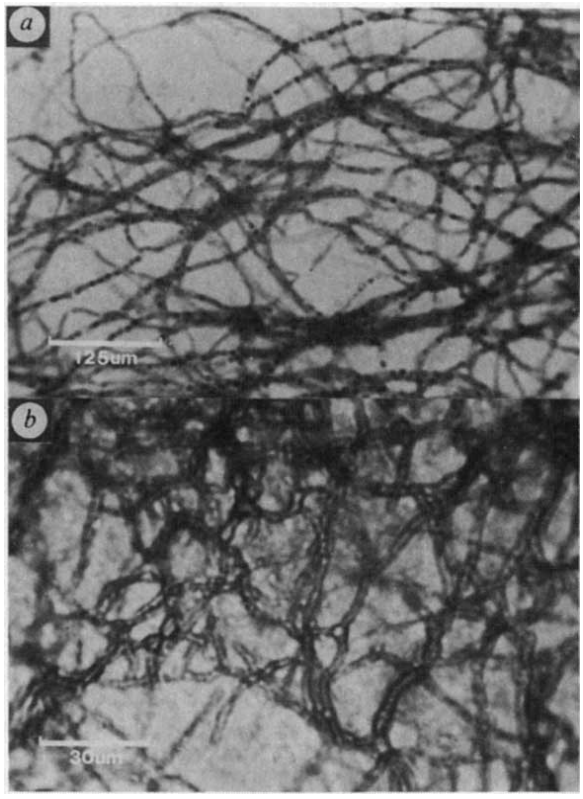

FIG. 4 Light microscopy micrographs of cultured microorganisms, stained with methylene blue. *a*, Morphology of filaments, scale bar 125 μm; *b*, light microscopy micrograph showing detail of branching filaments and possible spores, scale bar 30 μm. Oil immersion.

result of relatively recent surface-related processes. Experiments to demonstrate the potential for active uranium bioaccumulation on these microbes were outside the scope of this study. The high levels of toxic elements (uranium, selenium and bismuth) in these structures, however, indicate that post-mortem accumulation is more plausible[13].

The association of uranium with cell walls of the microbes from Needle's Eye is similar to that described from laboratory experiments of uranium bioaccumulation[1,3,6,19]. Site selectivity of metal uptake in bacteria is a recognized phenomenon related to ion-exchange or complexation between metal ions and active functional groups (such as amine, phosphate, phosphodiester and carboxyl groups) in polymers comprising the cell walls[1,3,4,6–8,13]. The cell walls of many bacteria, including actinomycetes (which have a strong affinity for uranium[9]), are electronegative[8,33,34] and are therefore able to act as ion-exchange resins[7,8,13], sequestering cations such as UO_2^{2+} from solution. Further uptake may result from inorganic adsorption or precipitation reactions[13]. Unlike previous accounts of either experimentally stained microorganisms or naturally mineralized microbes, the biomineralization in individual organisms from Needle's Eye is polymetallic. This is related to the complex geochemistry of the ground waters derived by leaching of polymetallic primary mineralization, in contrast with the simple chemistry of solutions used in experimental studies. Differences in the biogeochemical microenvironments associated with biofilms, cells walls and cell interiors may account for the different metal species deposited in these specific sites. □

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Infection of phytoplankton by viruses and reduction of primary productivity

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NATURAL marine waters contain roughly 10^6 to 10^9 virus particles per ml, yet their role in aquatic ecosystems and the organisms that they infect remain largely unknown. Electron microscopy has been used to study interactions between viruses and their hosts, focusing mainly on pathogens to prokaryotic organisms[1–5]. Here we demonstrate that viral pathogens infect a variety of important marine primary producers, including diatoms, cryptophytes, prasinophytes and chroococcoid cyanobacteria. Also, addition to sea water of particles in the 0.002–0.2 μm size range, concentrated from sea water by ultrafiltration, reduced primary productivity ([^{14}C]bicarbonate incorporation) by as much as 78%. These results indicate that, in addition to grazing and nutrient limitation, infection by viruses could be a factor regulating phytoplankton community structure and primary productivity in the oceans.

Relatively large volumes of water (20–100 litres) were prefiltered through glass-fibre and membrane filters (142-mm diameter; 0.2-μm pore size) to remove zooplankton, phytoplankton and most bacteria. The remaining particulate matter in the filtrate was concentrated to 10–30 ml using Amicon hollow-fibre or spiral cartridge filters (molecular weight cutoff, 100,000 and 30,000, respectively). Negative-staining electron microscopy[6] confirms that these methods yield diverse assemblages of virus-like particles essentially free of bacteria, clays and other particulates of non-viral appearance. We used the fluorochrome 4′-6-diamidino-2-phenylindole (DAPI) to

estimate the concentration of particulates in the concentrates that were associated with double-stranded DNA. Correcting for the concentrating factor of the cartridges, the number of DAPI-positive particles smaller than 0.2 μm (presumed to be viruses) in the sea water ranged from 10^6 to 10^7 ml^{-1} (Table 1). These concentrations are comparable to electron microscopy estimates[2-5].

Isolation and screening of viruses infecting phytoplankton was achieved initially in liquid culture. Phytoplankton were grown exponentially (for several transfers) in batch culture using microwave-sterilized f/2-enriched[7] natural sea water. To test for lytic viruses, growth rates and yields of unperturbed cultures were compared with cultures to which aliquots of natural viral communities, concentrated by ultrafiltration, had been added. Growth rates of the phytoplankton were monitored *in vivo* by chlorophyll fluorescence (an approximate measure of phytoplankton chlorophyll and hence biomass). When the additon of viral concentrate resulted in lower growth rates or cell lysis, the pathogen was purified by inoculating a small amount of supernatant from a centrifuged infected culture into an uninfected culture. The process was repeated many times to dilute non-replicating viruses from the orignal virus addition and to ensure that the effect could be propagated. The nature of the pathogen was confirmed by repeating these experiments using 0.2 μm-filtered culture lysate as inoculum. Samples of culture lysate were also preserved for electron microscopy. Using this method we were successful in isolating several viruses that infect eukaryotic phytoplankton of diverse taxonomy, including a pennate and a centric diatom (both very small and of uncertain taxonomy), a cryptophyte (*Rhodomonas* sp.) and a prasinophyte (*Micromonas pusilla*). (We were unable to propagate the *Rhodomonas* virus, but lysis of the experimental cultures and the observation of viral-like particles in electron micrographs indicated that viruses probably were responsible for the treatment effect.) In all cases addition of the viruses to cultures of their respective hosts caused dramatic decreases in fluorescence as the cultures approached stationary phase (Fig. 1). As far as we are aware, the only virus previously known to infect a marine eukaryotic phytoplankton was found in British Columbia coastal waters and, interestingly, was also a pathogen of *Micromonas pusilla*[8,9]. Viruses have also been isolated that infect freshwater *Chlorella* spp. that are endosymbiotic in *Paramecium* and *Hydra*[10,11]. We have also used ultrafiltration to isolate a phage that infects a chroococcoid cyanobacterium. As the alga and phage could withstand the temperatures required to pour top agar, this phage was further purified by combining centrifuged culture lysate with algae, embedding the mixture in a soft top layer of agar medium and screening for plaque formation[12].

As it was clear that particles in the 0.002-0.2 μm size range (assuming a molecular weight of 30,000 corresponds to a particle diameter of about 0.002 μm) contained pathogens that infected a variety of phytoplankton, we investigated whether this fraction could affect rates of primary productivity. The 0.002-0.2 μm fraction was concentrated from sea water and added back at a range of dilutions to 50-ml seawater samples from the same location. Samples were labelled with 5 μCi of [^{14}C]bicarbonate and incubated under fluorescent lights at an irradiance of 120 μmol m^{-2} s^{-1} for 4 h. Primary productivity, estimated from the radioactivity incorporated into particulate material, revealed that the rates of photosynthesis were as low as 22% of the controls without concentrate (Fig. 2). The inhibitor was also sensitive to autoclaving. We have repeated this experiment five times: on four occasions the results were similar to those

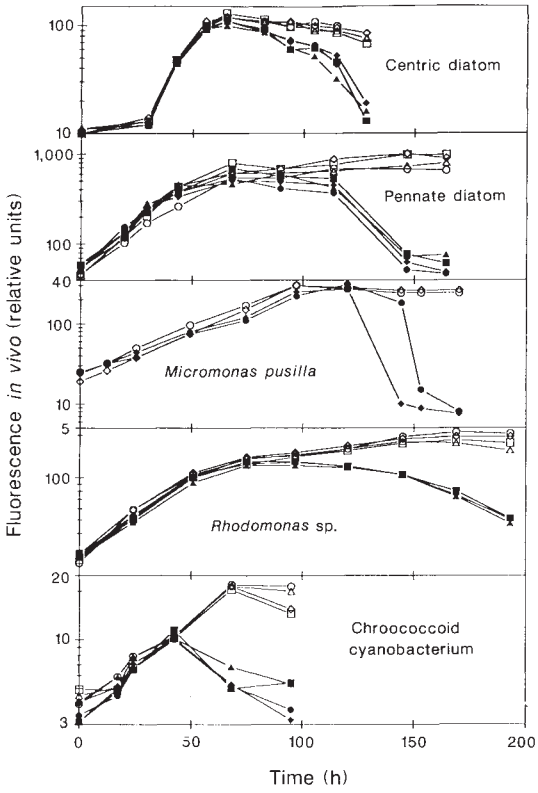

FIG. 1 Effect of adding naturally occurring marine viruses to cultures of their phytoplankton hosts. *In vivo* fluorescence gives a rough measure of chlorophyll *a* and hence of relative phytoplankton biomass. Each symbol represents a different replicate culture: open symbols, controls; closed symbols, cultures to which viruses have been added.

TABLE 1 Concentration of DAPI-positive particles in 0.2-μm filtered sea water

Location	Water type	DAPI-positive particles per ml	*n*
Laguna Madre	Hypersaline	3.3×10^7 (1.90×10^7)	6
MSI Pier/Harbour	Estuarine to clear coastal	1.4×10^7 (0.75×10^7)	6
Gulf of Mexico	Oligotrophic	2.0×10^6 (1.11×10^6)	3

Epifluorescence microscopy was used to obtain means and standard deviations of DAPI-positive particles in 0.2-μm filtered sea water in which the remaining particulate material was concentrated by ultrafiltration (*n*, number of sampling dates). The data are from several sampling dates between March and September 1989. Samples from Laguna Madre, a large (1,660 km^2) hypersaline lagoon separated from the Gulf of Mexico by a barrier island were collected at 27°28.6′ N, 97°19.2′ W. The Marine Science Institute (MSI) Pier and Harbour are situated adjacent to Aransas Pass on the north end of a barrier island (27°50.2′ N, 97°00.5′ W). Gulf of Mexico samples were obtained 70 km offshore from Aransas Pass. Estimates are from direct epifluorescent microscope counts of known volumes of DAPI-stained[18] samples collected by ultrafiltration of sea water, which was prefiltered through glass-fibre and 0.2-μm membrane filters. Electron microscopy revealed that the concentrates were virtually free of bacteria or other particulate material that might interfere with the DAPI-counting method. Unstained samples did not contain fluorescent material. The DAPI-positive material was destroyed by heating to 95 °C for 20 min. Samples were also treated for 30 min with protease-free DNase (250 units ml^{-1}) to remove dissolved free DNA and DNA not protected by a protein coat. The method was tested by digesting a sample to which DNA had been added with DNase and monitoring the change in absorbance at 260 nm. The accuracy of this method in enumerating viruses was tested using known titres of two double-stranded DNA marine bacteriophages that we have isolated. Estimates of the efficiency of recovery were made by adding known numbers of marine bacteriophage to 20 litres of ultrafiltered (particle size < 0.002 μm) sea water and subjecting it to our prefiltration and concentration procedures, then determining the concentration of DAPI-positive particles and plaque-forming units recovered. These results were compared with controls without bacteriophage. The overall concentration efficiencies of the spiral and hollow-fibre cartridges were 35 and 44% respectively.

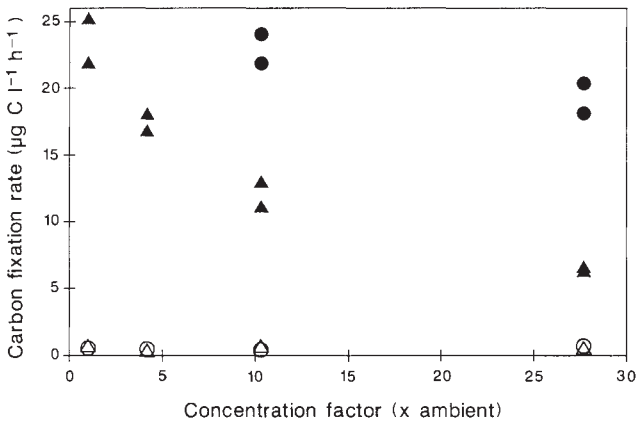

FIG. 2 Effect on phytoplankton photosynthetic rates of adding sea water from the 0.002–0.2 μm size fraction concentrated by ultrafiltration. Closed symbols represent samples that were incubated in the light and to which either autoclaved (circles) or non-autoclaved (triangles) concentrates were added. Open symbols represent samples that were incubated in the dark and to which either autoclaved (circles) or non-autoclaved (triangles) concentrates were added. On the basis of DAPI counts in the concentrate and the concentrating efficiency of the spiral cartridge, the ambient concentration of DAPI-positive particles in the size range 0.002–0.2 μm fraction was 2.1×10^7 ml^{-1}.

described, with primary productivity being reduced to an average 44% (s.e. 21%), but in one instance there was no effect on photosynthesis when concentrate was added.

These data do not prove that viruses were responsible for the decrease in carbon-fixation rate: proteins of relatively high molecular weight would probably also be concentrated by our procedure, are heat labile and can have antibiotic activity. Whatever the causative agents, they were able to inhibit the principal primary producers in our system. If host-specific viruses were responsible, they evidently occur in close spatial and temporal proximity to the phytoplankton they infect.

It is significant that the phytoplankton for which we have isolated viral pathogens are representative of groups of algae that are important primary producers. Chroococcoid cyanobacteria are the most abundant primary producers in the oceanic environment and account for a substantial proportion of the total primary productivity in the nutrient-depleted waters that make up much of the world's oceans[13]. *Micromonas pusilla* is a small flagellate (~1 μm in diameter) which is found throughout the world in both coastal and oceanic habitats and which commonly exceeds 10^6 cells per litre[14]. Centric diatoms are the principal primary producers in upwelling and coastal areas, the most productive regions of the world's oceans. By contrast, pennate diatoms are major benthic primary producers and also constitute a significant component of marine fouling communities. *Rhodomonas* and other cryptomonads are a common element of coastal phytoplankton communities but remain poorly studied.

Virus–host isolates provide model systems by which to study the interactions of indigenous marine viruses with phytoplankton. Such systems will help us in determining the effect of viral infection on the primary productivity and structure of natural phytoplankton communities. As viruses can infect a variety of marine phytoplankton, they may affect pathways of nutrient and energy flow in the ocean, restrict or terminate phytoplankton blooms, maintain phytoplankton species diversity and serve as vectors for the transfer of genetic material between phytoplankton. The presence of algal viruses in sea water may also explain previous reports of lysis of natural phytoplankton communities[15] and difficulties in balancing rates of primary productivity with loss rates for phytoplankton and sinks for organic carbon[16,17]. □

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A terrestrial field experiment showing the impact of eliminating top predators on foliage damage

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CERTAIN ecological models[1,2] predict that the impact of top predators on plants (producers) usually depends on the number of trophic levels in the food web: with three levels the impact is positive, whereas with four it is negative. These models assume that each of the nonbasal levels eats only the next level down. Indeed, freshwater pelagic systems show the predicted response when a fourth trophic level (piscivorous fish) is experimentally added to a three-level food web[3,4]. In terrestrial systems, however, the top predator, if insectivorous, typically feeds on more than one trophic level[5]. This 'closed-loop omnivory'[6], which may also characterize certain aquatic systems, necessitates a different model. *Anolis* lizards are often such top predators on small islands in the Bahamas[7], eating both carnivorous and herbivorous arthropods[8–10]. Numbers of web-spiders on islands without lizards are about 10 times those on islands with lizards[11,12] and in experimental enclosures with lizards removed are 3 times those in enclosures with lizards at natural densities[13,14]. Total leaf damage (by area) to buttonwood (*Conocarpus erectus*) on islands without lizards is 1.5 times that on islands with lizards[15]. These results indicate that lizards reduce the overall damage from herbivorous arthropods, even though they also reduce the numbers of web spiders which eat some herbivorous arthropods. Here we report on an experiment with lizards, spiders, herbivorous arthropods and sea grape, that indicates that the impact of top predators on producers depends on the relative strengths of interactions among the upper tropic levels. The impact is predicted to be positive if herbivorous arthropods are more prone to be eaten by lizards than by spiders (Fig. 1, model A) and negative if vice versa (model B). Experimental removal of lizards both increased scar damage on sea grape leaves, produced by homopterans and other organisms, and decreased damage produced by gall midges, indicating that the food web is a composite of models A and B (Fig. 1, model C). Contrary to the experiments in freshwater pelagic systems with four trophic levels, the net effect of top predators on producers was positive, apparently because the model A pathway predominates.

Details of the study site and experimental design are given elsewhere[14]. Briefly, in spring 1985 we staked out nine 83.6-m^2