Marine Viruses: Truth or Dare

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Abstract

Over the past two decades, marine virology has progressed from a curiosity to an intensely studied topic of critical importance to oceanography. At concentrations of approximately 10 million viruses per milliliter of surface seawater, viruses are the most abundant biological entities in the oceans. The majority of these viruses are phages (viruses that infect bacteria). Through lysing their bacterial hosts, marine phages control bacterial abundance, affect community composition, and impact global biogeochemical cycles. In addition, phages influence their hosts through selection for resistance, horizontal gene transfer, and manipulation of bacterial metabolism. Recent work has also demonstrated that marine phages are extremely diverse and can carry a variety of auxiliary metabolic genes encoding critical ecological functions. This review is structured as a scientific "truth or dare," revealing several well-established "truths" about marine viruses and presenting a few "dares" for the research community to undertake in future studies.

INTRODUCTION

Phage: a virus that infects a bacterial host; short for bacteriophage

Lysogeny: lifestyle of temperate phages, which integrate into the host's genome or are maintained as plasmids until an induction event triggers the lytic cycle

The first phage isolated from the marine environment was reported more than 50 years ago (Spencer 1955), but not until the abundance of viruses was recognized in the late 1980s did scientists begin to consider their ecological impacts in the oceans (Bergh et al. 1989). It is now well accepted that viruses are abundant and ecologically important components of the marine environment. Although viruses infect all organisms from bacteria to whales, this review focuses on marine phages (viruses that infect bacteria) in the water column (for a review on benthic viruses, see Danovaro et al. 2008a). To celebrate the field of marine virology reaching its early twenties, this review plays a scientific version of "truth or dare," detailing several "truths" about marine viruses that have emerged over the past two decades of intensive study and presenting a few "dares" to the research community regarding exciting avenues for future study.

TRUTH: VIRUSES ARE THE MOST ABUNDANT BIOLOGICAL ENTITIES IN THE OCEANS

It is difficult to find a paper in marine viral ecology that does not begin with a statement about the sheer abundance of viruses. In fact, it was the recognition of the abundance of viruses that drew attention to the field of marine virology in the late 1980s (Bergh et al. 1989, Børsheim et al. 1990, Proctor & Fuhrman 1990). Before this time, the presence of viruses in seawater was known (Anderson et al. 1967, Moebus & Nattkemper 1981, Torrella & Morita 1979); however, viruses were not thought to be abundant enough to have significant ecological impacts. In the past two decades, methods for direct counts have evolved from transmission electron microscopy to epifluorescence microscopy and flow cytometry, and viruses have been enumerated from thousands of samples throughout the world's oceans (Suttle & Fuhrman 2010).

There are an average of 10^7 virus-like particles per milliliter of surface seawater. This makes viruses approximately an order of magnitude more abundant than prokaryotes, which are the second most abundant biological group (Wommack & Colwell 2000). With total estimated numbers of $\sim 10^{30}$ in the oceans, viruses are by far the most abundant predators in the marine environment. For example, compare their average concentration of 10^7 viruses per milliliter to the average number of great white sharks per milliliter, which is only 10^{-19}. Viral concentrations in the oceans are approximately 17 femtomolar (fM). By contrast, if the entire human population (6.9 billion people) went swimming in the oceans simultaneously, humans would reach a concentration of only 10^{-20} fM in the oceans. Despite their small size (~100 nm; 10–200 fg), viruses compose the ocean's second largest biomass, exceeded only by the total biomass of prokaryotes (Suttle 2005).

Viral abundance is generally highest in the euphotic zone and then decreases exponentially with depth, although subsurface maxima in viral abundance have been observed at several sites (Boehme et al. 1993, Hara et al. 1996, Parsons et al. 2011, Wommack & Colwell 2000). Typically, viral abundance is higher in coastal environments than in offshore waters (Cochlan et al. 1993, Marchant et al. 2000). Seasonal variations in viral abundance, the virus-to-bacteria ratio, and the degree of lysogeny have also been observed, demonstrating the dynamic nature of marine viral communities (Paul 2008, Weinbauer et al. 1995, Wommack & Colwell 2000).

DARE: EXPAND THE SPATIAL AND TEMPORAL SCOPE OF VIRAL STUDIES

Most studies of marine viruses examine a small number of samples, each representing a snapshot of the viral community composition and activity in time and space. Because viral and bacterial

communities are extremely dynamic in nature, snapshots of the viral community are inadequate for describing the microbial ecology of marine systems (Hewson et al. 2006), especially without an understanding of the degree of spatial and temporal variability that exists.

Temporal Variability

Since the first studies that enumerated viruses, it has been known that there are seasonal variations in viral abundance (Bergh et al. 1989, Jiang & Paul 1994). Typically, viral abundance is higher in summer and autumn than in winter (Wommack & Colwell 2000). Although seasonal variability has been demonstrated at several marine sites, few studies have followed the seasonal changes over more than one year to determine whether these patterns repeat annually. The only multiyear time series to examine viruses in the open ocean demonstrated seasonally recurring patterns in viral abundance in the Sargasso Sea that corresponded to changes in water-column stability and the distribution of specific bacterioplankton lineages (Parsons et al. 2011). Overall, marine virology would strongly benefit from the incorporation of viruses into more long-term time-series programs.

At the other end of the temporal spectrum, little work has been done to examine variability in the viral community over short time periods. A study in the Indian Ocean demonstrated that the number of culturable *Synechococcus* phages from surface waters varied significantly over a 24-h period, with a peak in abundance at 0100 hours (Clokie et al. 2006). These temporal dynamics are likely determined by host metabolic processes and sunlight-induced decay rates; however, future work needs to determine if the temporal dynamics are similar for other phage-host pairs. Winget & Wommack (2009) demonstrated significant variations in viral production rates over 24-h cycles in the Chesapeake Bay; however, they did not observe a correlation between viral production and time of day (Winget & Wommack 2009). A study in the North Sea determined that the frequency of infected cells was generally higher at night than in the daytime and suggested that infection occurred mainly during the night, with viral lysis of bacteria occurring in the afternoon (Winter et al. 2004a). Viral production and cell lysis generally corresponded with times of high bacterial activity, which the authors suggested was a strategy to optimize the number of viral progeny. Studies examining total viral abundance over diel cycles using epifluorescence microscopy have produced conflicting results in terms of the time of maximal viral abundance. In the northwest Mediterranean Sea, viral abundance was maximal at midday (1800 hours) (Bettarel et al. 2002). By contrast, studies in Tampa Bay, Florida, (Jiang & Paul 1994) and in the northern Adriatic Sea (Weinbauer et al. 1995) demonstrated that peaks in viral abundance followed peaks in bacterial abundance with no clear diel patterns. Despite these conflicting results, there is clearly strong temporal variability in viral dynamics, and differences over short time scales (i.e., the time of day that samples are collected) likely have significant implications for comparing data from viral abundance studies.

Spatial Variability

Although individual studies have examined viral distribution, diversity, and dynamics at various sites throughout the world's oceans, temporal effects and differences in methodologies make it difficult to compare these studies. Owing to the large efforts and funding required, there is a paucity of large-scale studies encompassing multiple oceanic regions. In one such study, Yang et al. (2010) used flow cytometry to examine the distribution of viruses in surface waters throughout the Pacific and Southern oceans. This study found that the integrated viral abundance in the upper 200 m of the water column was high in the subtropical and tropical regions and lower in the Antarctic region. Variation in the abundances of photoautotrophic picoplankton (*Synechococcus*, picoeukaryotes, and *Prochlorococcus*) accounted for ~57% of the variability in

viral abundance, suggesting that cyanophages are an important component of marine viral assemblages. Similarly, Bettarel et al. (2002) found linkages between *Synechococcus* and total viral abundance in the northwestern Mediterranean Sea and a decadal time series in the Sargasso Sea revealed a strong correlation between the abundances of viruses and *Prochlorococcus* (Parsons et al. 2011). By contrast, an extensive study in the Adriatic Sea determined that viral abundance was independent of autotrophic biomass or other environmental parameters, instead correlating with turnover times of the bacterial community (Corinaldesi et al. 2003). These differing results may be due to sampling location, as the correlations between viral abundance and several other biotic variables vary between different oceanic regions (Yang et al. 2010).

Although viral infection dynamics are typically assessed in "bulk seawater," the oceans are extremely heterogeneous and should be envisioned as a continuum of particles rather than as clear blue water (Azam 1998, Simon et al. 2002). Indeed, significant heterogeneity in viral abundance occurs over extremely small spatial scales. Using flow cytometry, Seymour et al. (2006) demonstrated microscale patchiness in viral and bacterial abundance at centimeter-scale spatial resolution in marine systems. Unlike the fairly stable virus to bacteria ratio (VBR) of approximately 10 that is observed on bulk scales, at the centimeter-scale, there was no correlation between bacterial and viral abundance, with VBRs varying more than tenfold (Seymour et al. 2006). The authors suggested that local areas of elevated viral abundance (i.e., "hot spots") could result from the association of viruses with patches of host organisms, the adsorption of viruses to particulate matter, or the slower diffusion of viruses relative to bacterial motility away from patches. In some cases, high local viral abundance correlated with low local bacterial abundance, potentially reflecting a recent lysis event. This study demonstrated that averaging over large spatial scales may provide an inaccurate view of phage-host interactions (**Figure 1**). Despite the challenges of sampling on small

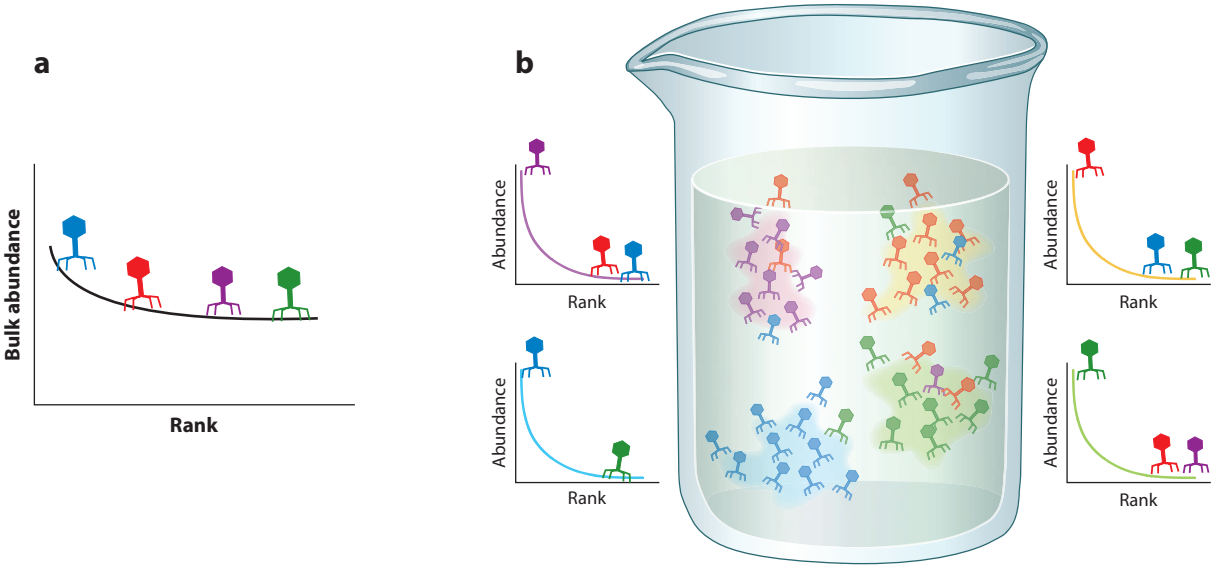

Figure 1

Parameters measured in bulk seawater samples may not accurately reflect phage-host interactions on local scales owing to the high degree of spatial heterogeneity in the oceans. (*a*) Most seawater samples appear to have extremely even viral communities; however, this may be an artifact of integrating over many microniches. (*b*) For example, consider a seawater sample that contains four particles, each dominated by a different phage type. The rank-abundance curve for the bulk sample in panel *a* does not accurately portray local phage diversity or interactions.

spatial scales, it is important to examine microscale variability in viral abundance and diversity because host distribution is highly heterogeneous (Long & Azam 2001) and patchy distributions will affect specific virus-host interactions.

The presence of viruses and infected bacterial cells on particles such as marine snow was recognized extremely early in the history of marine virology (Peduzzi & Weinbauer 1993, Proctor & Fuhrman 1991); however, methodological limitations have made the study of viral ecology on particles extremely difficult. Recent work combining confocal laser-scanning microscopy with lectin and nucleic acid staining demonstrated high viral abundances (ranging from 10^5 to 10^{11} viruses per milliliter) on suspended oceanic particles (Weinbauer et al. 2009). In a laboratory-based model system, Riemann & Grossart (2008) found that bacterial colonization of particles significantly increased phage production. Because bacteria attached to particles are typically present at higher densities and often have higher cell-specific activities than free-living bacteria (Grossart et al. 2007, Simon et al. 2002), particles may serve as hot spots of phage production in the oceans (Riemann & Grossart 2008). In addition, continued observation of these particles revealed that, after an initial lysis event, the particles were recolonized by phage-resistant bacterial strains, demonstrating that the acquisition of phage resistance may be enhanced on particles and may affect temporal phage-host dynamics (Riemann & Grossart 2008).

Overall, initial data suggest that particles serve as scavengers of viruses rather than viral factories (Weinbauer et al. 2009). The interactions between viruses, hosts, and particles are complicated, with many potential feedbacks (reviewed in Weinbauer et al. 2009). For example, viral lysis of hosts influences the formation of particles, the presence of particles affects host growth (which affects virus replication), and viral processes influence the fate of particles. If particles scavenge viruses from the water column, viral lysis among the free-living community may be reduced; however, these particles may then serve as viral reservoirs for subsequent infections. Some data suggest that viral decay rates may be high on aggregates (Simon et al. 2002, Suttle & Chen 1992). The net effect of particles on marine viral ecology is likely to be extremely variable, depending on specific characteristics of the particles (e.g., composition, quality, size, age) as well as environmental parameters such as residence time (Weinbauer et al. 2009).

The spatial scale on which marine viral communities are examined is often determined by methodological limitations (e.g., needing to concentrate many liters of water to obtain enough biomass for analysis). The degree of small-scale spatial variability in viruses is, therefore, largely unknown, and as a result, the appropriate spatial scales for examining marine viral communities have not been determined (**Figure 1**). For example, the narrow VBR that is consistently observed in bulk seawater may actually be an average of integrating over a large number of microniches in which the VBR varies drastically (Seymour et al. 2006). As methods improve, future studies need to dissect the distribution of viral particles and processes in the ocean to gain an understanding of the relevant scales for making these measurements.

TRUTH: VIRUSES ARE EFFICIENT KILLERS, AND THROUGH LYSING THEIR HOSTS, MARINE VIRUSES IMPACT GLOBAL CARBON AND NUTRIENT CYCLING

On the most basic level, viruses exist to replicate themselves, a process that most often involves killing their hosts. The majority of the viruses in the oceans are believed to be phages. By killing bacteria, phages control microbial abundance and release dissolved organic matter, influencing global biogeochemical cycles (Weinbauer 2004, Wommack & Colwell 2000). Marine viral communities are extremely dynamic, with production rates ranging from 10^8 to 10^{11} viruses per liter per day, translating into turnover times of 0.09 to 3.5 days (Jacquet et al. 2010, Weinbauer 2004,

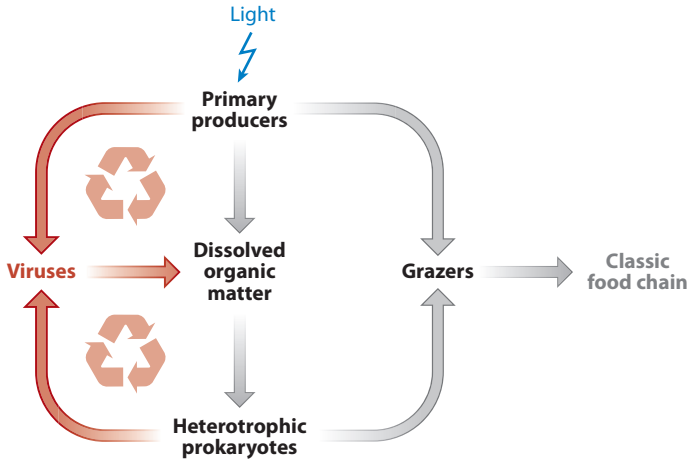

Figure 2

Simple diagram of the marine microbial food web, with the viral shunt highlighted in red. Consumption by grazers shuttles carbon up the traditional food chain into higher trophic levels. By contrast, the viral shunt produces dissolved organic matter that can be consumed by other prokaryotes, essentially serving as a marine microbial recycling program that stimulates nutrient and energy cycling.

Wommack & Colwell 2000). Each day, an estimated 10^{28} viral infections occur in the world's oceans, releasing up to 10^{9} tons of carbon from biological cells (Suttle 2007). In contrast to consumption by grazers, phage lysis of bacteria diverts carbon from the classical food chain (**Figure 2**). This process, termed the viral shunt, produces dissolved organic matter that can be consumed by other prokaryotes. It is estimated that 25% of the carbon fixed through photosynthesis cycles through the viral shunt (Wilhelm & Suttle 1999), which essentially functions as a marine microbial recycling program that stimulates nutrient and energy cycling (Fuhrman 1999, Suttle 2005, Wilhelm & Suttle 1999).

TRUTH: PHAGES ARE NOT JUST RUTHLESS KILLERS; SOME WAIT BEFORE LYSING THEIR HOSTS

Phages are most commonly thought of as killers (**Figure 3**). However, not all phages immediately lyse their hosts upon infection. During lysogeny, temperate phages form a stable interaction with their hosts, either by integrating into the bacterial chromosome or through maintenance as plasmids. The phage genome is maintained in this "silent" form of infection within a bacterial lysogen until some condition triggers the prophage to become lytic (a process termed induction).

Wilcox & Fuhrman (1994) concluded that lytic processes were more important than lysogeny in the oceans, but several studies have found inducible prophages in a high proportion of culturable marine bacteria. As determined by chemical induction, approximately half of bacterial isolates from a diverse range of marine environments contain prophages (Jiang & Paul 1994, 1998a; Leitet et al. 2006; McDaniel et al. 2006; Paul 2008). Some studies have even induced multiple prophages from single bacterial isolates, indicating that polylysogeny may be common in the oceans (Leitet et al. 2006). Bioinformatic analyses have also resulted in the identification of prophage-like elements in ~50% of marine bacterial genomes (Paul 2008). This may be an underestimate because prophage induction has been experimentally demonstrated in some marine bacteria where no prophages could be identified on the basis of sequence information (Zhao et al. 2010). Interestingly, a survey of the genomes of temperate marine phages indicated a lack of recognizable integrases, suggesting

Viral shunt: the transfer of energy and nutrients from living organisms into the dissolved phase through the process of viral lysis

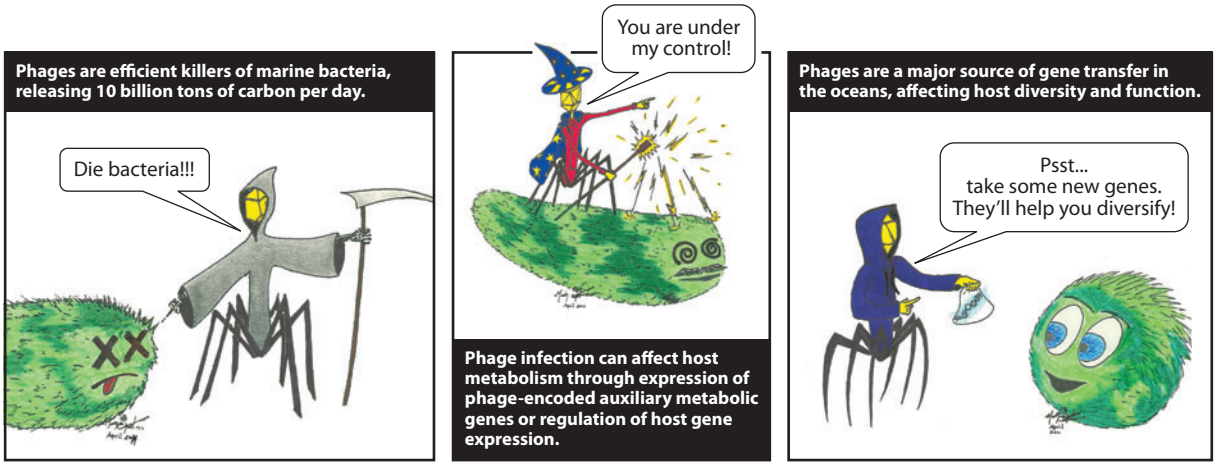

Figure 3

A cartoonist's depiction of the diversity of phage-host interactions in the oceans, ranging from cell lysis to manipulation of host metabolism to gene transfer. Illustrations by Mark Squitieri.

that unique mechanisms may be important for lysogeny in marine systems (Paul & Sullivan 2005). Accordingly, several plasmid-like prophages have now been identified in the oceans (Mobberley et al. 2008, Oakey et al. 2002).

In general, lysogeny is more prevalent during times of low host abundance and productivity. In Tampa Bay, Florida, the prevalence of lysogens is inversely related to temperature, host abundance, and bacterial and primary production (Paul 2008). Whereas lysogeny is prevalent in oligotrophic waters of the Gulf of Mexico, prophage induction is much lower in the highly productive waters of the Mississippi River plume (McDaniel et al. 2006). From the phage's perspective, lysogeny presents a survival advantage by allowing phages to "hide out" during unfavorable conditions (Paul 2008). Being intracellular, prophages are protected from UV inactivation and proteolytic digestion. Although the process is a little less straightforward, lysogeny may also be advantageous for the bacterial host (Paul 2008). Homoimmunity protects lysogens from infection by closely related phages (Brussow et al. 2004), although studies in the Gulf of Mexico have failed to find a correlation between the occurrence of prophage induction and general resistance to infection by lytic phages (McDaniel et al. 2006). Paul (2008) recently proposed that marine prophages may contribute to host survival in unfavorable environments through the suppression of unnecessary metabolic activities (**Figure 3**). In support of this hypothesis, a higher incidence of transcriptional regulatory and repressor-like proteins was found in marine prophages than in lytic phages (Paul 2008). Laboratory experiments with cultured phage-host systems have also demonstrated that lysogens have substrate utilization profiles different from those of uninfected hosts (Long et al. 2007, Paul 2008).

The factors triggering induction and controlling the lytic/lysogenic decision in marine systems are largely unknown. UV irradiation can cause prophage induction, a factor that may be especially important in surface waters. A variety of organic pollutants (pesticides and PCBs) can cause induction in natural populations of marine bacteria (Cochran et al. 1998). It has also been suggested that the concentrations of particular nutrients (e.g., phosphate) could control lysogeny in marine bacteria; however, experiments have produced contradictory results, perhaps owing to

the particular strains used or the environmental conditions under which the experiments were carried out (Paul 2008).

DARE: EXAMINE INTERACTIONS BETWEEN VIRAL INFECTION AND GRAZING

The most common link made between viruses and eukaryotic grazers (i.e., protists) is that both these predator groups account for substantial levels of bacterial mortality. Additional direct interactions exist between the two groups through intraguild predation, in which grazers feed on viruses or eukaryotic viruses can infect grazers (Miki & Jacquet 2008, 2010). Grazing is generally thought to be a less specific form of predation than viral infection; however, grazing can be selective depending on criteria such as bacterial cell size (Hahn & Hoefle 1999). By comparison, phages are often extremely host specific, and mortality due to phage infection is phylogenetically selective. High levels of grazing can reduce phylogenetic diversity among bacteria (Weinbauer et al. 2007), which may then result in higher susceptibility to phage infection (Simek et al. 2001).

Although phages and grazers are both significant top-down forces of bacterial mortality, they are often studied in isolation, with few studies examining the interactions between these two predator groups. The vast majority of research regarding linkages between viral lysis and grazing has been performed in freshwater systems. In many of these studies, the presence of grazers led to an increase in viral abundance and production (e.g., Weinbauer et al. 2007). Even though grazing leads to death of the prey, it is thought to stimulate the overall growth rate of the coexisting nongrazed cells through regeneration of nutrients and the reduction of bacterial density, which in turn reduces competition for resources (Miki & Jacquet 2010). However, the net effect of viral infection and grazing varies seasonally (Jacquet et al. 2007, Personnic et al. 2009), and in some studies the presence of grazers reduces viral activity (Maranger et al. 2002). In marine systems, most top-down predation studies have focused on the relative amount of bacterial mortality caused by phages versus protists (Boras et al. 2009, Fuhrman & Noble 1995, Weinbauer & Peduzzi 1995). Fewer studies have directly assessed the potential synergism of the two groups of predators (Bonilla-Findji et al. 2009, Longnecker et al. 2010, Ory et al. 2010).

Two recent studies have suggested that host susceptibility to grazing can be affected by current or previous interactions with viruses. A study with the coccolithophore *Emiliania huxleyi* demonstrated that cells undergoing lytic viral infection were preferentially grazed by the heterotrophic dinoflagellate *Oxyrrhis marina* (Evans & Wilson 2008). Another interesting study found measurable differences in the grazing susceptibility of a phage-resistant *Synechococcus* mutant compared with the wild-type strain (Zwirglmaier et al. 2009). This phage-resistant mutant had a modified lipopolysaccharide layer, suggesting that the changes in the cell-surface properties of the bacterial host that provided resistance to phage infection also modulated that cell's susceptibility to grazing by heterotrophic nanoflagellates. Generally, viral infection is thought to convert host biomass into the dissolved phase, where it continues to fuel the microbial loop. However, preferential grazing of infected cells would instead transfer this carbon to higher trophic levels. These studies provided unique linkages between the two modes of top-down mortality that should be investigated further.

TRUTH: PHAGES INFLUENCE BACTERIAL COMMUNITY COMPOSITION AND DIVERSITY

Through horizontal gene transfer and the lysis of specific hosts, phages influence bacterial community composition and diversity (Paul 2008, Weinbauer & Rassoulzadegan 2004). The primary

result of lysis is the removal of specific hosts from the community, but lysis can also create open niches (which may enable less-competitive species to grow) or stimulate growth of certain members of the microbial community through the release of organic substrates from biological cells into the dissolved pool (Middelboe & Lyck 2002).

The "Kill the Winner" (KtW) hypothesis extends classical Lotka-Volterra predator-prey dynamics to the microbial world by describing the interactions between phages and hosts (Thingstad & Lignell 1997, Winter et al. 2010). Bacterial communities can be thought of as containing competition specialists (r-strategists) and defense specialists (k-strategists), with both groups competing for the same limited resources. In the absence of predators, the competition specialists would dominate, sequestering all of the limiting resource. However, the presence of predators allows the defense specialists to gain a share of the resources. Under KtW dynamics, the abundance of the most active bacterial hosts is controlled by phage predation.

KtW can create cycles where the abundances of a specific bacterial host population and its corresponding phages oscillate in a classical predator-prey fashion. In this case, an increase in host abundance is followed by an increase in its phages, which then results in a decrease of the susceptible host and subsequently in a decline of the phages because they have fewer hosts to infect. However, another implication of KtW is that some of the most actively growing bacteria may be rare in ambient communities owing to high levels of phage pressure (Suttle 2007, Winter et al. 2010). Likewise, the dominant bacteria in ambient communities may contain defense specialists that are less competitive but gain a selective advantage in the environment as a result of phage predation. In support of this idea, experimental manipulations in mesocosms demonstrated that many rarer hosts became dominant when viral pressure was reduced (Bouvier & del Giorgio 2007).

The applicability of KtW in natural, heterogeneous systems is still not clear, because experiments designed to measure the effects of phage predation on marine bacterial community composition have produced highly variable results (Bouvier & del Giorgio 2007, Hewson & Fuhrman 2006, Schwalbach et al. 2004, Winter et al. 2004b). This may stem from the fact that the experiments were performed in different locations, because the balance between competition specialists and defense specialists is expected to vary on the basis of environmental conditions (Winter et al. 2010). The discrepancy between these different studies may also be due to the level of resolution on which microbial community composition was measured. There is currently no method for measuring the diversity of individual phage-host susceptibility pairs in a natural community. Instead, host diversity is often based on marker genes (such as 16S rDNA), which is problematic because phylogenetically identical bacteria can display vastly different phage-susceptibility patterns (Holmfeldt et al. 2007). Rodriguez-Brito et al. (2010) examined KtW in four aquatic ecosystems and found that, although each environment had stable species composition and metabolic potential over time, each experienced rapid changes at the level of viral genotypes and microbial strains. These results suggest that a great deal of functional redundancy exists within bacterial communities but that phage predation drives rapid fluctuations in the abundance of different strains.

Kill the winner (KtW): scenario describing the trade-offs between competition and defense specialists, where the abundance of the most active bacterial hosts is controlled by phage predation

Gene transfer agent (GTA): virus-like particles that transfer random fragments of the host bacterial genome to recipient cells in a process similar to generalized transduction

GENE TRANSFER

Gene transfer, accomplished through many different mechanisms, is another way viruses can affect bacterial community diversity (**Figure 3**). Viruses can transduce DNA between hosts, a process that is significant in the marine environment (Jiang & Paul 1998b). Viral lysis also produces a large amount of dissolved DNA, which is then available for transformation (Jiang & Paul 1995). Finally, gene transfer agents (GTAs) produced by α-proteobacteria have recently gained recognition within the marine realm (Lang & Beatty 2007, McDaniel et al. 2010, Paul 2008). Although they physically resemble small, tailed phages, GTAs indiscriminately package host genomic DNA (~4.5 kb), which is then transferred to a recipient host cell (Lang & Beatty 2007).

Compared with generalized transducing phages, which also transfer random fragments of host DNA, GTAs have a much higher frequency of transducing particles because they serve only to donate host DNA to a recipient cell without lysing that cell. McDaniel et al. (2010) demonstrated that GTAs produced by α-proteobacteria are capable of extremely high-frequency (6.7×10^{-3} to 4.7×10^{-1}) horizontal gene transfer to natural microbial communities in estuarine, coastal, open ocean, and coral reef environments (McDaniel et al. 2010).

Host range: the suite of bacterial hosts that an individual phage can infect

DARE: LINK PHAGES WITH THEIR HOSTS BY CHARACTERIZING HOST RANGE, RECEPTOR TYPE, AND RESISTANCE MECHANISMS

Existing methods for studying phage-host interactions rely heavily on having a cultured phage-host system to study in the laboratory. Because the vast majority of marine bacteria cannot be cultured using standard techniques (and the ones that are easy to obtain in culture are often not representative of the dominant bacteria in the oceans), many questions remain regarding the host range, receptor specificity, and development of resistance in natural marine environments.

Even though it is generally stated that phages are host-specific predators, the true host range for most marine phages is completely uncharacterized. Variation in the breadth of host range among cultured marine phages has been documented since the early 1980s (Moebus & Nattkemper 1981). More recent studies accentuate the complexity of marine phage-host interactions, where a single phage isolate could infect as few as one or as many as 20 host strains tested, with up to six orders of magnitude difference in the sensitivity of various hosts to the same phage isolate (Holmfeldt et al. 2007). Many cultured marine phages have extremely narrow host ranges (not infecting even closely related strains; Rohwer et al. 2000), whereas some cultured marine phages can infect multiple strains of the same species (Wichels et al. 2002), different related species (Comeau et al. 2006), or vastly different species (Chiura et al. 2009). Both phage type and isolation host seem to affect the breadth of host range observed in laboratory studies. For example, all the cyanophages isolated by Sullivan et al. (2003) on high-light-adapted *Prochlorococcus* were *Podoviridae* that had extremely narrow host ranges, whereas the cyanophages isolated on *Synechococcus* were primarily *Myoviridae* with the ability to infect a broader range of cyanobacterial hosts. Because these results are based on cultured isolates, the "natural" hosts for these phages in the water samples they were isolated from are unknown, as is the true range of hosts that they are capable of infecting in the environment. Polyvalency (i.e., the ability to infect multiple hosts) would be an effective survival strategy in the marine environment, where host densities are fairly low and host diversity is fairly high. Along these lines, a recent study of viral and host communities observed a concurrent increase in bacterial diversity and decrease in viral diversity when moving from shallow to deeper waters, suggesting that deep-sea viruses may have wider host ranges than those in surface waters (De Corte et al. 2010).

There are many mechanisms by which hosts can become resistant to phage infection, such as via the prevention of phage adsorption by altering surface receptors, destruction of incoming phage DNA using restriction-modification systems or the CRISPR/Cas system, blockage of phage infection through superinfection exclusion, and loss of both phage and host through abortive infection (Labrie et al. 2010). Studying these mechanisms is difficult in the marine environment owing to the lack of basic information on infection (such as the receptors used by most marine phages); however, studies have begun to detail the resistance mechanisms seen in cultured marine phage-host pairs. Several recent studies of phage RDJLΦ1, which infects the aerobic anoxygenic phototroph *Roseobacter denitrificans*, have provided some insight into possible resistance mechanisms. The phage DNA is unaffected by several commonly used restriction enzymes, leading to the idea that RDJLΦ1 may contain modified DNA bases designed to avoid restriction modification

HOST RESISTANCE TO PHAGE...

(Zhang & Jiao 2009). In addition, adsorption kinetics of a phage-resistant mutant suggested that adsorption inhibition was potentially responsible for resistance, and comparative proteomics of the susceptible and resistant host strains revealed significant changes in membrane proteins (Huang et al. 2010). In particular, several membrane proteins, possibly those that serve as phage receptors, were significantly downregulated in the phage-resistant strain. Adsorption kinetic assays comparing strains of *Synechococcus* resistant to one or more cyanophages suggested that resistance was most likely due to changes in host receptors that limit phage attachment (Stoddard et al. 2007). In the most comprehensive study of marine phage resistance to date, Avrani et al. (2011) analyzed 77 substrains of *Prochlorococcus* that were selected for resistance to 10 podophages. The majority of the resistant strains contained mutations in nonconserved, horizontally transferred genes involved in phage attachment to the cell surface that were localized in a hypervariable genomic island. Adsorption assays confirmed impaired phage attachment to the resistant *Prochlorococcus* strains. Both the Stoddard et al. (2007) and Avrani et al. (2011) studies demonstrated that selection for resistance to one phage sometimes resulted in cross-resistance to other phages. Cross-resistance to multiple phages through surface-receptor modification would be highly advantageous in the marine environment, where bacteria are exposed to a high diversity of phages (**Figure 4**).

Phage resistance that is accomplished through loss or modification of receptors can negatively affect host metabolism (e.g., by reducing uptake of certain nutrient sources), thereby decreasing the competitiveness of the host (Bohannan et al. 1999). Although one could imagine that this is especially critical in the low-nutrient marine environment, the costs of phage resistance in the oceans are not well described. In a chemostat experiment with a marine *Flavobacterium*, evolving resistance to phage infection was associated with a reduction in the ability of the host to utilize various carbon sources (as measured by Biolog assays) (Middelboe et al. 2009). This suggests that one cost of phage resistance may be reduced physiological capacity and shows that phage infection can generate functional diversity within a host population. Lennon et al. (2007) demonstrated a cost of resistance in phage-resistant *Synechococcus* equivalent to an approximately 20% reduction in fitness compared with susceptible strains. In addition, this cost of resistance was dependent

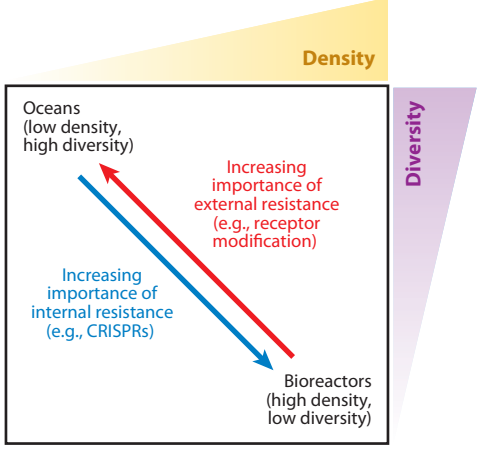

Figure 4

Hypothesis describing how the levels of phage and host diversity and density may influence the importance of different phage-resistance mechanisms in an environment. External resistance mechanisms such as receptor modification may be more important in low-density, high-diversity environments such as the oceans than internal resistance mechanisms such as clustered regularly interspaced short palindromic repeats (CRISPRs).

on the particular phages to which the strain had acquired resistance, suggesting that the overall effects of resistance on *Synechococcus* are influenced by the composition of the co-occurring phage community. In addition to a reduction in growth rate, Avrani et al. (2011) demonstrated a novel "enhanced infection dynamics" cost of resistance in *Prochlorococcus*, where certain phage-resistant mutants were subject to more rapid infection by other phages.

Clustered regularly interspaced short palindromic repeats (CRISPRs): adaptive microbial immune system that can provide resistance to phage infection

Recently, clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR-associated (Cas) genes were demonstrated as an adaptive microbial immune system that can yield resistance to phage infection (reviewed in Horvath & Barrangou 2010). CRISPR/Cas-mediated immunity has been documented in a wide range of ecosystems; however, the majority of these studies have focused on extreme environments (Tyson & Banfield 2008) or high-density bioreactors (Barrangou et al. 2007). The CRISPR/Cas system is highly specific, creating the hypothesis that this mechanism of resistance may dominate in environments with high host densities and/or low diversity, because both of these scenarios result in frequent contact between the same phages and susceptible hosts (**Figure 4**). However, the oceans have low density and high levels of diversity, and the importance of the CRISPR/Cas system in the marine environment is largely unknown. A bioinformatic survey of metagenomic data from the Global Ocean Sampling Expedition demonstrated almost 200 reliable CRISPR cassettes, at a total density similar to that observed in completely sequenced genomes (Sorokin et al. 2010). However, the highest densities of CRISPRs occurred in a hypersaline sample (Punta Cormorant Lagoon, Floreana) and a freshwater sample (Lake Gatun), both of which were relatively closed environments (Sorokin et al. 2010). CRISPR/Cas systems have been detected in the genomes of some cultured marine bacteria (Thomas et al. 2008), yet these genes appear to be underrepresented among the most abundant genomes found in marine metagenomes (Lauro et al. 2009, Yooseph et al. 2010). In cases where the CRISPR/Cas system is present in the marine environment, it presents a unique opportunity for following the history of phage infections and linking phage sequences with the hosts they infect (Andersson & Banfield 2008).

Insight into the mechanisms by which marine bacteria develop resistance to phages can also be gained through scaffolding of metagenomic sequences onto the complete genomes of cultured bacteria. Rodriguez-Valera et al. (2009) examined "metagenomic islands" (areas of bacterial genomes that are absent or underrepresented in metagenomes) and discovered that these were dominated by genes whose products are expressed extracellularly, which are likely to be phage receptors (e.g., the variable O chain of the lipopolysaccharide, exopolysaccharide biosynthesis clusters, genes involved in sugar modifications of extracellular structures). The high level of divergence among genes encoding potential phage receptors was seen in a variety of marine microbes, including extremely compact genomes such as "*Candidatus* Pelagibacter ubique," suggesting that avoiding phage predation is critical in the marine realm. A number of intracellular genes for phage defense (e.g., restriction-modification systems and CRISPRs) were also found to be highly variable. These bioinformatic analyses led to the proposal of the constant-diversity dynamics model, in which the diversity of prokaryotic populations is maintained by phage predation, because the best-adapted microorganisms are selected against by density-dependent phage predation (Rodriguez-Valera et al. 2009). Experimental evidence indicating that genes involved in phage attachment to the cell surface are preferentially located in genomic islands was recently provided for *Prochlorococcus* (Avrani et al. 2011). The diversity generated in these genomic islands by selective pressure for phage resistance effectively reduces the size of the susceptible host population for a given phage, facilitating phage-host coexistence in the environment. The intense bacterial-phage warfare seen from the side of the bacterial population is likely matched with corresponding rapid evolution in marine phage genomes. A study comparing multiple closely related strains of roseophages isolated over a 12-year period demonstrated that, although the genomes shared 97% overall nucleotide

identity, the largest number of differences between the strains occurred in the region encoding tail proteins, which are often involved in receptor binding and host specificity (Angly et al. 2009).

TRUTH: MARINE VIRUSES ARE INCREDIBLY DIVERSE

Because viruses often exhibit high levels of host specificity, it is critical that we understand the types of viruses present in the oceans. Examination of cultured phages from the marine environment has provided significant insight into marine phage ecology (Paul & Sullivan 2005), but it is estimated that >99% of all environmental bacteria cannot be cultured using standard techniques. Furthermore, the bacteria that grow readily in the laboratory are rarely the dominant bacteria present in the natural environment, and not all phages produce identifiable plaques on bacterial lawns. Amplification and sequencing of the 16S rDNA gene revolutionized our understanding of bacterial diversity (Olsen et al. 1986). However, there is no single genetic element that is conserved in all phage genomes that can be used in a similar manner (Rohwer & Edwards 2002). The small size and low DNA content of viruses also pose significant barriers to microscopic and molecular studies of diversity.

The recent examination of viral communities without the biases associated with culturing has significantly advanced our understanding of marine viral diversity. These methods include pulsed-field gel electrophoresis to separate viruses on the basis of genome size (Steward et al. 2000), amplification of "signature genes" conserved among a group of viruses (Breitbart & Rohwer 2004), randomly amplified polymorphic DNA PCR (Winget & Wommack 2008), and metagenomic sequencing (Angly et al. 2006). Regardless of methodology, these studies have revealed a great wealth of viral diversity and demonstrated that the vast majority of this diversity is novel. In addition, these studies have shown that many dominant marine viruses are not well represented in culture collections (Breitbart & Rohwer 2004). Environmental viruses are almost assuredly the largest reservoir of genetic diversity on the planet.

The biogeography of individual viruses or viral sequences is an unresolved question. As a result of methodological constraints, most studies of viral diversity examine only a limited number of samples. Even in a single sample, viral diversity is extremely high. For example, the first metagenomic survey of the viral community in a 200-liter sample of surface seawater predicted the presence of several-thousand viral genotypes (Breitbart et al. 2002). The viral community was extremely even, with the most abundant virus composing only 2%–3% of the total community (**Figure 1*a***). To extrapolate to an understanding of viral diversity in the oceans, it is necessary to know whether the 200-liter water sample is representative of that oceanic region and how similar viral communities from different oceanic regions are to each other. If each water sample contains a distinct viral community, then overall global marine viral diversity will be astronomically high; however, if there is significant overlap between the samples and regions, then global diversity (though still extremely high) will be much lower. To address this question, Angly et al. (2006) sequenced viral metagenomes consisting of more than 180 samples from four oceanic regions. Although each region contained a distinct viral community structure (e.g., cyanophages dominated in the Sargasso Sea and prophages dominated the Arctic sequences), the vast majority of the viral genotypes were common to all the locations (Angly et al. 2006). Therefore, all marine locations appear to share access to a common gene pool, supporting previous findings that identical phage sequences are found in geographically distant marine samples (Breitbart & Rohwer 2004, Short & Suttle 2005). Despite extremely high levels of diversity on local scales, a shared marine viral gene pool constrains global levels of viral diversity, with the differences between sites driven by enrichment of specific viral types through local selective pressure (Angly et al. 2006, Breitbart & Rohwer 2005).

GLOBAL GENE POOL SOMEWHAT MYSTERIOUS, NO?

DARE: SEARCH FOR GROUPS OF VIRUSES THAT TRADITIONAL METHODS MAY HAVE OVERLOOKED

Single-Stranded DNA Viruses

The general consensus is that most marine viruses are double-stranded DNA (dsDNA), tailed phages belonging to the *Podoviridae*, *Myoviridae*, and *Siphoviridae* families (Weinbauer 2004, Wommack & Colwell 2000), the vast majority of which contain genomes ranging from 25 kilobases (kb) to 70 kb in size (Steward et al. 2000). However, the recent discovery of single-stranded DNA (ssDNA) viruses in different marine environments suggests these viruses are more widespread than previously recognized.

Owing to methodological limitations, the ssDNA subset of the viral community has been overlooked in marine viral ecology, potentially leading to an underestimation of viral diversity and abundance. Because the small genomes of ssDNA viruses (<9 kb) produce a weak fluorescence signal below the detection limit of current instruments, these viruses are most likely not enumerated during viral counts performed with epifluorescent microscopy or flow cytometry (Tomaru & Nagasaki 2007). ssDNA viruses would also be overlooked in many diversity studies because their small size and circular genomes prevent their inclusion in pulsed-field gel electrophoresis studies, their small particle size may lead to loss during concentration by tangential flow filtration, and early large-scale metagenomic surveys of marine viruses recovered only dsDNA genomes.

As early as 2005, marine ssDNA viruses were obtained in culture. The first ssDNA virus to be discovered in the marine environment was the *Chaetoceros salsugineum* nuclear inclusion virus, which infects a bloom-forming diatom (Nagasaki et al. 2005). Subsequently, other ssDNA viruses have been isolated from the marine environment, including a prophage induced from a *Synechococcus* isolate from the Gulf of Mexico (McDaniel et al. 2006) and another diatom virus (Tomaru et al. 2008). Knowledge of ssDNA viruses in the oceans was significantly expanded through the incorporation of multiple-displacement amplification (MDA) into the preparation of viral metagenomes. Although originally touted as unbiased, MDA selectively amplifies circular ssDNA genomes by 2–3 orders of magnitude in a mixed community (Kim et al. 2008). In the past five years, MDA and sequencing of viral metagenomes have led to the discovery of novel ssDNA viruses (both phages and eukaryotic viruses) in different marine environments, including corals (Vega Thurber et al. 2008), stromatolites (Desnues et al. 2008), estuaries (Rosario et al. 2009), and the open ocean (Angly et al. 2006, Rosario et al. 2009, Tucker et al. 2011). The marine ssDNA viruses are only distantly related to known viruses, and some contain previously undescribed genome architectures (Nagasaki et al. 2005, Rosario et al. 2009, Tucker et al. 2011). The hosts for the ssDNA viruses identified through metagenomics remain unknown, and as with any newly discovered group, there are numerous unanswered questions regarding the abundance, diversity, distribution, and ecology of marine ssDNA viruses. Initial data suggest that marine ssDNA phages may have narrower geographic and depth distributions than those of dsDNA phages (Tucker et al. 2011), suggesting fundamental differences in distribution between ssDNA and dsDNA phages, possibly owing to differences in host range, host distribution, virion stability, or evolution rates and mechanisms.

RNA Viruses

For many years, essentially nothing was known about RNA viruses in the oceans. Numerous RNA viruses have now been obtained in culture (Nagasaki 2008), and PCR and metagenomic studies have revealed a high diversity of RNA viruses in marine systems (Culley et al. 2003, 2006). The diversity and roles of RNA viruses in the oceans were recently the subject of an excellent review (Lang et al. 2009), so the topic is not addressed here in any detail. However, it is noteworthy that

the vast majority of marine RNA viruses described to date infect eukaryotes (reviewed in Lang et al. 2009, Nagasaki 2008), with only one documented RNA phage (Hidaka & Ichida 1976).

Archaeal Viruses

Although originally thought to thrive only in extreme environments, archaea have been detected throughout the marine water column. Archaeal abundance increases with depth, and archaea can comprise the majority of the prokaryotic community below 1,000 m (Karner et al. 2001). Extrapolations from measurements of archaeal abundance in depth profiles suggest that the world's oceans contain approximately 10^{28} archaeal cells (Karner et al. 2001). Despite their abundance in the oceans, there are currently no published studies documenting the impact of viruses on marine archaea. This research is hindered by the difficulty of obtaining mesophilic marine archaea in pure culture. To date, the only sequenced marine archaeal virus infects the hyperthermophilic euryarchaeota *Pyrococcus abyssi*, which was isolated from the deep sea (Geslin et al. 2007). Virus PAV1 is a lemon-shaped particle with an 18-kb dsDNA circular genome that is present as a high-copy plasmid within the host cytoplasm and released spontaneously without lysing the cell (Geslin et al. 2007).

WEIRD!!!

Auxiliary metabolic genes (AMGs): phage-encoded metabolic genes that were previously thought to be restricted to cellular genomes

TRUTH: MARINE PHAGES CARRY AUXILIARY METABOLIC GENES THAT ENHANCE THEIR SUCCESS IN THE OCEANS

An exciting advance in marine virology is the discovery of auxiliary metabolic genes (AMGs), which are phage-encoded metabolic genes that were previously thought to be restricted to cellular genomes (Breitbart et al. 2007) (**Figure 3**). Typically, AMGs represent critical, rate-limiting steps of host metabolism, and it is believed that expression of these genes is critical to the success of certain phages in the oceans. AMGs involved in photosynthesis [e.g., genes involved in photosystem II (*psbA*, *psbD*), photosynthetic electron transport (PTOX, *petE*, *petF*), photosynthetic pigment biosynthesis (*ho1*, *pebS*, *cpeT*, *pcyA*), and synthesis of high-light inducible proteins (*hli*)], nucleotide metabolism [e.g., genes involved in ribonucleotide reduction (*nrdA*, *nrdB*, *cobS*), purine biosynthesis (*purH*, *purL*, *purM*, *purN*), and pyrimidine biosynthesis (*pyrE*, *thyX*)], carbon metabolism [e.g., genes involved in the pentose phosphate pathway (*talC*, *gnd*, *zwf*)], phosphate metabolism (e.g., *phoA*, *phoH*, *pstS*), and stress response (e.g., *mazG*) have been found embedded in phage genomes with clear relationships to well-studied phages that infect *Escherichia coli* (Millard et al. 2009; Rohwer et al. 2000; Sullivan et al. 2005, 2010; Thompson 2010; Weigele et al. 2007). Analysis of metagenomic sequences has subsequently expanded the list of known AMGs to include genes involved in other pathways such as photosystem I, antioxidation, and translational/posttranslational modification (Dinsdale et al. 2008; Sharon et al. 2009, 2010; Williamson et al. 2008b).

Perhaps the best-studied AMG is the *psbA* gene found in a large proportion of cyanophage genomes (Lindell et al. 2004, Mann et al. 2003, Millard et al. 2004, Sullivan et al. 2006). *PsbA* encodes the D1 protein, which is part of the photosystem II reaction center and, owing to its rapid turnover rate, is often a rate-limiting step of photosynthesis. Expression of the phage *psbA* is believed to replace damaged host D1 proteins, helping to maintain photosynthesis during the phage lytic cycle and to provide more energy for phage production. During infection, the inhibition of host transcription leads to a decrease in host-encoded D1 proteins, which is countered by an increase in the expression of the phage-encoded *psbA* (Lindell et al. 2005). Studies amplifying cyanophage *psbA* from aquatic samples demonstrate that this gene is widespread in both marine and freshwater systems and that *psbA* sequences can discriminate phages on the basis of their type (i.e., myoviruses versus podoviruses) as well as their host (i.e., *Prochlorococcus* versus *Synechococcus*) (Chenard & Suttle 2008). Phages can carry these genes between environments, ensuring their own

success while acting as genetic reservoirs that maintain diversity and contribute to lateral gene transfer (Sullivan et al. 2006).

Bathypelagic: deep waters of the ocean, typically between 1,000 m and 4,000 m

A modeling study by Bragg & Chisholm (2008) predicted that expression of the cyanophage *psbA* could produce up to a 4.5% increase in the number of phage genomes produced during a lytic cycle; however, that advantage was approximately equal to the cost of increasing the genome length to include the *psbA* gene. However, under higher irradiance conditions where protein D1 would be degraded more rapidly, expression of the phage *psbA* could present a greater advantage. Therefore, the fitness advantage for a phage to encode the *psbA* gene varies greatly depending on infection conditions, ranging from significantly positive under high-light stress to negative if infection occurs in the dark.

Some AMGs have been studied in detail, with the goal of understanding their functional roles during infection and the resulting ecological effects. Several studies have shown that the phage AMGs mimic their known functional role in the host, in some cases accomplishing a given function more efficiently (Dammeyer et al. 2008). However, the function of many AMGs in the phage genomes has yet to be assessed experimentally. Most AMGs seem related to sustaining the host's metabolism, providing more energy for phage production. Genes are more likely to be found in environments where their products will present fitness advantages. Although many cyanophages contain the AMGs described above, not all do, and the distribution of AMGs among different phage genomes is not well understood. It has been suggested that phages with short latent periods are less likely to contain AMGs because they spend a minimal amount of time in the host cell (Bryan et al. 2008). Many phage AMGs do not cluster phylogenetically with their host counterparts (i.e., the phages form distinct subclusters from their hosts), suggesting that the AMGs are not acquired directly from their primary hosts and that all phages may share access to a common gene pool (Bryan et al. 2008).

DARE: EXAMINE VIRAL DYNAMICS IN THE BATHYPELAGIC

Approximately 70% of the total volume of the oceans is located deeper than 1,000 m (Nagata et al. 2010). However, our knowledge of viral processes in the deep sea is significantly weaker than that of such processes in surface waters, mostly as a result of difficulties in obtaining and processing samples. Deep-sea virology has been a topic of recent increased research focus, with several interesting findings. Although hosts are at least an order of magnitude less abundant in bathypelagic waters than in surface waters (Tanaka & Rassoulzadegan 2002), viral abundances still remain relatively high, in the range of 0.6 to 60 $\times$ 10^8 viruses per liter (Nagata et al. 2010, Parada et al. 2007). This results in a high VBR in the bathypelagic waters of some oceanic regions (Nagata et al. 2010, Parada et al. 2007). The mechanism for maintaining high viral abundances in the bathypelagic is not clear because limited studies indicate that the decay rate of viruses in the deep sea is higher than can be supported by rates of viral production, which are believed to be relatively low (Parada et al. 2007). One potential viral input to the deep sea is through sinking particles, which may transport large numbers of viruses (Hara et al. 1996, Parada et al. 2007). Interestingly, deep-sea sediments exhibit extremely low VBRs (often <1) (Danovaro et al. 2002), providing an exception to the high VBR in the deep sea.

Determining relative rates of viral lysis and grazing is a challenging task anywhere in the oceans, but especially so in the bathypelagic, owing to the difficulty of replicating deep-sea conditions for incubations. Despite this obstacle, initial studies examining bacterial mortality in the bathypelagic waters of the Mediterranean Sea suggest that grazing rates by heterotrophic nanoflagellates are significantly higher than viral-induced mortality (Fonda-Umani et al. 2010). However, further research is needed because the relative impact of grazing versus viral lysis differed significantly

between locations (Fonda-Umani et al. 2010). The relative roles of viral infection versus grazing have not been examined concurrently in deep-sea sediments; however, a worldwide study of deep-sea sediments demonstrated high levels of viral production, with virus-induced bacterial mortality of approximately 90% at depths below 1,000 m (Danovaro et al. 2008b).

Very little is known about the diversity of viruses in the deep sea. A study utilizing RAPD-PCR (random amplified polymorphic DNA–polymerase chain reaction) demonstrated that the composition of bathypelagic viral communities changed substantially over time (Winter & Weinbauer 2010). When comparing samples from throughout the water column, the strongest linkages between changes in viral and host communities were observed in the bathypelagic region, suggesting codevelopment of virus and host communities (Winter & Weinbauer 2010). Linkages between viral and host communities are most often driven by the production of lytic viruses, and a study in deep-sea sediments suggested that the vast majority of viral infections were lytic (Danovaro et al. 2008b). However, several studies have described high prophage induction frequencies in both bathypelagic waters and deep-sea hydrothermal vents, suggesting that lysogeny may be an important lifestyle in the deep sea (Weinbauer et al. 2003, Williamson et al. 2008a).

SUMMARY POINTS

1. At concentrations of approximately 10^7 viruses per milliliter of surface seawater, viruses are the most abundant biological entities in the oceans. There are an estimated 10^{30} viruses in the global oceans.
2. Viruses are efficient killers. Through lysing their bacterial hosts, marine phages control bacterial abundance and impact global biogeochemical cycles.
3. Phages influence their hosts in a myriad of ways, including cell lysis, DNA transfer, and manipulation of host gene expression and metabolism. In addition to having effects on the about-to-be-lysed hosts, phages influence the remaining community through the release of dissolved organic matter, the introduction of novel genetic material, and the selection for resistance.
4. Marine viruses are extremely diverse and can carry a variety of auxiliary metabolic genes encoding critical ecological functions. Recent work suggests that all marine viruses share access to a common gene pool, with local conditions and host composition selecting for the dominant viruses at different sites.
5. Over the past two decades, marine virology has progressed from a curiosity to a well-established scientific field with critical importance for oceanography. In addition, viruses in the oceans have been studied more extensively than those in other ecosystems, so marine virology has the potential to inform numerous other fields, ranging from the ecology of other ecosystems (e.g., freshwater, terrestrial) to medicine (e.g., studies of the human microbiome). With so many significant breakthroughs over the past 20 years, there is no telling what the future of this field holds!

FUTURE ISSUES

1. The spatial and temporal variability of marine viral communities are not well characterized. Future studies should expand the spatial and temporal scales on which viral dynamics are measured to capture small-scale heterogeneity and elucidate large-scale patterns.

2. Although numerous studies have addressed the relative importance of phages versus grazers on bacterial mortality, there is a paucity of data regarding the interactions between viral infection and grazing in the marine environment. Future research should examine the potential synergism between the predator groups.
3. A rapid increase in the knowledge of both viral and bacterial diversity in the marine environment has been gained in recent years. However, our understanding of the linkages between phages and their susceptible hosts is lacking as a result of the inability to culture most marine bacteria. Future work should aim to develop novel methods for measuring host specificity, identifying phage receptors, and determining the mechanisms and costs of resistance without the requirement of culturing.
4. Knowledge of marine viruses is highly biased toward dsDNA phages that infect culturable bacteria. Future studies should target viruses with other nucleic acid types (e.g., RNA or ssDNA viruses) and attempt to discover viruses infecting alternate hosts (e.g., mesophilic marine archaea).
5. Viral dynamics in the harder-to-reach portions of the ocean (e.g., the bathypelagic) are less understood than those in surface waters. Recent work has suggested that deep-sea viral communities are dynamic and important components of the marine ecosystem, and this realm needs further exploration.

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The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Errata

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