

Fundamental changes in light scattering associated with infection of marine bacteria by bacteriophage

Abstract—Bacteria and phytoplankton are key determinants of the ocean's inherent optical properties. Despite their high abundance, marine viruses have generally been thought to play a minor role in ocean optics because of their small scattering cross-sections. Nevertheless, the role of specific viral infection on the optical properties of bacteria and phytoplankton has remained unknown (i.e., as viruses disrupt micron-sized host cells to produce submicron cell debris). Here, we used laboratory and mesocosm cultures of marine bacteria for virus infection experiments in which growth conditions and host-virus specificity were controlled. We report that the chief optical impact of viruses is associated with infection and lysis of their hosts. We quantitatively describe, for the first time, two optical changes associated with infection and lysis of marine bacteria by bacteriophage: (1) rapid, strong shifts in the magnitude and shape of the optical volume scattering function and (2) rapid production of colored dissolved organic material. Qualitatively, these changes result in nearly complete clearing of turbid host bacterial suspensions. Although some optical differences would be expected between infection of bacteria in laboratory cultures versus field populations (mainly because of differences in cell size), these results are applicable to the field, especially for dense host suspensions such as in blooms. Even in nonbloom situations, as long as the host bacteria contribute a significant amount of the total particle backscattering, we expect that virus-induced backscattering changes would be detectable by use of satellite or aircraft remote-sensing techniques.

Viruses are the most abundant biological particles in the sea (Lewin 1960; Proctor et al. 1988; Boersheim et al. 1990; Hara et al. 1991; Paul et al. 1991), with typical concentrations ranging from 10^9 to 10^{10} L^{-1} (Bergh et al. 1989; Bratbak et al. 1993, 1996; Fuhrman 1999). Viruses likely play a significant role in the ecological control of planktonic microorganisms (Suttle and Chan 1993). Despite their high abundance, marine viruses generally have been thought to play a minor role in ocean optics because of their small scattering cross-sections (Stramski and Kiefer 1991; Balch et al. 2000). Within the field of virology, however, it has long been known that bacterial viruses (bacteriophage) can rapidly lyse their hosts and, in some cases, clear turbid host cultures (Stent 1963; Lamanna et al. 1973). It would be expected, then, that the infection process would cause detectable changes in host shape, size, abundance, or refractive index, which would translate into changes in the inherent optical properties (IOPs; e.g., absorption, scattering, and attenuation).

The IOPs of seawater affect light penetration within, and reflection from, the sea. Thus, the source of the IOPs is of great interest in optical oceanography. Phytoplankton, bacteria, flagellates, and ciliates account for only ~50% of the observed particle scattering in the sea, with an unknown source for the remaining scattering. Much less is known

about backscattering (b_b) in the sea, with only ~10% of oceanic b_b attributable to the above particle classes (Morel and Ahn 1991; Stramski and Kiefer 1991). Clearly, other types of particles are responsible for the observed ocean light scattering.

In marine optics, “scale closure”—defined as the agreement between bulk IOPs versus the sum of the IOPs of each optically significant component (*see* p. 141 of Mobley 1994)—is often not achieved. It assumes that particles have known IOPs and that changes in bulk IOPs are associated with changes in particle concentrations. Scale closure does not accommodate the effect of biological, chemical, or physical transformations that might convert optically characterized particles into noncharacterized particles with different shapes, sizes, and refractive indices. Rapid particle transformations such as aggregation (Chin et al. 1998) and grazing (Huntley et al. 1987) are well known and may contribute to the lack of scale closure. Viral infection of optically significant, biological particles represents another process that might rapidly transform planktonic cells into new types of optically significant material (i.e., colored dissolved organic material [cDOM] and cell debris) and, hence, have major effects on ocean optical properties and scale closure. Bacteria are ideal subjects for examining the role of viral infection on host light-scattering properties, given (1) their large contribution to optical scattering (Stramski and Kiefer 1991) and (2) their high abundance (which means that they are more likely than eukaryotic hosts to encounter a virus) (Fuhrman 1999).

Materials and methods—Methods for isolation, enumeration, and propagation of marine bacteriophage and host cells from ambient waters have been described elsewhere (Balch et al. 2000). For time-course experiments, single samples were evaluated at each time point for plaque and plate counts, as well as counts of virus and bacteria that used the SYBR green technique (Molecular Probes, Inc.; Noble and Fuhrman 1998). In brief, samples were initially fixed in 2% formalin (final concentration) and refrigerated at 4°C prior to staining with SYBR green. Filter preparation and methods for SYBR-green counts were done as described elsewhere (Balch et al. 2000). Two hundred particles were counted for each concentration determination (virus or bacteria). For a subset of samples, triplicate counts were performed to assess the statistical resolution of the SYBR green technique. For 112 triplicate bacterial and virus counts with SYBR green, the average coefficient of variation was $17.4 \pm 12\%$ (range, 3%–49%). Statistics for virus and bacterial counts were identical. A list of the bacterial and virus strains used in laboratory experiments, including isolation sites, media type, and virus sizes, is provided in Table 1. Flasks were gently agitated during growth and infection. Size distribution of

submicron particles was measured with flow field–flow fractionation (FFFF; FFFractionation, LLC), a chromatographic-like separation technique (Vaillancourt and Balch 2000). Unpolarized volume scattering of 514 nm light from the bacterial/virus suspensions was measured with a Dawn Laser Light Scattering Photometer (Wyatt Technologies). The incident beam of this instrument is an argon-ion laser with a vertically polarized light beam. Ideally, for measurement of the standard volume scattering function (VSF), the detectors should view unpolarized volume scattering from an incident light source that is nonpolarized (Mobley 1994). Nevertheless, because of randomness of particle distributions in our suspensions, differences between measurements of volume scattering with a polarized versus unpolarized light source were minimal according to comparisons of calibration standards against values from the literature. Instrument drift and calibration was checked by use of a solid, bead-impregnated, standard supplied with the instrument and cross-checked against 0.02 μm -filtered, distilled water. Scattering from a seawater or culture media blank was always subtracted from the total scattering of experimental samples, to estimate the scattering from suspended particles. For each determination, 2,000 VSFs were sampled over a 10-s period, between 35.5° and 144.5° (Balch et al. 1999, 2000). Samples were initially swirled but not subsequently mixed during this 10-s period. Backscattering was estimated from the volume scattering data as described by Balch et al. (1999). In brief, the volume scattering data were first integrated from 90° to 144.5° by use of trapezoidal integration. To estimate the remainder of the backscattering between 144.5° and 180°, the Beardsley-Zaneveld function (Beardsley and Zaneveld 1969; Gordon 1976) was fitted to the volume scattering data at 45°, 90°, and 135°, and the integral of this function between 155° and 180° was added to the 90–155° integral to calculate total backscattering.

For transmission electron microscopy (TEM), purified virus concentrates were placed onto 200 mesh copper grids (Ted Pella, Inc.) supported with carbon-coated Formvar film. The sample was then drawn through the grid by use of absorbent tissue. After drying at room temperature, grids were stained with 1% phosphotungstic acid (Sigma) and examined at magnifications of $\times 20,000$ – $\times 50,000$ in a Zeiss transmission electron microscope (model 902A).

Laboratory infection experiments were performed by adding diluted host bacteria to two sterile flasks. Host bacteria were always derived from log-phase cultures and maintained at 25°C. Host-specific virus was added to one of the flasks at a multiplicity of infection of 1.0, as determined by preexperimental virus and host counts. At regular intervals, aliquots were withdrawn for volume scattering measurement, bacteria/virus enumeration (by use of plaque/plate counts and SYBR-green counts), and FFFF analysis. Flasks then were returned to reduced-light conditions at 25°C until the next sampling.

A mesocosm experiment was conducted with use of *Pseudomonas perfectomarina*, grown outdoors under environmentally relevant conditions of temperature and light, to examine changes in b_b and attenuation during infection. Experiments were performed in 100-liter, autoclavable plastic bags; the experimental design included control (no virus-

Table 1. Summary of host and virus strains used in these experiments, viral capsid diameter (based on transmission electron micrographs), physical cross-section, backscattering cross-section, propagation rate, host mortality rate, change in backscattering after infection, and time to “clear.” Negative host mortality rates indicate a decrease in host numbers over time. Note that *Synechococcus* sp. virus could not be concentrated sufficiently to measure its backscattering cross-section. Media types and isolation sites given as footnotes.

Host bacterium	Viral strain	Virus capsid diameter (nm)*	Virus geometric cross section (m ²)	Virus-specific backscattering cross-section (514 nm; m ² virus ⁻¹)	Viral propagation rate (h ⁻¹)	Host mortality rate (h ⁻¹)	Decrease in b_b after infection (m ⁻¹)	Time to clear (h)
<i>E. coli</i> †	MS-2	25–30	5.94×10^{-16}	1.13×10^{-20}	7.2–8.5	-4.39 to -8.99	0.002–0.06	0.75–0.83
<i>E. coli</i> †	T-4	100	1.54×10^{-14}	5.86×10^{-18}	4.6–7.1	-6.2 to -20.2	0.04–0.2	1.0–1.7
<i>Pseudomonas nautica</i> ‡	C-2	110	9.5×10^{-15}	5.83×10^{-18}	2.4–2.8	-0.2 to -0.85	0.03–0.06	2.8–23.0
<i>Photobacterium</i> spp.§	K-4	50	1.96×10^{-15}	2.32×10^{-18}	1.56–9.2	-1.43 to -5.34	0.01–0.05	1.5–4.8
<i>Vibrio harvey</i> §	K-5	48	1.81×10^{-15}	1.776×10^{-19}	2.09–2.63	-0.52 to -1.93	0.16–0.22	2.0–4.5
<i>Pseudomonas perfectomarina</i> ‡	LS-05	45	1.59×10^{-15}	2.134×10^{-19}	4.17–6.8	-0.7 to -5.34	0.02–0.05	1.0–3.8
<i>Synechococcus</i> sp. Str 1333	S-1	NA	NA	NA	NA	-0.001	0.15	170–190

* Based on transmission electron microscopy.

† Data taken from Balch et al. (2000). Grown in tryptone yeast agar. Source: American Type Culture Collection.

‡ Isolated off Bigelow Laboratory dock; grown in 2216 marine broth (Difco).

§ Isolated from Saco River, Biddeford, ME; grown in 2216 marine broth.

|| Source: Provasoli-Guillard National Center for Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences; originally isolated from Long Island Sound, CT. Grown in F/2 media. NA, not applicable.

es added) and experimental (virus-infected) bags. The bags transmitted light as follows: >90% from 300 to 700 nm, 85% at 250 nm, and 25% at 200 nm. Bags, fitted with a sampling tube, were autoclaved prior to use and filled with sterile media ($f/2 + 0.05\%$ yeast extract) via a peristaltic pump. To protect bags from wave action, each was tethered inside a 208-liter polyethylene cylindrical tank (open on top) that was extensively perforated with 2-cm holes to let ambient seawater freely circulate around the bag. Media in the bags was allowed to equilibrate for 20 h prior to initiation of the experiment. Host bacteria were added to both bags at an initial concentration of 10^7 ml^{-1} . LS-05 virus was added to the experimental bag 2 h later. One-liter aliquots were removed periodically for optical measurements and particle counts. Samples were pumped (peristaltic pump) through a Wet Labs (Philomath) ac-9, to measure absorption (a) and attenuation (c) at nine visible wavelengths (Mueller and Austin 1995), for the total suspensions, as well as that fraction passing a $0.2\text{-}\mu\text{m}$ cartridge filter (cDOM).

Results and discussion—Viral infection caused a pronounced change in the light-scattering properties of the marine bacterial populations in all of our experiments. Qualitatively, relative to turbid host controls, infected cultures became as clear as media blanks. Quantitatively, this change was observed as a sharp drop in the backscattering coefficients several hours after infection (Fig. 1A,D,G). This was attributed to two factors: (1) a sharp decrease in volume scattering values (Fig. 2A–D); and (2) for three host species (*Vibrio harveyi*, *P. perfectamarina*, and *Photobacterium* sp.), a pronounced flattening of the VSF in the forward and backward directions (Fig. 2E–G). Accompanying these changes were simultaneous increases in virus concentrations in infected samples and decreases in host bacteria after a short infection period (Fig. 1B,E,H). Uninfected control bacteria exhibited standard logarithmic growth followed by a stationary phase. FFFF (Vaillancourt and Balch 2000) revealed the presence of peaks close to, but not necessarily identical to, the virus size, as estimated by TEM (Fig. 1C,F,I; Table 1). Phage propagation and host mortality rates, backscattering values, and culture clarification times are summarized in Table 1. For comparison, we include results of *Escherichia coli* infection experiments with coliphages, MS-2 and T-4, for which scattering properties already have been described (Balch et al. 2000).

Infection of a cyanobacterium (*Synechococcus* sp.; Bigelow CCMP 1333) also resulted in backscattering reduction (Table 1). Six days after virus inoculation, backscattering decreased to 0.03 m^{-1} , which was significantly clearer than the original media blank. Relative to the control, the infected sample lost its characteristic green reflectance, with large cell aggregates appearing at the bottom of the vessel. Volume scattering of the infected sample was markedly lower than the control, but no significant changes in VSF shape were detectable because of a low signal-to-noise ratio for angles $>90^\circ$ (Fig. 2D,H).

In the outdoor mesocosm experiment, the virus concentration increased ~ 1000 times after 24 h and then stabilized at 10^{10} ml^{-1} . Host cell concentration in the infected bag increased more slowly than in the control, and no sharp de-

creases in bacterial abundance were noted except 5 h after infection (Fig. 3A). The most notable change was an increase in beam attenuation (c) of the $<0.2\text{-}\mu\text{m}$ fraction at 412 nm from 30 to 46 h in the experimental bag. The increased c of the dissolved material was associated with an increase in phage concentration ~ 24 h earlier (Fig. 3B). The spectral attenuation of this cDOM (after correction for the media blank) decreased exponentially with increasing wavelength (least-squares fit equation: $c_{\text{cDOM } \lambda} = c_{\text{cDOM } 412} \exp[-0.0043(\lambda - 412)]$; $r^2 = 0.99$; data not shown). The low slope showed it to be a weakly colored DOM as opposed to cDOM found in natural environments, which usually has a slope of 0.015–0.020 (Mobley 1994). Indeed, absorption of the $<0.2\text{-}\mu\text{m}$ fraction was low, with most of the increase in attenuation predominantly due to scattering (data not shown). These results must be interpreted cautiously, however, because of the VSF shape change after infection, which would have affected the ac-9 scattering correction and thus complicated the interpretation of the absorption (and scattering) estimates in experimental and control bags. It is also worthy of note that in the bag with infected bacteria, FFFF data revealed an initial 90-nm particle peak, the same size as earlier infection experiments with LS-05 phage. However, 46 h after infection, a secondary broad peak developed at ~ 450 nm.

The most notable observation from these experiments was the rapidity with which turbid marine bacteria suspensions were clarified after viral infection, which was directly attributed to decreases in volume scattering and flattening of the VSF. Culture clearing times of 1–5 h were common for heterotrophic host species, whereas the cyanobacterial host required 6 d to clear (with the actual clearing process confined to ~ 30 h). Within the context of optical changes in the sea, the clearing of infected bacterial suspensions is the same, or faster, than increases in backscattering due to cell growth. In the field, rapid clearing might be expected for phage infection of a low-diversity microbial population, such as in a bloom. This is because a bloom provides a large host population that can be rapidly infected and lysed by viruses rather than more diverse nonbloom host populations that have a low but constant proportion of infected cells. Thus, the magnitude of clearing likely would be inversely proportional to the diversity of the host assemblage.

It should be noted that our experimental design specifically addressed infection in laboratory-propagated cultures of indigenous marine bacteria. This allowed absolute control and reproducibility of the growth and infection conditions (including host-virus specificity). This stands in contrast to many in situ observations of bacteria and viruses in seawater, which cannot guarantee host specificity. Because of the optimal culture conditions in laboratory media, our host bacteria may have been larger than their field counterparts (Morita 1997). Sieracki et al. (1985), using image analysis, demonstrated that natural populations of bacteria increased their physical cross section by ~ 2.9 times 24 h after nutrient enrichment. The populations were far from monodisperse; they showed that the mode of the equivalent spherical diameter (ESD) of natural, control bacteria cells was $\sim 0.7 \mu\text{m}$ (with the majority of cells between $0.5\text{--}0.9 \mu\text{m}$ diameter). For nutrient-augmented populations, the mode ESD increased over 29 h to $\sim 1.2 \mu\text{m}$ (with the majority of cells

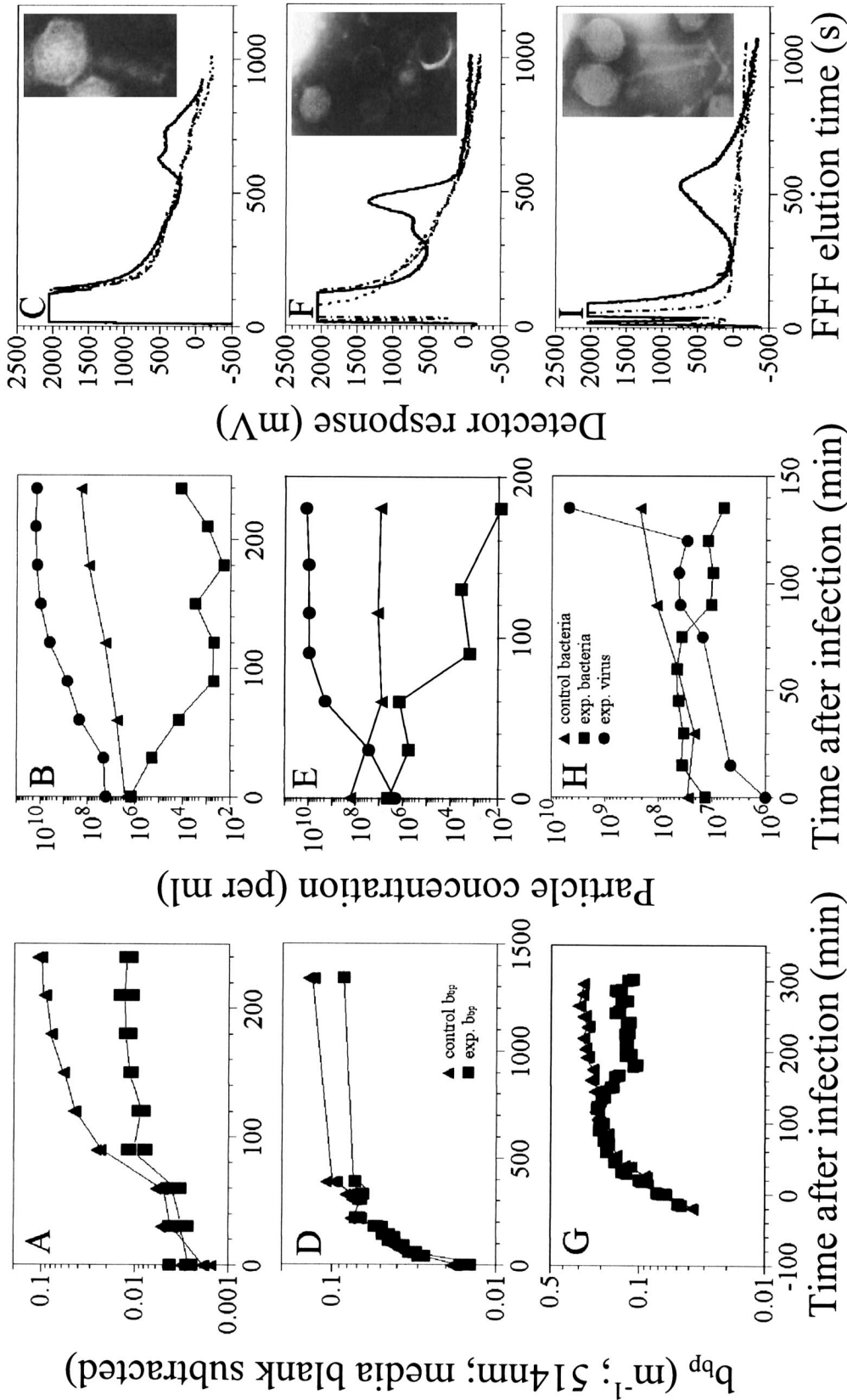


Fig. 1. Results of infection experiments. (A–C) *V. harveyi* infected by K-5 phage, (D–F) *Photobacterium* spp. infected by K-4 phage, and (G–I) *P. perfectomarina* infected by LS-05 phage. Panels A, D, and G show 514-nm backscattering as a function of time for control (triangles) and infected samples (squares). Data reported herein have had a media backscattering blank subtracted. Panels B, E, and H show particle concentration for control bacteria (triangles), infected bacteria (squares), and viruses (circles). Panels C, F, and I show results of the FFFF for zero time, control, and experimental samples, several hours after infection. For these three panels, the fine dotted line shows the particle size distribution in the cultures just prior to infection. The heavy black line shows the size spectrum within the cultures after infection (2.5, 7, and 3 h for panel C, F, and I, respectively). The dashed dotted line shows the particle size distribution in the uninfected, control cultures for the same experimental times. FFFF was calibrated each day with latex beads of known diameters. In panel C, the broad peak of particles in the uninfected sample was 0.15–0.20 μm . In panel F, the peak corresponded to 0.090- μm particles, and, in panel I, the peak corresponded to 0.14- μm particles. Transmission electron micrographs of each virus are shown as insets. The capsid diameter is given in Table 1.

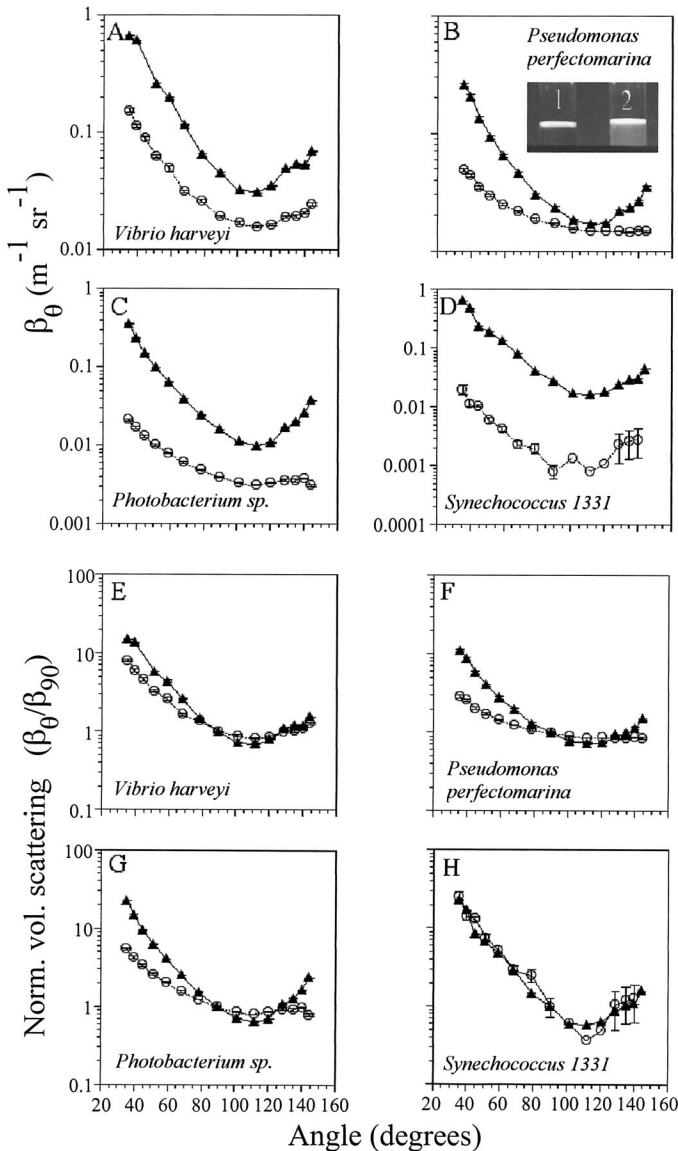


Fig. 2. (A–D) VSFs of uninfected and infected bacterial species. A media blank has been subtracted from all samples. Triangles connected by solid lines show the VSF for control bacterial suspensions, and the open circles connected by dotted lines show postinfection VSFs (after sample had cleared). Error bars represent standard deviations (often smaller than the symbols). (E–H) Same as for above panels, except that VSFs have been normalized to a 90° volume scattering value to better discern changes in VSF shape. Panel B (inset) qualitatively demonstrates typical decrease in scattering after virus infection of marine bacteria; it shows two 20- μ l scintillation vials containing samples, illuminated from below in order to best highlight differences in light scattering: (1) experimental vial of *P. perfectomarina* cells, 5.5 h after infection (in which the bulk of the sample showed negligible scattering except for reflections from the upper meniscus) and (2) control vial of uninfected *P. perfectomarina* cells (which show high scattering throughout the sample).

between 0.7 and 1.3 μ m; see fig. 8C of Sieracki et al. 1985). The expected backscattering of bacteria can be approximated as

$$b_b = N Q_{bb}(r, n, \lambda)G$$

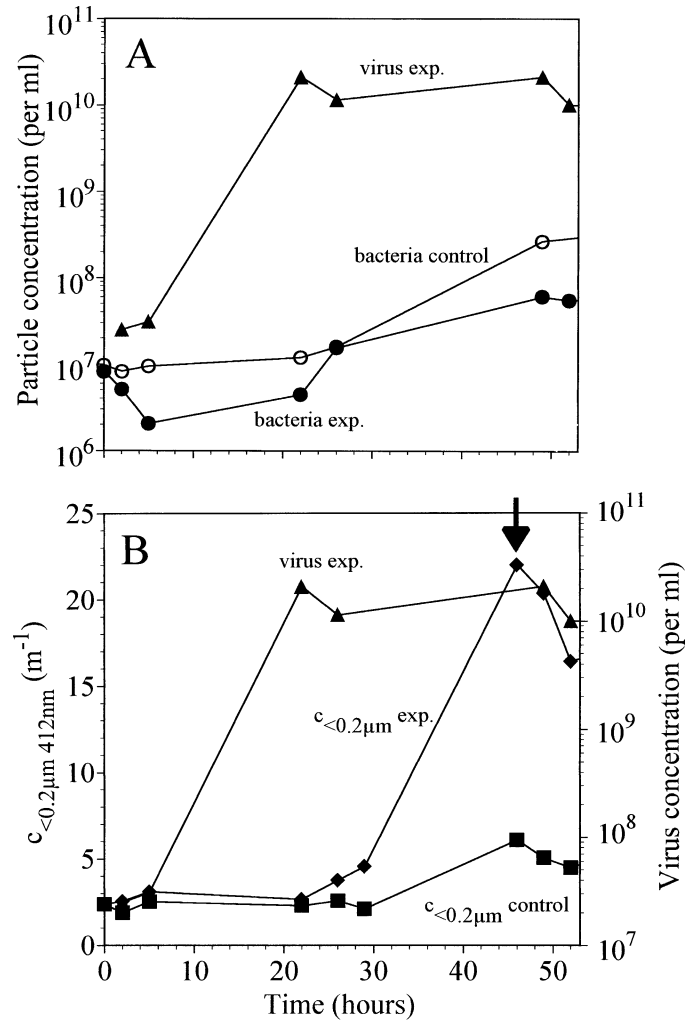


Fig. 3. Results from an outdoor mesocosm infection experiment in which *P. perfectomarina* was infected by LS-05 virus: (A) concentration of *P. perfectomarina* in control (open circles) and experimental (solid circles) bags, plus LS-05 virus concentration in experimental bag (triangles), (B) time series for attenuation of <0.2 μ m size fraction in control (squares) and experimental (diamonds) bags, along with virus concentration (triangles). The arrow designates the time that 0.45- μ m particles appeared in the 0.2 μ m-filtered fraction of the experimental bags. Attenuation data reported herein have had a blank subtracted to correct for attenuation by the media.

where N is the particle concentration (m^{-3}), Q_{bb} is the efficiency of backscattering (which is a function of particle radius (r), relative refractive index (n), and wavelength of incident light (λ), and G is the particle cross-sectional area. If we assume a monodisperse population of spherical bacteria (admittedly unlikely but useful for example), then Mie theory predictions of Q_{bb} , based on 514 nm light and a relative refractive index of 1.05, show increases of ~9% as the cell diameter increases from 0.7 to 1.2 μ m. If we assume a polydisperse population of spheres (closer to reality) with a uniform size distribution of small cells (0.5–0.9 μ m) or large cells (0.7–1.3 μ m), then Q_{bb} increases ~4% from small to large cells. Thus, the net effect of using lab-cultured bacteria

in these infection experiments, rather than bacteria from the field, would be that the backscattering per cell would have been approximately three times larger for lab-cultured cells (2.9 times larger G and 1.04 times larger Q_{bb}). It follows, then, that infection of cultured bacteria could have caused a three times larger drop in b_b than infection of nutrient-starved, field bacteria.

Notwithstanding the above issue of bacteria size, our laboratory results remain relevant to the field. First, whether bacteria have an ESD of 0.7 or 1.2 μm , they still will behave as particle scatterers (with a size equal to or bigger than the wavelength of visible light). Second, a decrease in backscattering due to lysis of bacteria by viruses should be visible as a decrease in remote sensing reflectance (R_{rs}), provided that (1) marine bacteria hosts contribute a significant percentage of total ocean particle backscattering (Morel and Ahn 1990) and (2) viral infection can be epidemic and catastrophic to a host bacterial population (as it was in our cultures). The field relevance is more obvious when one considers that R_{rs} in case (1) waters is proportional to $b_b/(a + b_b)$ (Gordon et al. 1988). For a natural mixture of nonabsorbing, heterotrophic bacteria and other particles, removal of the backscattering of bacterial hosts lowers R_{rs} in approximately the same proportion that the host bacteria contributed to the original total backscattering. For example, if the host bacteria contributed 25% of total backscattering, then their removal by an infection event will lower the R_{rs} by $\sim 25\%$ (with some nonlinearities because of the relative importance of absorption and backscattering). Differences in the degree of eutrophy would probably have the greatest effect on the kinetics of host infection by the virus, because the probability of viruses contacting their hosts would be greater in a more densely populated, eutrophic suspension.

It is useful to evaluate the contribution of viruses per se to the observed backscattering after infection. Virus concentrations in the sea range from 10^3 to 10^{10} L^{-1} in eutrophic regions (Bergh et al. 1989; Bratbak et al. 1993, 1996; Fuhrman 1999). Our experiments began with bacteriophage concentrations typical for eutrophic areas (10^9 – 10^{10} L^{-1}). However, at the conclusion of each experiment, bacterial abundance decreased dramatically, and viral concentrations typically approached $\sim 10^{13}$ L^{-1} . Moreover, their backscattering cross-sections (Table 1) were comparable to those measured elsewhere (Balch et al. 2000). Thus, at the end of the infection experiments, K-5, K-4, and LS-05 virus concentrations were high enough (0.4 – 2×10^{16} m^{-3} ; Fig. 1) to contribute $\sim 30\%$, 39% , and 0.6% of the final particle backscattering in the infected cultures, respectively. The remainder of the backscattering likely came from host fragments or aggregates (Fig. 1; Table 1). However, extrapolated to field virus concentrations of 10^{10} L^{-1} , the direct virus contribution to backscattering is expected to be minimal, with the largest optical effect associated with lysis of optically significant hosts. Before infection, host cells had backscattering cross-sections between $\sim 10^{-15}$ and 10^{-14} m^2 cell^{-1} (see Fig. 1), and, after infection, they were transformed to a mixture of dissolved compounds and submicron intracellular organic debris.

On the basis of attenuation measurements from the LS-05 phage mesocosm experiment, the postinfection optical shift

was associated with a release of blue-attenuating material from the host that passed through a 0.2 - μm filter (Fig. 3B). On the basis of our previous results, this would have caused a shift from particle scattering, dominated by intact cells, to molecular scattering, also known as “Rayleigh” scattering (Mobley 1994), which is dominated by particles smaller than the wavelength of light. Of interest, the increase in dissolved attenuation (which implies the release of cDOM from host cells) actually lagged behind the increase in viral concentration by ~ 24 h (Fig. 3B), which suggests that the optical change of the suspension, although likely caused by host lysis, involved some process in addition to simple leakage of cDOM. Further clues to this alternate process were seen in the FFFF results, which showed a 0.09 - μm particle size peak in the infected sample 26 h after infection. At 46 h, the <0.2 μm -filtered fraction showed an additional, unexpected particle size peak at 0.45 μm (see arrow in Fig. 3B). We suspect that such particles were sufficiently flexible to pass through the 0.2 μm pore-size filter and caused the increase in blue attenuation (due to moderate increases in absorption and large increases in scattering, compared with the control bag).

The timescale for the appearance of these 0.45 - μm particles suggests that they might have been polymer gels (Chin et al. 1998; Orellana and Verdugo unpubl.), assembled from smaller polymers released during host lysis 24 h earlier. This is consistent with observations elsewhere in which assembly of 0.5 μm diameter polymer gels occurred after ~ 12 h (Chin et al. 1998), with sizes up to 4 μm possible. It has been hypothesized elsewhere that viral-induced lysis could be a major factor in the production of dissolved organic carbon in the field (Fuhrman and Suttle 1993; Bratbak et al. 1998; Fuhrman 1999; Jacobsen 2000). What is new in the mesocosm observations is that viral-induced lysis not only initiated a rapid optical shift from particle scattering to Rayleigh scattering but that viral lysis also appears to have provided the building blocks necessary to produce another group of particle scatterers—polymer gels.

In closing, these are the first quantitative optical observations of virus-induced clarification of marine bacterial suspensions. Although viruses themselves typically contribute little to particle backscattering, their infection and lysis of bacterioplankton can rapidly alter light scattering. Fast optical transformations of particle scattering by viruses could confound attempts to achieve scale closure, such that the IOPs of known particles and dissolved materials do not sum to the bulk IOPs. This would be particularly true given the short transformation timescales that we observed here (hours), which can be as short as the time needed for collection and preservation of water samples at sea for particle counts. Our observations extend the understanding of the important role of viruses in the turnover of plankton populations (Fuhrman 1999) and the optical consequences of their infection. Moreover, the above-mentioned optical changes might provide a mechanism for studying viral infection, especially if coupled with observations of DOM and virus enumeration, inside and outside the host. Viral control of low-diversity “blooms” of heterotrophic bacteria has been described before (Lewin 1960; Lewin et al. 1964; Yager et al. 2001). Moreover, monospecific blooms of prokaryotic or

eukaryotic algae are well known (Balch et al. 1991; Bidigare et al. 1997; Morel 1997), and their control by viruses has been postulated (Bratbak et al. 1993, 1995, 1996; Brussaard et al. 1996; Suttle 1996). We hypothesize that viral infection in optically-significant blooms provides an ideal mechanism for (1) rapid reduction in scattering due to profound changes in the VSFs of the particle suspension (as the scattering of the suspension changes from particle scattering-dominated to molecular scattering-dominated), (2) changes in other IOPs of particulate and dissolved fractions as lysed cells release their contents, and (3) possible aggregation of DOM into "Koike" particles/polymer-gels (Koike et al. 1990; Chin et al. 1998; Wells 1998). Polymer gels, as has already been pointed out by Stramski and Kiefer (1991), may themselves be a potentially important source of scattering in the sea.

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