bottom depth

at −20 °C in the dark until counted. Counting was performed with an Olympus BH-2 epifluorescence microscope using blue excitation (ca 450 nm). Counts for both orange-fluorescing cells (presumably phycoerythrin-containing cyanobacteria) and red-fluorescing cells were made.

Chlorophyll *a*. Samples for chlorophyll *a* were filtered onto Whatman GF/F filters and stored at −20 °C until analysis. Chlorophyll *a* was extracted with methanol and determined fluorometrically (Holm-Hansen & Riemann 1978).

Photosynthetic carbon fixation. ^{14}C-carbon fixation was only measured at Stn 10 and then only in samples taken from the euphotic zone (top 100 m). Samples were incubated in 500 ml acid-washed, autoclaved polycarbonate flasks. Samples were incubated in a deck-top incubator and the light intensity at each depth approximated by neutral density screening and blue acetate filters. Samples were amended with $NaH^{14}CO_3$ (1.92 GBq mmol^{-1}, Amersham Radiochemicals) at a final radioactivity of 0.5 µCi ml^{-1}. Duplicate subsamples were filtered at t = 0 and t = 2 h onto Millipore GS (0.2 µm) filters. The filters were added to scintillation vials containing 0.5 ml 0.5 N HCl, incubated overnight to remove unincorporated ^{14}C-bicarbonate, and the vials counted the next day in a TM Analytic Delta 300 liquid scintillation counter. Counting efficiency of standards treated similarly was 72.8 %. Net light fixation was determined by subtracting dark fixation values from samples treated similarly but incubated in the dark.

Particulate and dissolved DNA. Particulate DNA was measured by the method of Paul & Myers (1982). Samples were filtered onto Millipore GS filters and stored frozen in 3 ml SSC (0.15 M NaCl, 0.015 M Na_3citrate, pH 7.0) until processing. DNA was extracted by sonication and determined fluorometrically using the Hoechst 33258 method (Paul & Myers 1982).

Dissolved DNA was determined on 0.2 µm filtrates of water samples as previously described (DeFlaun et al. 1986). Filtrates were ethanol precipitated and the precipitates harvested by centrifugation. At most stations, duplicate samples were processed and a third was spiked with an internal standard (calf thymus DNA) to determine efficiency of recovery. DNA was measured in the concentrated extracts fluorometrically using the Hoechst 33258 method (Paul & Myers 1982, DeFlaun et al. 1986). Subsamples of concentrated extracts were DNAase digested to correct for nonspecific fluorescence.

Data analysis. Multiple correlation and multiple regression analysis was performed on data sets using the 'Regress' software by Human Systems Dynamics. *t*-tests and analysis of variance testing were performed as described in Zar (1974).

RESULTS

Distribution of viral populations

Fig. 2A shows the distribution of viral abundance along a transect in the SE Gulf of Mexico. Viral direct counts were greatest in Tampa Bay (4.6×10^6 to 2.7×10^7 VLP ml^{-1}). All Gulf of Mexico surface waters, ranging from coastal oceanic environments to offshore oligotrophic waters, had similar viral direct counts (3.8 to 8.5×10^5 VLP ml^{-1}; mean of $5.5 \pm 1.3 \times 10^5$). Subsurface euphotic zone measurements (≤ 118 m) had similar (Stns 4, 5, 8 & 9) or significantly greater viral direct counts (Stn 3 and the 50 m depth sampling of Stn 10) than corresponding surface waters. The results of a profile at Stn 10 appear in Figs. 3 & 4. Temperature, salinity, dissolved oxygen, and *in situ* fluorescence data, as determined by CTD, for the top 100 m at Stn 10 appear in Fig. 3A, B. Maximum *in vivo* fluorescence occurred at 68 m, indicative of a deep chlorophyll *a* maximum (Fig. 3B). The viral direct count maximum occurred at 50 m, above the chlorophyll *a* maximum, and corresponding to the depth of the maximal particulate DNA, dissolved DNA, picocyanobacteria, and photosynthetic carbon fixation (Fig. 4A). The lowest viral direct counts were found in waters below the euphotic zone (> 100 m) and ranged from 1.4 to 4.7×10^4 ml^{-1} (average = $2.7 \pm 2.3 \times 10^4$, n = 7).

Heterotrophic bacterial populations

Bacterial direct counts were also greatest in the estuarine waters, ranging from 2.8×10^6 to 5.3×10^6 ml^{-1} (Fig. 2B). Coastal oceanic surface waters (Stns 3 & 4) averaged $5.7 \pm 2.9 \times 10^5$ cells ml^{-1}, which was significantly greater than the oligotrophic oceanic surface waters, Stns 5 to 10 (average = $2.5 \pm 0.8 \times 10^5$ cells ml^{-1}). In the euphotic zone, there was no consistent pattern of bacterial direct counts with depth. At Stns 2 & 5, subsurface euphotic samples had more bacterial direct counts, while at Stns 6 & 9, subsurface waters had less counts, and at Stns 8 & 10, subsurface waters (taken at the *in situ* fluorescence maximum) had similar bacterial counts as surface waters (Fig. 4B). Thus, light penetration seemed to have little effect in the vertical stratification of bacterial abundance, as compared to chlorophyll *a* or other photoautotrophic parameters. Deep subsurface (> 118 to 2500 m) water averaged $2.6 \pm 1.8 \times 10^4$ cells ml^{-1} (range: 0.5 to 5.5×10^4).

Photoautotrophic populations

Enumeration of the photoautotrophic population as autofluorescent cell counts indicated that these organisms are nearly 2 orders of magnitude less abundant

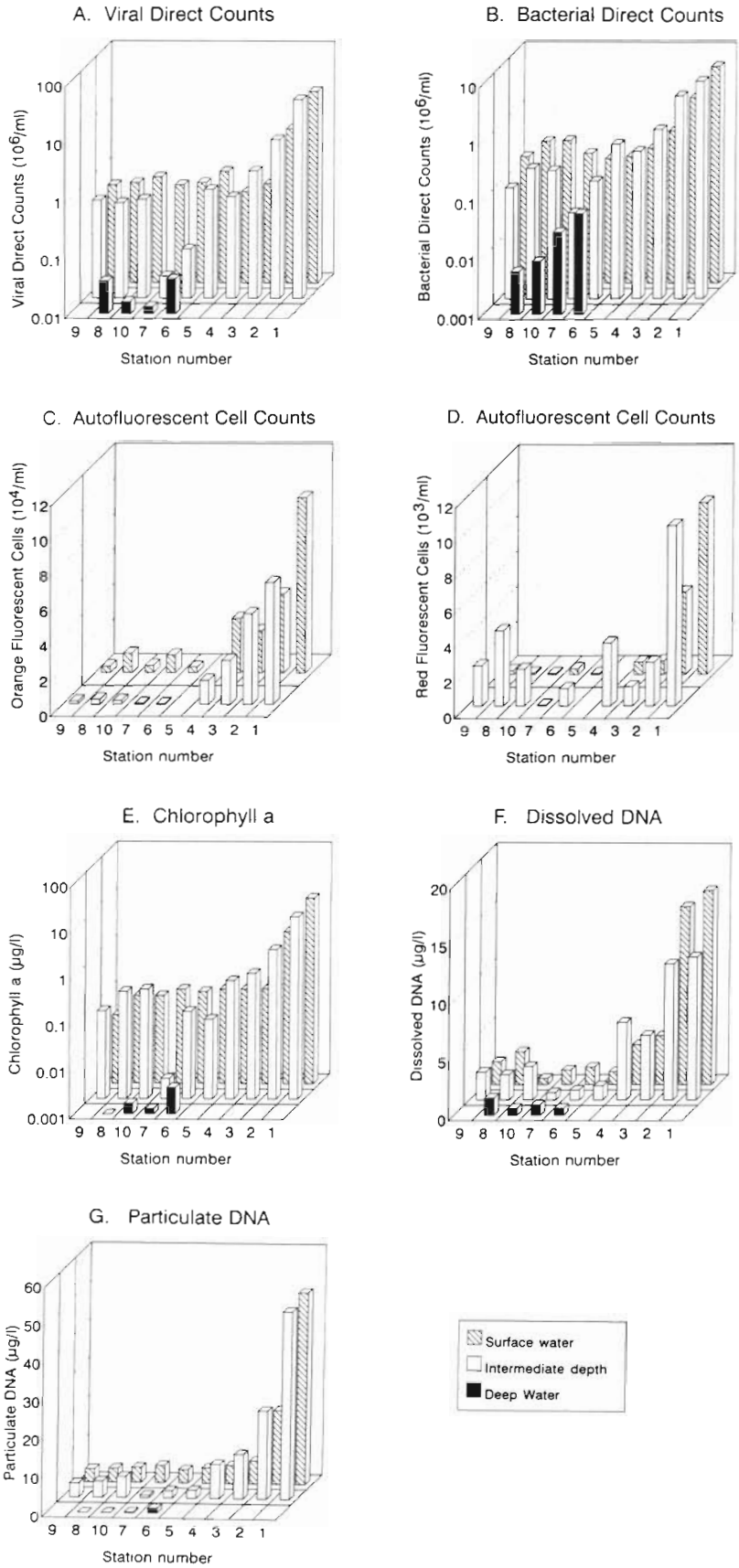

Fig. 2. Values for biological parameters measured at the stations indicated in Fig. 1. Surface waters are those indicated by the 'A' depth in Fig. 1B (usually 3 m or less); intermediate waters refer to all 'B' depths in Fig. 1B, except for Stn 10, which was the 'C' depth; and all deep samples refer to 'C' depths in Fig. 1B, except for Stn 10, which was depth 'H' (2500 m). (A) Viral direct counts; (B) bacterial direct counts; (C) orange-autofluorescing phytoplankton counts; (D) red-autofluorescing phytoplankton cells; (E) chlorophyll *a*; (F) dissolved DNA; (G) particulate DNA

than the total bacterial population as enumerated by DAPI direct cell counts (Fig. 2C, D). No autofluorescent cell counts were made for samples from depths greater than 100 m, so no deep water cell counts appear in Fig. 2C, D. Orange-fluorescing cells [phycoerythrin (PE)-containing *Synechococcus*-like organisms] ranged from 4.5 to 10 × 10^4 cells ml^{-1} in estuarine environments, 2.4 to 3.1 × 10^4 cells ml^{-1} in coastal oceanic waters, and 0.36 to 1.1 × 10^4 cells ml^{-1} in oligotrophic offshore waters (Fig. 2C). The subsurface euphotic zone had the same or significantly less *Synechococcus* cells. Red-fluorescing cells (Fig. 2D) were also enumerated, and were on the average an order of magnitude less abundant than the *Synechococcus*-like cells. Estuarine waters had 2.5 to 10.3 × 10^3 cells ml^{-1}, coastal oceanic waters had between 700 to 800 cells ml^{-1}, and offshore red cell counts ranged from 62 to 330 cells ml^{-1}. Unlike the picocyanobacteria, the red-fluorescing cells were most abundant in the deeper portions of the euphotic zone (Fig. 4B; $0.005 < p < 0.001$). These red-fluorescing cells probably contributed to the *in situ* fluorescence maximum found in the profile at Stn 10 (Fig. 4B) and other stations.

Chlorophyll *a* values were greater in subsurface waters (< 100 m) compared to surface waters for the euphotic zone (Figs. 2E & 4C). Chlorophyll *a* values ranged from 1.7 to 10.2 µg l^{-1} for Tampa Bay waters, from 0.03 to 0.112 (mean of 0.084 ± 0.03) µg l^{-1} for all other Gulf of

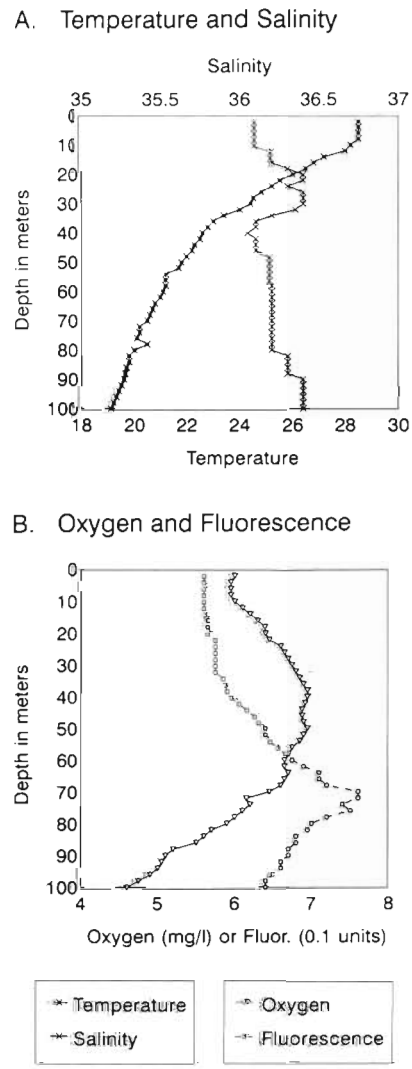

Fig. 3. (A) Salinity and temperature and (B) oxygen and *in situ* fluorescence data obtained with by CTD at Stn 10 in the top 100 m

Mexico surface waters, and from 0.079 to 0.52 µg l^{-1} for the subsurface euphotic zone (Fig. 2E). Deep aphotic zone (200 to 2500 m) chlorophyll *a* measurements ranged from 0.72 to 3.7 ng chlorophyll *a* l^{-1} (mean = 1.8 ± 1, n = 7). Although photosynthetic carbon fixation was not measured at all stations, there was a subsurface peak in carbon fixation at 50 m, corresponding to the peak in other microbial parameters measured (Fig. 4C).

Dissolved and particulate DNA

Dissolved (<0.2 µm) DNA concentrations ranged from 11.9 to 16.8 µg l^{-1} in Tampa Bay, 3.5 to 6.7 µg l^{-1} in coastal oceanic waters (Stns 3 & 4), and 0.53 to 2.8 µg l^{-1} for oligotrophic oceanic waters (Fig. 2F).

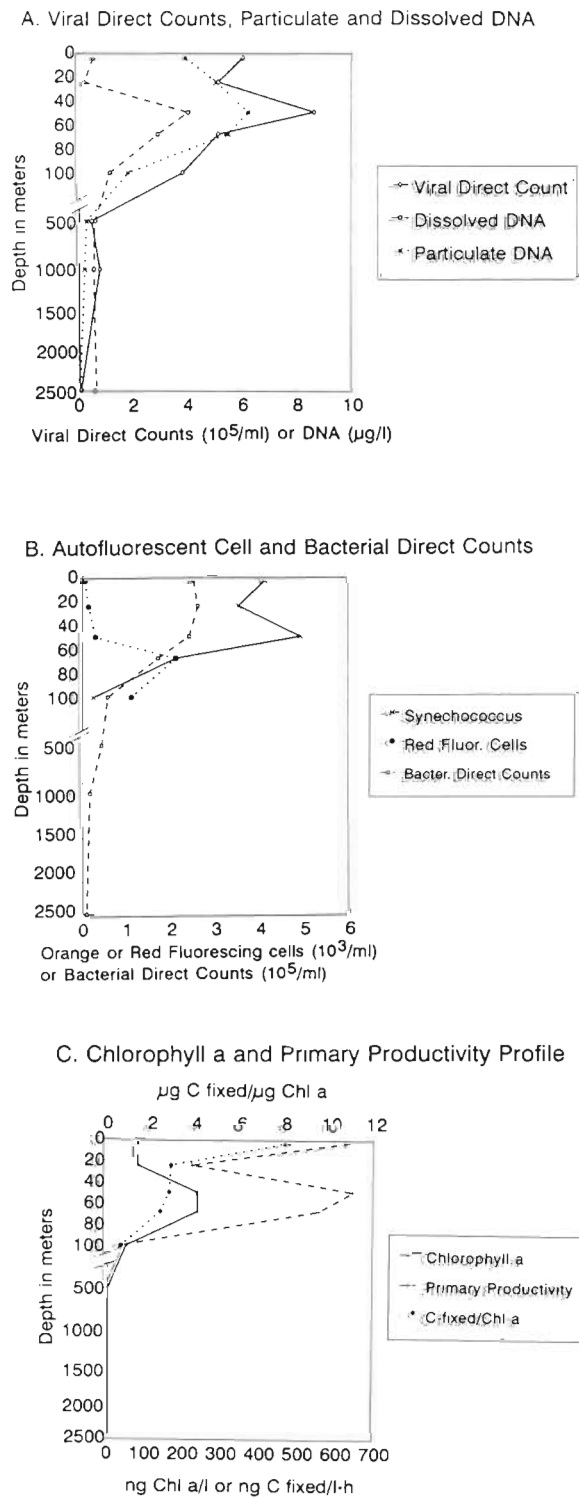

Fig. 4. Depth profile of biological parameters measured at Stn 10. (A) Viral direct counts (——), dissolved DNA (- - - - -) and particulate DNA (· · · · ·). (B) Orange-autofluorescing cells (——), red-fluorescing cells (· · · · ·) and bacterial direct counts (- - - - -). (C) Chlorophyll *a* (——), ^{14}C-fixation (- - - - -) and carbon fixed µg^{-1} chlorophyll *a* (· · · · ·)

These values are consistent with those reported for estuarine, coastal oceanic, and offshore environments (DeFlaun et al. 1987). Subsurface euphotic waters had statistically similar concentrations of dissolved DNA to those found in the surface waters. The only exception to this occurred at Stn 10 (Fig. 4A), where the 50 m depth had a significantly greater dissolved DNA concentration than the surface ($0.005 < p < 0.01$).

Particulate DNA (i.e. associated with particles >0.2 µm) concentrations were greatest in Tampa Bay, ranging from 19.2 to 50 µg l^{-1} (Fig. 2G). Coastal and offshore surface waters ranged from 4.8 to 6.0 and 3.4 to 4.5 µg l^{-1}, respectively (Fig. 2G). For surface euphotic zone waters, particulate DNA concentrations exceeded dissolved DNA values by about a factor of 2 (mean = 2.3 ± 1.4, n = 21). No consistent pattern of vertical stratification in particulate DNA was observed for waters in the euphotic zone. For example, subsurface particulate DNA concentrations exceeded surface water concentrations for Stns 3, 4 & 10 (Fig. 4A), while at Stns 5, 6 & 7 values were less. Particulate DNA values measured in waters sampled at the deep chlorophyll *a* maximum at Stns 8 & 9 had the same values for particulate DNA as the corresponding surface waters. In all subsurface aphotic zone samples (>100 m) particulate DNA values were far below the euphotic zone concentrations (mean = 0.38 ± 0.4 µg l^{-1}).

To assess what components of the microbial flora contributed most significantly to the particulate DNA, an estimate of bacterial DNA for each station was obtained by multiplying the bacterial direct counts by an estimated average DNA content (i.e. 5.66×10^{-15} g $cell^{-1}$; Paul et al 1985). The latter figure was originally obtained from cellular DNA values for the <1 µm fraction of oceanic bacterioplankton. Because the current data set includes the >1 µm bacterioplankton and attached bacterioplankton, values obtained may be an underestimate. The average bacterial DNA content for all stations was 51 ± 33% of the total particulate DNA (range: 11.7 to 138%). Thus, bacterioplankton contributed significantly to the particulate DNA signal in the water column. Estimation of phytoplankton DNA content based on conversion factor of Holm-Hansen (1969; 2.0 µg DNA μg^{-1} chlorophyll *a*) increased the amount of DNA accountable by both phytoplankton and bacterioplankton to 58.7% (range: 16.6 to 157%). Undoubtedly there are uncertainties in using such correction factors, particularly when applying common factors to both offshore and estuarine populations. Nonetheless, these data indicate that most of the particulate DNA can be accounted for by bacterioplankton, with a smaller proportion being contributed by phytoplankton. The unaccounted proportion may be caused by error in the conversion factor, error in the measurement of the parameters, and the presence of non-chlorophyll-containing microorganisms (i.e. ciliates, flagellates) which were not counted because of their poor preservation in samples fixed for bacterial direct counts. Other possibilities for more DNA accountable by bacterioplankton and phytoplankton may be dead (achlorophyllous) phytoplankton cells or adsorbed DNA.

A similar calculation was made for dissolved DNA to determine the proportion of dissolved DNA comprised of DNA in phage capsids. Assuming an average phage DNA content of 9×10^{-17} g DNA $phage^{-1}$ (Freifelder 1987), and that all VLPs observed contained dissolved DNA, about 4 ± 5.8% of the dissolved DNA could be attributed to viral genomes in intact viral particles. This may be an overestimate of the viral contribution to dissolved DNA, because the viral direct counts were made on unfiltered samples, and the dissolved DNA measurements were made on 0.2 µm filtrates of samples. Our previous studies (Paul et al. 1991) indicated that 0.2 µm prefiltration reduced viral direct counts on the average by two-thirds.

Multiple correlation analysis and multiple regression analysis were performed on all data and euphotic and offshore subsets of the data to determine which parameters might be correlated or co-dependent. Analysis of all data indicated high correlations for nearly all microbial parameters, probably because of onshore-offshore effects or surface to depth effects. Viral direct counts were most highly correlated with chlorophyll *a* (r = 0.97) followed by particulate DNA (r = 0.96) and bacterial direct counts (r = 0.94; Fig. 5A to C). Viral direct counts showed no correlation with depth or temperature. Dissolved DNA was most highly correlated with picocyanobacterial counts (r = 0.93) followed by bacterial direct counts (r = 0.91) and particulate DNA (r = 0.88). Particulate DNA was most highly correlated with bacterial direct counts (r = 0.98). Multiple regression analysis of this data set indicated that the variation in viral direct counts could be explained by the following 4 independent variables: chlorophyll *a*, bacterial direct counts, dissolved DNA, and red-fluorescing cell counts (multiple correlation coefficient = 0.99). The variation in dissolved DNA could be explained by only one independent variable, chlorophyll *a*.

Because the correlations noted could have been the result of grouping relatively elevated surface values with the low subsurface (>100 m depth) values, a subset of the data only including euphotic zone data was subjected to similar regression and correlation analysis. Virtually all correlation coefficients remained the same as in the complete data set. In terms of multiple regression analysis, viral direct counts in the euphotic

zone data subset required only chlorophyll *a* as the independent variable and a constant to explain variation (r = 0.98).

Because the high correlation between microbial parameters may have been caused by nearshore/offshore effects, a further subset of the data was devised by omitting the estuarine Stns 1 & 2 and the coastal Stn 3. Multiple correlation analysis of this data set indicated that viral direct counts no longer correlated significantly with any other parameter (all r ≤ 0.32). Dissolved DNA now correlated most significantly with chlorophyll *a* (r = 0.81) followed by particulate DNA (r = 0.77). The latter may have been influenced by the strong correlation between particulate DNA and chlorophyll *a* (r = 0.86). Stepwise multiple regression analysis indicated no significant regression when viral direct counts was the dependent variable. The variation in dissolved DNA (dependent variable) could be explained by that in chlorophyll *a* and picocyanobacterial direct counts (r = 0.89).

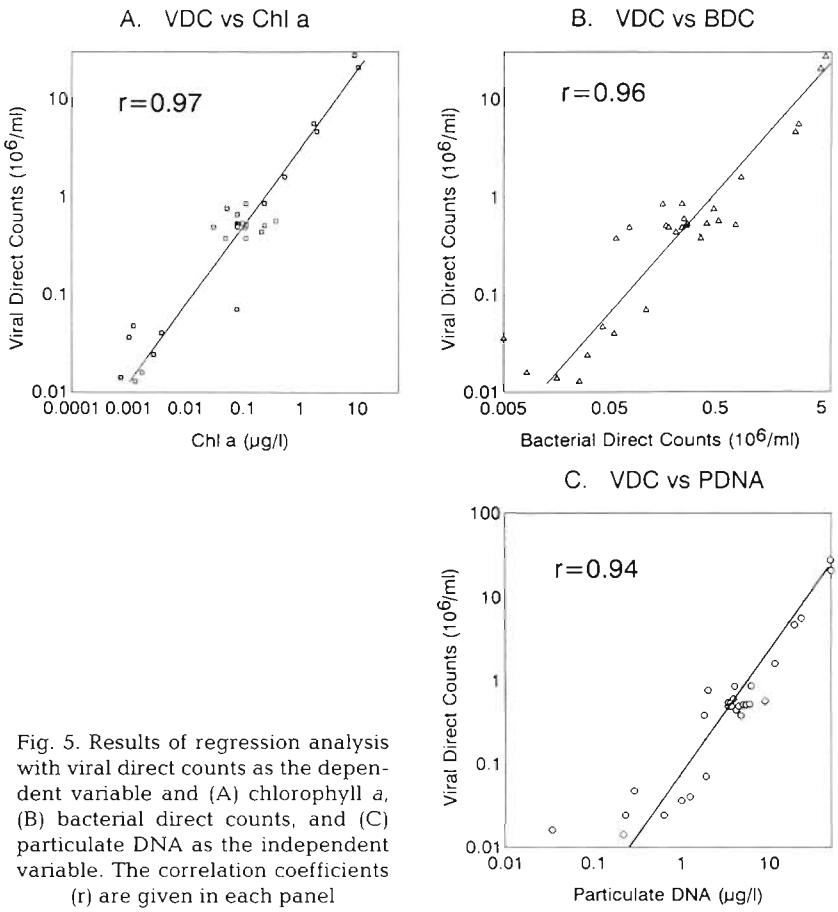

Fig. 5. Results of regression analysis with viral direct counts as the dependent variable and (A) chlorophyll *a*, (B) bacterial direct counts, and (C) particulate DNA as the independent variable. The correlation coefficients (r) are given in each panel

DISCUSSION

A major objective of the current study was to understand the distribution of viruses in oligotrophic oceanic environments. Our review of the literature indicated that prior to this work, only 10 offshore oceanic viral direct count measurements had been published in 5 papers, including a report for the Kurashio current (Hara et al. 1991), the Atlantic Ocean (Bergh 1989), the eastern and western Caribbean (Proctor & Fuhrman 1990), and the Gulf of Mexico (Paul et al. 1991, Suttle et al. 1991). These reports are usually single station, single sampling observations from widely differing environments, and not detailed studies of oceanic transects or profiles. Additionally, no attempt has been made to correlate viral abundance to other microbial parameters. The previously reported oceanic values for viral direct counts ranged from 3×10^3 VLP ml^{-1} (Sargasso Sea; Proctor & Fuhrman 1990) to over 4.6×10^8 in the Gulf Stream (Proctor & Fuhrman 1990), with most values in the 10^5 to 10^6 VLP ml^{-1} range. Our values for the oligotrophic surface waters of the southeastern Gulf of Mexico were fairly constant (3 to 8×10^5 ml^{-1}), with deep-sea environments containing ca 10^4 ml^{-1}. At certain stations, subsurface maxima in viral direct counts were found.

In general, viral direct counts correlated highly with chlorophyll *a* and multiple regression analysis indicated that chlorophyll *a* concentration was an important variable in explaining the distribution of viral direct counts. This could be interpreted that the majority of viruses observed were pathogens of marine phytoplankton (Sieburth et al. 1988, Suttle et al. 1990, 1991). Estimates of viral infection based upon TEM observation of intracellular mature phage particles indicated that picocyanobacteria and heterotrophic bacteria were equally infected (Proctor & Fuhrman 1990). The sheer excess of bacterioplankton cell abundance over autotrophic cell abundance (by 2 orders of magnitude) favors bacteriophage as the abundant viral type in these environments. Similarly, viral direct count subsurface maxima occurred at Stns 3 & 10, and subsurface maxima were observed at Stns 3, 4 & 10 for particulate DNA and 10 for dissolved DNA. A subsurface maxima for chlorophyll *a* and red-fluorescing cells was observed for all euphotic zone stations, while picocyanobacteria were always more abundant

in surface waters. Thus, the vertical abundance of viruses seems more related to the distribution of particulate (cellular) DNA than phytoplankton biomass. Particulate DNA has been previously shown to be the result of bacterial genomes in the water column (Paul & Carlson 1984, see below). The correlation noted between viral direct counts and chlorophyll *a* may demonstrate the dependence of viral infection on the presence of active bacterioplankton; bacterioplankton populations in the presence of elevated phytoplankton populations will undoubtedly be growing faster than bacterial populations in the absence of such populations.

The ratio of virus particles to bacteria (VBR; Hara et al. 1991, Womack et al. 1992) is apparently lower for offshore stations than estuarine stations. For example, Womack et al. (1992) reported values as high as 12.6 and 25.6 in the Chesapeake Bay at times when viral titers were highest and bacterial populations lowest. These incidences were thought to coincide with times of bacterial population control by viral lysis. Hara (1991) reported an average VBR of 6.37 in the coastal and oceanic waters of Japan, with lower values (3.7) for offshore waters. The average value measured in our study, 2.6 ± 2.0, is low compared to coastal values but perhaps reasonable for offshore oligotrophic waters. Coastal waters with large, rapidly growing bacterial populations would seem ideal for phage infection and growth compared to oligotrophic offshore environments, which are characterized by slow bacterial growth and long generation times.

In a vertical profile at the oligotrophic Stn 10 (surface chlorophyll $a = 0.078\ \mu g\ l^{-1}$), viral direct counts showed a subsurface maxima at 50 m, which corresponded with maxima for particulate and dissolved DNA, and primary productivity. This active microbial zone was situated above the *in situ* fluorescence maxima (68 m). Subsurface maxima in phytoplankton parameters have been well documented (Joint & Pomroy 1986, Li & Wood 1988, Furuya 1990). In general, the cyanobacterial maximum (i.e. PE-containing *Synechococcus*-like procaryotes) is usually situated above the chlorophyll *a* max (Iturriaga & Mitchell 1986, Glover et al. 1988, Iturriaga & Marra 1988) although sometimes it is coincident with the chlorophyll *a* max (Olson et al. 1990). In all samples examined in the euphotic zone, the deep euphotic samples had highest counts of red-fluorescing cells. No attempt was made to characterize or identify these organisms. Others (Chisholm et al. 1988, Olson et al. 1990, Veldhuis & Kraay 1990) have detected abundant levels of prochlorophytes at such depths, usually corresponding to or just below the deep chlorophyll maximum. Prochlorophytes in unconcentrated samples are usually not easily detected by epifluorescence microscopy, although they have been observed by microscopy in samples concentrated by flow cytometry (Chisholm et al. 1988). We do not know if the red-fluorescing cells observed in the present study were prochlorophytes, microeucaryotes, or non-PE-containing cyanobacteria.

The preponderance of bacterial biomass in oligotrophic oceanic environments has been well documented (Fuhrman et al. 1989). For example, Fuhrman et al. (1989) showed that heterotrophic bacteria contained 70 and 80% of the microbial C and N, respectively, in the Sargasso Sea water column, and that cyanobacteria, photosynthetic and heterotrophic nanoplankton contained only 7 and 17% of the total C and N. Our work here demonstrates that heterotrophic bacterioplankton account for at least 50% of the particulate DNA for all samples. This is in agreement with previous work for these waters (Paul et al. 1985) which indicated that bacterioplankton accounted for at least 49 ± 15% of the total particulate DNA. Estimation of phytoplankton DNA in the present study increased the amount of the water column DNA that could be accounted for to 58.7 ± 35.7%. Multiple regression analysis (stepwise solution) generated the following model to describe the distribution of particulate DNA in the water column:

$$\mathrm{PDNA} = 4.85\,\mathrm{BDC} + 2.32\,\mathrm{chl}\,a + 0.113\,t - 0.173$$
$$(n = 29,\ r = 0.99,\ F = 718)$$

where PDNA = particulate DNA ($\mu g\ l^{-1}$); BDC = bacterial direct counts ($10^9\ l^{-1}$); chl *a* = chlorophyll *a* ($\mu g\ l^{-1}$); and t = temperature (°C). A similar analysis was performed on the euphotic zone only subset of the data (all samples from depths ≤100 m) and yielded the following relationship:

$$\mathrm{PDNA} = 4.94\,\mathrm{BDC} + 2.31\,\mathrm{chl}\,a + 2.77$$
$$(n = 22,\ r = 0.99,\ F = 833)$$

This relationship is independent of temperature, presumably because of the somewhat homogeneity of temperature in the euphotic zone. In either model, the coefficients for the bacterial and chlorophyll terms are remarkably close to bacterial DNA contents (5.66 μg DNA 10^{-9} cells; Paul et al. 1985) and chlorophyll to DNA conversion factors (2 μg DNA μg^{-1} chlorophyll *a*; Holm-Hansen 1969). Thus, this model may have predictive value in determining DNA content in waters where only chlorophyll *a* and bacterial direct counts have been measured, 2 determinations usually measured in any water column microbial study.

In contrast, DNA encapsulated in viral particles could not account for a significant proportion of the dissolved DNA (mean = 4 ± 5.75%). These values are

in agreement with the few simultaneous measurements of viral direct counts, estimates of viral DNA content, and dissolved DNA previously reported (Paul et al. 1991). For example, estimation of the viral contribution to dissolved DNA for 2 estuarine stations, 1 freshwater lake, and 2 coastal environments indicated an average of 3.7 % of the dissolved DNA could be the result of DNA in viral particles. Not surprisingly, of all biological parameters measured in the present study, viral direct counts yielded the lowest correlation coefficients with dissolved DNA (0.74 to 0.76). However, others have found viral DNA to be a significant component of the dissolved DNA pool. Beebee (1991) reported that 78 to 85 % of the dissolved DNA in streams and lakes was macromolecular, behaved like DNA in viral particles, and was attributed to viral DNA. Maruyama et al. (1993) recently reported that >90 % of the DNA in the < 0.2 µm fraction of Tokyo Bay water was of high molecular weight and coated, not being susceptible to DNAase digestion. These workers concluded that this was viral DNA. Neither of these studies measured viral abundance. In the latter paper, estimates of viral abundance based on the dissolved DNA content ranged from 3 to 6×10^8 ml^{-1}, which is, to our knowledge, the highest report of viral abundance yet published.

Viral infection and lysis could be a mechanism of production of dissolved DNA, however. Dissolved DNA was found to correlate with a variety of parameters, including picocyanobacterial counts, bacterial direct counts, and particulate DNA. In offshore environments, dissolved DNA was found to correlate with chlorophyll *a* and particulate DNA. Not surprisingly, the distribution of source organisms (presumably bacterioplankton and phytoplankton, collectively represented as particulate DNA) determines the abundance of dissolved DNA. Previous studies have found significant correlations between dissolved DNA, bacterial direct counts, bacterial production, and particulate DNA (DeFlaun et al. 1987).

In summary, the distribution of viral particles seems to follow the distribution of other microbial parameters, particularly particulate DNA. The majority of the particulate DNA could be accounted for by bacterioplankton DNA, whereas DNA in viral particles was a small proportion of the dissolved DNA. Viruses may be instrumental in the production of dissolved DNA by involvement in microbial lysis, yet viruses are apparently a minor component of the dissolved DNA.

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