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Effect of concentrating the virus-rich 2–200-nm size fraction of seawater on the formation of algal flocs (marine snow)

Abstract—Particle aggregates >0.5 mm in diameter (marine snow) are an important component of marine environments. Viruses are now recognized as active members of the marine microbial food web, infecting both planktonic primary producers and bacteria. The effect of adding material from the 2–200-nm size fraction, concentrated from seawater with ultrafiltration, on the development of phytoplankton biomass and formation of algal flocs was examined. At higher concentrations of material in this size fraction (2.3 times enrichment with viruslike particles), the buildup of phytoplankton biomass was initially delayed. After 8 d, these enrichments resulted in the formation and persistence of up to 5 times higher numbers of larger aggregates (>1-mm equivalent spherical diameter) concomitant with even higher phytoplankton biomass. Both the size distribution of algal aggregates and primary production can be affected by material in the size range of 2–200 nm containing large numbers of viruses.

The formation of algal flocs in natural seawater depends on physical, chemical, and biological factors including cell size, cell concentration, differential settlement, and shear (Riebesell 1991*a* and references therein). However, while these conditions act as prerequisites, the degree and ultimate course of aggregation is thought to be determined by the biology of the organisms involved (Riebesell 1991*b*). Some factors causing aggregation include stickiness, growth of colonial and chain-forming species, increased activity of heterotrophic organisms, and production of amorphous mucus during bloom conditions due to increasing concentrations of dissolved organic C (e.g. Riebesell 1992). It has been demonstrated recently that viruses can be active partners in microbial trophodynamics, for example infecting primary producers (Bratbak et al. 1990; Suttle et al. 1990; Heldal and Bratbak 1991), although the actual role remains a topic of debate (Olofsson and Kjelleberg 1991). The significance of the viral size fraction to marine ecological processes is now a matter of considerable interest.

To test the influence of the 2–200-nm size fraction on aggregate formation during phytoplankton bloom conditions, we incubated unfiltered seawater from the northern Adriatic Sea (an environment exhibiting a high potential to form marine snow aggregates; Herndl and Peduzzi 1988) in rotating cylindrical tanks (3.5 rpm). This method produces aggregates quite similar to those found in the sea and keeps them in suspension during the experiments (Shanks and Edmondson 1989). The water was collected in January 1992 off the Laboratorio di Biologia Marina at Aurisina, Gulf of Trieste.

A series of eight 10-liter tanks was exposed in a temperature-controlled room to light (300 μEinst m^{-2} s^{-1}, 12:12 L/D cycle) and temperature (17–18°C) conditions representative of spring bloom periods in the area. Before use, the tanks (of transparent acrylic glass) were acid washed and thoroughly rinsed with deionized and sample seawater. In three tanks, the concentration of viruslike particles (VLPs) was increased 2.3 times by back-adding a concentrate produced from the same but prefiltered (1.2-μm Millipore "Isopore" RTTP and 0.2-μm GTTP membrane filters to remove plankton and most bacteria) water body with ultrafiltration technology following the protocol of Suttle et al. (1991). Amicon spiral cartridges (molecular weight cutoff, 30,000) were used, assuming that 30,000 MW equals 2 nm (Suttle 1992). Ultracentrifugation methodology and uranyl acetate-staining electron microscopy (Bratbak et al. 1990) revealed VLPs to be by far the most abundant visible material. Initial concentrations in the tanks were 20.4×10^6

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VLPs ml^{-1} in the enriched treatments and 8.9 $\times 10^6$ ml^{-1} in the unaltered incubations. These concentrations can be considered average values well within the range observed during an annual cycle (free virus particle concentration in the northern Adriatic Sea in 1991–1992 ranged from 0.1 to 95.5 $\times 10^6$ virions ml^{-1}; Weinbauer and Peduzzi in prep.). As a control for abiotic floc formation, cyanide was added to the water of two tanks (final concn, 2 mM) to inhibit activity of microorganisms and production of new virus particles (Heldal and Bratbak 1991).

Total suspended matter (TSM) and Chl *a* were measured by withdrawing aliquots of the incubation media from the tanks, filtering onto GF/F filters (Whatman), and using standard methods to determine TSM (as dry wt, 60°C oven-dried) and Chl *a*. Virus particles were counted in a Zeiss EM 902 transmission electron microscope after ultracentrifugation of fixed samples (2% formaldehyde) onto formvar-coated grids (Bratbak et al. 1990). Marine snow aggregate dimensions and size distributions were determined in all tanks by taking photographs against a black background with lateral strobe illumination. Photographs were enlarged for counting and sizing particles according to Shanks and Edmondson (1989) and Riebesell (1991*b*). The lower detection limit of this method was found to be particle diameter of 50–100 μm. Particle size is expressed as the equivalent spherical diameter (ESD; i.e. the diameter of a sphere with equivalent volume to nonsperhical-shaped particles). Oval to round shape was assumed for aggregates on the photographs with the third dimension (depth) being equal to the shorter axis (Riebesell 1991*a*). Since other studies have demonstrated that the maximum dimension of aggregates in the ocean remains on average ~1-mm ESD (e.g. Riebesell 1991*a*), we applied this as a threshold size to our data.

In the poisoned treatments the size of initially present, small aggregates (always <0.5 mm) as well as concentrations of free virus particles, Chl *a*, and TSM (Fig. 1) did not change during the entire incubation period (400 h). The unpoisoned treatments exhibited a gradual increase in TSM (Fig. 1A); in tanks with added virus-rich concentrates, the initial increase appeared to be slightly delayed. However it was not significant. A time lag was more clearly observable in the development of the Chl *a* fraction of TSM, indicating inhibition of phytoplankton biomass during the first 170 h of incubation (Fig. 1B). On day 9, however, phytoplankton biomass reached levels slightly higher than in unaltered treatments up until the bloom collapsed. This mixed bloom consisted largely of diatoms (*Chaetoceros* sp., *Coscinodiscus* sp., and *Nitzschia* sp.).

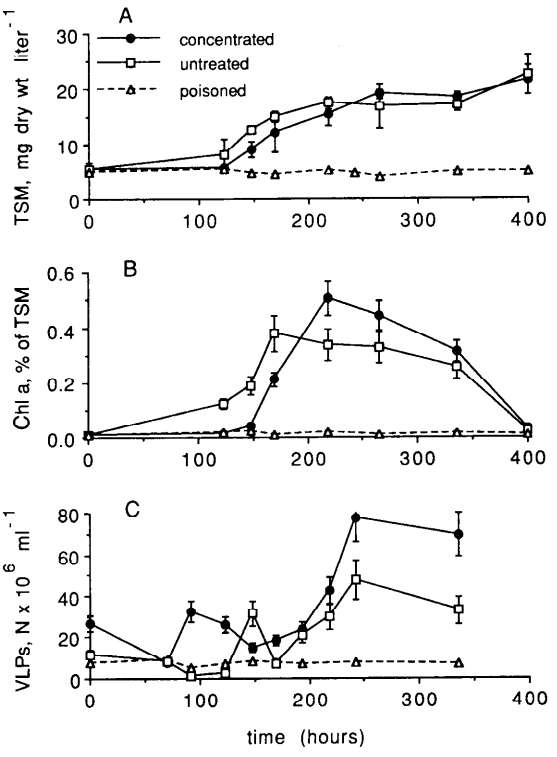

Fig. 1. Effect of adding the virus-rich 2–200-nm size fraction concentrated from seawater and of cyanide poisoning on the development of total suspended matter, the percentage of Chl *a* in TSM, and the change in abundance of viruslike particles (VLPs). Values are means of duplicate (poisoned treatments) or triplicate incubations; viruses were counted in one poisoned, two enriched, and two untreated cultures. Vertical bars indicate mean deviations.

The abundance of free VLPs exhibited a dynamic pattern with typically higher numbers in the virion-enriched treatments (Fig. 1C). Highest values were observed during the decline of the bloom, with maximum concentrations of up to 77.5 $\times 10^6$ VLPs ml^{-1}. From ~200 h on, a marked difference in the size

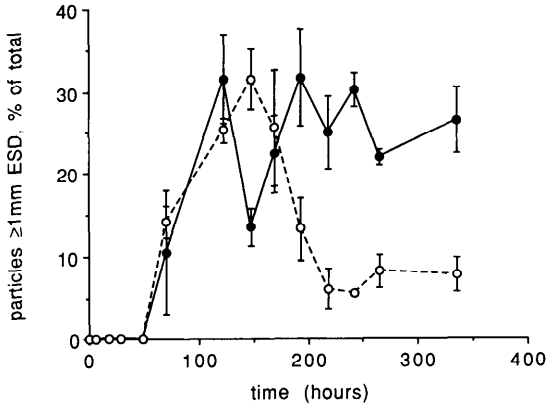

Fig. 2. Development of the abundance of aggregates ≥ 1-mm ESD expressed as percent of total particles present in natural seawater incubated in rotating cylindrical tanks. In three tanks, the concentration of VLPs was increased 2.3-fold (●) and compared to untreated natural seawater (○). Values are means of triplicate incubations.

distribution of marine snow particles could be observed between enriched and unaltered treatments (Fig. 2). Whereas in the untreated incubation <10% of the total aggregate numbers (countable by the method we used) belonged to a size class ≥1-mm ESD, ~30% of the aggregates were ≥1-mm ESD (up to 4 mm and more) in treatments to which the concentrate was added. During the entire second half of our experiment, marine snow particles ≥1-mm ESD were between 1.3 and 4.9 times more abundant in tanks receiving concentrates. The number of marine snow particles ranged from not detectable by our method to 594 aggregates liter^{-1}. In general, fewer marine snow particles were present when the size of aggregates was larger. The particles were largely spherical to ovoid. In unaltered seawater the observed decreasc in the percentage of marine snow particles ≥1-mm ESD could be attributed to both disaggregation of large marine snow and the preponderance of small-sized, newly formed aggregates.

Phytoplankton biomass in terms of Chl *a* concentration was higher at the same time that formation and persistence of large aggregates was favored in tanks with added concentrates. Due to this coincidence and the yellow-greenish color of large aggregates during this period, it can be assumed that in enriched treatments a significant proportion of phytoplankton was bound to these large particles. Our findings strongly suggest that the elevated concentrations of material in the 2–200-nm size fraction caused this shift toward formation and longer persistence of higher numbers of algal flocs ≥1-mm ESD concomitant with relatively high phytoplankton biomass.

Estimates of viral abundance in marine snow particles based on ultrathin sections and transmission electron microscopy revealed ~3 orders of magnitude higher concentrations of viruses in aggregates than in the ambient water. Unfortunately we were unable to provide data on virus dynamics within the aggregates due to the enormous time involved in sample preparation and counting. In flocs taken when the experiment ended, however, 8.7×10^{10} virus particles cm^{-3} were found in virion-amended treatments vs. 6.2×10^{10} in unaltered incubations. Similar values were found in natural marine snow aggregates in summer 1991 when the average virus abundance in marine snow aggregates was 5.6×10^{10} viral particles cm^{-3} (Weinbauer and Peduzzi in prep.).

We have shown that adding concentrated material from the virus-rich 2–200-nm size fraction can influence the time-course of particle aggregation during algal bloom events in natural seawater cultures: the formation and persistence of higher numbers of larger aggregates is favored. Our experimental results agree with the theoretical assumptions of others who suggested biological processes as important, poorly understood mechanisms determining floc formation within given physical boundaries (e.g. Alldredge et al. 1990; Riebesell 1991*a*,*b*). Although we do not yet understand the specific processes underlying our observations, one can suggest that cell lysis products originating from virus-mediated mortality of both phytoplankton and bacteria may act as biological glues (Proctor and Fuhrman 1991). Similar experiments showed that bacterial abundance was repressed by up to 50% beyond 150 h of incubation when 2–200-nm concentrates were added, suggesting increased presence of lysed cells and lysis products at this time (Peduzzi and Weinbauer in press). On the other hand, there is evidence that aggregation can provide favorable conditions for planktonic organisms in some situations (Herndl and

Peduzzi 1988; Gotschalk and Alldredge 1989). Virus-mediated lysis might increase recycling of organic material in a cell–virus-dissolved organic material loop (Bratbak et al. 1990).

We have further demonstrated that the inhibiting effect of elevated concentrations in the virus-rich 2–200-nm size fraction on primary producers may be only transient, possibly modifying the community structure and distribution patterns of planktonic organisms. Primary production may even be enhanced at times. Although colloidal particles were extremely scarce in our samples, other substances present in the 2–200-nm size fraction, like free proteins, humic or fulvic acids, colloids not detectable by our methods, etc., cannot be ruled out as possible agents influencing biological processes and aggregate formation (Wells and Goldberg 1991; Suttle 1992). However, the bioactive nature of this size fraction, also confirmed by our experiments, should be of global significance when the effect of aggregate size on primary production, sinking rates, and changes in light climate, as well as on cycling of energy and nutrients in the sea, is considered.

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