

# Viruses rule the waves – the smallest and most abundant members of marine ecosystems

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In his authoritative book *Marine Microbiology*, ZoBell (1946) stated that ‘...since bacteriophage is generally found associated with large numbers of rapidly multiplying bacteria, it is very doubtful if the sparse bacterial population characteristic of the open ocean is conducive to the development or activity of bacteriophage’. ZoBell’s view on bacteria in sea water was based on the numbers obtained with plate counts (around 100–1000 c.f.u. ml<sup>-1</sup>) and his reasoning concerning viruses was logical, but in the late 1970s the total abundance of bacteria in sea water was found to be 1000 times higher, i.e. about 10<sup>6</sup> cells ml<sup>-1</sup>. The organisms are quite active, growing at a rate of about one division per day. The basis for ZoBell’s view on viruses was thus proved wrong and we had to revise the general textbook wisdom that viruses are unimportant in natural waters. We now know that viruses outnumber bacteria by a factor of 10, at 10<sup>7</sup> ml<sup>-1</sup>.

By the end of the 1980s, when much data on bacterial biomass and production and on protozoan grazing had accumulated, it appeared that bacterial production and removal were not always in balance. This could have been due to methodological inaccuracies, but it was also possible that other removal mechanisms such as cell lysis and viruses were involved. At this time centrifugation was used to harvest bacteria directly onto electron microscope grids for transmission electron microscopy (TEM) analysis and we reasoned that if viruses were present in significant numbers we could harvest them too just by increasing the speed of the centrifuge. With some trial and error, and a wrecked ultracentrifuge rotor, we came up with convincing electron microscope images of native virus communities (Fig. 1) and quantitative results on the abundance of viruses.

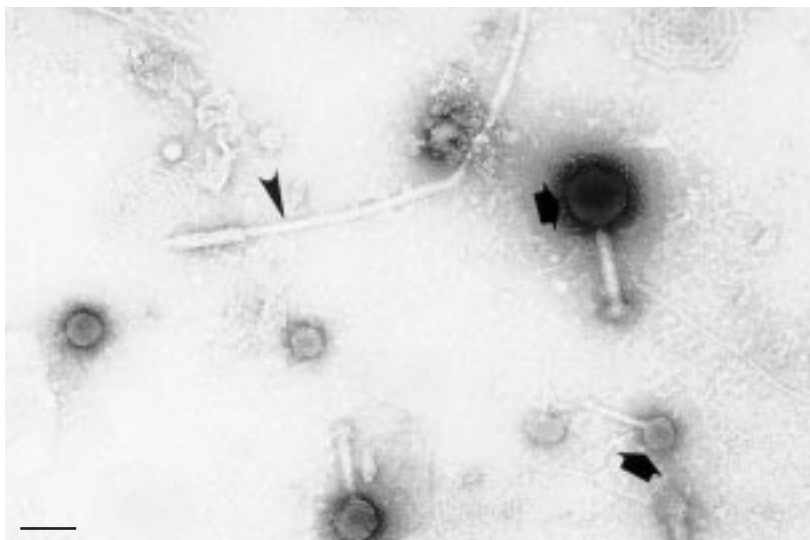
We did not discover viruses in the sea and nor was the ‘novel’ method we invented for counting viruses actually new. The presence of viruses, i.e. bacteriophages, in sea

water has been known for a long time (at least since the 1950s) and high abundance was also reported in 1979. Unfortunately for these early researchers, their findings were not recognized and appreciated when published. Our publication appeared at a time, 10 years ago, when viruses fitted in as the last (?) piece in the puzzle. It provoked an immediate response. Publication in *Nature* gave the story publicity and the high number (billions in a teaspoon of water!) of viruses (dangerous and scary!) was considered newsworthy by the press. The notion that virus activity essentially is a question of ‘sex and crime’ might also have been a factor in this interest. At the time, we thought that the method we had developed for counting viruses in the transmission electron microscope was simple, elegant and new. Later we became aware of no less than five papers published between 1949 and 1986 describing procedures for counting viruses by the same approach.

Counting viruses, was of course not enough, and in the years after the ‘discovery’ we and many other research groups have worked to understand their ecological significance. First of all, what we include in viral total counts are not actually viruses but Virus-Like Particles (VLPs), i.e. electron-dense particles with a hexagonal to round shape and a diameter of 30–200 nm (Figs 2 and 3). This distinction is important, as there is no way to ascertain that each and every particle we observe in the microscope really is a virus. They may in fact not be viruses at all, they may be DNA blebs, ink particles from squid, or some other particle of biological origin that has not been described yet. On the other hand, we may miss a lot of viruses that are not hexagonal or round. In our TEM preparations we sometimes observe rod-shaped and filamentous particles that may well be viruses (Figs 3 and 4), but how can we tell?

One of the first questions we asked ourselves – what do the viruses do in the ocean? – was in fact quite naïve. They obviously do the same as they do in cultures, i.e. lyse cells or make them lysogenic. Like other DNA-based entities, they struggle to survive and transfer their DNA to the next generation as best they can. The basic questions in microbial ecology – who, how many, and how fast? – may sound simple but they have nevertheless proved difficult to answer and indeed this has also been the case in virus ecology. The effects of viruses on population dynamics, community

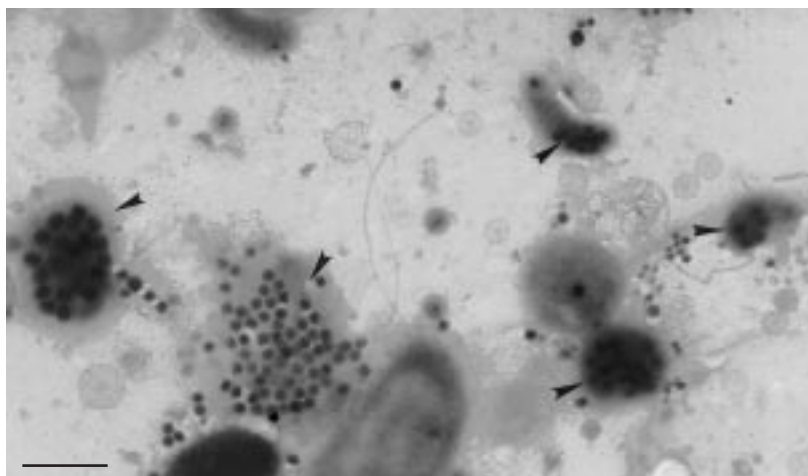
Some 10 years ago a series of letters was published in *Nature* showing that bacteriophages were both abundant and active in natural waters. These reports boosted research on the ecological significance of viruses and the field is now well established as an integral part of aquatic microbial ecology.



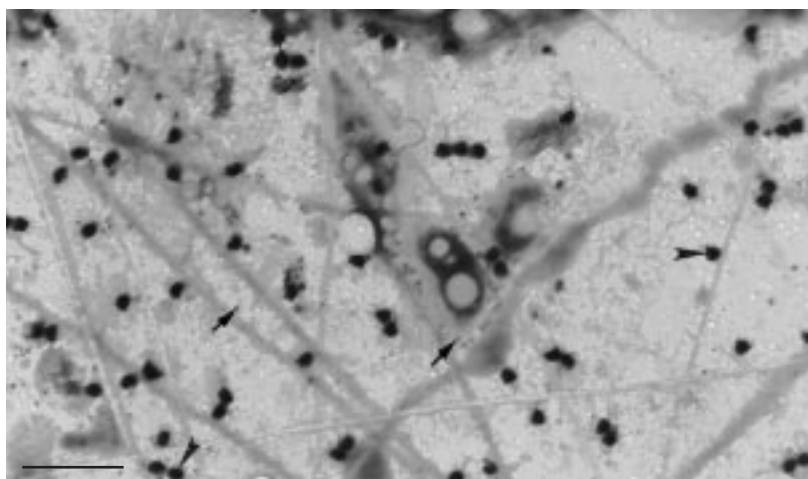
LEFT: Fig. 1. Tailed bacteriophages (arrows) from coastal sea water. The wide range in head diameter indicates a diverse community. Other structures (arrowhead) may perhaps relate to filamentous phages. The particles were harvested directly onto nickel grids (400 mesh) by centrifugation (200,000 g, 30 min.), and negatively stained with uranyl acetate (2% in water). Bar, 0.1 µm.

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RIGHT (UPPER):  
**Fig. 2.** Five bacteria (arrowheads) at different stages of viral maturation and lysis. These virus particles may be more difficult to recognize as viruses compared with the tailed viruses in Fig. 1. Note the variation in size of virus particles and in numbers of particles per cell. Preparation was as described for Fig. 1, but the sample was positive-stained with uranyl acetate. Bar, 0.5  $\mu$ m.  
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RIGHT (LOWER):  
**Fig. 3.** Algal virus (arrowheads) and phages (arrows) in a coastal sea water sample. The thread-like structures are presumably from *Phaeocystis* sp. (small arrows), but a lot of particles of unknown identity and origin are also seen. Preparation as for Fig. 2. Bar, 1  $\mu$ m.  
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BELOW:  
**Fig. 4.** TEM image demonstrating the diversity of particles that may be found in coastal seawater. The bacterium (b) is easily recognized, but what are all the round and filamentous particles in the background? Preparation as for Fig. 1. Bar, 1  $\mu$ m.  
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structure, diversity, nutrient flow, biogeochemical cycles and climate are far from obvious and the research requires the combined effort of many disciplines.

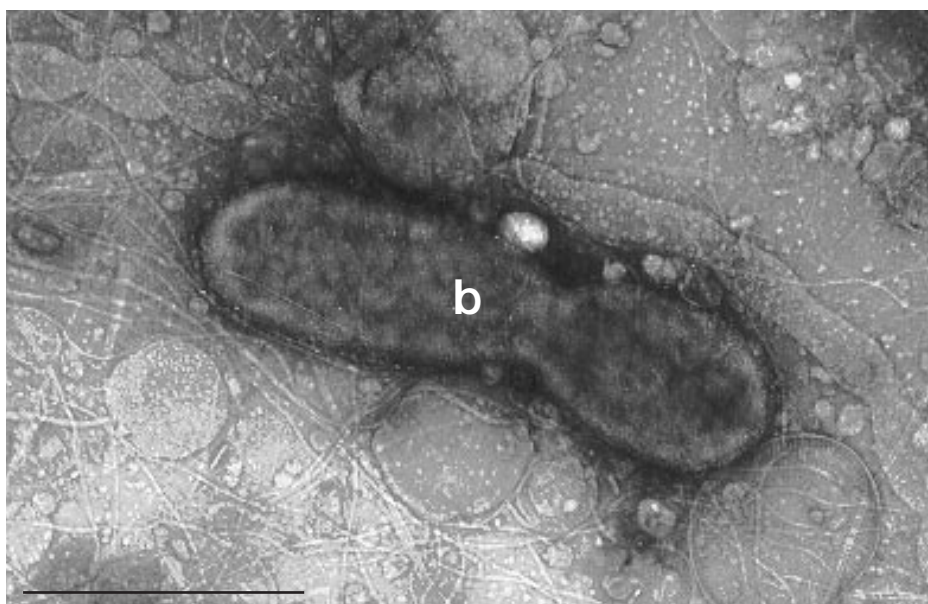
Based on the simple observation that bacteria are the most abundant possible hosts in the sea, it is assumed that most of the viruses are bacteriophages. The problem with the bacteria in this context is their general lack of morphological traits, which makes it difficult to distinguish one possible host population from another, and that less than 1% of them are easily isolated and cultured in the lab. We thus know very little about individual populations in mixed natural marine communities. What we know is mainly based on the use of molecular methods and ribosomal RNA analysis. We have just begun to unravel the diversity of bacterial communities and the distribution and dynamics of individual bacterial populations. It has therefore been difficult to demonstrate how viruses affect native bacterial populations in natural environments. Some phytoplankton species are, in contrast, easily recognized by trained taxonomists and several studies have now demonstrated that

phytoplankton blooms may be terminated, and the succession driven, by viral infections.

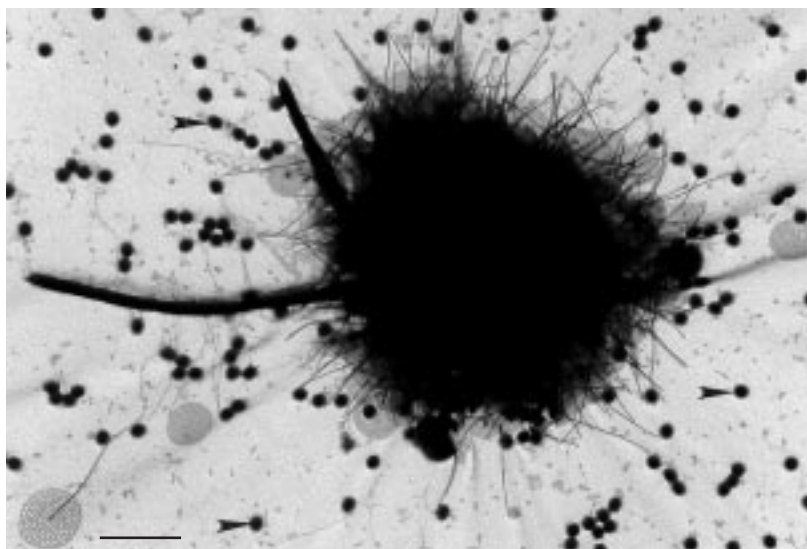
What controls viral activity in natural waters is still a matter of debate. Some experiments indicate that the majority of viruses are produced as a consequence of a lytic infection. The rate of virus production will thus be controlled by the abundance of hosts multiplied by the abundance of virus. The alternative is that the host is lysogenic, or somehow carries the virus or the virus genome in an inactivated stage. This hypothesis would explain how the virus 'survives'

when the host population is gone, where it comes from when the host population returns and how the host population protects itself from being exterminated by the virus. Our guess is that both strategies and all possible intermediates are used by different populations in the bacterial community. If we look for lytic infection we will find it and if we look for lysogeny we will find that too. What is important is the question of timescale and the nexus of cause and effect, and thus the experimental design.

Population growth in natural environments is controlled and limited by food supply and by predation.



These factors may be selective but they are not specific because they to some degree affect all organisms in the community, depending on the properties of the organisms involved. Those with high nutrient affinity and high growth efficiency may, for example, perform better under nutrient limitation than others. Very small, very long and very large cells may experience less grazing pressure than average-sized cells. The population control exerted by viruses



is very different. Viruses are population-specific and by attacking one single population (i.e. the host) they make life easier for competing populations, which of course also may have their viruses. Thus, food limitation and predation selects for those that are able to cope with given environmental conditions, i.e. those that are most fit. The rate of virus infection depends on host-cell abundance and virus replication requires actively growing cells. Successful populations with active and abundant cells are thus more vulnerable to viral infection than those which are small or inactive. Food limitation and predation selects the winner – and the viruses kill the winner (Fig. 5).

The most frequently cited figures suggest that 20–30% of bacterial production and 2–10% of phytoplankton primary production is channelled through ‘the viral shunt’ in the microbial food web. Cell lysis implies that organic material is lost from the grazing food chain, where the organisms depend on particulate food, and becomes available to bacteria thriving on dissolved organic material and nutrients. The net effect of this is increased nutrient recycling and respiratory loss of organic carbon in the lower parts of the food chain. As a quantitative significant process, viral activity does have direct implications for the carbon budget of the ocean, and hence for the global climate. Moreover, virus infection in algae has been found to cause increased production of dimethylsulfoniopropionate (DMSP) and dimethylsulphide (DMS). When DMS escapes to the atmosphere it causes increased cloud formation, which in turn affects global radiation and thus global warming. If this was not enough, DMS also causes acid rain.

We all have viruses and know what they do to us; we try to avoid them and have doctors to cure us. We know that bacteria and algae have their pests. There are also a few other examples of the ecological significance of viruses in the sea that have attracted public attention. In

1988–89 a morbillivirus epizootic killed >18,000 seals around the North Sea and in 1990 hundreds of dolphins died of the same virus in the Mediterranean. Diseases caused by viruses like ISAV and IPNV caused significant economic loss in salmon farms before efficient vaccines became available. The obvious question then is what about all the other creatures in the sea – herring, mackerel, cod, anchovies, whales, etc.? Can food limitation, predation, fishing and hunting really explain all the large fluctuations in population density that have been observed for these species? They all have viruses and it is tempting to suggest that virus infection may play some role in controlling their lives.

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LEFT:

**Fig. 5.** Blooms of the marine phytoplankton *Emiliana huxleyi* have often been observed to be terminated by viral infection and the concentration of *E. huxleyi* viruses (arrowheads) in the sea may become very high. Viruses are host-specific and the flagellate shown here does not have to worry about the surrounding *E. huxleyi* viruses. On the contrary, it may rejoice at less competition from *E. huxleyi*: the *Emiliana* bloomed, but a virus killed the winner! Preparation as for Fig. 2. Bar, 1 µm. COURTESY M. HELDAL AND G. BRATBAK

## Further reading

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