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The abundance, distribution, and correlation of viruses, phytoplankton, and prokaryotes along a Pacific Ocean transect

Abstract—Concentrations of virus-like particles (VLP), prokaryote-like particles (PLP), chlorophyll *a,* and zeaxanthin were determined at 9–12 depths (0–250 m) for each of 13 stations along a 3,800-km transect from the coastal waters of Monterey Bay, California (36.62°N, 122.25°W) to the open ocean Hawaii Ocean Time-Series (HOTS) station near Hawaii (22.69°N, 158.12°W). We collected VLP and PLP, the latter of which included heterotrophic bacteria and cyanobacteria, in glutaraldehyde-fixed samples on 0.02-μm Anodisc filters. The samples were stained with the nucleic acid dye Yo-Pro-1 and quantified by epifluorescence microscopy. Measurements of Chl *a* and zeaxanthin were used as indicators of total phytoplankton and cyanobacteria biomass, respectively. With the exception of the most coastal station, depth-integrated VLP and PLP abundance was similar at all stations along the transect; all stations showed a decrease of VLP and PLP with depth. Standard multiple regression analysis showed that logarithmically transformed PLP abundance ($sr^2 = 0.64$) was the only variable that contributed significantly to the prediction of (log of) VLP. These results confirm and extend results elsewhere that indirectly suggested that prokaryotes are the major host organisms for viruses throughout the ocean. Simple linear regression suggests that VLP abundance is ~15 times that of PLP across the central eastern Pacific transect surveyed in this study (VLP $ml^{-1} = 14.7 \times$ PLP $ml^{-1} + 9.6 \times 10^5$; $r^2 = 0.80$, $n = 90$).

Marine virus distribution and ecology has been investigated with culturing, optical, and molecular techniques. These studies indicated that viruses are consistently the most abundant biological entities in the ocean and that they affect autotrophic and heterotrophic planktonic communities (Wommack and Colwell 2000). Because viruses affect all major groups of the microbial food web, they play an important role in the biogeochemical cycling of carbon and nitrogen in the ocean (Fuhrman 1999; Wilhelm and Suttle 1999). Determining the distribution and abundance of viruses and the variation of these parameters in different oceanographic regions is a critical step toward understanding this role.

Marine viruses are most abundant in productive, nearshore waters and least abundant in deeper, offshore waters. A transect from Tampa Bay, Florida, to an oceanic station in the Gulf of Mexico found nearshore surface viral abundance to be two orders of magnitude greater than offshore surface viral abundance (Boehme et al. 1993). In depth profiles of viral abundance from the Bering and Chukchi Sea (Steward et al. 1996), Northern Pacific (Hara et al. 1996), Gulf of Mexico (Boehme et al. 1993), and central Pacific Ocean and coastal Southern California (Cochlan et al. 1993; Noble and Fuhrman 1998), viral abundance was greatest at the surface and declined with depth. In a majority of marine coastal and oceanic studies, viral abundance was highly correlated with bacterial abundance (Cochlan et al. 1993; Weinbauer et al. 1993; Hara et al. 1996; Steward et al. 1996), which indirectly suggests that bacteria are the major host group of marine viruses in the ocean. However, the statistical comparison of data from environments of widely differing trophic status is compromised by the nonuniform methods of viral enumeration that have evolved over the last decade.

In this study, we report the abundance of viruses from 13 stations sampled from Monterey, California, to Honolulu, Hawaii. We relate viral abundance to the distribution of prokaryotes, cyanobacteria, and phytoplankton. The purpose of the study was to provide a comprehensive data set, assayed with uniform methods, that covers a wide range of environments transitioning from coastal to open-ocean oligotrophic sites.

Sample collection—Samples were collected during a cruise aboard the RV *Moana Wave* from 2 to 28 September 1998. Thirteen stations were sampled (Fig. 1), and 9–12 depths were sampled per station. Water samples were collected with 10-liter polyvinylchloride Niskin bottles (General Oceanics) mounted on a 24-position rosette equipped with a Seabird SBE9 conductivity-temperature-depth (CTD) profiler that was used to determine salinity and temperature. Sample depths were chosen on the basis of in vivo fluorescence profiles acquired from a Sea Tech in situ fluorometer mounted to the CTD package.

Hydrographic data—Salinity and temperature were processed into 1m average bins by use of standard Seabird software (Seasoft, version 4.236) and plotted as T-S diagrams to identify changes in hydrographic water mass conditions along the coastal to oceanic transect; the T-S signatures were used to identify neritic, transitional, and oceanic stations (Fig. 2). Depth-integrated viral, prokaryote, chlorophyll *a,* and zeaxanthin crops were estimated by use of trapezoid rule (Hornbeck 1975), integrated to 175 m for all stations.

Enumeration of viruses and prokaryotes—Virus-like particles (VLP) and prokaryote-like particles (PLP) were enumerated by epifluorescence microscopy after being stained with Yo-Pro-1 [4-[3-methyl-2,3-dihydro-(benzo-1,3-oxazole)-2-methylmethyledene]-1-(3′-trimethylammoniumpropyl)-quinolium diiodide] (Molecular Probes) according to

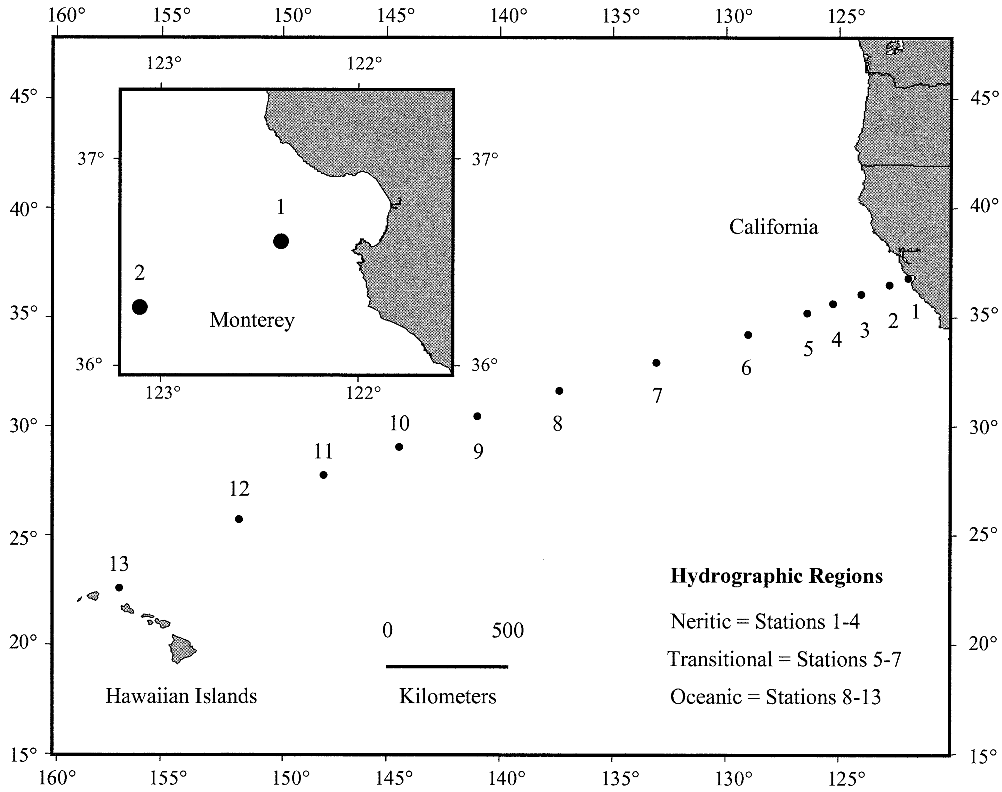


Fig. 1. Station locations. Samples were collected in September 1998.

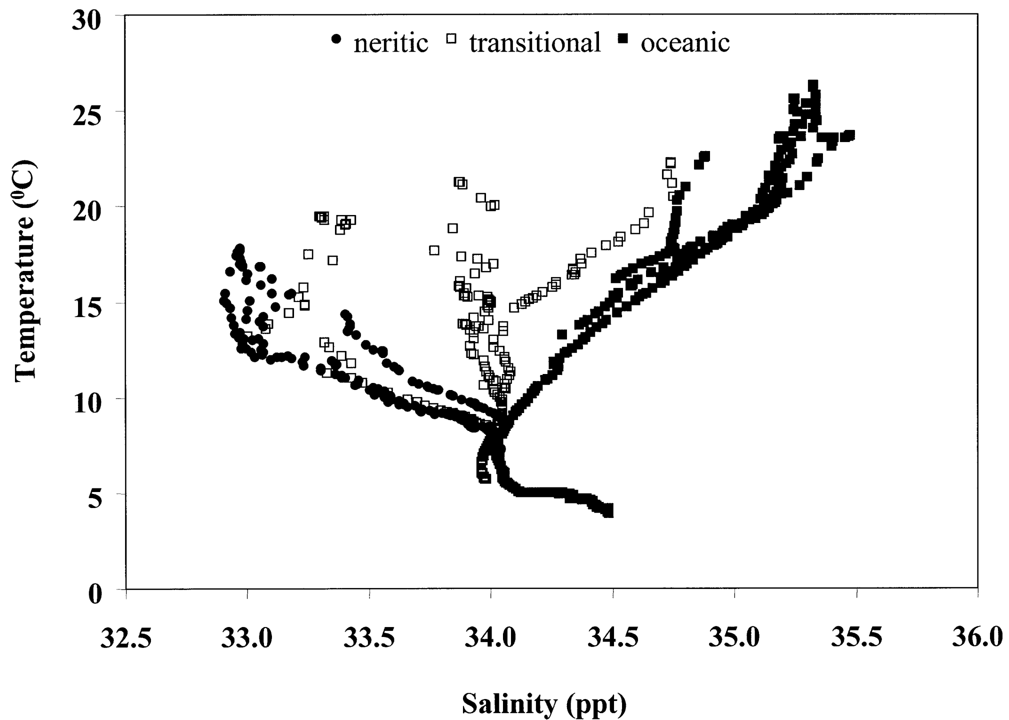


Fig. 2. T-S diagram of all stations sampled along the transect. Regions were determined on the basis of the apparent trends in these data.

the method of Hennes and Suttle (1995) as modified by Xenopolous and Bird (1997). Once collected, the water for VLP and PLP counts was transferred into sterilized 30-ml polypropylene bottles and immediately fixed with 0.02 μm–filtered gluteraldehyde at a final concentration of 2%. While at sea, each fixed sample was diluted threefold with 0.02 μm–filtered milli-Q water and filtered (400 mmHg) on to a 0.02-μm pore size AL_2O_3 Anodisc membrane filter (Whatman). We quantitatively filtered 1 ml of sample in coastal waters and 2–4 ml of sample in more oligotrophic waters. Filtered Milli-Q water, without the addition of sample water, averaged 6.2×10^3 VLP ml^{-1} and 42 prokaryotes ml^{-1}; seawater VLP and PLP counts were at least three orders of magnitude greater than the blank values above for all samples processed.

Yo-Pro staining was achieved as follows. The stock solution of the cyanine-based dye (delivered from the manufacturer as 1 mM Yo-Pro-1 in aqueous dimethyl sufoxide) was diluted to a final concentration of 50 μM in 0.02-μm filtered Milli-Q water. Prior to filtering samples, an 80-μl drop of the stain was placed in the bottom of each pyrex Petri dish used for each sample. Filters were placed sample side up on the drop of Yo-Pro-1. The Petri dishes were enclosed in an opaque container and irradiated on a turntable in a microwave for 3.5 min at the lowest setting (600 W, Sanyo), then allowed to cool for 10 min. Excess stain was removed by placing filters face up on the filter tower and exposing them to vacuum (400 mmHg) for 10 s. Subsequently, the Anodisc filters were mounted on glass slides with mounting medium composed of 0.5% ascorbic acid in 50% glycerol/50% phosphate-buffered saline (0.05 M Na_2HPO_4, 0.85% NaCl [pH 7.5]) and a coverslip (Noble and Fuhrman 1998). Slides were stored at -20°C until processed.

During counting, we selected 5–20 fields on each filter randomly and a total of >200 viruses and >100 prokaryotes were counted at 1000× on an Olympus BH2 epifluorescence microscope with an acridine orange filter set (excitation <490 nm, dichroic filter 500 nm, and barrier filter >515 nm). VLP particles emitted a bright green florescence and were distinguished from PLP because of their relatively small size and dimmer fluorescence. When replicate samples from Monterey Bay were used, the coefficient of variation of the Yo-Pro method was 11% ($n = 4$).

Pigments—Chl *a* was determined fluorometrically on board ship according to the protocol of Welschmeyer (1994). Zeaxanthin was used in this study as a chemotaxonomic marker for cyanobacteria biomass under the assumption that the zeaxanthin content per cell remains constant under a wide range of photoadaptive states, as shown by Kana et al. (1988). Zeaxanthin was determined with high-performance liquid chromatography (HPLC) according to methods similar to those described by Strom and Welschmeyer (1991). We quantitatively filtered 1–5 liters of sample onto a 25-mm GF/F filter (Whatman) and immediately froze the sample in liquid nitrogen, where it was held until processing in the lab. Sample filters were extracted in 1.2 ml of 90% acetone in microcentrifuge tubes for at least 24 h in the dark at -20°C. The samples were vortexed, compressed to the bottom of the centrifuge tube with a stainless steel spatula, and centrifuged prior to HPLC analysis. A Gilson 231 autosampler, cooled to 12°C, was used to autoinject samples on to a Varian model 5060 ternary HPLC. The autosampler was programmed to dilute and mix each sample just prior to injection, yielding a quantitative dilution of two parts sample and one part milli-Q water. The aqueous dilution reduced the sample solvent strength, which prevented band spreading in the early eluting peaks. A Microsorb 25-cm C-8 column was used primarily for its ability to separate divinyl Chl *a* from monovinyl Chl *a*; however, lutein coelutes with zeaxanthin on this C-8 column/solvent system. We therefore independently analyzed subsets of samples for the presence of lutein by use of the HPLC method for lutein/zeaxanthin described in Wright et al. (1991); lutein was absent in the samples analyzed here. The linear ternary gradient delivery system was set at a flow rate of 1.5 ml min^{-1}. The three solvents were A, methanol: aqueous 0.5 M ammonium acetate, 85 : 15, vol : vol; B, acetonitrile; and C, acetone. The gradient delivery was as follows: 0 min, 100%A; 8 min, 100%B; 13 min, 70%B and 30%C; 17 min, 20%B and 80%C; 19 min, 20%B and 80%C; and 20 min, return to 100%A. Eluting peaks were monitored with a Linear model 200 absorbance detector set at a fixed wavelength of 440 nm. Zeaxanthin standards were purified from laboratory *Synechococcus* cultures according to methods described in Strom and Welschmeyer (1991) to determine a zeaxanthin retention time and to calibrate zeaxanthin peak areas. An absorption coefficient of 234 liters g^{-1} cm^{-1} (Aasen and Liaaen-Jensen 1966) was used to determine the concentration of zeaxanthin standards; HPLC peak areas were quantified by use of Maxima 820 integrating software (Waters). During the cruise, replicate samples from Monterey Bay yielded a coefficient of variation of 1% for the HPLC method described herein ($n = 3$).

Statistical analysis—We performed a standard multiple regression using VLP abundance as the dependent variable and PLP abundance, Chl *a* concentrations, and zeaxanthin concentrations as the independent variables. Analysis was performed by use of SPSS REGRESSION and SPSS FREQUENCIES software for evaluation of assumptions. The analysis was based on the multiple-regression procedure described in Tabachnick and Fidell (2001).

Hydrographic data—Three distinct hydrographic regions were identified on the basis of a comparison of T-S plots for each station (Fig. 2). The neritic region included four stations and was characterized by relatively cooler, less salty water (Sta. 1–4), Sta. 5–7 were considered transitional, whereas the oceanic region included six stations and was characterized by relatively warmer, saltier water (Sta. 8–13).

Distribution and abundance—VLP abundance ranged from 1.5×10^6 ml^{-1} (200 m, Sta. 11) to 15.0×10^6 ml^{-1} (15 m, Sta. 2). PLP counts also ranged over an order of magnitude from 0.08×10^6 ml^{-1} (Sta. 12, 200 m) to 0.9×10^6 ml^{-1} (Sta. 1, 16 m). Surface VLP and PLP abundance tended to be higher in the neritic region than in the oceanic region and were highest in surface waters and progressively decreased with depth (Fig. 3A–F).

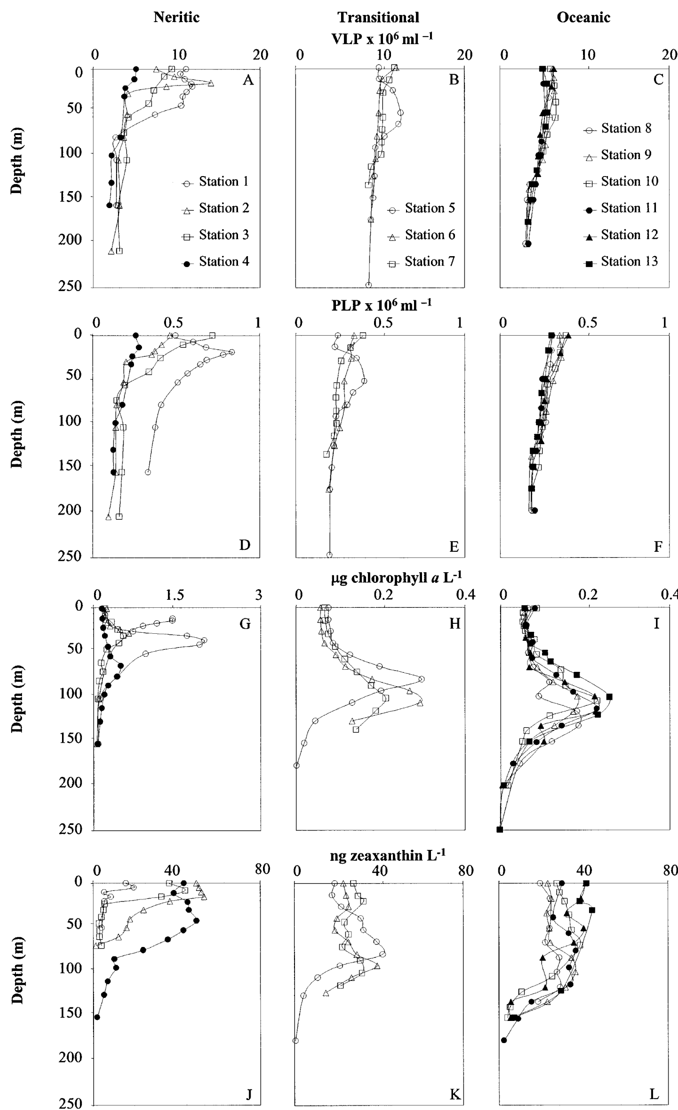


Fig. 3. Depth profiles of VLP, PLP, Chl *a,* and zeaxanthin for each station grouped by neritic, transitional, and oceanic region.

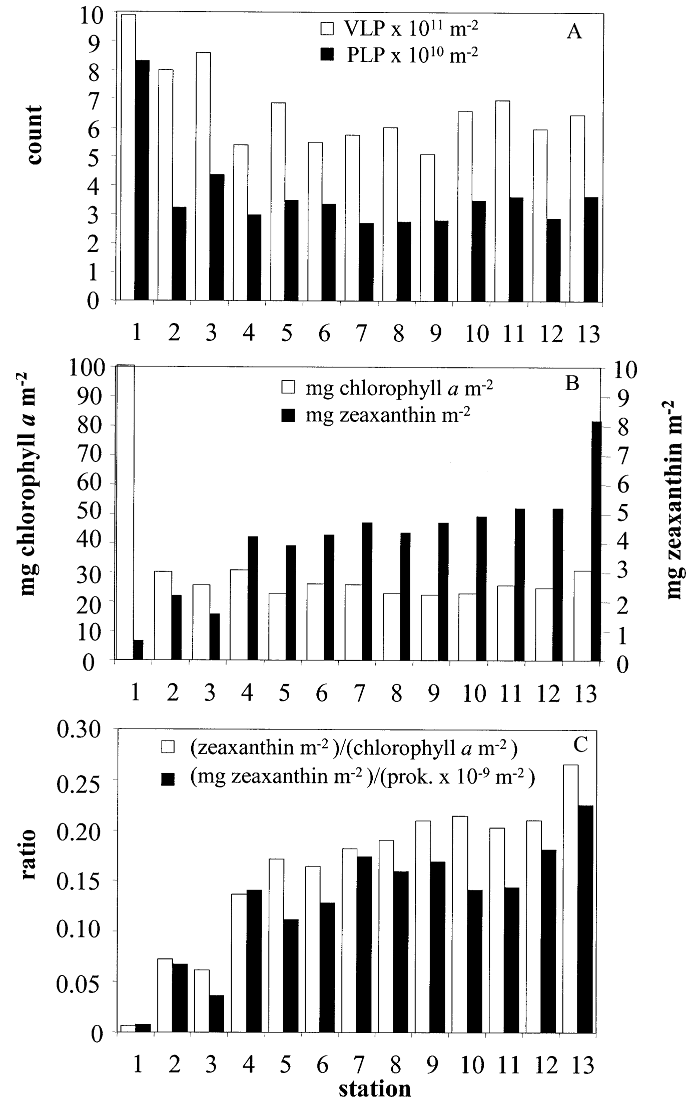


Fig. 4. VLP, PLP, Chl *a,* and zeaxanthin integrated abundance for each station. Variables were integrated to 175 m. (C) Ratios of zeaxanthin to Chl *a* and zeaxanthin to PLP for each station.

Neritic Chl *a* depth profiles showed distinct subsurface maxima from 27 (Sta. 2) to 63 m (Sta. 4), whereas transitional and oceanic subsurface Chl *a* maxima (SCM) ranged from 79 (Sta. 5) to 132 m (Sta. 8) (Fig. 3G–I). The depth of the SCM increased with distance from shore. VLP concentrations generally decreased with depth and showed no vertical relation to the subsurface chlorophyll maxima observed throughout the cruise (Fig. 3). Zeaxanthin concentration was higher at the surface and declined with depth (Fig. 3J–L). However, in contrast to Chl *a,* the transition from onshore to offshore was characterized by a progressive increase in surface zeaxanthin concentration, which indicates an increase in cyanobacterial abundance offshore.

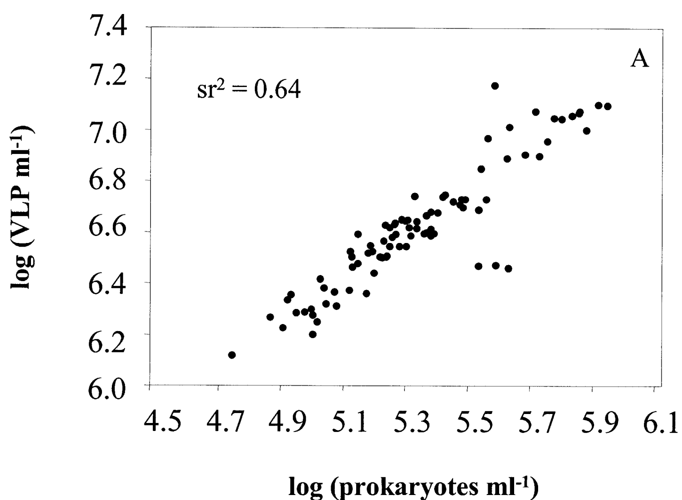
With the exception of Sta. 1, depth-integrated VLP, PLP, and Chl *a* standing crops remained relatively constant throughout the transect (Fig. 4A). Integrated VLP crops ranged from 5.1×10^{11} m^{-2} (Sta. 9) to 9.9×10^{11} m^{-2} (Sta. 1). Integrated PLP abundance ranged from 2.7×10^{10} m^{-2} (Sta. 7) to 8.3×10^{10} m^{-2} (Sta. 1) and integrated Chl *a* was lowest at Sta. 9 (22.5 mg m^{-2}) and highest at Sta. 1 (100 mg m^{-2}). Although stations in the neritic region were hydrographically similar (Fig. 2), Sta. 1 surface Chl *a* concentrations were typical of those observed in Monterey Bay water during the summer (Pennington and Chavez 2000), whereas Chl *a* concentrations from Sta. 2–4 were typical of California coastal water (Hayward and Venrick 1998). Total integrated zeaxanthin tended to increase with distance from shore (Fig. 4B), with values ranging from 0.7 (Sta. 1) to 8.2 mg m^{-2} (Sta. 13). As a result, the integrated zeaxanthin to Chl *a* and integrated zeaxanthin to PLP abundance ratios progressively increased with distance from the coast, reaching maximum values at Sta. 13 (Fig. 4C).

Correlation—VLP abundance, PLP abundance, Chl *a* concentration, and zeaxanthin concentration were log transformed to reduce skewness, reduce the number of outliers,

Table 1. Standard multiple regression of the independent variables prokaryote-like particle abundance (PLP), Chl *a* concentration, and zeaxanthin concentration (zeax) on virus-like particle abundance (VLP).

Variables	log VLP (DV)	log PLP	log Chl *a*	log zeax	*B* (unique)	β	sr²
log PLP	0.94				0.860*	0.92	0.64
log Chl *a*	0.34	0.27			0.060	0.10	0.01
log zeax	0.40	1.45	0.12		−0.011	−0.03	0.00
Mean	6.61	5.33	−0.82	1.38		adjusted	$R^2 = 0.88$†
Standard							$R^2 = 0.88$
deviation	0.18	0.19	0.29	0.39			$R^2 = 0.94$†

* $P < 0.01$.
† Shared variability = 0.24.



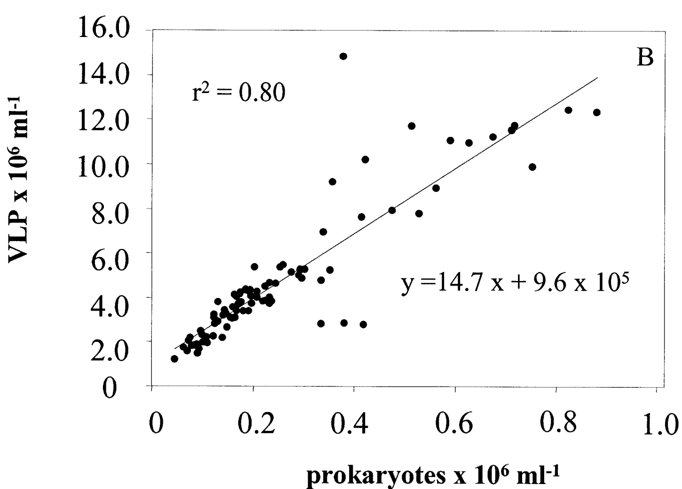


Fig. 5. Scatter plots of log-transformed and nontranformed VLP vs. PLP. (A) Squared semipartial correlation (sr²) of log-transformed VLP vs. PLP. (B) Trend line and equation for the simple regression of nontransformed VLP vs. PLP.

and improve normality, linearity, and homoscedasticity of residuals (Tabachnick and Fidell 2001). After transformation, no cases had missing data, and no outliers or suppressor variables were found.

Table 1 shows the correlations among the variables, the unstandardized regression coefficients (*B*), the standardized regression coefficients (β), the squared semipartial correlations (sr²), R^2, and adjusted R^2. Regression (*R*) was significantly different from zero: $F_{3,71} = 181.04$ and $P < .01$. Only the regression coefficient for (log of) PLP abundance and its relation to VLP differed significantly from zero. The 95% confidence limits for (log of) PLP abundance were 0.773–0.946. Logarithmically transformed PLP abundance (sr² = 0.64) was the only variable that contributed significantly to the prediction of (log of) VLP. When combined, the three independent variables contributed an additional 0.24 in shared variability; 88% of the variability in (log of) VLP abundance was predicted by the three logarithmically transformed independent variables. The correlation between (log of) PLP abundance and (log of) zeaxanthin concentration was significantly different from zero: $F_{3,71} = 5.99$ and $P < .01$. However, (log of) zeaxanthin concentration did not contribute significantly to the regression.

A strong correlation between virus and prokaryote abundance has been reported elsewhere. For example, Steward et al. (1996) reported a strong correlation ($r = 0.83$, $n = 43$) between viruses and bacteria in the Bering and Chukchi Seas. The significant contribution of PLP abundance to the prediction of VLP abundance, and not Chl *a* concentration (Table 1), suggests that the majority of free marine viruses may have prokaryotic hosts. This conclusion is also supported by the results from direct transmission electron microscope analysis of the virioplankton that showed that most marine viruses possess a phage-like morphology (Wichels et al. 1998) and capsid diameter (Steward et al. 2000). Furthermore, marine viruses are nonmotile obligate intracellular pathogens and must rely on diffusive transport to reach a new host; therefore, the frequency of contact with the appropriate host is a critical factor in viral reproduction. On the basis of an estimation of the frequency of contact necessary to support observed viral abundance, Murray and Jackson (1992) concluded that the numerically dominant bacterioplankton are most likely the major host group of viruses in the ocean. Scatter plots of log-transformed and nontransformed VLP and PLP relations are given in Fig 5.

The nontransformed linear regression for our eastern Pacific transect suggests that VLP abundance is ~15 times that of PLP for all stations sampled (VLP $ml^{-1} = 14.7 \times$ PLP $ml^{-1} + 9.6 \times 10^5$; $r^2 = 0.80$, $n = 90$).

The lack of a significant correlation between VLP abundance and zeaxanthin concentration (Table 1) suggests that cyanophages do not influence the variability in the total abundance of viruses in the water column. However, we were unable to distinguish between small cyanobacteria and other prokaryotes, and the PLP abundance and zeaxanthin concentration correlation value ($r = .45$) was significantly different from zero ($F_{3,71} = 5.99$, $P < .01$). Therefore, small cyanobacteria may have contributed to PLP abundance.

The lack of a significant correlation between VLP abundance and Chl *a* concentration (Table 1) suggests that phytoplankton do not influence the variability in the abundance of free viruses. Cochlan et al. (1993), Weinbauer et al. (1993), and Hara et al. (1996) reported similar results. However, the variation in the carbon : Chl *a* ratio from surface to depth and between the neritic and oceanic region may limit the use of Chl *a* as a phytoplankton biomass marker, particularly in oligotrophic waters.

In this study, we present an extensive survey of viral abundance across a section of the Pacific Ocean. In both coastal and oceanic regions, viral abundance was greatest at the surface and declined with depth. The results of this study support suggestions elsewhere that the primary hosts of marine viruses are prokaryotes. Furthermore, it appears that cyanophages and algal viruses do not significantly contribute to total viral abundance.

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