

# PREDATION ON PROKARYOTES IN THE WATER COLUMN AND ITS ECOLOGICAL IMPLICATIONS

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**Abstract** | The oxic realms of freshwater and marine environments are zones of high prokaryotic mortality. Lysis by viruses and predation by ciliated and flagellated protists result in the consumption of microbial biomass at approximately the same rate as it is produced. Protist predation can favour or suppress particular bacterial species, and the successful microbial groups in the water column are those that survive this selective grazing pressure. In turn, aquatic bacteria have developed various antipredator strategies that range from simply 'outrunning' protists to the production of highly effective cytotoxins. This ancient predator-prey system can be regarded as an evolutionary precursor of many other interactions between prokaryotic and eukaryotic organisms.

## PELAGIC HABITAT

The parts of a lake, river and ocean that make up the water column.

## OLIGOTROPHIC

An aquatic environment that has low levels of nutrient and algal photosynthetic production (for example, high mountain lakes).

## PHAGOTROPHY

The uptake of particles by eukaryotic cells.

## BACTERIOPLANKTON

Bacteria that inhabit the water column of lakes and oceans, either freely suspended or attached to particles.

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The size of a population of organisms in the environment is determined by the balance between their specific cell growth and mortality rates. Microorganisms are no exception to this ecological truism. Bacteria and archaea in PELAGIC habitats can grow in OLIGOTROPHIC conditions<sup>1</sup> and can survive extended periods of starvation<sup>2</sup>. It is therefore unlikely that they die predominantly from a lack of resources. The main sources of microbial mortality in the water column are currently considered to be viral-mediated lysis and grazing by PHAGOTROPHIC protists<sup>3</sup>. Viruses and protists have been deemed the most important cause of microbial death by both environmental virologists and protozoologists, respectively<sup>4,5</sup>, sometimes in conspicuous agreement with their respective research focuses. It is also possible that autolysis (for example, owing to ultraviolet (UV) radiation damage)<sup>6</sup> is a source of mortality in some habitats and, furthermore, genes that are involved in programmed cell death are present in numerous prokaryotic species<sup>7</sup>.

The interplay between viruses and protists in the control of aquatic prokaryotes is still poorly understood. There are indications that virus-induced mortality is more substantial when bacterial productivity is enhanced<sup>8</sup> or in anoxic conditions<sup>9,10</sup>. Sometimes,

the rate of viral infection of aquatic bacteria seems to be higher during periods of intense protistan grazing<sup>8</sup>, however protists themselves can also be targets for viral attack<sup>11,12</sup>.

Theoretical models and empirical surveys indicate that protistan predation might often be most influential in limiting the total abundance and biomass of the BACTERIOPLANKTON, whereas viruses are considered to more profoundly affect prokaryotic community diversity<sup>3,5,13</sup>. The unequal effects of these two mortality sources on microbial assemblages are probably due to the OMNIVORY of HETEROTROPHIC protists on the one hand (they can feed on a range of different prokaryotic species) and the typically narrow host range of viruses on the other. Moreover, viral-associated mortality is highly density dependent — viruses will primarily affect the largest, most rapidly growing bacterial populations in mixed assemblages (known as the 'killing the winners' phenomenon)<sup>3</sup>. By contrast, protistan predation can act on less abundant bacteria in mixed assemblages. This is owing to selective foraging — although many protists are omnivorous, some important groups, particularly flagellates, might nevertheless show a strong preference for particular prey species<sup>14–16</sup> or morphotypes<sup>17,18</sup>.

**Phylogenetic affiliations of predators and prey**  
**Predators.** The identification of the BACTERIVOROUS NANOFLAGELLATES that form large populations *in situ* is often difficult, owing to the paucity of distinct morphological features, the destruction of their delicate forms by fixation procedures and the selectivity of culturing approaches<sup>19,20</sup>. Accordingly, only a few ecological studies have reported the population dynamics of individual taxonomic groups and their relative contributions to total bacterivory<sup>20–22</sup>.

Heterokont taxa (that is, those with unequal flagellae, mainly chryomonads and bicoseocids) constitute 20%–50% of heterotrophic nanoflagellate (HNF) biomass in both marine and freshwater pelagic communities, followed by choanoflagellates (5–40%) and kathablepharids (10–>25%)<sup>23</sup>. Members of the genus *Spumella* seem to be particularly abundant in lakes<sup>21</sup>. Numerous phylogenetic lineages of novel unicellular eukaryotes have been described recently in the oceans by comparative sequence analysis of 18S ribosomal RNA genes<sup>24</sup>. At least two of these lineages comprise small (2–3 mm) phagotrophic organisms that can seasonally form a substantial fraction of all HNFs in coastal waters<sup>25</sup>. The important ciliated bacterivores are small oligotrichs, peritrichs and scuticociliates in freshwaters<sup>26</sup>, and aloricata (naked) oligotrichous forms in marine environments<sup>27</sup>.

**Prey.** Over the past 15 years, cultivation-independent molecular approaches have provided a first insight into the taxonomic composition of the heterotrophic prokaryotic assemblages in the water column of lakes and oceans<sup>28,29</sup>. Members of the SAR11 clade of the  $\alpha$ -proteobacteria are probably among the most common prokaryotes in marine plankton<sup>30</sup>. These bacteria form >50% of the total number of bacteria in the surface waters of the Sargasso Sea. A large fraction of prokaryotes in the deeper waters of the open oceans are related to Crenarchaeota of 'marine group I'<sup>31</sup>. The  $\alpha$ -proteobacteria that are affiliated to *Roseobacter* species are another common component of coastal and offshore microbial assemblages<sup>32</sup>, and they seasonally constitute 10–25% of marine PICOPLANKTON<sup>33</sup>. The  $\gamma$ -proteobacteria that are related to the NOR5 and SAR86 lineages make up another 5–10% of bacteria in coastal waters<sup>33</sup>. Such habitats, moreover, host numerous bacteria that are affiliated to the *Cytophaga-Flavobacterium-Flexibacter* lineage of the Bacteroidetes<sup>34</sup>. Other clades from this large phylogenetic group are typically encountered in freshwaters<sup>29,35</sup>. Several lineages of  $\beta$ -proteobacteria<sup>29,36,37</sup> and Gram-positive Actinobacteria<sup>38</sup> are common in the water column of lakes and rivers<sup>29,36,37</sup>. By contrast,  $\alpha$ - and  $\gamma$ -proteobacteria are usually rare. Both marine and freshwater systems also contain many small unicellular cyanobacteria<sup>39</sup>.

**Bacterivores in aquatic microbial food webs**

The microbial food webs in the water column of lakes, rivers and oceans are composed of viruses, chemoorganotrophic prokaryotes and cyanobacteria, as well as autotrophic and heterotrophic unicellular eukaryotes.

Most organic carbon is produced and consumed in these microbial food webs, which themselves are embedded in the 'classic' grazing food chain (algae-zooplankton–fish).

The 'microbial loop' concept<sup>40</sup> refers to the production of dissolved organic material (DOM) in aquatic food webs during the flux of particulate matter towards larger organisms, and the reincorporation of this DOM by heterotrophic bacteria and archaea. Bacterivorous nanoflagellates and, to a lesser extent, small ciliates 'close' this loop by forming a bridge between the DOM-consuming microorganisms of the picoplankton, which have cell lengths <1–2 mm, and those planktonic organisms (such as crustacean zooplankton, rotifers, larger ciliates and dinoflagellates) that can only feed on cells >3–5 mm in diameter<sup>41</sup>. Protists, moreover, can induce 'short-circuits' in the microbial loop<sup>22</sup> by either releasing DOM<sup>42</sup> in a process that is graphically described as 'sloppy feeding'<sup>43</sup>, by competing for DOM with bacteria<sup>44</sup> and by directly feeding on DETRITAL PARTICLES<sup>45</sup> or viruses<sup>46</sup>. Furthermore, protists also feed directly on bacterial PRIMARY PRODUCERS from the AUTOTROPHIC picoplankton<sup>22,47,48</sup>, that is, on free-living unicellular cyanobacteria that are mainly affiliated to the genera *Synechococcus* and *Prochlorococcus*, and on small eukaryotic algae<sup>49</sup>. In the EUPHOTIC ZONE of the world's oceans, at an ecosystem level, this protistan HERBIVORY might be even more important than the consumption of heterotrophic bacteria<sup>50</sup>.

Protistan grazing on bacteria is also an important mechanism of NUTRIENT REGENERATION, in particular, of nitrogen and phosphorus. These two elements (as well as iron and silicium) limit the growth of prokaryotic and eukaryotic autotrophs in many aquatic systems<sup>51</sup>. Aquatic bacteria are better competitors for phosphorus than eukaryotic algae at low ambient nutrient concentrations<sup>52</sup>. Therefore, bacteria can incorporate the nutrients that are required for the proliferation of primary producers in their biomass. However, bacteria also depend on the release of photosynthetically fixed organic carbon that is overproduced during phytoplankton growth<sup>40</sup>. Consequently, the high bacterial affinity for nutrients has a negative effect on their main source of organic carbon. This 'lose–lose' aspect of bacterial and algal coexistence is counterbalanced by the activity of the bacterivorous protists. Compared with eukaryotes, prokaryotes have a higher nitrogen and phosphorus concentration per volume of biomass, owing to their higher ratio of proteins and nucleic acids to total cell mass<sup>53</sup>. Protists that graze on picoplankton cells release into the environment excess nutrients that are not required for growth, for example, ammonium<sup>54</sup> or dissolved amino acids<sup>55</sup>. This nutrient recycling can stimulate the proliferation of both primary producers<sup>56</sup> and other bacteria<sup>57</sup>.

The abundance of HNFs in different pelagic habitats varies greatly, but typically ranges from 100 to 10,000 cells ml<sup>-1</sup> in the plankton of lakes, rivers and marine surface waters<sup>23</sup>. It has been claimed that the typical ratio of HNFs to heterotrophic bacteria is

OMNIVORY

Ability of animals to feed on different types of prey.

HETEROTROPHY

The acquisition of metabolic energy by consumption of particulate or dissolved organic matter.

BACTERIVOROUS

NANOFLAGELLATES

Small, flagellated protists that range in size from 3 to 15 mm and that can feed on bacteria.

PICOPLANKTON

Organisms suspended in the water column that are less than 2 mm in size.

DETRITAL PARTICLES

Dead organic material suspended in the water column.

PRIMARY PRODUCERS

Organisms that are the original source of organic material in an ecosystem — plants, algae or chemosynthetic microorganisms.

AUTOTROPHY

The acquisition of metabolic energy from the fixation of inorganic carbon, for example, by photo- or chemosynthesis.

EUPHOTIC ZONE

Upper realms of the oceans that are penetrated by sufficient amounts of light for the growth of photosynthetic organisms.

HERBIVORY

The consumption of plants.

NUTRIENT REGENERATION

Processes by which nutrients that are bound in organismic biomass are retransformed into their inorganic form.

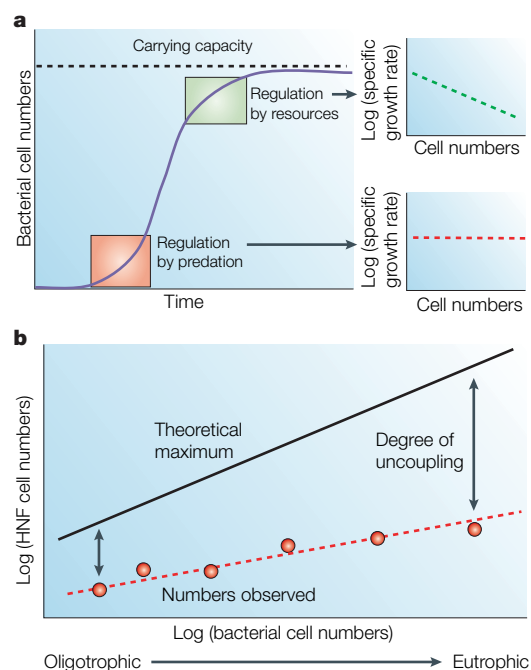
approximately 1:1,000 (REF. 58), but this estimate has been disputed<sup>59</sup>. HNF biomass can change seasonally by a factor of 100. The contribution of larger heterotrophic flagellates (>15 µm) to total bacterivory is unknown. The relative importance of grazing by ciliated protists seems to be highest in very productive waters<sup>26,60,61</sup>.

#### 'Top-down' or 'bottom-up' control?

Is the world of aquatic microorganisms a mirror of the macroscopic (terrestrial) world as outlined by Hairston and colleagues 45 years ago<sup>62</sup>? In this classic ecology paper, the authors argue that "the world is green" because plant biomass is controlled by the availability of resources (for example, light), not by animal grazing, whereas the abundance of herbivores is controlled by predators. If this concept applies to aquatic microbial food webs, the biomass of prokaryotes in the water column should be limited by competition for carbon and nutrients, and the biomass of their protistan grazers should be limited by predation<sup>63</sup>. Alternatively, microbial standing stocks might be controlled by mortality, and protists might be starved at the typical concentrations of prokaryotic cells found in marine and freshwater plankton.

Currently, there seems to be no consensus about which hypothesis is correct, and the factors that regulate bacterial standing stocks must be distinguished from those that limit prokaryotic growth. At a community level, the biomass of heterotrophic aquatic prokaryotes seems to be more tightly controlled by grazing mortality compared with the biomass of other planktonic organisms, and the annual fluctuation in numbers is much less than, for example, that of chlorophyll *a* or microzooplankton<sup>64,65</sup>. This might be a consequence of the similar ranges of growth rates of HNFs and their bacterial prey. By contrast, the extremely low ambient levels of labile organic carbon<sup>66</sup> or growth-limiting nutrients in aquatic systems, and the rapid export of newly produced, particulate organic matter to the sediments<sup>67</sup>, indicate that the growth rate of at least the freely-suspended bacteria in the water column should be mainly limited by resources.

There are indications that the issue of picoplankton 'TOP-DOWN' CONTROL by protistan grazing or 'BOTTOM-UP' CONTROL by the availability of organic carbon and nutrients might be related to overall ecosystem productivity both in marine and freshwater microbial food webs<sup>63,68</sup>. Comparative analyses show that the abundance ratios of HNFs to prokaryotes tend to be higher in nutrient-poor, oligotrophic systems than in more productive regions of the oceans<sup>65</sup> (FIG. 1). Other empirical observations<sup>5</sup> and theoretical models<sup>69</sup> also predict that bacteria are more tightly controlled by protistan predation in highly oligotrophic systems, whereas their growth is limited by competition for nutrients in more productive waters. This might sound counter-intuitive at first, but must be understood in the framework of the food web: top-down control of the prokaryotic assemblages



**Figure 1 | Two models that address the relative importance of predation in oligotrophic and eutrophic conditions.**

**a** | The density-dependent logistic model proposed by Wright and Coffin<sup>147</sup>. If bacterial abundance approaches the carrying capacity of the environment (resource limitation), a negative correlation between bacterial-specific growth rates and abundance is predicted. By contrast, if bacterial abundance and specific growth rates are unrelated, predator control is assumed.

**b** | The empirical model proposed by Gasol<sup>68</sup> reflects the observation that bacterial abundance tends to rise more steeply than predator abundance as the trophic state of the habitat increases (compared with theoretical predictions derived from energetic calculations and measured grazing rates), and therefore the coupling between predator and prey decreases. Bacteria are therefore regarded as resource-limited in eutrophic systems and predation-limited in oligotrophic systems. The graph shows the theoretical maximum abundance of HNFs (black solid line) and the actual observed abundance of HNFs (red dashed line) in different environments. The distance between these two lines reflects the degree of uncoupling between the theoretical maximum and the measured concentration, which, in turn, reflects the degree of uncoupling between HNFs and their bacterial prey. Adapted with permission from REF. 65 © (2002) Springer.

in nutrient-poor habitats implies that the growth of protistan predators themselves is limited by the low availability of prey, that is, it is bottom-up controlled. By contrast, the more nutrient-rich EUTROPHIC systems can sustain a richer community of top predators — larger protists, zooplankton and fish larvae — that control the abundance of the bacterivorous HNFs, thereby releasing the prokaryote community from predation control.

However, such an unambiguous relationship between system productivity and community control might be a feature only of marine waters. In freshwater

#### TOP-DOWN CONTROL

Ecological scenario in which the abundance or biomass of organisms is mainly determined by mortality owing to predation.

#### BOTTOM-UP CONTROL

Ecological scenario in which the abundance or biomass of organisms is mainly determined by a lack of resources and mortality owing to starvation.

#### EUTROPHIC

Aquatic systems with high availability of dissolved organic matter from photosynthetic production or other sources. Examples include shallow lowland lakes and coastal estuaries.

systems, the pattern seems to be substantially modified by other parameters, which include the influx of organic material from rivers or terrestrial sources<sup>70</sup> and cascading effects from higher levels of the food web (that is, the feeding modes of the dominant zooplankton species)<sup>71</sup>.

### Protists attached to particles

Productive and unproductive habitats in aquatic environments are not separated by large geographical distances. A significant proportion of microbial activity in the pelagic zone occurs on, or near, particles such as senescent algae or macroscopic organic aggregates >0.3–0.5 mm in diameter that are formed by the flocculation of phytoplankton and detritus (known as marine snow<sup>72</sup> or lake snow<sup>73</sup>). Both the CHEMOSPHERE and particle surfaces are preferential sites of colonization by mobile microbial species that are guided by CHEMOTAXIS<sup>74</sup>. However, suspended organic aggregates offer no refuge from protistan predation<sup>75</sup>. HNFs are also found at elevated concentrations on and around such aggregates, where they form communities that can differ from those in the ambient water<sup>76</sup>. The colonization of marine snow particles by HNFs occurs in hours to days of aggregate formation<sup>77</sup>, and contact is favoured by both random mobility and chemotaxis<sup>78</sup>. Attached protists might control the abundance of attached bacteria and, hence, influence the rate of aggregate degradation and the export of organic matter from the water column to the sediments.

The attachment of HNFs or ciliates to suspended particles or large algae<sup>79,80</sup> can also offer other advantages. For hydrodynamic reasons, protists are more efficient in collecting suspended bacteria if they are attached to a surface<sup>81,82</sup>. Larger particles might moreover represent a refuge from zooplankton predation. Considering the spatial heterogeneity of bacterial productivity, alternation between the attached and free-living lifestyle in response to prey availability<sup>82</sup> seems to be highly advantageous for bacterivorous protists in the water column of aquatic systems. Such a dual lifestyle is indeed adopted by many pelagic HNFs.

### Feeding behaviour

To understand how aquatic bacterivorous protists shape the composition of microbial assemblages, it is crucial to study their feeding strategies. FILTER FEEDING is particularly advantageous for attached protists<sup>81</sup>, but this strategy is also adopted by free-living marine and freshwater flagellates (for example, choanoflagellates) and small ciliates<sup>26</sup>. Bacterivory by INTERCEPTION FEEDING is a multi-step process that is as complex as the feeding habits of multicellular predators. First, the prey is drawn to the flagellate by a feeding current that is induced by the beat of one flagellum. It is then captured between the flagella and brought into contact with a sensitive area of the cell surface. A phagocytotic vesicle is formed at this morphologically inconspicuous spot and the prey is ingested. During the digestive

process, the food vacuole is transported towards the posterior end of the protist and undigested remains are subsequently expelled by exocytosis<sup>83</sup>.

Owing to the specific mechanisms of particle uptake and handling, protistan predators cannot feed on all sizes of bacteria with equal efficiency. Furthermore, hydrodynamic considerations also indicate that the rates of encounter between flagellates and bacteria are minimal at bacterial cell diameters of ~0.5 mm<sup>84</sup>. Consequently, microbial cells that range in length from 1 to 3 mm are preferentially consumed by many planktonic HNFs<sup>85,86</sup> and bacterivorous ciliates<sup>18</sup>, whereas smaller or larger cells benefit from reduced loss rates<sup>87,88</sup>.

The processing of microorganisms that have been engulfed in food vacuoles depends not only on temperature<sup>89</sup> but also on the bacterial species. Such differential digestion is an important mechanism that is responsible for the apparently selective grazing pressure of HNFs on mixed bacterial assemblages. For example, it seems to be much more time-consuming to digest a Gram-positive than a Gram-negative bacterial cell<sup>90</sup>. As a consequence of the various aspects of feeding, the growth efficiencies of protists can depend strongly on the available bacterial prey species and its nutritional value<sup>14</sup>.

However, finality might not always be an optimal foraging strategy for a suspension-feeding omnivore. Active selection might only be advantageous at high particle concentration, that is, if the formation of new food vacuoles is the rate-limiting step in feeding. At low bacterial concentrations, the selectivity of interception-feeding flagellates markedly decreases, as it is energetically more effective to feed on all particles that can be morphologically ingested and then to attempt to digest them<sup>91</sup>. This is the case, for example, in oligotrophic waters: assuming a random distribution, 1,000,000 bacteria ml<sup>-1</sup> occupy only ~0.1 millionth part of this volume. Accordingly, protists need to collect prey with astonishing efficiency, and an individual protist can clear the particles from at least 10<sup>5</sup> times its own body volume of water every hour<sup>92,93</sup>. Moreover, as for many animals, uptake selectivity can depend on the nutritional state of the grazer: whereas a population of satiated flagellates readily discriminated between bacteria and similarly sized latex beads, a starved population of the same species did not<sup>94</sup>. Protists might encounter such a situation in aquatic habitats that are rich in inedible mineral particles<sup>95</sup>, for example, from terrestrial influx.

Diverging reaction norms between clones seems to be an inherited trait of protistan populations. There can be significant interspecific and intraspecific variability in grazing rates and feeding-related parameters such as growth efficiency<sup>96</sup>. Moreover, protistan feeding can be highly variable over the entire cell cycle<sup>81,82</sup>. For example, individual flagellates can cease to ingest bacteria during cell division. Therefore, it is debatable whether the levels of protistan bacterivory found in short-term measurements can be extrapolated to periods of days or more.

#### CHEMOSPHERE

Zone of elevated concentration of organic molecules that diffuse from the surface of a suspended particle.

#### CHEMOTAXIS

Ability of microorganisms to follow a chemical gradient.

#### FILTER FEEDING

Feeding mode that filters particles from the water by means of a sieving structure. Usually the prey is very small compared with the predator.

#### INTERCEPTION FEEDING

The capture of individual bacteria or particles by direct random contact with a protistan cell. Usually the sizes of the predator and prey are similar.

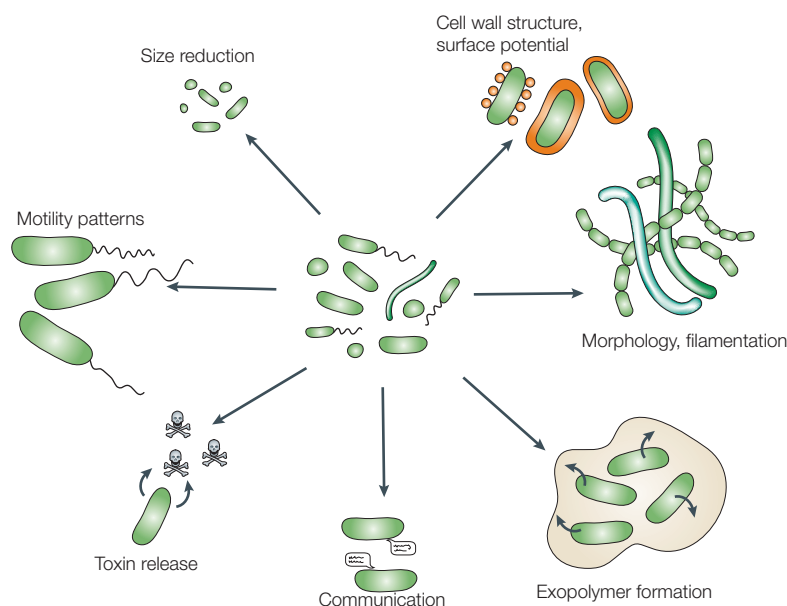


Figure 2 | Phenotypic properties of aquatic bacteria that might provide protection from predation by heterotrophic protists. The different strategies are discussed in the text.

### Mixotrophy

Bacterivorous PHOTOTROPHS occur in various freshwater and marine habitats<sup>97</sup>. The term 'MIXOTROPHY' refers to a broad array of ecological strategies that range from a predominantly phototrophic to an almost exclusively heterotrophic lifestyle. The metabolic costs of forming and maintaining a phagotrophic apparatus in addition to the photosynthetic machinery seem to be rather low (<10% of total metabolic expenditure) and are easily matched by the potential benefits<sup>98</sup>. Mixotrophy is an efficient means of acquiring limiting nutrients in oligotrophic pelagic environments<sup>99</sup>, and might also provide a further source of organic carbon at light-limiting growth conditions<sup>100</sup>. If the higher affinity of prokaryotes for some inorganic nutrients is considered, phagotrophy in phototrophic eukaryotes is understood conceptually as a strategy of 'eating your competitor'<sup>101</sup>. In some systems such as nutrient-poor, high mountain lakes, mixotrophic nanoflagellates can even be the dominant primary producer species. Such mixotrophs severely affect the productivity of the prokaryotic assemblages, and they create a scenario for bacteria of commensalism and predation that has been poetically described as 'neither with nor without you'<sup>102</sup>.

### Microbial antipredator strategies

Several phenotypic features of aquatic bacteria have been interpreted as adaptations to escape protistan grazing pressure (FIG. 2). Some of these features, such as exopolymer secretion and filament formation, could be the consequence of physiological conditions that are unrelated to predation, such as growth rate<sup>103</sup>. Others, such as high-speed motility<sup>104</sup>, cell miniaturization<sup>87</sup> and toxin production<sup>105</sup>, seem to have specifically evolved in response to grazing mortality.

During the past decade, an increasing number of bacteria have been isolated from phylogenetic lineages that also occur in high densities in the water column of lakes and oceans<sup>33,106,107</sup>. Many of these isolates are so-called ULTRAMICROBACTERIA. Although these bacteria might not be entirely protected from flagellate predation, they are nevertheless close to the lower size limits of ingestibility<sup>87</sup>. Therefore, the loss rates of ultramicrobacteria are substantially reduced compared with other bacterial isolates. Currently, there are no convincing alternative suggestions why such a small cell size should be advantageous for microbial growth and survival in aquatic habitats.

Some bacterial strains show considerable morphological plasticity at different growth conditions. For example, some species can form large thread-like morphotypes at enhanced growth rates<sup>103</sup>. Filament formation in subpopulations can also occur at sub-optimal growth conditions or during starvation<sup>108</sup>. Some bacterial species in freshwater habitats are of a permanently filamentous morphotype<sup>35,109</sup>. Filamentous bacteria either exceed the size range of cells that most bacterivorous protists can feed on, or filaments are grazed at substantially lower rates than smaller bacteria<sup>88,110,111</sup>. Filament formation is therefore regarded as an efficient means of protection from protistan predation (but thread-like morphotypes might nevertheless be vulnerable to grazing by larger zooplankton<sup>71</sup>). It is conceivable that such morphological shifts could be triggered by external chemical cues that might even be released by the predator itself.

Aquatic bacteria produce a wide range of extracellular polymeric substances (EPS) that comprise polysaccharides, proteins, nucleic acids, lipids and other biological macromolecules<sup>112</sup>. EPS secretion significantly enhanced bacterial survival on exposure to intense HNF grazing in experiments with EPS-deficient mutants<sup>113</sup>. EPS-producing planktonic bacteria typically develop subpopulations of single cells and microcolonies that are embedded in an EPS matrix<sup>114</sup>, and the larger microcolonies are protected from flagellate predation owing to their size. The shift to the colonial cell type may be a passive consequence of selective feeding on single cells<sup>114</sup>, but microcolony formation can also be specifically induced in the presence of predators by cell-cell communication (QUORUM SENSING)<sup>113</sup>.

Protection from protistan grazing might moreover be mediated by properties of the bacterial cell wall. Gram-positive enteric bacteria are consumed at significantly lower rates by protists than are Gram-negative strains<sup>115</sup>. The consumption of naturally occurring Gram-positive freshwater Actinobacteria is selectively avoided by HNFs, as deduced from analysis of protistan food vacuoles<sup>116</sup>. An ultramicrobacterial Gram-positive isolate from a lake was found to be completely protected from grazing<sup>107</sup>. Bacterial cell surface charge and hydrophobicity have also been suggested as parameters that might influence grazing losses<sup>117,118</sup>. This has been explained on physicochemical grounds

#### PHOTOTROPHS

Organisms that fix inorganic carbon using light energy.

#### MIXOTROPHS

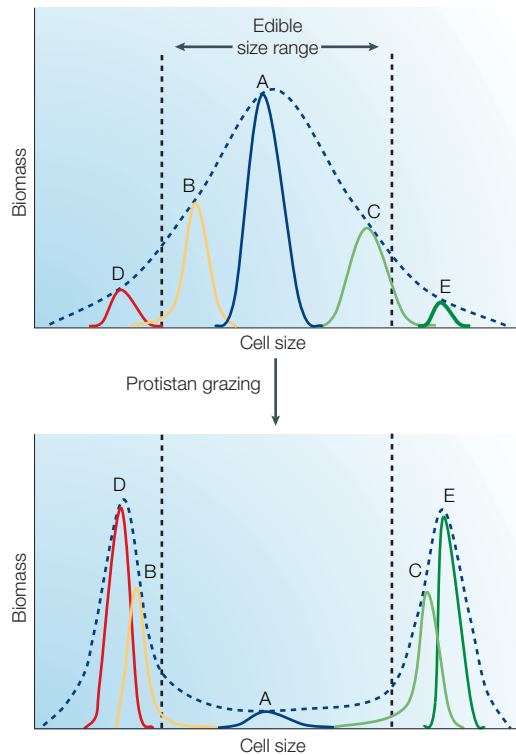
Organisms that are part autotrophic and part heterotrophic, for example, carnivorous plants.

#### ULTRAMICROBACTERIA

Bacteria that maintain cell volumes of <0.1 mm<sup>3</sup> even during exponential growth on rich media.

#### QUORUM SENSING

Bacterial communication system based on the secretion and detection of a quorum, which is a substance that increases with population density and that induces expression of specific genes in the population above a threshold concentration.



**Figure 3 | The effect of predation on microbial community structure.** The effects on biomass size distributions of individual populations (depicted in different colours) are shown. Bacterial species that are in the size range that can be readily ingested by protists (A, B and C) are disproportionately reduced by grazing (shown in the upper panel), unless they can change their morphotype (B, C) as shown in the bottom panel. Populations outside the edible size range (D, E) will be favoured by grazing. Combined from concepts by several authors<sup>127,134,136</sup>.

as the effect of molecular interaction forces on the probabilities of contact between predator and prey<sup>118</sup>. Hydrophobicity is viewed as important in regulating vulnerability to phagocytosis by mammalian leukocytes<sup>119</sup>, but at present, it is unclear if such surface properties have a role as grazing protection mechanisms for aquatic bacteria. A more hydrophobic strain of the marine cyanobacterium *Prochlorococcus* showed significantly higher vulnerability to predation than a similar-sized, but more hydrophilic, strain<sup>118</sup>. By contrast, the feeding rates of three freshwater flagellate species on various strains of heterotrophic bacteria were not significantly dependent on either prey hydrophobicity or cell-surface potential<sup>117</sup>.

Bacterial motility seems to be a two-sided coin. On the one hand, the probability of random contacts between predators and prey increases with bacterial swimming, and motile bacteria can therefore be consumed at higher rates by HNFs<sup>120</sup>. On the other hand, the capture efficiency of rapidly swimming bacteria (>25 mm s<sup>-1</sup>) by flagellates is decreased compared with less rapid swimmers and some bacteria may reach an ‘escape velocity’ that is high enough to effectively protect them from flagellate grazing<sup>104</sup>.

**APOPTOTIC RESPONSE**  
Phenotypic changes that occur during programmed cell death in eukaryotic cells, for example, cell shrinkage.

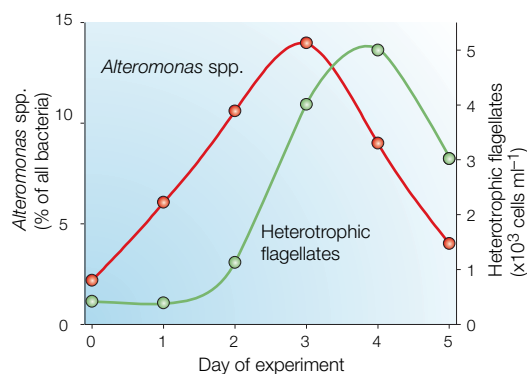
Some bacteria can even poison their predators in a concerted manner: *Chromobacterium violaceum* specifically induced the eukaryotic APOPTOTIC RESPONSE after being ingested by a flagellate<sup>105</sup>. The constitutive production of the toxic agent, violacein, was only observed in strains that were capable of quorum sensing. Toxin secretion related to quorum-sensing abilities has also been reported for mature biofilms of *Pseudomonas aeruginosa*<sup>113</sup>.

**Community-level effects of predation**

The current conceptual model of the impact of predation on the morphological structure of bacterioplankton implies that flagellate grazing eliminates mainly medium-sized cells and shifts the size-structure of bacterial assemblages towards smaller and/or larger cells<sup>121</sup> (FIG. 3). Blooms of protist-inedible filamentous or microcolony-forming bacteria are typically encountered in the spring communities of productive lakes<sup>35,122,123</sup> and in acidified lakes with high levels of grazing by mixotrophic flagellates<sup>124</sup>. Similar phenotypic changes in the bacterioplankton can be induced experimentally by food-web manipulation<sup>125</sup>. The growth of resistant bacteria sometimes completely compensates for the overall losses in bacterial biomass owing to grazing mortality<sup>126,127</sup>.

The small average cell size of aquatic bacteria is usually thought to be a result of starvation<sup>2</sup> or protection against size-selective protistan feeding. This might not necessarily be a contradiction. One of the less obvious consequences of HNF grazing is its negative influence on the activity of bacterial assemblages. Starvation of aquatic bacteria often results in a marked decrease in cell size, and non-growing bacteria from many culturable genera tend to be smaller than growing cells<sup>2</sup>. HNFs feed on these small starving cells at lower rates<sup>85,120</sup>. They seem to control the overall productivity of bacterioplankton by specifically feeding on the larger, most rapidly growing<sup>64</sup> or dividing<sup>128</sup> cells in the assemblage (for example, cells with high nucleic acid content<sup>129</sup>) rather than by unselectively cropping bacterial standing stocks.

The formation of grazing-resistant filamentous morphotypes in some freshwater bacteria has been described as a side effect of enhanced growth rates<sup>103</sup>. Therefore, the response of bacterioplankton assemblages to HNF predation might also be related to the supply or qualitative composition of dissolved organic substrates<sup>130</sup>. There are even indications that different growth-limiting factors might favour the development of specific types of grazing-resistant bacteria in mixed assemblages. When freshwater bacteria in phosphorus-limited, continuous culture were exposed to HNF grazing, large filamentous cells were dominant. If the same experimental assemblages were limited by organic carbon, small, highly motile bacterial morphotypes prevailed that could escape from predation<sup>131</sup>. Such observations invite speculation as to whether the rareness of filamentous bacteria in marine picoplankton — compared with freshwater picoplankton — might be related to a different interplay of resource limitation and predation in the two types of habitat.



**Figure 4 | 'Killing the winner' by predation.** Protistan predation can counterbalance the growth of invasive bacterial species in coastal marine waters. Rare bacteria, such as *Alteromonas* spp., rapidly overgrow the original microbial assemblages at artificially reduced grazing pressure (such as in dilution cultures). These species typically form cells that are larger than other planktonic bacteria and are therefore disproportionately reduced during the regrowth of the flagellate population. The vulnerability of such bacteria to grazing probably accounts for their rarity in the bacterial water column. Data compiled from REF. 16.

### Do protists affect diversity?

The impact of predators on the diversity of the prey community is one of the fundamental ecological discoveries of the early twentieth century: in 1925, Tansley and Adamson described the invasion and dominance of rare plant species after the fencing-out of rabbits from plots of chalk grassland<sup>132</sup>. By contrast, theoretical models of microbial food-web interactions have deemed protistan predation of minor importance for bacterioplankton community composition<sup>3,69</sup>. However, as these analyses were based on the unrealistic assumption of totally unselective protistan feeding, there are legitimate doubts as to whether this is the final word on the subject.

Experimental investigations and field studies both indicate that the community structure of pelagic bacterial assemblages can be shaped substantially by protistan predation. The addition of protistan grazers to experimental bacterial assemblages or the manipulation of predator densities in natural microbial food webs can induce profound shifts in community composition<sup>37,127,133</sup>. Moreover, the bacterivores leave a direct imprint on the taxonomic composition of natural aquatic microbial assemblages: phylogenetic groups of ultramicrobacteria occur in high densities both in lakes and in the ocean<sup>30,36</sup>, and the Gram-positive Actinobacteria, which are allegedly better protected from grazing, constitute a prominent fraction of the bacterioplankton in many freshwater assemblages<sup>38</sup>.

For practical reasons, it is currently not possible to directly investigate whether predation affects the SPECIES RICHNESS in natural microbial assemblages. Laboratory experiments nevertheless indirectly indicate that the microdiversification of a single freshwater bacterial species into strains with different cell sizes might be related to size-selective protistan grazing<sup>87</sup>.

There is more direct evidence that predation mortality can cause substantial changes in bacterioplankton COMMUNITY EVENNESS. High HNF predation induced distinct blooms of an ultramicrobacterial Gram-positive species<sup>133</sup> and a flock-forming *Pseudomonas* strain<sup>134</sup> in experimental assemblages of freshwater bacteria. During periods of intense HNF grazing, a single filamentous bacterial species transiently constituted >40% of total bacterioplankton biomass in a lake<sup>35</sup>.

Shifts in bacterial phylogenetic composition can also occur when grazing pressure is relieved<sup>135</sup>. In temperate lakes, this is a natural and recurrent phenomenon: during the spring, 'clear-water' phase, bacterivorous protists (as well as algae and filamentous bacterial morphotypes) are suppressed by filter-feeding zooplankton of the genus *Daphnia*<sup>71</sup>. Experimental removal of this top-predator from a eutrophic pond rapidly induced a bloom of HNFs, followed by taxonomic and phenotypic successions in the microbial assemblages towards protist-inedible bacterial species<sup>136</sup>.

Protistan grazing can selectively reduce the relative abundance of some bacterial groups, for example, unicellular cyanobacteria<sup>48</sup> or specific freshwater  $\beta$ -proteobacteria<sup>37</sup>. Some HNF species in coastal North Sea plankton can suppress planktonic blooms of large, metabolically active bacteria that pursue a 'FEAST OR FAMINE' growth strategy (for example, *Alteromonas*, *Vibrio* and *Pseudoalteromonas* species (FIG. 4)<sup>16</sup>. It is possible that selective protistan predation might also control the free-living subpopulations of related pathogenic species with similar morphological characteristics, for example, *Vibrio cholerae*<sup>137</sup>.

The complex feeding strategies of protists indicate that different co-occurring bacterivorous species probably show a high degree of functional diversity<sup>138</sup>. There are indications that the benefits of bacterial adaptations that protect against grazing might be partly predator specific, and that the taxonomic composition of HNF communities might influence the diversity of bacterial assemblages. This has been shown in experimental microbial assemblages that were exposed to grazing by a flagellate or a ciliate predator species (or both)<sup>133</sup>. It has been argued that a diverse HNF community might not allow the dominance of a single bacterial species as individual microorganisms follow strategies that are only effective against some predator species<sup>139</sup>.

The interactions of top-down and bottom-up factors in shaping and stabilizing the community structure of aquatic microbial assemblages are still poorly understood. It has been suggested that the magnitude of shifts in bacterial community composition might be deduced from changes in the ratios of overall bacterial mortality rate to growth rate<sup>140</sup> (FIG. 5). This concept predicts that communities with an apparently stable taxonomic composition can be encountered at very different levels of total microbial productivity or mortality, and that rapid community changes are induced by sudden shifts from top-down to bottom-up control, or vice versa. Although there is empirical evidence for a significant relationship

**SPECIES RICHNESS**  
Number of species that are present in a community.

**COMMUNITY EVENNESS**  
Balance of the respective number of individuals in each species of a community.

**FEAST OR FAMINE**  
Growth strategy of microorganisms that rapidly proliferate if conditions are optimal and that can survive extended periods of starvation.

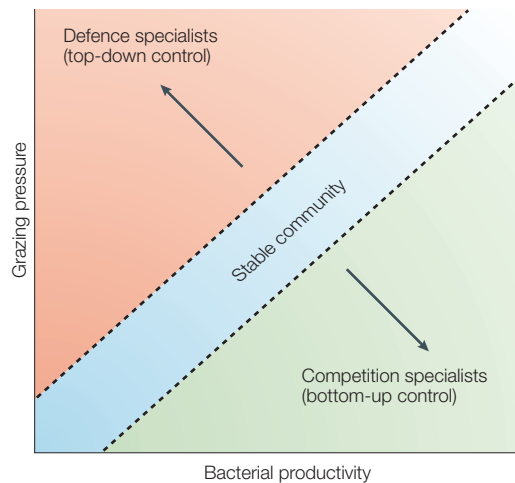


Figure 5 | **A simple conceptual model that relates changes in microbial species composition to community-level growth and loss.** This model, proposed by Simek *et al.*<sup>140</sup>, predicts that stable communities of microbial species will exist at different levels of microbial productivity if there is an approximate balance between bacterial production and protistan bacterivory. Changes in species composition of the microbial assemblage are triggered by rapid shifts from ‘top-down’ to ‘bottom-up’ control. Depending on the direction of such shifts, bacterial species are favoured that are able either to minimize predation losses (‘defence specialists’) or to respond most rapidly to favourable changes in growth conditions (‘competition specialists’).

between growth-to-mortality-rate ratios and taxonomic community shifts after experimental manipulation in the field or in mixed cultures<sup>140</sup>, it remains to be investigated whether this can also be verified in natural microbial assemblages.

The study of predator effects on the community structure of aquatic prokaryotes is currently favoured by the rapid advance of methods in the field of microbial ecology. The community composition and feeding behaviour of aquatic protists can be addressed by a combination of ECOPHYSIOLOGICAL and MOLECULAR BIOLOGICAL approaches<sup>25,116,141</sup>. Moreover, techniques have become available to determine the physiological activities of defined prokaryotic taxa *in situ* without prior cultivation<sup>142</sup>. A combination of these approaches will eventually allow us to assess whether selective predation on particular prokaryotic species can influence the magnitude and stability of specific biogeochemical processes. For example, members

of the *Roseobacter* clade are important mediators of the turnover of organic sulphur compounds in the marine water column<sup>143</sup>, but it is unknown how the abundance of these bacteria is controlled by the food web. Furthermore, investigations of microbial predator–prey interactions might also be stimulated by more applied motivations. As outlined above, the elimination rates of some pathogenic bacterial species might be related to the structure of the microbial food web<sup>137</sup>. It is, moreover, conceivable that the conspicuously unsuccessful release of specific (for example, genetically modified) microbial strains for bioremediation purposes could be a consequence of their selective removal by protistan predation.

**Evolution of microbial predator–prey systems**

Predation could be one of the oldest interactions between the prokaryotic and eukaryotic worlds<sup>144</sup>. The uptake and subsequent trapping of particulate organic matter in cell vacuoles allows for the evolutionary development of specific digestive processes. These digestive processes are probably more economical for controlled and flexible processing of engulfed prokaryotic cells inside eukaryotic predators than for the direct secretion of exoenzymes into the environment.

However, prokaryotes are the ‘senior players’ in the evolutionary ‘arms race’. From a bacterial perspective, a eukaryote might pose a threat, but might also represent a habitat, a food patch, or both. Microorganisms have developed various pre-ingestion defence mechanisms and effective toxins against phagocytosis by unicellular eukaryotic cells, which can be aquatic protists or leukocytes<sup>105,119</sup>. Some bacteria have evolved means to take control of the food vacuole by circumventing digestion, giving rise to fundamental ecological phenomena such as phototrophy in eukaryotes<sup>145</sup> and other forms of endosymbiosis<sup>146</sup>. Symbiosis and parasitism are evolutionarily related lifestyles, for example, the typical bacterial endosymbionts of amoebae are phylogenetically closely related to pathogens<sup>146</sup>. It therefore seems to require only a small step to progress from the adaptations that free-living bacteria use for protection against protistan grazing to the elaborate, specific mechanisms that promote bacterial pathogenicity. In summary, it is conceivable that the invention of bacterivory by eukaryotic protists has shaped microbial evolution as profoundly as, for example, oxygenic photosynthesis.

ECOPHYSIOLOGICAL APPROACHES  
Determination of protistan physiological properties under field conditions, for example, of feeding rates through uptake of surrogate particles.

MOLECULAR BIOLOGICAL APPROACHES  
Cultivation-independent identification of protists in environmental samples by sequencing of rRNA genes and fluorescence *in situ* hybridization with rRNA-targeted probes.

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#### Competing interests statement:

The author declares no competing financial interests.

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## CORRIGENDUM

# PREDATION ON PROKARYOTES IN THE WATER COLUMN AND ITS ECOLOGICAL IMPLICATIONS

*Jakob Pernthaler*

*Nature Reviews Microbiology* **3**, 537–546 (2005), doi:10.1038/nrmicro1180

In the above article, the following passage of text on page 542 might have been misleading: ‘Some bacteria can even poison their predators in a concerted manner: *Chromobacterium violaceum* specifically induced the eukaryotic APOPTOTIC RESPONSE after being ingested by a flagellate<sup>105</sup>. The constitutive production of the toxic agent, violacein, was only observed in strains that were capable of quorum sensing.’ It should have read: ‘Some bacteria can even poison their predators in a concerted manner: *Chromobacterium violaceum* caused rapid cell death after being ingested by a flagellate<sup>105</sup>. The toxic agent violacein is known to induce the APOPTOTIC RESPONSE in eukaryotic cells<sup>105a</sup>. Its constitutive production was only observed in strains that were capable of quorum sensing.’

The author apologizes to readers for any confusion caused.

105a. Melo, P. S., Justo, G. Z., de Azevedo, M. B., Duran, N. & Haun, M. Violacein and its  $\beta$ -cyclodextrin complexes induce apoptosis and differentiation in HL60 cells. *Toxicology* **186**, 217–225 (2003).

## ERRATUM

Also in this article, a global formatting problem unfortunately resulted in all units in  $\mu\text{m}$  being mistakenly changed to mm. We wish to apologize to the author, and to readers, for any confusion caused.