Causative Agents of Bacterial Mortality and the Consequences to Marine Food Webs

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ABSTRACT

It has been known for about 20 years that production of planktonic heterotrophic bacteria represents a large fraction of the flow of energy and matter in aquatic systems. We also know that prokaryotic photoautotrophs, including cyanobacteria and prochlorophytes, are very important in global primary production. It is critically important to know the fates of these organisms, specifically the causative agents of their mortality, in order to understand the patterns of energy flows and nutrient cycles in such systems. Starvation as a cause of mortality is functionally different from actual removal, because starved cells must still be removed at a rate similar to the starvation rate, or they would accumulate and continue to increase over time (not observed). A decade ago, it was generally believed that protists, primarily small flagellates, were the only significant agents for the removal or disappearance of bacteria. However, a broad variety of evidence accumulated over the past decade suggests that viruses are also responsible for considerable amounts of microbial mortality, apparently of a magnitude comparable to protist-based mortality. Viral infection leads to cell lysis and release of the cell contents which then become available for subsequent bacterial uptake. Thus, this mode of mortality leads to a closed loop that increases bacterial production and respiration while regenerating inorganic nutrients. Models show that this robs matter and energy from larger organisms and forces more of the "action" of the system into the bacteria. Viruses may also exert a strong influence on plankton species composition and lead to increased biodiversity, because viral infection is density-dependent and largely species-specific. This mortality contrasts with that caused by protists, which apparently can graze most any bacterium and can reduce even a highly diverse mixture of bacteria to low levels. Other, less known, mortality mechanisms include antibiosis, predatory bacteria, and aggregation followed by sinking.

Introduction

It is now clear that microbes are critical and quantitatively significant components of aquatic food webs and have numerous roles [3, 8]. While much attention has focused on the production of bacterial biomass, it is often more important to understand the fate of that biomass, particularly the bacterial mortality. This brief review will summarize the various causative agents and discuss what is known about their significance in planktonic systems.

Possible Causative Agents of Mortality

Protist Grazing

This mechanism has been known for decades to be significant, and for many years it was considered to be the primary cause of bacterial mortality [13]. Grazing on individual bacteria is thought to be dominated by flagellates due to the small size of the prey, although ciliates and some sarcodines also participate [13]. Grazing is thought to be relatively indiscriminate in that protists can feed on diverse bacterial assemblages. Flagellates usually intercept their prey by collision and capture it into a vacuole. Digestion within the vacuole is relatively rapid and often completed in tens of minutes or less. Undigested material is released in what is sometimes called 'pico pellets'. Because flagellates have fast potential growth rates (doubling a few times per day), they are capable of responding to varying conditions and can grow fast enough to keep bacteria in check under most natural aquatic conditions.

Viral Infection

Although known from pure cultures for several decades (reviewed by Fuhrman and Suttle [10]) viruses of aquatic microbes have been considered quantitatively significant components of aquatic systems for only about a decade [4, 17, 18]. Viruses are thought to be specific for their hosts (usually species or strain), and are density-dependent because they rely on diffusion to pass from host to host. Lytic infection results in bursting of the host cell with release of the cell contents and progeny viruses. Whether this cell debris resembles bacterial 'ghosts' or simply amorphous material is a matter of debate. Although the existence of chronic infection is known, in which the progeny viruses are released continuously (without bursting the host), its significance in nature is unknown. There is evidence from marine systems of the common occurrence of lysogeny, in which a viral genome is integrated into the host genome and reproduced with it over many generations [12]. However, it appears that induction of the lytic cycle from lysogens is relatively rare compared to successive lytic infection [23]. Viruses are capable of rapid proliferation and can quickly decimate populations of susceptible bacteria, as is readily shown in pure culture.

   Defenses against viruses may include extracellular proteases and nucleases, mutated or missing cell surface receptors, intracellular restriction enzymes, genetic incompatibility, and immunity via prophage [1]. Viruses may counter these defenses, and resistance may reduce a cell's growth rate, so bacteria and viruses may be thought of as being in a constant battle. Interestingly, attempted viral infection of an immune bacterium may feed the bacterium.

Predatory Bacteria

Some bacteria attack and feed on other bacteria by a variety of mechanisms. Examples are *Bdellovibrio, Vampirococcus, Daptobacter*, and there are almost certainly many unknown others. In natural aquatic systems, their significance is poorly known. The species range of bacterial prey varies, depending on the predator. The remains of the prey are usually cell debris, which is probably not usually recognizable as a cell.

Starvation

This mechanism is often discussed [15], but there are few data on its quantitative significance in nature. Though many species in culture produce small cells upon starvation,

it does not necessarily follow that all small bacteria are starved. The common ability of bacteria to survive for long times upon starvation in some kind of semi-resting state [15] suggests that mortality from this mechanism is probably rare. A starved cell, even if dead, may be ordinary-looking by standard microscopy, and is still suitable (albeit low-calorie) food for grazers, although small molecules would leak out and storage reserves would be exhausted.

Antibiosis ("Microbe-wars")

Antibiotics may come from other microbes, including protists, and higher eukaryotes. Their significance in aquatic food webs is not known. Some are bacteriostatic, with a temporary effect on growth, while others are bacteriocidal, and kill the target cell. Cells affected by antibiotics may appear normal, or may be enlarged, elongated, distorted, or broken up. Defenses against antibiotics include hydrolytic enzymes, mutated targets, and modified transport systems.

Virus-Related Processes Not Leading to Viral Reproduction

There are a variety of mechanisms by which viruses or viral components may damage cells without having the virus reproduce [7]. They are poorly understood, especially in natural systems. Cells that fall victim may be intact or broken up. The range of target cells may be wide or narrow.

Aggregation Followed by Sinking and Grazing

Aggregation of particles, including bacteria, is a common process in aquatic systems [2]. Such aggregates often sink, transporting material downward. Aggregates are large enough to be grazed by metazoa, including fish.

Ecological and Biogeochemical Consequences

Protists

Grazing leads to trophic transfer, and due to inherent inefficiencies, there are significant respiratory 'losses' (concomitant with nutrient regeneration). Such losses always occur in food webs, so they are not particularly high for heterotrophic microbes (i.e., the protist-bacteria part of the microbial loop is not necessarily a sink).

Viruses

As discussed extensively [5, 8, 17], the release of cell contents and progeny viruses can lead to a semi-closed cycle of bacterial uptake and release of organic matter. The net result is to oxidize organic matter (a sink) and release inorganic nutrients. Quantitative models show the result of having 50% of bacteria mortality from viruses (compared to 0%) is to increase the bacterial production and respiration rate by about one third, depriving energy from higher trophic levels and regenerating nutrients [8]. Note that the bacteria, viruses, and dissolved substances do not sink, so this process helps maintain nutrients (N, P, Fe, etc.) higher up in the water column.

Recent experiments confirm that lysis products are available to bacteria [14, 16]. In a series of experiments in California, native viral lysis products (viruses plus cell debris) were digested rapidly, but the digested tritium-labeled macromolecules were accumulated slowly,

if at all, into bacterial biomass. However, with P labeled viral lysis products in the Mediterranean (Villefranche Bay, France), almost all the label was taken up into particles within a few hours [16], consistent with P-limitation.

These results suggest that measurements of bacterial respiration rates are probably overestimates because viruses are included in most attempts to separate the bacteria from other organisms. Respiration is usually measured for a bacteria-virus mini-ecosystem that includes recycling of bacterial matter and extra respiratory losses. Similarly, bacterial gross growth efficiency is underestimated.

Viruses also affect biodiversity, because they are species-specific and density-dependent. Dense hosts are more susceptible to infection, and rare ones are less so. The net effect is that viruses work in opposite direction of competitive dominance of host organisms, so they tend to flatten out the species distribution curve and help maintain rare types [10]. Furthermore, genetic transfer may be virus-mediated [7].

Predatory Bacteria

The ecological effect is superficially similar in effect to viral lysis - a loop within the bacteria. But this is not a repeating cycle, so it is more of a one-time extra trophic transfer with respiratory losses.

Starvation

Dead starved cells are probably acceptable food for grazers, offering less nutrition. They may be counted as a cell by epifluorescence. Autolysis is a possibility, but some reports of it occurring may actually describe lysogenic induction.

Antibiosis and Non-reproductive Virus-like Processes

If cells remain intact, the ecological effect is probably like starvation, but with more nutritious food for a grazer. If the process breaks up the cell, the effect is like viral lysis, with the cell debris available for other bacteria to consume.

Aggregation/Sinking/Grazing

This transports material and nutrients to deeper waters. Microbes degrade and solubilize particles while possibly enhancing nutrition [2]. Grazing by metazoa skips intermediate trophic steps and increases trophic transfer efficiency.

Budgets

To budget mortality among the various mechanisms, rates must be quantified. Protist grazing can be quantified two ways: (A) **Fluorescently Labeled Bacteria** (FLB) can be used as tracers of the native bacterial community, so their specific clearance rate can be extrapolated to the entire community. They should ideally be the same size as the native bacteria because clearance rates vary linearly with prey diameter. Motility matters as motile cells collide more often and are grazed faster. Thus, killed FLBs can yield underestimates. (B) **Specific clearance** estimates can be applied to measured protist counts or (preferably) protist biovolume. Given the likely variability among protists, such calculations represent only an approximation. Mortality from viral infection has been measured four ways: (1) **Frequency of Infected Bacteria (FIB)** TEM is used to observe assembled viruses within the bacteria, representing the final irreversible stage of infection. To interpret the

results, a model must be used, incorporating the fraction of the infection cycle that has visibly-infected cells and also the time of the infection cycle in comparison to the cell generation time [17].

(2) **Viral Decay Rate** For this approach, production of new viruses is eliminated with a poison such as cyanide, and decay is measured [6]. Comparison to a control shows the production rate, assuming no effect of cyanide on decay.

(3) **Virus Production** The viral nucleic acid synthesis rate can be estimated from radioisotope incorporation, with either thymidine or phosphate [21], calibrated with laboratory data.

(4) **Fluorescently Labeled Native Viruses** Viruses are concentrated, labeled with SYBR Green, and added back to a sample [16]. Because the tracers are removed like ordinary viruses, but newly produced viruses are not labeled, the total counts and proportion of labeled viruses allow calculation of both loss and production rates. This method is sensitive enough for oligotrophic waters.

Methods 2, 3, and 4 require a burst size estimate to calculate bacterial mortality. Lastly, there is 1 non-specific method [19], in which bacterial DNA is labeled with tritium (with a pulse of thymidine that is all taken up). The DNA disappearance rate is then measured over time to approximate the mortality rate. It is possible to remove protist-caused mortality by size fractionation, and thus look for mortality from other causes.

There are about five studies comparing bacterial mortality and production that also partition mortality between viruses and protists. Bratbak et al. [6] made the first budget attempt in Danish coastal waters. Viral decay was used to estimate production and mortality, and FLB to estimate grazing. Virus rates alone appeared to be six times bacterial production, and grazing another two times bacterial production. It is uncertain why. Fuhrman and Noble [9] studied two mesocosms of Southern California coastal waters using multiple methods (virus production, FIB, FLB, Servais) with consistent results between methods. Thymidine and leucine agreed for estimates of production and the mesocosms allowed calculation of total mortality independent of mechanism. Budgets agreed to within about 30% (totals of protist plus virus rates were low, perhaps suggesting other mechanisms). Viruses caused about 40-50%, and protists about 50-60%, of the sum of the two mortality rates. Guixa-Boixareu et al. [11] examined solar salterns adjacent to the Mediterranean. Using FIB and FLB (with cultured, relatively large cells) they found that viruses caused a small percentage of mortality in the lowest salinity, but a high percentage in the highest salinity ponds (372 ppt), especially in the mortality of square archaea. The high salinity ponds essentially lacked protists. Steward et al. [20] carried out studies at several locations and depths in the Bering and Chukchi Seas. They used FIB for viruses, and abundance and clearance for protists. Viruses and protists had similar mortality impact (with a large range and mode near the 1:1 ratio). The viral proportion of mortality was highest in the euphotic zone. The total attributed mortality was typically half or less of bacterial production. Weinbauer and Höfle [22] studied eutrophic Lake Plussee. They looked at whole cell and thin sections FIB, correcting for ca. 25% empty cells ('ghosts'). Flagellate abundance and clearance rates from the literature were used to calculate grazing. Viruses caused 8-41% of total mortality in the epilimnion, but 51-91% in the metalimnion and 88-94% in the hypolimnion. Their budget of production and summed mortality was

reasonable in two zones - 67-128% in the epilimnion, 43-103% in the hypolimnion, but a low 22-57% in the metalimnion.

Summary

There are numerous potentially important mortality mechanisms, but of these only grazing by protists and viral lysis are well studied and quantified. Often these two mechanisms seem to account for most of the mortality, but in some instances they do not. The latter may be a true result or just a problem in accuracy. Most often the amount of mortality caused by these two mechanisms is similar in magnitude. Conditions that are inhospitable for protists (low oxygen, high salinity) obviously favor viruses. Under some conditions grazing by protists dominates, but reasons for this are unknown (perhaps relatively inhospitable for viruses?). Mortality from viruses and protists has different ecological consequences. Protists are relatively indiscriminate grazers and can pass material to higher trophic levels. Viruses are relatively host-specific, help regulate species composition, and lead to dissipation of energy and retention of nutrients in the smallest, nonsinking forms. Despite considerable research to date, there are still many unanswered questions about microbial mortality.

Acknowledgments

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