

Trade-Offs between Competition and Defense Specialists among Unicellular Planktonic Organisms: the “Killing the Winner” Hypothesis Revisited

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INTRODUCTION

Organisms face the dilemma of how to allocate the limited resources available to them. In general terms, resources can be used to increase growth and reproduction or to counteract stress (e.g., predators, parasites, decay, unfavorable environmental conditions, or competitive ability). Thus, substantial investments into stress defense or avoidance strategies may result in a reduction in growth and reproduction and vice versa (39). This trade-off has been reported frequently in the literature for plants (27, 90) and for animals such as insects (22, 49), sponges (111), or birds (9, 97). It is less well known that such a trade-off between reproduction and stress defense holds even for viruses infecting prokaryotes (organisms lacking a nucleus, comprised of the domains *Bacteria* and *Archaea*; this grouping does not imply a phylogenetic relationship). De Paepe and Taddei (23) have shown that the mortality of a diverse array of viruses infecting *Escherichia coli* (viruses infecting prokaryotes are often referred to as phages) is positively correlated to their multiplication rate. In other words, viruses with a high multiplication rate degrade quickly, because under resource-limited conditions these viruses cannot invest higher levels of resources into the stability of their capsids but rather invest them

into numbers of offspring. Those authors further demonstrated that the capsid thickness and the density of the packaged genome in the virus capsids account for the majority of the variation in mortality between the different viruses. Thus, De Paepe and Taddei (23) gave a mechanistic explanation for the trade-off between reproduction (multiplication rate) and stress defense (capsid thickness and density of packaged genome exerting pressure onto the capsid) in a very simple biological system. Nyström (67) went a step further and proposed that the trade-off between growth and stress (maintenance of cellular functions) in cells of *Escherichia coli* is facilitated by the intracellular availability of RNA polymerases (a limited resource) and the competition between different transcription factors. If correct, the hypothesis by Nyström (67) would extend the concept toward the molecular level. Given the many examples of a trade-off between strategies maximizing growth and minimizing losses, from the very small and simple to the very large and complex, one cannot avoid concluding that such a trade-off appears to be a fundamental property of evolving biological entities existing in environments with limited resources (10).

More than 20 years ago it was discovered that conventional culturing techniques grossly underestimate the abundance and number of different types (richness) of prokaryotes in natural environments (“great plate count anomaly”) (88). Soon thereafter it became evident that viruses are abundant and active members of aquatic food webs and constitute a major source of mortality for prokaryotes (7, 73). Since those early days, tremendous improvements in our understanding of the roles of

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both planktonic prokaryotes (4, 47) and viruses (91, 100) have been made. However, one can still claim that our understanding of the mechanisms regulating prokaryotic and viral abundance, community composition, and population dynamics in natural aquatic environments remains inadequate.

The concept of negative frequency-dependent selection states that the fitness of an organism (e.g., success in reproduction) decreases as its frequency (relative abundance) increases. If correct, this mechanism selects for rare types and thus maintains high diversity (see, e.g., references 28 and 99). Soon after the discovery of high viral abundance in the ocean, it was argued that viruses might be instrumental in maintaining high prokaryotic richness (29, 93), because the viral infection rate depends on, among other parameters, the abundance of host cells. Thus, as suggested by negative frequency-dependent selection, abundant prokaryotic types will be exposed to strong viral pressure. Eventually, this and other ideas were incorporated into what is now known as the “killing the winner” hypothesis (KtW) (92, 94), where “winner” refers not necessarily to the most abundant but to the most active prokaryotic population. KtW was mathematically formalized in the context of an idealized food web comprised of prokaryotes, viruses, and protozoans grazing nonselectively on prokaryotes and is based on Lotka-Volterra-type equations (8). The concept has some potential as a testable theoretical framework that can be challenged with observations and experiments, and it has indeed been used as such by a number of investigators. However, in many cases the comprehension of KtW appears to be based more on a heuristic and not always correct understanding than on an appreciation of the assumptions and limitations of the highly idealized mathematical versions of the hypothesis. For example, the concept of negative frequency-dependent selection is very appealing and can be understood almost intuitively; however, it is only one aspect of KtW, albeit the most prominent one. In order to further stimulate a fruitful dialogue between experimental or observational work and the development of the conceptual framework, our objectives are (i) to review the basic ideas and assumptions of KtW; (ii) to conceptually extend the framework by introducing size- or shape-selective grazing by protozoans, leading to a Russian doll-like hierarchy with KtW principles at work on different levels; (iii) to evaluate which aspects of present experimental and observational knowledge appear to be in agreement or disagreement with KtW; (iv) to point out fundamental limitations of KtW in its present form; and (v) to list challenges for the future that currently prevent a thorough test of the theory.

BASIC IDEAS AND ASSUMPTIONS OF KtW

KtW in its general form is illustrated in Fig. 1. The assumption is that two populations (competition specialist and defense specialist) compete for a shared limiting resource (e.g., phosphate). This limiting resource either can exist in the free form that is directly available to the two competing populations or is being sequestered by the competitors and a predator or parasite in the form of biomass. Heuristically, one can see that if the selective loss of the competition strategist from predation or parasitism prevents this population from sequestering all of the resource, there will be more of the limiting resource available to the defense specialist. The optimal strategy in terms of

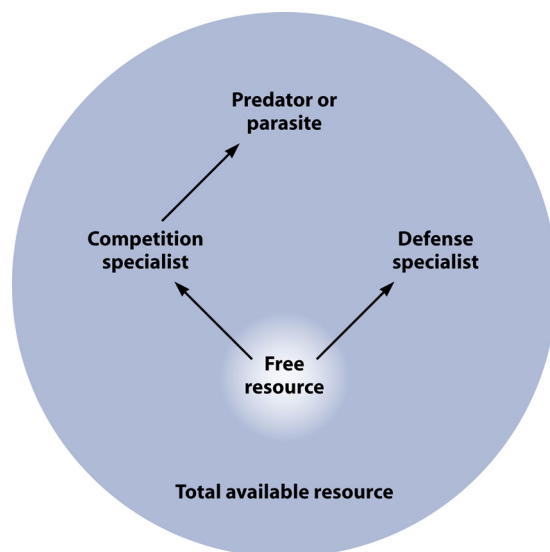


FIG. 1. Schematic of the general structure of the “killing the winner” hypothesis.

dominating biomass thus depends on the environmental conditions. In an oligotrophic environment (low total available resource level) the competition specialist would be expected to dominate, whereas in a eutrophic environment (high total available resource level) the system would be dominated by the defense specialist. It is important to realize that the “winner” in KtW refers to the competition specialist, which may or may not correspond to the most abundant population. This simple three-population structure (Fig. 1) contains some of the basic elements that need to be incorporated into a general ecological framework. It links trophic interactions to biogeochemistry and food web dynamics to organism properties and strategies. Thus, KtW appears to be relevant for the present debate on a trait-based ecology (30, 54). Since it also contains the arms race perspective between the three populations, there is a clear evolutionary aspect implicit in this generalized structure. By allowing the two populations to coexist on one common limiting resource, KtW provides a simple solution to Hutchinson’s paradox (40) without the need to invoke particular assumptions about spatial and/or temporal heterogeneities.

KtW ON DIFFERENT LEVELS: A RUSSIAN DOLL-LIKE HIERARCHY

Not surprisingly, most attention to KtW has come from studies of the influence of lytic viruses on their host populations (see, e.g., references 11, 12, and 13). However, KtW in its general form (Fig. 1) is much more widely applicable. In the immediate trophic neighborhood of the pelagic food web, the prokaryote-phytoplankton-protozoan case has been explored both theoretically and in an idealized experimental system (70, 95). In more complex systems, the balance between edible and inedible populations as representatives of the competition and defense specialists, respectively, has been explored for the prokaryote-protozoan case (46, 71), as well as for the phytoplankton-mesozooplankton case (58). Thus, the principle appears to work, not only at different trophic levels in the food web but also for different levels of

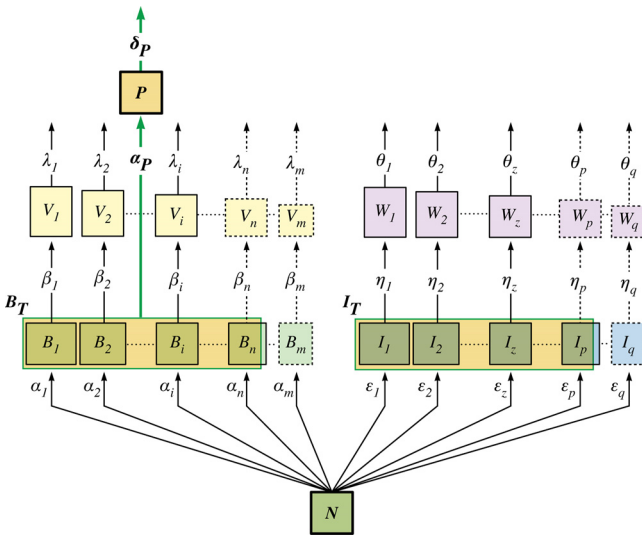


FIG. 2. Schematic of the mechanisms underlying the “killing the winner” hypothesis, also incorporating size- or shape-selective grazing by protozoa. There are potentially m different edible and q different inedible prokaryotic types that are infected by as many different virus types. The populations B_n and I_p do not grow fast enough to sustain stable virus populations V_n and W_p (indicated by excluding part of the rectangles referring to the population sizes of B_n and I_p). Thus, population B_n is only subject to mortality due to grazing, and I_p is controlled solely by resource limitation. Populations not able to establish stably in the system are indicated by dashed lines. The meanings of the symbols are given in Table 1.

aggregation (e.g., species versus communities of edible and inedible types). Based on this observation, we conceptually expand the system analyzed by Thingstad (92) by introducing size- or shape-selective grazing due to protozoa, as illustrated in Fig. 2. While host-specific parasitism allows different prokaryotic types to coexist on one limiting resource, selective grazing by protozoa leads to the establishment of a group of inedible prokaryotic types (subject to resource availability). However, in natural environments selective grazing is likely to lead to a number of different groups of populations differing in the degree of their accessibility to the selective predator; thus, Fig. 2 illustrates the simplest case. The basic requirement is that the sum of the biomasses of all organisms in the system and the amount of free resource N must equal the amount of the total available resource N_T (Table 1 gives a list of symbols used in the formulas and their meanings):

$$N_T - N - P = B_T + V_T + I_T + W_T \quad (1)$$

Using the equations given by Thingstad (92) for the total biomass of edible prokaryotes (B_T) and their viruses (V_T) and following analogous arguments for the case of inedible prokaryotic types (I_T) and their viruses (W_T), we get

$$N_T - N - P = \left(\frac{\delta_p}{Y_p \alpha_p} \right) + \left(N \sum_{i=1}^{n-1} \frac{\alpha_i Y_{B_i} - \alpha_n Y_{B_n}}{\beta_i} \right) + \left(\sum_{z=1}^{p-1} \frac{\theta_z}{\eta_z Y_{W_z}} + I_p \right) + \left(N \sum_{z=1}^{p-1} \frac{Y_{I_z} \epsilon_z}{\eta_z} \right) \quad (2)$$

The system in Fig. 2 is analogous to a Russian doll, where KtW

principles are at work on an inner level (viruses) and an outer level (protozoan grazing). More intriguing, this hierarchy bears a resemblance to the self-similarity principle of fractal geometry (57), where the same structure is repeated at different levels of resolution within the system.

PREDICTIONS OF KtW

In this section we use key equations describing the biomasses of specific prokaryotic and viral populations in the case of nonselective (92) and size- or shape-selective protozoan grazing (Fig. 2) together with the equations for total prokaryotic and viral biomass given above to evaluate the evidence for and against specific predictions of KtW.

$$B_i = \frac{\lambda_i}{\beta_i Y_{V_i}} \quad (3)$$

$$I_z = \frac{\theta_z}{\eta_z Y_{W_z}} \quad (4)$$

The equations state that the prokaryotic abundance or biomass B_i (edible prokaryotes) or I_z (inedible prokaryotes) is directly proportional to the virus-specific decay rate λ_i or θ_z and indirectly proportional to the product of the fraction Y_{V_i} or Y_{W_z} of the limiting resource in B_i or I_z that is transferred to the virus population V_i or W_z and the virus-specific adsorption constant β_i or η_z (Fig. 2).

$$V_i = \frac{N(\alpha_i Y_{B_i} - \alpha_n Y_{B_n})}{\beta_i} \quad (5)$$

$$W_z = \frac{N Y_{I_z} \epsilon_z}{\eta_z} \quad (6)$$

In a system with nonselective protozoan grazing (92) and given a limiting resource N , the abundance V_i of a specific viral

TABLE 1. Explanation of symbols

Symbol	Meaning
N	Concn of free limiting resource
N_T	Total available limiting resource
B_T and I_T	Total prokaryotic biomass of edible and inedible types
B_i and I_z	Prokaryotic biomass of edible and inedible types
V_T and W_T	Total viral biomass infecting edible and inedible prokaryotic types
V_i and W_z	Biomass of viruses infecting edible and inedible prokaryotic types
m_i and l_z	Burst size of viruses infecting edible and inedible prokaryotic types
P	Protozoan biomass
Y_{V_i} and Y_{W_z}	Fraction of resource in prokaryotic host transferred to viruses
Y_{B_i} and Y_{I_z}	Fraction of resource transferred to prokaryotes
α_i and ϵ_z	Prokaryotic affinity for limiting resource of edible and inedible types
β_i and η_z	Viral adsorption constants of V_i and W_z
λ_i and θ_z	Viral decay rates of V_i and W_z
Y_P	Fraction of limiting resource transferred to protozoans
α_p	Protozoan grazing rate
δ_p	Protozoan loss rate

TABLE 2. Specific predictions of the “killing the winner” hypothesis

Equation(s) for indicated parameter(s)	Mechanism	Prediction
B_i and I_z	Increase in λ_i and θ_z	B_i and I_z increase, no. of different prokaryotic types decreases
B_i and I_z	Increase in β_i and η_z	B_i and I_z decrease, no. of different prokaryotic types increases
B_i and I_z	Increase in Y_{V_i} and Y_{W_z} , increase in m_i and l_i	B_i and I_z decrease, no. of different prokaryotic types increases
B_i and I_z	Change in N	No. of different prokaryotic types does not depend on N
B_T	Increase in δ_P	Total prokaryotic abundance B_T increases
B_T	Increase in Y_P	Total prokaryotic abundance B_T decreases
B_T	Increase in α_P	Total prokaryotic abundance B_T decreases
I_T and model	Increase in N	Increase in I_T
V_i and V_T , W_z and W_T	Increase in N	Increase in V_i and W_z , increase in total viral abundance V_T and W_T
V_i and V_T	Increase in $\alpha_i Y_{B_i} - \alpha_n Y_{B_n}$	Increase in V_i and V_T
V_i and V_T , W_z and W_T	Increase in $\alpha_i Y_{B_i}$ and $\epsilon_z Y_{I_z}$	Increase in V_i and V_T , W_z and W_T
V_i and V_T , W_z and W_T	Increase in β_i and η_z	Decrease in V_i and V_T , W_z and W_T
Model	Change in host community composition	Change in viral community composition
Model	High-productivity environment/high N	Predation regulates prokaryotic community composition

population is directly proportional to the difference in the products of the fraction Y_B of the limiting resource that is taken up by prokaryotic hosts and the prokaryotic affinity α for the limiting resource of the i th and n th populations and is indirectly proportional to the virus-specific adsorption constant β_i (Fig. 2). Size- or shape-selective protozoan grazing creates a niche for inedible prokaryotic types. Given a limiting resource N , the biomass W_z of a specific viral population infecting inedible prokaryotic types is directly proportional to the fraction Y_z of the limiting resource transferred into prokaryotes and the prokaryotic affinity ϵ_z and is indirectly proportional to the virus-specific adsorption constant η_z (Fig. 2).

These equations give rise to a number of predictions (Table 2). For example, an increase in the virus-specific decay rate λ_i increases the biomass B_i of a specific prokaryotic population (equation 3) and thus reduces the total number of prokaryotic types that can become established in the system (equations 1 and 2). Thus, an increase in the virus-specific decay rate λ_i causes a decrease in the evenness and richness of the prokaryotic assemblage, since less well adapted populations are becoming extinct due to a shortage of the available resource in the system (Fig. 2). However, the model as a whole is more complicated than the sum of its parts and gives rise to a number of less recognized predictions. In the case of nonselective protozoan grazing, the total viral biomass V_T and the biomass of specific viral populations V_i depend on the difference in the adsorption coefficients α of the prokaryotic host populations (equations 1, 2, and 5). In other words, large differences between fast- and slow-growing prokaryotic host populations in a system result in a high total viral biomass V_T . The biomasses V_i and W_z of viruses with high adsorption constants β_i and η_z are small because the size of the prokaryotic host population declines correspondingly (equations 3 to 6). Also, the biomass of edible prokaryotes B_T is controlled by grazing, whereas the biomass of grazing-resistant prokaryotes I_T depends on the amount of free resource N (equations 1 and 2).

SIZING UP THE EVIDENCE

Here we review the recent literature from 2000 onwards that is relevant to the KtW hypothesis to identify aspects of the theory that are in agreement or disagreement with experimen-

tal and observational work. Also, we will point out shortcomings of KtW that are revealed in the literature. In order to give a better overview, we distinguish experimental studies according to the nature of the manipulations and the type of organisms or environments studied (Table 3). Likewise, we have grouped observational studies according to the environments investigated (Table 4). For an overview of the general literature, not covered in this article, pertaining to all aspects of viral ecology, see reviews by Wommack and Colwell (110), Weinbauer (100), and Weinbauer and Rassoulzadegan (104).

The Issue of Resistance to Viral Infection

Studies focusing on the influence of viruses on prokaryotic isolates either alone or in competition experiments in the presence or absence of viruses could be considered the simplest type of experiment to conduct with respect to KtW. This type of experiment reveals aspects of the virus-host relationship that are difficult to attain in more complex setups. Many of the studies of isolates reviewed here demonstrate that the development of resistance to viral infection is rapid and that this mechanism can influence or completely change the clonal composition of the host population from vulnerable to resistant (6, 19, 20, 59, 60, 61, 75). However, in most cases the development of resistance was associated with a fitness penalty for the resistant population. This cost of resistance was investigated in detail by Lennon et al. (52) in the case of *Synechococcus* sp. isolates. The study found that when a cost of resistance was detected, it resulted in an ~20% reduction in fitness compared to the ancestral strains. Furthermore, the cost of resistance was unaffected by the total number of viruses for which resistance occurred. However, under competitive conditions, the cost of resistance was dependent on the identity of the viruses. The essence of KtW is a trade-off between competition and defense specialists; thus, these results are in agreement with KtW (equations 1 and 2). Topically related work by Middelboe et al. (61) focused on the genetic, structural, and physiological differences of a *Cellulophaga baltica* strain that developed as a response to the presence of two viruses infecting this strain in chemostat cultures. The *Cellulophaga baltica* strain was initially sensitive to 24 tested viruses. During the incubations, the authors found a succession of strains, with the dominant strains

TABLE 3. Experimental studies with relevance to the “killing the winner” hypothesis, grouped according to the nature of the experimental manipulation and the organisms or environments studied

Authors (reference)	Manipulations	Organisms and/or environments studied				Main parameter(s)
		Isolates	Marine	Freshwater	Estuary	
Brockhurst et al. (19)	Viruses	×				<i>Pseudomonas aeruginosa</i> adaptive radiation
Holmfeldt et al. (38)	Viruses	×				Host strain susceptibility; phage host range
Lennon et al. (52)	Viruses	×				Cost of resistance in <i>Synechococcus</i> spp.
Middelboe et al. (61)	Viruses	×				Prokaryotic and viral abundance, colony morphology, phage susceptibility; BIOLOG profiles, lipopolysaccharide profiles of prokaryotes, partial 16S rRNA gene sequences, universally primed PCR profiles of prokaryotes
Middelboe et al. (63)	Viruses	×				Competition between <i>Cellulophaga</i> spp. and <i>Photobacterium</i> sp., prokaryotic and viral abundance, prokaryotic morphology
Middelboe et al. (60)	Viruses	×				competition between 4 isolates, batch and continuous cultures, clonal composition (resistant vs sensitive)
Bouvier and del Giorgio (16)	Viruses		×			FISH for distinct prokaryotic groups, prokaryotic production, growth rates of the community and distinct prokaryotic groups
Hewson and Fuhrman (34)	Viruses		×			Prokaryotic and viral abundance, community composition of <i>Bacteria</i> , richness of <i>nifH</i> gene (diazotrophs)
Middelboe and Lyck (62)	Viruses		×			Influence of viruses on prokaryotic net growth and respiration, prokaryotic and viral abundance, prokaryotic production
Schwalbach et al. (82)	Viruses		×			Prokaryotic abundance, community composition of <i>Bacteria</i>
Winter et al. (109)	Viruses		×			Prokaryotic abundance, community composition of <i>Bacteria</i> and <i>Archaea</i>
Auguet et al. (3)	Viruses			×	×	Influence of auto- and allochthonous viruses on prokaryotes, prokaryotic and viral abundance, community composition of <i>Bacteria</i> and viruses, prokaryotic production, viral production
Hewson et al. (35)	Viruses				×	Benthic study, community composition of microalgae and phytoplankton, prokaryotic abundance, photosynthesis and carbon fixation rates
Bonilla-Findji et al. (15)	Prokaryotes			×	×	Influence of nonindigenous prokaryotes on viruses, prokaryotic and viral abundance, prokaryotic production, prokaryotic respiration, dissolved organic carbon, community composition of <i>Bacteria</i>
Šimek et al. (85)	HNF ^a			×		Community composition of <i>Bacteria</i> , mortality due to viruses, FISH for distinct prokaryotic groups
Sime-Ngando and Pradeep Ram (87)	HNF			×		Concn of nutrients, prokaryotic and viral abundance, prokaryotic production, frequency of visibly infected cells, FISH for distinct prokaryotic groups
Bonilla-Findji et al. (14)	Viruses, HNF		×			Prokaryotic and viral abundance, prokaryotic production, inorganic nutrients, community composition of <i>Bacteria</i> and <i>Archaea</i>
Zhang et al. (113)	Viruses, HNF		×			Prokaryotic abundance and production, community composition of <i>Bacteria</i> and <i>Archaea</i>
Jacquet et al. (42)	Viruses, HNF			×		Prokaryotic and viral abundance, concn of nutrients, grazing rates, viral infection and burst size
Jardillier et al. (44)	Viruses, HNF			×		Prokaryotic and viral abundance, abundance of protists, community composition of <i>Bacteria</i> , FISH for distinct prokaryotic groups, prokaryotic production, viral infection of prokaryotes
Šimek et al. (86)	Viruses, HNF			×		Effects of predators on <i>Flectobacillus</i> sp. populations
Weinbauer et al. (103)	Viruses, HNF			×		Prokaryotic abundance and production, community composition of <i>Bacteria</i> and <i>Archaea</i> , FISH for distinct prokaryotic groups
Benmayor et al. (6)	Viruses, productivity, disturbance	×				<i>Pseudomonas fluorescens</i> adaptive radiation
Brockhurst et al. (18)	Viruses, temp	×				Outcome of competition between <i>Pseudomonas fluorescens</i> and <i>Pseudomonas aeruginosa</i>
Brockhurst et al. (20)	Viruses, spatial heterogeneity	×				<i>Pseudomonas fluorescens</i> adaptive radiation
Evans et al. (25)	Viruses, HNF, nitrate, phosphate		×			Chlorophyll <i>a</i> , concn of nutrients, abundance and composition of phytoplankton (<i>Micromonas</i> spp.), viral abundance, grazing and lysis rates
Hewson et al. (36)	Viruses, phosphate, ammonia		×			Benthic study, prokaryotic abundance, community composition of <i>Bacteria</i> , photosynthesis
Malits and Weinbauer (56)	Viruses, turbulence		×			Prokaryotic and viral abundance, prokaryotic production, viral production, prokaryotic cell vol, no. of organic aggregates, community composition of <i>Bacteria</i> and <i>Archaea</i> , concn of nitrate and phosphate

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TABLE 3—Continued

Authors (reference)	Manipulations	Organisms and/or environments studied				Main parameter(s)
		Isolates	Marine	Freshwater	Estuary	
Weinbauer et al. (102)	Viruses, dissolved organic matter, dilution		×			Prokaryotic and viral abundance, prokaryotic production, abundance of <i>Vibrio</i> - and <i>Rhodobacter</i> -related populations using FISH
Bohannon and Lenski (11)	Glucose	×				Abundance of bacteria and viruses
Middelboe (59)	Different growth rates in chemostats	×				Host cell lysis and viral production, burst size, latent period, resistance
Riemann and Grossart (75)	Agarose beads	×				Abundance of bacteria and viruses
Jacquet et al. (43)	Nitrate, phosphate, glucose		×			Prokaryotic and viral abundance, prokaryotic cell size and morphology, distinction of prokaryotic and viral populations using flow cytometry
Larsen et al. (50)	Nitrate, phosphate		×			Abundance of prokaryotes, viruses, and algae; community composition of <i>Bacteria</i> and <i>Eukarya</i> ; viral community composition; primary production; chlorophyll <i>a</i> ; phytoplankton abundance
Øvreås et al. (68)	Nitrate, phosphate, glucose		×			Community composition of <i>Bacteria</i> and viruses, prokaryotic abundance and cell morphology, FISH for distinct prokaryotic groups
Riemann et al. (77)	Phosphate, silicate, nitrate, ammonia; induction of diatom blooms		×			Prokaryotic and viral abundance, HNF abundance, prokaryotic production, community composition of <i>Bacteria</i> , chlorophyll <i>a</i> , abundance of phytoplankton cells, ectoenzyme activity
Sandaa et al. (81)	Phosphate, glucose		×			Prokaryotic and viral abundance, chlorophyll <i>a</i> , community composition of <i>Bacteria</i> and viruses
Jardillier et al. (45)	HNF, zooplankton, nitrate, phosphate, ammonia			×		Concn of nitrate, phosphate, and ammonia; prokaryotic and viral abundance; community composition of <i>Bacteria</i> ; prokaryotic production; abundance of protists and metazooplankton; grazing rates; FISH for distinct prokaryotic groups
Pradeep Ram and Sime-Ngando (72)	HNF, glucose, nitrate, phosphate			×		Prokaryotic and viral abundance, prokaryotic production and respiration, mortality due to viral lysis, FISH for distinct prokaryotic groups
Šimek et al. (84) and Weinbauer et al. (101)	HNF, availability of phosphorus and DOC			×		Prokaryotic and viral abundance, prokaryotic production, viral production and lysis rate, prokaryotic cell vol, FISH for distinct prokaryotic groups

^a HNF, heterotrophic nanoflagellates.

eventually having acquired immunity to up to 22 of the tested viruses. At the end of the experiment, the cultures were dominated by strains resistant to either one or both of the viruses present during the incubations, with small populations of sensitive strains maintaining both viruses at abundances of above 10^6 ml^{-1} . Loss of sensitivity to virus infection was associated with a reduction in the ability to use various carbon sources, thus demonstrating that the actions of viruses can lead to a functional diversification of the host population. Nevertheless, the degree of resistance was not correlated to the loss of metabolic potential. Thus, both studies (52, 61) found that the cost of resistance is not proportional to the degree of resistance. A fitness penalty depending on the specific virus type rather than on the total number of different viruses to which resistance developed implies the existence of different mechanisms of resistance. Many viruses infecting prokaryotes use as a docking site surface proteins that are also involved in the uptake of nutrients by the host cell (24, 66). A structural change in these surface proteins or in their abundance per host cell may result in resistance to viral infection and at the same time cause a reduction in the capabilities for uptake of nutrients (53). Lysogenic viruses may also confer resistance by initiating the production of repressor proteins that ensure the host's immunity to additional viral infection (21). Nevertheless,

maintenance of such a repressor system is costly and incurs a fitness penalty for the cell. Recently, another highly specific mechanism of resistance to viral infection, based on clustered regularly interspaced short palindromic repeats (CRISPR), was described (5). The CRISPR system represents a DNA-encoded defense system based on genomic features that provides prokaryotes with immunity against viruses (and plasmids), likely employing RNA interference. While there presumably is a cost associated with running this defense system, the cost of adding a new virally derived CRISPR sequence to the library of the host cell seems minor. However, Anderson and Banfield (1) demonstrated that resistance to viral infection conferred by CRISPR sequences is rather short-lived due to recombination sufficiently shuffling the sequence motifs so that only the most recently acquired CRISPR sequences result in immunity. Based on the currently available knowledge, the CRISPR defense system appears to be difficult to incorporate into the KtW framework. The complexity of the issue of resistance to viral infection becomes even more apparent when considering a study by Holmfeldt et al. (38). Those authors investigated the susceptibility of 23 *Bacteroidetes* sp. strains to viral infection by 46 different viruses. Based on 16S rRNA gene and internal transcribed spacer DNA sequences, 21 of these bacterial isolates could be con-

TABLE 4. Observational studies with relevance to the “killing the winner” hypothesis, grouped according to the environments studied

Authors (reference)	Environment studied				Main parameter(s)
	Marine	Freshwater	Estuary	Other	
Hewson and Fuhrman (33)	×				Prokaryotic and viral abundance, community composition of <i>Bacteria</i> , richness of <i>nifH</i> gene (diazotrophs), viral production, prokaryotic production
Hewson et al. (37)	×				Prokaryotic and viral Abundance, community composition of <i>Bacteria</i> and viruses
Larsen et al. (51)	×				Concn of nutrients, prokaryotic and viral abundance, abundance of phytoplankton, community composition of phytoplankton, community composition of viruses and <i>Bacteria</i> , particulate organic phosphorus, dissolved organic carbon, prokaryotic production, alkaline phosphatase activity
Parada et al. (69)	×				Prokaryotic and viral abundance, viral production, prokaryotic production, viral community composition <i>in situ</i> and newly produced viruses
Winter et al. (107)	×				Prokaryotic and viral abundance, viral infection, community composition of <i>Bacteria</i> and <i>Archaea</i>
Winter et al. (108)	×				Prokaryotic and viral abundance, prokaryotic production, community composition of <i>Bacteria</i> and <i>Archaea</i>
Filippini et al. (26)		×			Prokaryotic and viral abundance, prokaryotic production
Tijdens et al. (96)		×			Prokaryotic and viral abundance; abundance of phytoplankton and flagellates; community composition of <i>Bacteria</i> , cyanobacteria, and viruses
Yoshida et al. (112)		×			<i>Microcystis aeruginosa</i> , viruses infecting this species
Riemann et al. (76)			×		Nutrients, total organic carbon, chlorophyll <i>a</i> , concn of dissolved DNA, prokaryotic and viral abundance, prokaryotic production, viral production, community composition of viruses, alkaline phosphatase activity, prokaryotic DNA uptake rates
Wang and Chen (98)			×		Abundance of cyanobacteria and viruses, abundance of viruses infecting <i>Synechococcus</i> sp., richness of <i>g20</i> genes (cyanomyoviruses)
Andersson and Banfield (1)				Mine biofilms	Community genomics
Sabet et al. (80)				Soda lake	Isolation of bacterial and viral strains, genetic analysis, probes targeting viral isolates

sidered strains of *Cellulophaga baltica* (*Flavobacteriaceae*). The study found that all of the bacterial isolates showed unique virus susceptibilities and differed by up to 6 orders of magnitude in sensitivity to the same titer of virus. The tested viruses themselves showed variations in host ranges, infecting between 1 and 20 bacterial strains. This study reveals a major shortcoming of KtW as presented in Fig. 2: each host population is subject to mortality due to multiple co-occurring virus populations, not just one. The data suggest that a higher resolution is required also within the population boxes of Fig. 2 representing prokaryotic “species,” so that each prokaryotic population consists of different strains with profiles of different resistance to viruses. Thus, the hierarchical structure of KtW may be extended to the level of subpopulations representing different strains, as demonstrated in Fig. 3. However, if the control by lytic viruses is at the strain level, it is not immediately obvious how population size is controlled at the “species” level. Also, only 5 out of 46 different viruses tested by Holmfeldt et al. (38) were host specific, questioning the validity of the generally assumed specificity of the virus-host relationship and thus also making incorporation into a simple KtW structure difficult. The study also reveals a more general methodological problem. Many investigators studying the influence of viruses on natural communities rely on the use of marker genes such as the 16S rRNA gene to monitor the prokaryotic com-

munity. However, Holmfeldt et al. (38) suggest that these techniques may severely underestimate the complexity of virus-host relationships (Fig. 3) and in some cases may completely miss the effects of viral lysis on the host populations.

Influence of Environmental Conditions on Virus-Host Relationships Studied with Isolates

KtW predicts that under highly productive conditions, predation is the major regulatory mechanism for community composition, whereas in environments with low productivity, competition drives community composition. This prediction has been confirmed in a model system consisting of two strains of *Escherichia coli* with different vulnerabilities to the virus T2 (11). However, investigators also tested the influence of other environmental variables on virus-host relationships. Benmayor et al. (6) found that the presence of viruses increased diversity at environmental extremes of disturbance and productivity by imposing selection for virus-resistant types but decreased diversity in less stressful environments. Thus, viruses appear to have a mitigating effect on the influence of other environmental variables on the community composition of their hosts. The disturbance treatment by Benmayor et al. (6) consisted of repeated reinoculation of the culture into fresh culture medium. However, KtW is based on the assumption of steady-

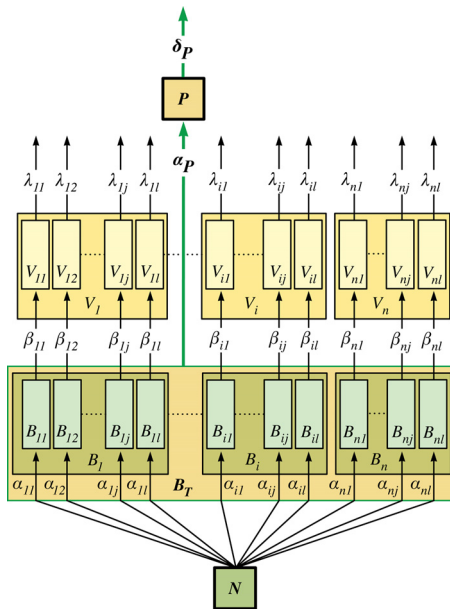


FIG. 3. “Killing the winner” mechanisms on three levels. Experimental data suggest that different strains of the same “species” may have profiles of different resistance to viruses. Thus, it appears that the influence of viruses on prokaryotes is on the strain level ($B_{ij}; j = 1, 2, \dots, l$) and not on the “species” level ($B_i; i = 1, 2, \dots, n$) commonly detected by PCR-based fingerprinting techniques targeting the 16S rRNA gene. This is illustrated here for the case of edible bacteria B_T ; however, similar arguments also hold for the case of inedible bacteria I_T . The figure suggests that the abundance of the prokaryotic community B_T is controlled by protozoan grazing and that the abundance at the strain level B_{ij} is controlled by viral lysis. However, the controlling mechanism for population size at the “species” level B_i is not immediately obvious from this model. The meanings of the symbols are given in Table 1.

state conditions so that it refers to the climax situation at the end of a string of successional developments. Such a steady state may or may not be locally stable, and there may or may not be oscillations in the form of successive replacements of virus-host pairs (Fig. 4). The successional replacement of the most abundant hosts due to viral lysis is therefore a possible result of KtW but not a definition of the concept as sometimes presented (see, e.g., reference 17). In another study, Brockhurst et al. (20) tested the influence of spatial heterogeneity and the presence of parasites on the evolution of *Pseudomonas fluorescens*. Viruses increased total diversity in homogenous environments but had no effect in heterogenous environments. Heterogenous environments offer a number of different niches that can be occupied by the competing populations. KtW considers the case of competition for one limiting resource and is thus not relevant for heterogenous environments. However, for the case of homogenous environments, the results reported by Brockhurst et al. (20) are in agreement with KtW (equations 1 and 2). Brockhurst et al. (18) conducted competition experiments using communities consisting of *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* and their viruses. The communities were incubated at two different environmental temperatures, which, without the presence of viruses, reversed the outcome of competition. The results show that coexistence was enhanced (evenness was increased) in the presence of

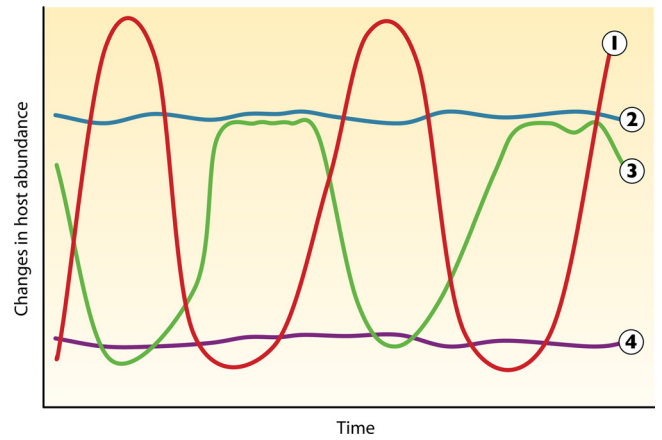


FIG. 4. Possible scenarios for temporal changes in host abundance as a consequence of viral lysis. In scenario 1, the abundance of the host population changes periodically, resembling predator-prey oscillations. Thus, viral lysis regulates numerically dominant populations (similar to negative frequency-dependent selection). In scenario 2, the host population is resistant to viral infection and thus can maintain high host abundance. This scenario is unlikely due to the transient nature of resistance and the associated costs of resistance. Scenario 3 depicts the case of a population that coevolves with its virus. The oscillations are due to a change in the population’s sensitivity to the virus. In scenario 4, the sensitive host population is kept at a low abundance due to strong viral control.

viruses infecting the superior competitor and in the presence of viruses infecting both bacterial strains. In accordance with KtW, viruses reduced the abundance of the superior competitor, allowing the weaker competitor to increase its density (equations 1 and 2). However, coexistence of the two strains was not equally stable at the two different environmental temperatures.

Feedback Loops Generated by Release of Lysis Products

Viral lysis of host cells releases not only progeny virus particles but also a cocktail of sugars, proteins and peptides, amino acids, nucleic acids, etc., that could serve as a source of nutrients for the surviving community. A number of studies investigated whether these lysis products are taken up by the survivors and also whether this effect would be of any significance (35, 59, 62, 63, 76). Middelboe (59) studied the dynamics of a marine virus-host (*Pseudoalteromonas* sp.) system at different steady-state growth rates in chemostat experiments. The author found that cell lysis and virus production were, in agreement with KtW, positively correlated with the host growth rate (equations 5 and 6). The results also demonstrated that the burst size increased while the latent period decreased with increasing host growth rate and that the release of viral lysates stimulated growth of noninfected resistant cells. Thus, although resistance to viral infection may have an associated fitness penalty (52), resource enrichment due to the lysis of vulnerable cells may have a mitigating effect. Also, Hewson et al. (35) concluded that in oligotrophic sediments, viruses stimulated carbon fixation due to increased nutrient availability as a consequence of viral lysis of prokaryotes. Further studies of the role of viral lysates for prokaryotic growth demonstrated that this effect is of quantitative importance not only in model

systems (63) but also for mixed marine prokaryotic communities (62, 76). In addition to liberating organic carbon bound in prokaryotic biomass, it has also been reported that 25% of the dissolved DNA pool in a phosphorus-limited estuary is derived from viral lysis and that the uptake of dissolved DNA accounts for 70% of the prokaryotic phosphorus demand in this environment (76). Based on the available evidence, the question of whether or not a feedback loop that routes resources from vulnerable (competition specialist) to resistant (defense specialist) populations exists can be answered in the affirmative, yet it is currently not included in KtW.

Availability of Resources

The effects of resource availability on mixed prokaryotic communities were investigated in studies of mesocosms inoculated with marine assemblages (43, 50, 68, 77, 81) or of a marine sediment-water mesocosm (36). These studies, although difficult to compare due to the distinct setups and inocula used, provide evidence relevant for specific aspects of KtW. Larsen et al. (50) followed the dynamics of phytoplankton, prokaryotes, and viruses in a seawater mesocosm enriched with nitrate and phosphate. Flagellates dominated the algal community, followed by the appearance of a number of different size classes of viruses. Similarly, Jacquet et al. (43) could link the development in the abundance of specific groups of autotrophic prokaryotes and eukaryotes to different groups of viruses in experiments with mesocosms enriched with nitrate, phosphate, and glucose. Thus, changes in the host community are followed by changes in the corresponding virus community, as predicted by KtW. Øvreås et al. (68) conducted mesocosm experiments to investigate the effects of the addition of inorganic nutrients (nitrate and phosphate) and of inorganic plus organic (glucose) nutrients on prokaryotic and viral communities. The authors found small changes in bacterial community composition upon addition of mineral nutrients alone; however, the combined addition of mineral nutrients and glucose resulted in major changes in the bacterial community composition. Also, in agreement with KtW, changes in the bacterial community caused similar changes in the virus community. Riemann et al. (77) studied the dynamics of prokaryotic activity, abundance, and bacterial community composition in mesocosms fertilized with inorganic nutrients to initiate a diatom bloom. Prokaryotic abundance abruptly decreased during the peak of the bloom concomitantly with the disappearance of three dominant phylotypes from the bacterial community. Increased flagellate and viral abundance during the diatom peak suggest that grazing and lysis could have caused this phylotype-specific mortality. Sandaa et al. (81) studied the effects of viral lysis and substrate limitation on the bacterial community in mesocosms amended with phosphate, glucose, and phosphate plus glucose. In agreement with KtW, these authors found that changes in the bacterial community composition were linked to changes in the viral community. Furthermore, data obtained by pulsed-field gel electrophoresis (PFGE) showed that the total number of virus populations was the same in all treatments; however, the composition of the viral community varied between the treatments. The authors concluded that the number of different virus-host pairs is controlled by viral lysis (top-down control, in agreement with KtW

[equations 1 and 2]), whereas the identity of these virus-host pairs is controlled by substrate limitation (bottom-up control). Finally, Hewson et al. (36) investigated the effects of virus infection and nutrient enrichment on the microbial compartment of oligotrophic carbonate-based sediments. The addition of nutrients (phosphate and ammonia) resulted in elevated virus abundance and increased bacterial richness. These results are in principle in agreement with KtW (equations 1 and 2).

Manipulating Viral Abundance in Experimental Incubations

Studying mixed prokaryotic communities instead of isolates clearly bears a higher resemblance to studying natural systems. However, the results of such experiments are often more difficult to interpret and suffer from other biases, such as the adverse effects of sample handling and containment. Also, it is plausible to assume that not all prokaryotic communities coming from different environments will display similar reactions to experimental manipulations of viral abundance, so it is difficult to directly compare the results. Schwalbach et al. (82) and Winter et al. (109) incubated marine prokaryotic assemblages with significantly reduced or enhanced viral abundances to study the effects on prokaryotic community composition. Both studies found significant effects of sample manipulation and containment on the prokaryotic communities. Schwalbach et al. (82) reported modest but significant effects on bacterial community composition in virus-reduced incubations but no effects in virus-enriched cultures. The results of Winter et al. (109) showed that the effects of viruses were detected on the level of individual bacterial and archaeal phylotypes. In another study, Hewson and Fuhrman (34) investigated the influence of viral infection on bacterial communities at 15 stations in the North Atlantic, North Pacific, and Gulf of Mexico and in southern California. The authors performed batch culture incubations that were enriched in or depleted of ambient viruses and used diazotrophs as model organisms for rare prokaryotic types, which were hypothesized to benefit from viral activity. The effects of viruses were not consistent between sampling locations. Also, there was no significant difference in the relative abundances of common and rare bacterial types in the response to virally enriched or depleted incubations. In a study investigating the sediment-water interface, Hewson et al. (36) found that the addition of viruses to benthic flocculent layer samples increased bacterial richness compared with the addition of heat-killed viruses as a control. In what could be called a virus-transplant experiment, Auguet et al. (3) investigated the potential effect of freshwater viruses on the composition and activity of bacterial communities in an estuary (Marennes-Oléron Bay, France). Those authors found that confinement and incubation temperature were the two principle experimental factors influencing bacterial community composition. However, the addition of freshwater viruses had a significant effect on the bacterial communities and reduced net prokaryotic growth compared to the addition of inactivated viruses or ambient viruses. The study suggests that prokaryotic communities are adapted to ambient viruses, while the community needs to change in order to adjust to the presence of allochthonous viruses. In a similar study, Bonilla-Findji et al. (15) cross-transplanted freshwater and marine prokaryotic communities into batch cultures mimicking marine and freshwater environ-

ments. Those authors found that prokaryotic production was stimulated in the transplanted marine and freshwater communities, while bacterial richness decreased. Furthermore, mortality due to viruses increased in the transplanted marine community but decreased in the transplanted freshwater community, suggesting that freshwater viruses appear to be able to infect marine prokaryotes. All of the above-mentioned studies except for that of Hewson and Fuhrman (34) are in principle compatible with KtW. However, according to Holmfeldt et al. (38), current methodological limitations in the analysis of mixed prokaryotic communities may result in a severe underestimation of the effects of viral lysis on the host communities. Also, studies of specific virus-host systems (see, e.g., reference 18) suggest that a multitude of other environmental factors affect the sensitive balance between viruses and their hosts, and these might explain the varying results. One of those environmental factors rarely studied is turbulence. Malits and Weinbauer (56) found that turbulence stimulated prokaryotic production, likely by enhancing the formation of microaggregates and nutrient availability. However, the presence of viruses appeared to reduce the number of microaggregates. Furthermore, turbulence together with viruses increased prokaryotic cell length. Those authors also report that specific phylotypes appeared to be inhibited or stimulated by turbulence and/or viruses. Weinbauer et al. (102) studied the response of a prokaryotic community to the additions of dissolved organic carbon and viruses and to dilution, with particular focus on *Vibrio*- and *Rhodobacter*-related populations. Their results suggest that *Vibrio*- and *Rhodobacter*-related populations can be described as r and K strategists, respectively. K strategists are considered to be strong competitors and have a low reproductive output, whereas r strategists invest in large numbers of offspring but do not have strong competitive abilities (55). However, both populations were only weakly susceptible to viral infection despite a 2-fold-higher growth rate of *Vibrio*- compared to *Rhodobacter*-related populations. Finally, Bouvier and del Giorgio (16) incubated marine bacterioplankton in batch cultures that were greatly depleted of ambient viruses. The result was a dramatic increase in the relative abundance of bacterial groups that were undetectable in the *in situ* bacterioplankton community. Thus, host susceptibility is not necessarily proportional to host density, as is often assumed, and rare marine bacterioplankton groups may be more susceptible to virus-induced mortality because these groups may actually be the winners in the competition for nutrients (Fig. 4, scenario 4). This is in agreement with KtW, predicting that viruses with fast-growing hosts should be most abundant and that the viral biomass of specific populations V_i and W_z (equations 5 and 6) and the total viral biomass V_T and W_T (equations 1 and 2) are independent of host biomass.

Combined Effects of Viral Lysis and Protistan Grazing

There are two experimental studies, both conducted in freshwater environments, that manipulated grazing pressure as the sole experimental factor (85, 87). Both studies found that prokaryotic and viral activity increased in the presence of grazers and suggest that this stimulation is due to grazer-mediated resource enrichment. Similar results were also obtained in a study manipulating grazer abundance and the concentration of nutrients (72). Thus, grazing appears to increase the amount of

resources available to the surviving populations in a feedback loop similar to viral lysis. Such a feedback loop is currently not included in KtW. However, in agreement with KtW, Sime-Ngando and Pradeep Ram (87) and Šimek et al. (85) also noted the appearance of grazing-resistant forms and suggested that the biomass of edible prokaryotes B_T is regulated by grazing (Fig. 2 and equations 1 and 2). Above we have introduced size- or shape-selective grazing to enhance the applicability of KtW (Fig. 2). As Fig. 2 makes clear, the presence of both viruses and grazers should result in the highest prokaryotic richness, subject to the availability of resource N . However, the experimental evidence is more differentiated than that. Jardillier et al. (44) investigated the influence of viruses and protistan grazing on freshwater prokaryotic communities in batch culture experiments. In agreement with KtW, those authors concluded that prokaryotic abundance was controlled mainly by protistan grazing, whereas viruses were mostly responsible for changes in the composition of the prokaryotic community (equations 1 to 4). Jacquet et al. (42) investigated the temporal variation of prokaryotic mortality due to viruses and grazers in a lake. Those authors found that mortality due to viruses increased and that due to grazers decreased during the summer compared to spring. Also, Evans et al. (25) found that mortality of *Micromonas* spp. due to grazing was similar to or exceeded mortality due to viral lysis. Šimek et al. (86) subjected bacterioplankton to additions of flagellates or viruses plus flagellates to identify the influence of these mortality factors on *Flectobacillus* sp. populations. Filamentous prokaryotic forms appeared in both treatments but were twice as abundant, long, and active in incubations with both predators than in the flagellate treatment alone. In a companion paper, Weinbauer et al. (103) found that the vulnerability to the two sources of mortality (viruses and flagellates) was distinctly different between prokaryotic groups (see also reference 38). However, in contradiction to KtW (Fig. 2), the results showed that prokaryotic richness was highest in the virus treatment and lowest when both predators were present and that flagellates reduced prokaryotic richness but increased viral production. In total, there are two marine studies investigating the effects of grazers and viruses on the prokaryotic communities in an offshore (14) and a coastal (113) marine environment. Bonilla-Findji et al. (14) used size fractionation to conduct batch culture experiments with virus, flagellate, and virus-plus-flagellate treatments. Those authors found that the addition of viruses or viruses plus flagellates increased prokaryotic abundance and richness but decreased prokaryotic production. However, the results were not consistent in the flagellate treatments compared to the control. Based on flow cytometric data, the relative abundance of large prokaryotic cells with a high nucleic acid content was lower in the virus and virus-plus-flagellate treatments than in the control or the flagellate treatment. The data suggest that viruses and viruses combined with flagellates sustain prokaryotic richness (equations 1 to 4) and control prokaryotic production by regulating the abundance of highly active members of the community (equations 5 and 6), in accordance with KtW. Similarly, Zhang et al. (113) found that the effects of viruses and flagellates combined were consistent and closely resembled natural conditions and that viral lysis and protistan grazing acted additively to reduce prokaryotic production and sustain prokaryotic richness. Overall, there is some variability

in the literature as to the effects of protistan grazing on prokaryotic communities, particularly in combination with viral lysis. However, as shown above for isolates (11), the availability of resources is another important factor modifying the complex interactions between grazers, prokaryotes, and their viruses. In the case of mixed prokaryotic communities, Šimek et al. (84) and Weinbauer et al. (101) studied the effects of resource enrichment and grazing on viral production and bacterioplankton communities. In contradiction to KtW, predation affected bacterial community composition in resource-limited prokaryotic communities but was of minor importance under resource-replete conditions. However, in agreement with KtW, the effect of virus-induced mortality was higher in resource-replete treatments, where prokaryotic and viral abundance, prokaryotic and viral production, and virus-induced lysis rates increased (equations 5 and 6). Also, grazing stimulated viral abundance, production, and virus-induced mortality, as found in other studies (72, 85, 87). Jardillier et al. (45) studied the influence of nutrients, grazing, and viral lysis on the prokaryotic community composition in an oligomesotrophic lake and in a eutrophic lake. In agreement with KtW, those authors found that the prokaryotic community in the oligomesotrophic lake was affected mainly by the availability of nutrients and that in the eutrophic lake by the concentration of nutrients and mortality due to grazing and viral lysis (equations 1 to 4). In summary, the availability of resources appears to modify the effects of grazing on prokaryotic communities.

Observational Studies

Although observational studies may not always provide the hard evidence obtainable through experimental manipulation, they are nevertheless important because investigators can gain information about how the complex relationships between prokaryotes and their environment play out in nature. In accordance with KtW, a number of studies in marine and freshwater environments found that virus and host communities of either specific populations or entire assemblages are linked (26, 37, 33, 51, 69, 96, 98, 107, 108, 112), and there is evidence suggesting similarly coupled prokaryotic and viral communities in a soda lake (80). The details vary, however. More specifically, Winter et al. (108) studied the relationship between prokaryotic richness and the abundance of prokaryotes and viruses, prokaryotic production, and other physicochemical parameters in the North Sea. The results showed that bacterial richness decreased with increasing viral abundance and prokaryotic production. The authors conclude that high prokaryotic production was sustained by a relatively small number of highly active bacterial populations that also maintained high viral abundance. These results are in principle in agreement with KtW, because viruses with fast growing hosts are predicted to be the most abundant ones (equations 5 and 6). In another study, Hewson et al. (37) investigated the relationship between viral and bacterial communities in oligotrophic waters of the West Florida shelf. Consistent with KtW, the study found that biomass and community richness were independent (equations 1 to 4) and that temporal patterns in the bacterial community were closely related to changes in the viral community. In a comparative study, Hewson and Fuhrman (33) investigated the covariation of viral parameters (abundance and production)

with bacterial community composition and richness in the water column and sediments of the North Pacific, Amazon River plume, North Atlantic, Gulf of Mexico, Southern California Bight, and Coral Sea. The results suggest that there appears to be no universally applicable relationship between viruses and their host community, probably due to the different effects of other environmental parameters. However, the community composition and richness of diazotrophs, as a model of rare but opportunistic organisms, were significantly negatively correlated to viral parameters across all sites. The authors concluded that viral lysis may have positive effects on bacterial richness in oligotrophic oceans, by regulating the abundance of dominant competitors. However, KtW predicts that predation/parasitism should be more important under eutrophic conditions, whereas under oligotrophic conditions competition is predicted to be the most important factor in shaping prokaryotic community composition (equations 1 and 2). The study also suggests that rare taxa may be more susceptible to viral attack due to opportunistic lifestyles (see also reference 16). Winter et al. (107) related bacterial and archaeal community composition and richness in the tropical Atlantic Ocean to the geographic distance between the sampling stations, the frequency of infected cells, and physicochemical parameters to identify factors influencing the prokaryotic community composition. Those authors found no detectable effect of geographic distance or differences between water masses on bacterial and archaeal community composition. Bacterial communities changed with depth, whereas changes in the archaeal community were related to temperature and the frequency of infected cells. Thus, the results suggest that KtW mechanisms are also relevant for archaeal virus-host relationships under natural conditions. Parada et al. (69) demonstrated that newly produced viruses represent only a fraction of the ambient viral community and that the *in situ* viral community was fairly stable over time. The results show that viral infection and lysis are dynamic processes changing on time scales of hours to days. In accordance with KtW, the results demonstrate the fine temporal scale at which changes in the host community are mirrored by changes in the virus community. Larsen et al. (51) studied the succession and community composition of algae, prokaryotes, and viruses in coastal waters around the time of the spring phytoplankton bloom. In agreement with KtW, those authors' results show that viruses were closely linked to the developments in the microbial community. Also, Wang and Chen (98) investigated the population dynamics of cyanovirus communities, and Yoshida et al. (112) studied the dynamics of viruses infecting the toxic bloom-forming cyanobacterium *Microcystis aeruginosa*. Both studies found that changes in the virus communities were related to changes in the host communities, supporting KtW. The link between viruses and hosts was further corroborated for entire freshwater assemblages by Filippini et al. (26) and Tijdens et al. (96). Filippini et al. (26) showed that changes in prokaryotic abundance were synchronized with changes in viral abundance. Tijdens et al. (96) found that viral abundance did not correlate with prokaryotic and cyanobacterial abundance during the entire study period. However, as suggested by KtW, viral community composition did correlate

with bacterial and cyanobacterial community composition during short periods of strong fluctuations.

FUNDAMENTAL LIMITATIONS OF KtW

KtW remains a model, and as such it is based on highly idealized relationships and assumptions that may not always be correct. Among its shortcomings is that the model relies on the assumption of steady-state conditions. Only very few environments actually remain in such a deadlocked situation for long. However, the problem is really one of temporal resolution. A good example is the study by Parada et al. (69), which found that although the ambient viral community is relatively stable over time, only a small number of viral types are actually produced at any given time. Thus, small and rapid changes in the activity of specific viral populations give rise to an overall stable viral community. Another obvious problem in the assumptions underlying KtW is that in most environments prokaryotic hosts are vulnerable to more than one co-occurring virus population. To complicate matters even more, the vulnerability of distinct host populations to viral infection varies widely (38) (Fig. 4). KtW also assumes that virus-host relationships are specific, i.e., that one virus infects only one host. The evidence, although based solely on isolates, suggests that this may not be correct (38) and that viruses with a wide host range may be more abundant than currently acknowledged. Another aspect of KtW that might be perceived as a limitation is that the model considers the case of competition for one limiting resource as stated in Hutchinson's paradox (40). Different resources limit the activity of the different prokaryotic populations in a community/assembly. As a whole, a prokaryotic community/assembly usually depends on the availability of a small subset of these resources (see, e.g., reference 68). The fact that KtW considers only one limiting resource therefore does not appear to be a major limitation. However, both viral lysis and protistan grazing create feedback loops by liberating resources that would otherwise be locked up in biomass. These freed-up resources further appear to stimulate growth of the surviving populations (defense specialist) and represent a conceptual problem for the trade-off between resource and competition specialists that is at the heart of KtW.

CHALLENGES TO BE OVERCOME FOR A THOROUGH TEST OF KtW

Over time, KtW has provided numerous testable hypotheses. Nevertheless, many aspects of KtW still remain untested or are not fully explored due to current methodological limitations. Essentially all of the studies investigating the influence of viral lysis and/or protistan grazing on prokaryotic community composition reviewed here used either (i) PCR-based fingerprinting techniques mainly targeting the 16S rRNA gene or the internal transcribed spacer region between rRNA genes or (ii) fluorescent *in situ* hybridization (FISH) targeting various groups of prokaryotes. However, particularly the study by Holmfeldt et al. (38) demonstrates that these techniques do not provide the resolution necessary to capture the changes occurring at the strain level in prokaryotic communities. It becomes even harder to obtain adequate data for viral communities, because viruses lack a universally conserved gene

that could be used for fingerprinting (32, 79). Here, data on the composition of the virus community were generally based on PFGE. PFGE separates the members of the viral community based on their genome size, and thus the data have no genetic aspect, in the sense of nucleic acid sequence information (89). Recently, Winget and Wommack (106) introduced randomly amplified polymorphic DNA PCR (RAPD-PCR) analysis to determine viral community composition by circumventing the need for a universally conserved gene. Although RAPD-PCR represents a significant advance in our capabilities to study viral communities, we do not expect that it is able to provide enough resolution to capture the relevant changes in the virus community. Thus, presently our methods allow for a rough overview of the processes regulating prokaryotic community composition by viruses and/or protistan grazing, but we are largely ignorant of the fine-scale picture. Currently, the most detailed data on prokaryotic and viral community composition are provided by metagenomics, that is, the genomic analysis of a population or community of microorganisms (31) and viruses (see, e.g., reference 2). However, metagenomic techniques still carry a large price tag, and the huge data sets obtained can be difficult to interpret (41). We expect that as the costs of metagenomic analysis of prokaryotic and viral communities drop and the bioinformatic methods to tame and analyze the flood of data mature, it will be possible to employ these large-scale sequencing techniques to study temporal and spatial developments of prokaryotic and viral communities in experiments and the natural environment. Even so, it will be necessary for more traditional microbial ecologists to start speaking "metagenomics" (48) and for the scientists familiar with these relatively novel techniques to look beyond nucleic acid sequences and consider ecological concepts in their work (74). First steps into this direction have recently been carried out by Andersson and Banfield (1), who matched viruses to their prokaryotic hosts by identifying the viral CRISPR sequences in a metagenomic data set, and by Rodriguez-Valera et al. (78), who suggested that the differences between strains of the same bacterial species detected by metagenomics appear to be caused mainly by the actions of lytic viruses.

The second and probably most daunting challenge for the future lies in obtaining rate measurements for individual prokaryotic communities (especially affinity for the limiting resource) and viral communities (adsorption and decay rates) necessary to constrain the formalized version of KtW. This has been done before for simple model systems employing chemostats (see, e.g., reference 59) but presently seems not to be feasible for complex mixed communities and even more so at the level of individual strains. It is difficult to foresee if and when suitable techniques will be available to achieve this ambitious goal; however, we think that in the coming years a fruitful approach would be to analyze the pool of mRNAs of entire communities (metatranscriptomics [83]) or even of proteins (metaproteomics [see, e.g., reference 105]) in combination with metagenomics. Such an approach should yield a detailed picture of prokaryotic and viral community composition as well as which genes are transcribed and translated. Nevertheless, particularly proteomics presently requires large amounts of proteins that are not easily obtainable from prokaryotic and viral com-

munities, and the amount of data generated is very large, demanding extensive bioinformatics skills and capabilities.

CONCLUDING REMARKS

Every model needs to strike a delicate balance between its complexity and its understandability. Thus, the trade-off for being as realistic as possible is a decrease in the model's usefulness, because overly complex models, although they may resemble natural systems more closely, will be harder to understand. KtW in its present form is a relatively simple model, and we think that the simplicity and intuitive understanding of some of its aspects led to the widespread dispersal of the idea. Despite all its problems, KtW in its simplicity appears to capture many aspects of the relationship between viruses, protozoan grazers, and prokaryotes, so that the basic principle of KtW (Fig. 1) is relatively well established. However, attempts to assemble this principle into a complete model for all the hierarchical levels that is consistent with observations still have a long way to go. Thus, we would like to caution the scientific community not to apply predictions based on KtW too rigorously and not to fall victim to the thought that the model is set in stone. Evidence will and always should be at the heart of research, yet KtW has proven to be very effective and useful in developing testable hypothesis. It is precisely the discrepancies between data and model that should be used to further develop KtW (64, 65) in order to better understand the underlying mechanisms.

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