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Predation in a Microbial World: Mechanisms and Trade-Offs of Flagellate Foraging

Thomas Kiørboe

Centre for Ocean Life, DTU Aqua, Technical University of Denmark, Kongens Lyngby, Denmark; email: tk@aqua.dtu.dk

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Abstract

Heterotrophic nanoflagellates are the main consumers of bacteria and picophytoplankton in the ocean and thus play a key role in ocean biogeochemistry. They are found in all major branches of the eukaryotic tree of life but are united by all being equipped with one or a few flagella that they use to generate a feeding current. These microbial predators are faced with the challenges that viscosity at this small scale impedes predator–prey contact and that their foraging activity disturbs the ambient water and thus attracts their own flow-sensing predators. Here, I describe some of the diverse adaptations of the flagellum to produce sufficient force to overcome viscosity and of the flagellar arrangement to minimize fluid disturbances, and thus of the various solutions to optimize the foraging–predation risk trade-off. I demonstrate how insights into this trade-off can be used to develop robust trait-based models of microbial food webs.

INTRODUCTION

Microbes are the engine of ocean biogeochemistry, and the structure and function of bacterial and phytoplankton communities are shaped through competition and predator–prey (and viral) interactions. Most biogeochemical models emphasize bottom-up regulation and competition, where the underlying resource acquisition kinematics has a firm mechanistic basis but where predation is included only as a closure term. MIT's Darwin model is a famous example (Follows & Dutkiewicz 2011). Yet predation is important in determining microbial population dynamics and food webs for at least three reasons. First, and most obvious, predation is what allows energy and matter to flow between organisms to form food webs. Second, predation (top down) is as important as resource acquisition and consequent growth rates (bottom up) in regulating prey biogeography and population dynamics, simply because (predation) mortality rates on average equal growth rates. In fact, in the realm of the simple classical Lotka–Volterra predator–prey model, the average abundance of the prey is entirely independent of properties of the prey itself (e.g., its resource uptake and growth rate) and depends only on properties of the predator (see the sidebar titled The Lotka–Volterra Predator–Prey Model). Finally, and maybe not commonly appreciated, all organisms face the fundamental dilemma of eating without being eaten, and the consequent fundamental foraging and defense trade-offs are likely the most important source of organismal diversity and, together with

THE LOTKA–VOLTERRA PREDATOR–PREY MODEL

To illustrate the effects of predation on prey populations, consider the simple Lotka–Volterra predator–prey model. The rates of change of the population sizes of bacteria (*B*) and flagellates (*F*) are given by, respectively,

$$\frac{\mathrm{d}B}{\mathrm{d}t} = \mu B - \beta BF,$$

$$\frac{\mathrm{d}F}{\mathrm{d}t} = \gamma \beta BF - \delta F,$$

where μ is the growth rate of the bacteria, β is the clearance rate of the flagellates, γ is the growth efficiency of the flagellates, and δ is the mortality rate of the flagellates. The steady-state concentrations of bacteria and flagellates are, respectively,

$$\hat{B} = \frac{\delta}{\beta\gamma},$$

$$\hat{F} = \frac{\mu}{\beta}.$$

Thus, surprisingly, the steady-state concentration of prey (bacteria) depends only on properties of the predator and not on its own properties (e.g., its growth rate, and thus resource availability). Inserting typical parameter values yields average concentrations of bacteria and flagellates on the order of 10^6 and 10^3 cells mL^{-1}, respectively, similar to concentrations found in the ocean (Kiørboe 2008).

The independence of bacterial abundance from the bacteria's properties is only apparent: The prey may defend itself and thereby reduce the predator's clearance rate on the prey. The better defended the prey is, the more of them there are, and—again surprisingly—the more of their predators there are, because the steady-state abundances of both predator and prey vary inversely with the predator's clearance rate. Thus, the distribution and population dynamics of microbes depend strongly on the underappreciated effects of predation, including the ability of the prey to defend itself.

the environment, the main determinant of the structure and function of (microbial) communities and ecosystems (Cadier et al. 2019, Thingstad 2022, Tilman 1990).

Biogeochemical trophic trait-based models of plankton communities that explicitly consider predation are only now beginning to emerge (Prowe et al. 2019, Serra-Pompei et al. 2020, Ward et al. 2012). However, they are typically based on heuristic descriptions of predator–prey interactions and have a weak mechanistic basis, and thus they cannot safely be extrapolated outside their calibration envelope. Also, foraging and defense trade-offs are rarely explicitly considered (see, however, Cadier et al. 2019; Våge et al. 2014, 2018), maybe because of the notorious difficulty in both demonstrating and quantifying them.

Predators of microbes range widely in size, from few-micron-sized flagellates to centimeter- or even meter-sized pelagic tunicates, and their prey capture mechanisms vary similarly. In this synthesis, I focus on the mechanisms of foraging and the associated trade-offs in flagellates. Flagellates are the main consumers of bacteria and picoeukaryotes in the ocean, include important primary producers, and are themselves prey for higher trophic levels. They have diverse resource acquisition modes—heterotrophic, mixotrophic, and autotrophic—that result from their evolutionary history and origin (Tikhonenkov 2020). My focus here is on predatory flagellates, but with necessary excursions to other foraging modes. In nutrient–phytoplankton–zooplankton-type models, heterotrophic pico- and nanoflagellates are often treated as one functional unit, but they are in fact a hugely diverse group, with representatives in all major branches of the eukaryotic tree of life (Adl et al. 2012, 2019; Burki et al. 2020; Tikhonenkov 2020) (**Figure 1**). While united by being small, unicellular, and equipped with one or a few flagella with a conserved internal structure, the number, position, beat pattern, and kinematics of the flagella are very diverse, as are the external structures of the flagella (**Figure 2**). The overarching hypothesis is that this diversity represents different solutions to the resource acquisition–predation risk trade-off, which in turn governs the structure and function of microbial communities. Other grazers on microbes (mainly pelagic tunicates) and viruses as a source of mortality and structuring are not considered here.

Predatory flagellates face three main challenges. First, the ocean is nutritionally dilute compared with terrestrial systems, and flagellates (and other zooplankton) need to daily clear a volume of water for prey that corresponds to approximately 10^6 times their own cell volume to maintain a population in the face of predation (Hansen et al. 1997, Kiørboe 2011). Second, at the spatial scale of flagellates, water appears as thick as syrup, and viscosity impedes predator–prey contact: A flagellate approaching a prey will—everything else being equal—tend to push away the prey. And third, flagellates generate a feeding current by the beating of their flagella, but this disturbance of the ambient water may attract their flow-sensing predators (Nielsen & Kiørboe 2021). This leads to a fundamental trade-off between eating and not being eaten, like that found in all other predators but underexplored in microbes.

I begin with a brief account of flagellate evolution. This will be followed by three sections, one that describes the mechanism of flagellate feeding, one that considers foraging trade-offs in flagellates, and one that briefly considers defense trade-offs in flagellate prey. I conclude with a brief account of the implications of trade-offs for the structure, functioning, and modeling of marine microbial food webs.

THE ORIGIN OF EUKARYOTES AND THE EVOLUTION OF FLAGELLATES

The first eukaryotic cell arose perhaps 2 billion years ago, when a lineage descended from marine Archaea developed the endomembrane system and a nucleus and later enslaved a bacterium that eventually evolved into the mitochondrion (Cavalier-Smith 2013). The first eukaryotic cell was likely an unflagellated amoeboid type. Early in the evolution of eukaryotes, the flagellum appeared,

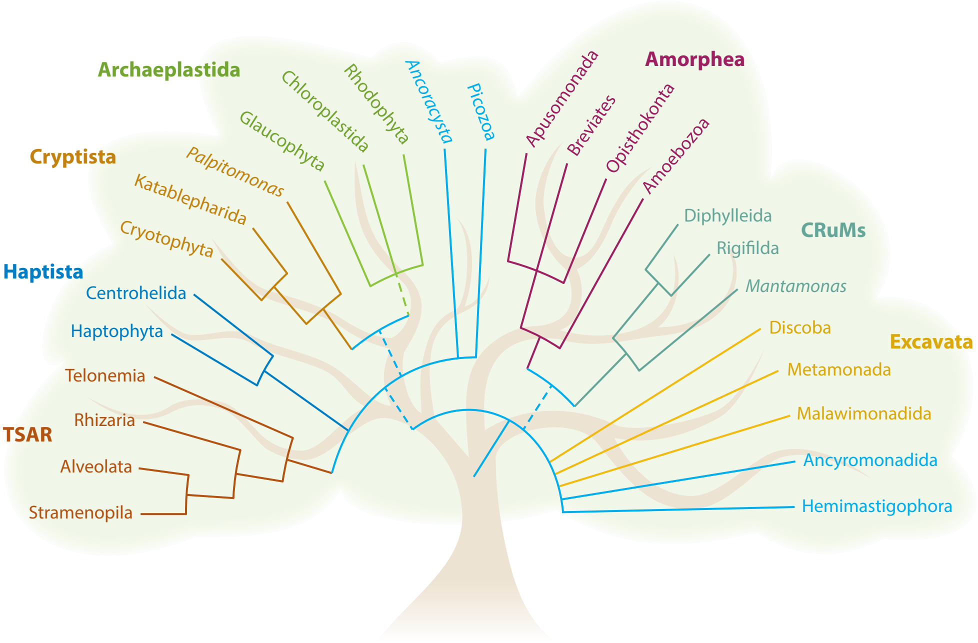

Figure 1

The eukaryotic tree of life. Phagotrophic flagellates are found among all of the branches of the tree. The higher plants evolved as a side branch among the Chloroplastida, and animals evolved as a side branch within the Opisthokonta as a sister group of the choanoflagellates. The position of the recently defined supergroup Provora is uncertain, but it may be a sister group to a supergroup comprising TSAR and Haptista (Tikhonenkov et al. 2022). Abbreviations: CRuMs, Collodictyonidae (also known as Dyphylleida), Rigifilida, and *Mantamonas*; TSAR, Telonemia, Stramenopila, Alveolata, and Rhizaria. Figure modified from Burki et al. (2020) (CC BY 4.0).

perhaps initially as a sensory organ (Jékely & Arendt 2006) that later also became an organelle that played a key role in propulsion and—maybe in particular—foraging in these early heterotrophic flagellates. The ability to generate a feeding current must have increased the foraging efficiency considerably. Cilia or flagella (which are the same thing) are found among all eukaryote groups, and this, together with their conserved 9+2 structure, shows that they evolved before the living eukaryote groups diverged. Considerable evidence suggests that the last common ancestor of living eukaryotes was, in fact, a biflagellated cell (Cavalier-Smith 2013).

The first biflagellated flagellates may have been unable to produce sufficiently efficient feeding currents to achieve the high clearance rates required in the modern ocean (Dölger et al. 2017). However, the ancient ocean was severely nutrient limited and able to support only very short food chains, and predators on the flagellates were thus largely missing. Hence, the requirement for efficient foraging was relaxed. In fact—and counterintuitively—lower foraging efficiencies (clearance rates) may lead to larger population sizes of both flagellates and their prey (see the sidebar titled The Lotka–Volterra Predator–Prey Model). Eukaryotic diversity remained low until approximately 1 billion years ago, before which the ocean oxygen content was also rather low. Nutrient enrichment of the ocean, or better oxygen conditions, then allowed eukaryotic diversification, likely facilitated by the addition of higher trophic levels and predators on flagellates.

Also, early in the evolution of eukaryotes, more than a billion years ago, a heterotrophic flagellate consumed a cyanobacterium that became internalized and evolved to become the chloroplast

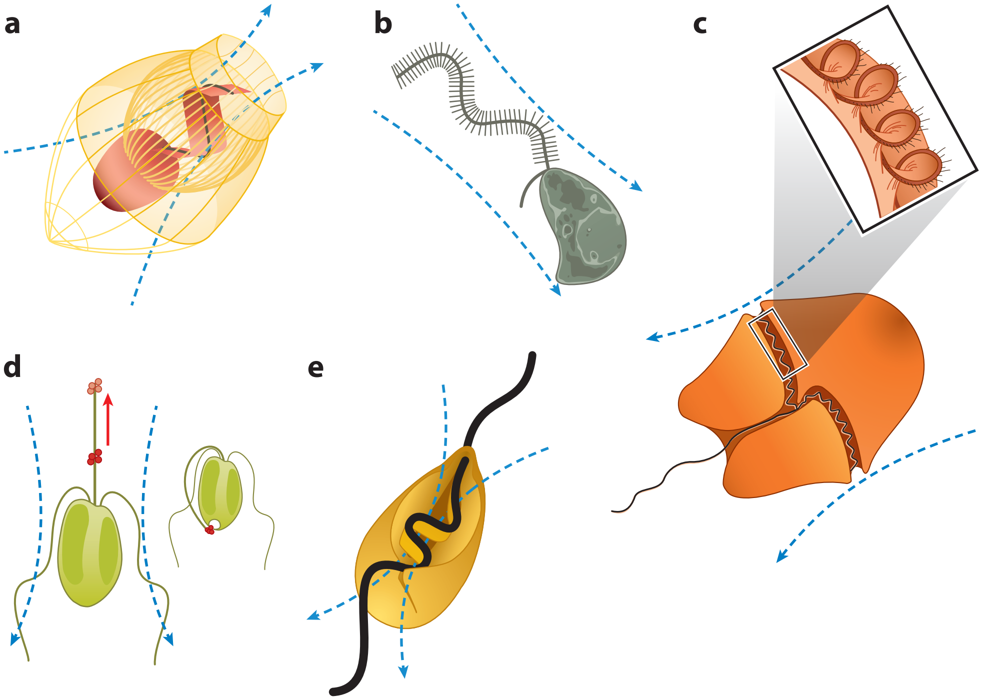

Figure 2

Five generic flagellate types to illustrate some of the morphological diversity of flagellates: (*a*) choanoflagellate (Opisthokonta), (*b*) stramenopile, (*c*) dinoflagellate (Alveolata), (*d*) haptophyte (Haptista), and (*e*) excavate. Figure based on Andersen (1988), Berdach (1977), Kawachi et al. (1991), Moestrup (1982), Nielsen et al. (2017), and Simpson & Patterson (1999).

(Ku et al. 2014, Parfrey et al. 2011). Eukaryotic photosynthetic organisms were born, and this unique event subsequently gave rise to all photosynthetic eukaryotes, including the higher plants. Several secondary (and even tertiary) symbiotic events, with one predatory flagellate consuming and internalizing a photosynthetic eukaryote, have followed during evolutionary history, further diversifying the photosynthetic flagellates (Keeling 2013, Keeling & Palmer 2008). The first photosynthetic flagellates thus evolved from predatory flagellates and were by default mixotrophic. In some lineages, photosynthesis became the primary or sole nutritional mode, e.g., the diatoms, which have lost the ability to phagocytize other cells as well as their flagella. Other photosynthetic flagellates—maybe most—remained mixotrophic, while others again eventually lost their photosynthetic ability and became purely heterotrophic. Thus, the different resource acquisition modes of flagellates—heterotrophic, mixotrophic, and autotrophic—have diverse evolutionary origins. Of course, the environment in which the organisms live, together with their cell sizes, determines what resource acquisition mode is the most beneficial (Chakraborty et al. 2017); nevertheless, the different flagellar arrangements characteristic of different lineages may have constrained their evolutionary potential (**Figure 2**). Kiørboe et al. (2014) hypothesized that the different flagellar arrangements found among flagellates may be differentially efficient for acquisition of prey (heterotrophs) or for nutrient molecules (autotrophs) and may expose the flagellates differently to rheotactic (flow-sensing) predators. Thus, the fundamental trade-off between resource acquisition and predation avoidance may depend on the flagellar arrangement (Nielsen & Kiørboe 2021). In turn, this could determine the dominant resource acquisition mode of different lineages and, hence, their role in microbial food webs.

HOW FLAGELLATES FEED

Predatory Resource Acquisition in Flagellates

In the vast oligotrophic ocean, most prokaryotes are small and nonmotile and hence subject to Brownian motion. Does this motility play a role for prey encounter in flagellates? The volume-specific clearance rate of the smallest known eukaryote grazer, *Minorisa minuta* (radius $a \sim 0.75\ \mu\mathrm{m}$) is approximately 10^6 cell volumes per day (del Campo et al. 2013b). The clearance rate based solely on Brownian diffusion of the smallest nonmotile bacteria ($a = 0.3\ \mu\mathrm{m}$; Brownian diffusion coefficient $D \sim 10^{-8}\ \mathrm{cm}^2\ \mathrm{s}^{-1}$) would amount to the equivalent of $3Da^{-2} \sim 0.5 \times 10^6$ cell volumes per day. If we assume that this small flagellate swims at $50\ \mu\mathrm{m\ s}^{-1}$, a typical value, then the enhancement of the clearance rate due to swimming (advection) is a factor of approximately 2 (see the simple equations in Kiørboe 2008). This produces an estimated specific clearance that matches the clearance rate calculated from data by del Campo et al. (2013b) and matches the clearance required to sustain a population in the open ocean. However, the contribution of Brownian motion to prey encounter decreases with the square of flagellate size, and this contribution is therefore negligible for larger nanoflagellates.

In more nutrient-rich areas, a significant fraction of the bacteria are large and motile, and here prey encounter may depend on bacterial swimming. The encounter rate due to bacterial swimming can be estimated as $\pi a^2 v$, where v is the bacteria swimming speed, and the specific clearance rate can be estimated as $\pi a^2 v(4/3\ \pi a^3)^{-1} = 3v/4a$. With a typical swimming speed of marine motile bacteria of $30\ \mu\mathrm{m\ s}^{-1}$ (Johansen et al. 2002), this suggests that cells with a radius smaller than $\sim 3\ \mu\mathrm{m}$ would not need a feeding current to make a living on motile bacteria, and conversely, larger forms would be unable to do so. An exception is larger forms with long prey-collecting pseudopodia, such as nonmotile helioflagellates (*Ciliophrys* spp.), which are passive ambush feeders that depend on the motility of their prey. In all other cases, predatory flagellates depend on advection due to swimming or a feeding current to encounter their bacterial prey.

Advection-based predation in the low-Reynolds-number world in which flagellates live is challenged by the fact that viscosity impedes predator–prey contact: The viscous boundary layer surrounding a swimming flagellate will tend to push away bacterial prey that are approached (Jabbarzadeh & Fu 2018), and overcoming the effect of viscosity requires a significant force. The beating flagella generate a feeding current that facilitates predator–prey contact, but formal fluid physics is often required to understand prey encounter mechanisms. The fluid physics of flagella and microswimmers has been intensively studied by physicists, but mainly from the perspective of propulsion (Lauga 2020). However, one can argue that resource acquisition is a more important fitness component than propulsion per se, and that the evolution of flagella and flagellates is governed more by resource acquisition optimization than by propulsion needs. Thus, propulsion may in some cases be considered a secondary adaptation of the beating flagellum, which is further emphasized by the fact that many heterotrophic flagellates are attached to a surface when feeding (Boenigk & Arndt 2002, Christensen-Dalsgaard & Fenchel 2003, Suzuki-Tellier et al. 2022). The fluid dynamics of the huge diversity in flagellar arrangements (**Figure 2**) has until recently only been poorly examined. Thus, a recent review on propulsion modes in microswimmers, including flagellates, considered only the naked flagellum trailing behind the cell while pushing it forward (Velho Rodrigues et al. 2021), even though this swimming gait is the exception rather than the rule for free-living flagellates. Also, as we shall see, such naked flagella appear unable to deliver the force required to generate a sufficiently efficient feeding current.

Most experimental, observational, and theoretical studies on foraging in predatory flagellates have concentrated on four groups (**Figure 2**): (*a*) the heterokont stramenopiles, which are characterized by having two dissimilar (heterokont) flagella, one naked and one equipped with stiff

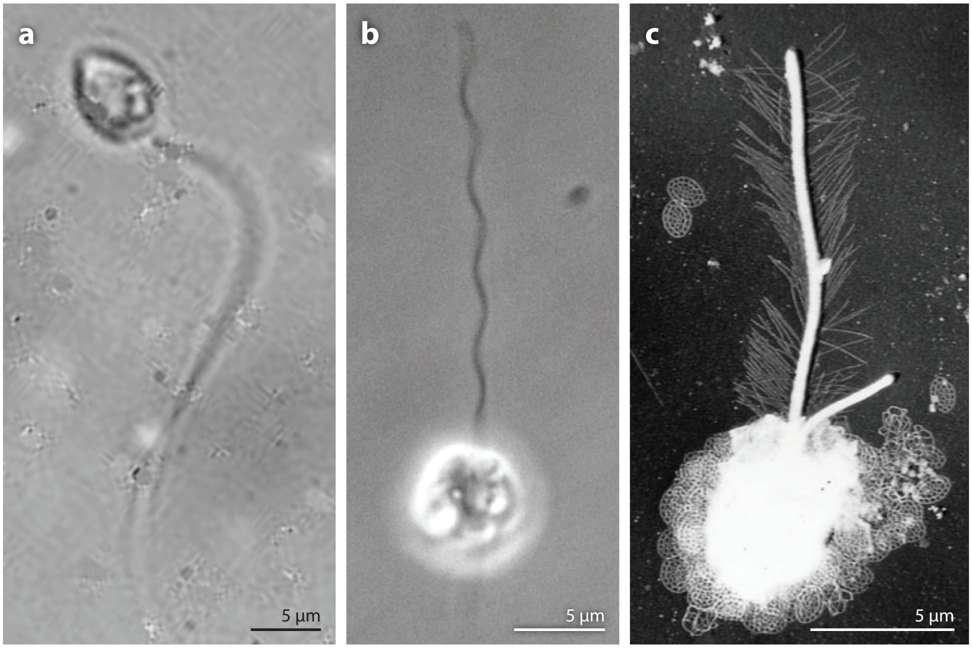

Figure 3

(*a*) Video still image of the swarmer stage of a choanoflagellate (*Salpingoeca rosetta*), (*b*) video still image of a swimming stramenopile flagellate (*Pseudobodo* sp.), and (*c*) transmission electron micrograph of a stramenopile flagellate (*Paraphysomonas* sp.) showing the hairs on the flagellum. Panels *a–c* provided by S. Asadzadeh, S. Suzuki, and T. Fenchel, respectively.

hairs (mastigonemes) (**Figure 3*b*,*c***); (*b*) the dinoflagellates, which also have two flagella, one that trails behind the swimming cell and one equatorial flagellum that is embedded in a groove; (*c*) the haptophytes, which have two left–right-symmetric flagella and are further equipped with a haptonema, a flagellum-like organelle that extends from the cell in the swimming direction and can be more than 20 cell diameters long; and, finally, (*d*) the choanoflagellates, which are characterized by the presence of a collar made up of 20–50 closely spaced microvilli that surrounds the single flagellum. All these groups are small forms, approximately 5 μm in diameter, except for the dinoflagellates, which may reach 100 μm or more.

The focus on these groups does not necessarily reflect their importance or abundance in the ocean; rather, it reflects that they can be cultured. Most forms in the ocean are uncultured and have thus escaped more detailed behavioral observations (del Campo et al. 2013a). However, some of the above groups appear to be quantitatively significant or even dominant in the ocean, like the stramenopiles and the choanoflagellates, which may account for more than 80% of the heterotrophic flagellates in the oligo- and mesotrophic Atlantic and Pacific Oceans (Kamennaya et al. 2022, Obiol et al. 2021). Studies on the foraging of unculturable forms are based mainly on morphological and molecular evidence, with limited possibility to achieve an understanding of the mechanisms and underlying fluid dynamics, but studies on the few cultured relatives may still be representative, except maybe in some cases (haptophytes; see the section titled Haptophytes and Other Left–Right-Symmetric Biflagellates, below).

In the following, I briefly describe the current understanding of how these four groups of flagellates forage. While there are differences among species within the groups, here I consider generic types. The understanding is based on direct microscopic observations using (high-speed) videomicrography, flow visualization through particle tracking or particle image velocimetry, and computational fluid dynamics (CFD) modeling as well as simpler analytical models.

Choanoflagellates. Choanoflagellates are obligately heterotrophic. In the typical choanoflagellate, the beating of the flagellum pushes the cell slowly forward—unless it is tethered—and drives a current past or through the collar (**Figure 2*a***). Bacterial prey are captured on the collar, which in most species functions as a filter (Andersen 1988, Fenchel 1986), and transported along the filaments to the cell surface, where they are phagocytized. Many species are equipped with an exoskeleton, the lorica, within which the cell is suspended. The collar filter represents a substantial resistance to the flow because the separation of the filter elements (microvilli) is only 0.1–0.3 μm. The magnitude of the resistance can be computed from simple models (Fenchel 1986). One can also provide estimates of the force that a naked flagellum can produce—crudely from its length and beat frequency, more accurately using CFD—and the resulting estimate is approximately 1–2 pN (Nielsen et al. 2017). However, the clearance rate that can be estimated by combining the force generated by the flagellum and the resistance of the filter is more than one order of magnitude less than the clearance rate that has been measured experimentally, either in incubation grazing experiments (Andersen 1988) or from direct measurements of flow through the collar (Nielsen et al. 2017). Larger forces than can be delivered by a naked flagellum appear to be required to overcome the impeding effect of viscosity. This led Nielsen et al. (2017) to suggest that the flagellum is equipped with a broad vane that spans the interior of the collar and that the vane undulating within the collar and driven by the flagellum thus functions like a peristaltic pump. With this assumed pumping mechanism, the clearance rates that can be calculated become similar to those measured, not only for their study species, but for six of the seven species for which data were available. At that time, a narrow vane had been found only in a few freshwater forms. Recently, however, a broad vane has been visualized in marine choanoflagellates using novel cryogenic electron microscopy (Pinskey et al. 2022).

The smallest prey that choanoflagellates can capture is governed by the spacing between the filter elements: Finer spacing can capture smaller particles, thus increasing the potentially available prey biomass. However, a finer filter also has a larger resistance, leading to a lower flow past the filter. There is, therefore, a filter spacing that yields the largest prey biomass encounter rate. In fact, the optimum filter spacing predicted from simple considerations matches very well with the observed filter spacing across a range of choanoflagellates and other true microbial filter feeders (Dölger et al. 2019, Nielsen et al. 2017).

The function of the elaborate exoskeleton (lorica) found in many species has remained elusive (Leadbeater et al. 2009). It has been suggested that the lorica may prevent recirculation of water and increase the drag, both of which are assumed to increase foraging efficiency (Andersen 1988). However, neither appears to be the case (Andersen & Kiørboe 2020, Asadzadeh et al. 2019). Rather, the presence of a lorica reduces the periodic reverse flow through the collar due to the lateral motions of the beating flagellum, as demonstrated by CFD modeling (Asadzadeh et al. 2019). This may prevent prey captured on the collar from being washed away, as has been observed in nonloricate choanoflagellates (Dayel & King 2014, Pettitt 2001).

Some choanoflagellates may cluster in colonies, with the individual cells pointing in different directions and with each cell generating its own feeding flow. These colonial forms have been studied in the context of the evolution of multicellularity in animals, since choanoflagellates appear to be the closest relatives of Animalia, including sponges. Sponges, in fact, are akin to colonial choanoflagellates, with the individual choanocytes very much resembling choanoflagellate cells. Coloniality poses the potential problem of competition among cells in the colony. Different theoretical studies have suggested that such competition may or may not occur (Kirkegaard & Goldstein 2016, Roper et al. 2013), while experimental studies have demonstrated that choanoflagellates that form colonies have per-individual prey capture rates that are similar to or larger than those of solitary cells of the same species (Fenchel 2019, L'Etoile & King-Smith 2020) and may have other benefits in the form of reduced predation mortality (Koehl 2021, Kumler et al. 2020).

Colonial choanoflagellates may produce swarmer cells that do not possess the collar and have a longer flagellum than the colonial morph. This long, naked flagellum pushes the cell through the water, much like a sperm cell, and thus offers a rare example of this propulsion mode among free-living flagellates. The beating flagellum typically has less than one wavelength, which is optimal for swimming by a naked flagellum (Higdon 1979) but very different from the multiple waves expressed by hairy flagella that instead pull the cell through the water (**Figure 3**; see the next section on Stramenopila).

Stramenopila. Stramenopiles include mixotrophic and purely heterotrophic species. The feeding current in the stramenopiles is generated by the beating of a hairy flagellum, while the second naked flagellum typically plays a role in handling captured prey or is used for attachment (Boenigk & Arndt 2000, Fenchel 1982, Suzuki-Tellier et al. 2022, Wetherbee & Andersen 1992) (**Figure 2*b***). The hairs are oriented in the beat plane rather than perpendicular to it, as intuition may suggest, and their presence reverses the direction of the feeding current (Jahn et al. 1964). This implies that the flow arrives at the cell after passing the beating flagellum. The flagellum—or the hairs on the flagellum—perceives individual prey, and the presence of hairs thus extends the region scanned (Christensen-Dalsgaard & Fenchel 2004, Suzuki-Tellier et al. 2022). In many species, the hairy flagellum also captures the prey, and the transport of prey to the cell body is thus further facilitated by the incoming flow.

A comparison of swimming speeds shows that the presence of hairs appears to increase the force that the flagellum produces by a factor of 5–10 compared with that of an equally beating naked flagellum (Nielsen & Kiørboe 2021). The presence of hairs thus allows the flagellate to produce an efficient feeding current and overcome the impeding effects of viscosity. Also, in many forms, the prey is captured by the flagellum some distance from the cell body (Suzuki-Tellier et al. 2022), thus reducing the effect of the viscous boundary layer around the cell.

Why does the presence of hairs reverse the flow? The functioning of a beating naked flagellum is due to drag anisotropy along the waving flagellum: For any short segment of the beating flagellum, the drag in the sideways motion is larger than the drag in the lengthwise motion, giving rise to a net force component perpendicular to the direction of motion. As the flagellum beats, it thus pushes the cell forward (Lauga 2020). Adding hairs in the beat plane that are perpendicular to the flagellum may reverse the direction of the flow by the same mechanism (Holwill & Sleigh 1967). However, this assumes that the flow generated by each hair does not interact with flow generated by neighboring hairs, which is unlikely given the short distance between hairs ($<0.2\ \mu$m). A CFD model that considers hydrodynamic interaction between hairs suggests that the row of hairs on each side of the flagellum instead functions as a flexible sheet. As the flagellum beats, the area of this sheet will be greater on the outside of bends in the flagellum than on the inside, thus generating a net force in the direction opposite that of the traveling wave (Asadzadeh et al. 2022). The density of hairs that is required for them to function as a sheet is 6–7 per micron, close to what is found in representative species. This proposed mechanism also explains the 5–10-times enhancement of the force compared with that of a naked flagellum. Because the force is generated at the crest of the bending flagellum, it also explains why hairy flagella typically have more waves than naked ones (**Figure 3**) as well as other morphological features. Also, the force estimated by the model (~10 pN for the study species) is in line with forces estimated from swimming speeds and flow quantification (Christensen-Dalsgaard & Fenchel 2003, Suzuki-Tellier et al. 2022).

The efficient feeding current of hairy flagellates, their extended scanning area due to the presence of hairs and the reversal of the flow, and their ability to select between prey (Matz & Jürgens 2001) make stramenopile flagellates very efficient foragers on picoplankton. This may be the reason that this group appears to dominate among heterotrophic flagellates in large regions of the ocean (Kamennaya et al. 2022, Obiol et al. 2021).

Dinoflagellates. Dinoflagellates are mainly mixotrophic, although there are obligately heterotrophic species. They are generally larger than the obligately heterotrophic species otherwise considered here, and they typically feed on prey that are larger (relative to their own size) than the pure heterotrophs. Most have a very special flagellar arrangement: a trailing flagellum and a coiled transverse flagellum that is running in a groove around the cell, typically near the equator (**Figure 2*c***). The length of the trailing flagellum is generally ∼2 cell lengths, similar to that of other flagellates; however, the transverse flagellum is much longer, on the order of 10 cell lengths or even more, distinctly different from that of other biflagellated cells. The transverse flagellum has a rigid structure (paraxial rod) running along the coiling axoneme (the 9+2 microtubules) within the flagellar membrane. The beating flagellum thus drives an undulating sheet (Berdach 1977). The flagellum is also equipped with hairs with an unknown three-dimensional orientation (Moestrup 1982). The swimming cell rotates around its own axis but—counterintuitively—in the same direction as the traveling wave of the undulating transverse flagellum, likely due to the presence of hairs, as discussed above (Miyasaka et al. 2004). This special flagellar arrangement propels the dinoflagellates through the water and generates a distinct feeding current that directs the flow toward the prey capture area on the cell surface. Quantifications of the feeding currents in a range of species demonstrate that these flows are sufficient to efficiently overcome the impeding effect of viscosity, thus allowing clearance rates of $\geq 10^6$ cell volumes per day and the purely heterotrophic foraging mode found in some species (Nielsen & Kiørboe 2015).

Whether the trailing or the transverse flagellum drives the swimming has been a topic of discussion: Some authors have argued that propulsion is due mainly to the trailing flagellum and that the transverse flagellum is used to control orientation (Cachon et al. 1991, Fenchel 2001), while others have inferred the opposite (Miyasaka et al. 2004). A simple argument may help settle the controversy. A 30-μm dinoflagellate, *Prorocentrum reticulatum*, may swim at up to 250 $\mu\text{m s}^{-1}$. A conservative estimate of the drag force is $F = 6\pi\mu a v \sim 70$ pN, where μ is the dynamic viscosity, a is the radius of the cell, and v is the swimming speed. This is the drag force acting on a sinking sphere. The force necessary to propel the flagellate, however, is larger than this because streamlines come closer to the cell body, hence increasing the drag. One can similarly provide an upper estimate of the force produced by the flagellum from its length (55 μm), wavelength (22 μm), amplitude (5.5 μm), and beat frequency (45 Hz) using resistive force theory: ∼25 pN (for equations and application, see Suzuki-Tellier et al. 2022). While these two estimates differ by less than a factor of three, they are lower and upper estimates, respectively, thus suggesting that the transverse flagellum accounts for most of the propulsion and for generating and directing the feeding current. This is also supported by simulation studies (each with its own limitations) (Miyasaka et al. 2004, Nguyen et al. 2011).

The dinoflagellates overlap in size and swimming speeds with ciliates (Schavemaker & Lynch 2022) and, like ciliates, create efficient feeding currents that bring prey directly to the cell surface. This requires significant forces. While ciliates create forces of several hundred piconewtons with their many cilia (Pepper et al. 2021), dinoflagellates produce forces of similar magnitude (R. Schuech & T. Kiørboe, unpublished CFD simulations) with just two flagella. It is the very special flagellar arrangement that allows this biflagellate into the size class with ciliates, their close relatives among the eukaryotes.

Haptophytes and other left–right-symmetric biflagellates. Flagellates from several branches of the eukaryotic tree of life (e.g., the haptophytes and the chlorophytes) have two left–right-symmetric flagella that extend from the front of the cell and then along either side. These forms all have chloroplasts, but many, especially among haptophytes, are also capable of phagocytizing prey and, hence, are mixotrophs. The flagella may be relatively short and beat in a ciliary fashion with power and recovery strokes (breaststroke swimming) or may be longer and beat in a sinusoidal pattern with a continuously produced wave. In some species, the flagella are of unequal length. Here, I discuss the biflagellate haptophytes as examples of this group (**Figure 2*d***). The haptophytes include bloom-forming species, such as coccolithophores and colonial *Phaeocystis* spp., both of which have very short haptonemas and appear to be almost exclusively phototrophic, as well as mixotrophic species (e.g., among *Chrysochromulina* spp.), which in the open ocean at times can be quantitatively important grazers and have significant foraging abilities (Li et al. 2022). I focus on the mixotrophic haptophytes, for which we have some information on their foraging behavior.

Haptophytes with a long haptonema use it for prey collection (**Figure 2*d***): Bacterial prey captured on the haptonema are transported to its tip, and the haptonema can then bend and bring the prey to the posterior end of the cell, where they are phagocytized (Kawachi et al. 1991). Dölger et al. (2017) compared the observed flow field generated by swimming haptophytes with that derived from a simple fluid mechanical model and found very good correspondence between the two. They could then use the model to estimate clearance rates from flow fields. For *Prymnesium polylepis*, a species with a long haptonema and long flagella, the estimated clearance capacity was low ($<10^5$ cell volumes per day) even though prey may be encountered by both the haptonema and the cell body (and the capacity would be lower still for a species with a short haptonema). Motile prey may be cleared at a higher rate, although less than the $>10^6$ body volumes per day required for living freely in the open ocean water column. These values are largely consistent with clearance rates measured for haptophytes in coastal environments ($\sim10^5$–10^6 d^{-1}) and consumption rates that account for only a small fraction of the daily needs (Unrein et al. 2014). The conclusion is that phototrophy dominates energy acquisition in these species.

The above results are in stark contrast to recent findings by Li et al. (2022) that some species of haptophytes isolated from the tropical Pacific appear to be able to clear cyanobacteria at very high rates, with one 2–3-μm *Chrysochromulina* species having specific clearance rates of $>10^7$ cell volumes per day. The species were unidentified, and their morphology (dimensions of flagella and haptonema) and foraging behavior (flagellar beat pattern and kinematics) are unknown, but it appears that there is a huge discrepancy between the understanding derived from the behavioral observations above and data derived experimentally in these poorly known species. Haptophytes have their cell body covered with scales, which in one species, *Chrysochromulina spinifera*, are modified into long spines that extend from the body surface. In this species, the beating flagella create a feeding current, and bacterial prey are captured by the spines and subsequently phagocytized (Kawachi & Inouye 1995). Such morphological features may dramatically increase clearance rates. There is clearly room for improvement in our understanding of foraging in these species.

Conclusion. The above has demonstrated that advection-based predation in heterotrophic flagellates typically requires forces that exceed what a naked flagellum can deliver. Each group considered above has shown special adaptations of the flagellum in the form of vanes or hairs that increase the force production or are based on more elaborate pumping mechanisms. Another example may be delivered by excavates, where typically a vane-equipped flagellum pumps water through a groove, in which prey appear to be captured (**Figure 2*e***). The foraging mechanisms in other purely heterotrophic forms with no apparent (known) adaptation of the flagella and sometimes strange motility patterns—such as biflagellated *Telonema* species and Rhodelphidia (a sister group to red algae), which prey on eukaryotes (large prey items) (Gawryluk et al. 2019, Tikhonenkov 2020) and picoeukaryotes such as the widespread Picozoa, which appear to feed on colloids (Moreira & López-García 2014)—remain poorly described and, hence, poorly understood, as are the reported very high clearance rates in some haptophytes.

Autotrophic Resource Acquisition in Flagellates

While many heterotrophic flagellates are attached to a surface when feeding, likely because prey availability is high there (Andersen & Kiørboe 2020), mixo- and autotrophic flagellates remain free swimming. This allows them to move to small-scale patches of inorganic nutrients via chemotaxis (Seymour et al. 2009) or, for the larger powerful swimmers, to perform diel vertical migration to overcome the vertical separation of their resources—light near the ocean surface, nutrients at depth—thus facilitating resource acquisition (Peacock & Kudela 2014). Also, for forms larger than ~20 µm, motility itself may enhance the advective transport of nutrients toward the surface of the cell (Langlois et al. 2009, Magar & Pedley 2005, Schavemaker & Lynch 2022), e.g., by a factor of ~2 in the 60-µm dinoflagellate *Dinophysis acuta* (Nielsen & Kiørboe 2015). The flow fields that benefit prey capture, with streamlines coming close to the cell body, are also beneficial to nutrient uptake.

FORAGING TRADE-OFFS

Advection-based foraging in flagellates comes with costs in terms of elevated predation risk: Swimming increases the risk of encountering a predator, and the feeding current generated by a flagellate attracts flow-sensing predators. The latter include adult copepods that forage on nano- and micro-sized flagellates and their juvenile nauplii, which also feed on smaller flagellates (Berggreen et al. 1988). Copepods are arguably the most abundant metazoans in the ocean, and their predation is therefore likely a strong selective force. Regardless of whether the copepods themselves produce a feeding current from which they harvest prey or are ambush feeders that wait for motile prey to pass within the sensory sphere of the copepod, prey are perceived from the fluid disturbance that they make (Bruno et al. 2012, Jiang & Paffenhöfer 2008, Kiørboe et al. 2009). In response to predation risk, flagellates, like other prey, have evolved costly defense mechanisms.

Flow Architecture and Stealth Behavior

The first line of defense is to avoid being detected by a predator, and this depends on the architecture, extension, and intensity of the flow field generated by the beating flagella. The flow field that minimizes predation risk may not necessarily be the flow field that optimizes propulsion and feeding. For example, a force positioned near the surface of the cell (e.g., a short flagellum or the transverse flagellum in dinoflagellates) pulls streamlines close to the cell surface and thus promotes feeding, but it will also result in a larger drag than a force of the same magnitude positioned far from the surface (e.g., a long flagellum), which instead optimizes propulsion (Langlois et al. 2009). Also, as I elaborate on below, forces positioned near the equator of the cell (like the transverse flagellum of a dinoflagellate) minimize noise and optimize propulsion efficiency (Jiang 2011), while a puller arrangement with force positioned near the front of the cell (like in the stramenopiles) may optimize prey encounter (Dölger et al. 2017). Thus, the design of the flow architecture may be a compromise between conflicting fitness components and is governed by the position of the flagella. Indeed, the flow fields that optimize feeding also lead to the highest predation risk. Stealth behavior comes with the cost of reduced feeding efficiency (**Figure 4**).

To explore this further, I will use highly idealized simple point force models to examine three situations representing the foragers described above: a tethered flagellate, a freely

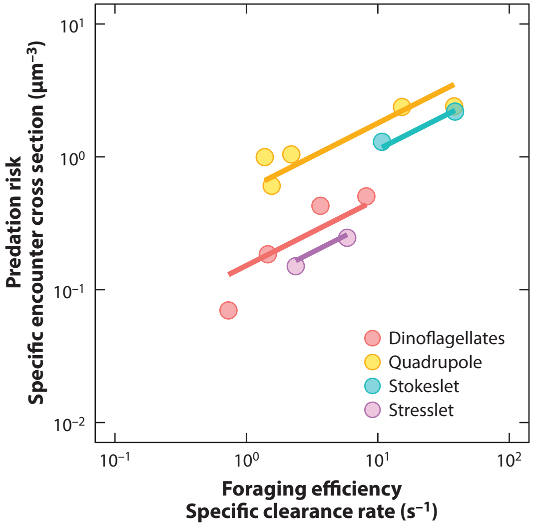

Figure 4

The relation between foraging efficiency (quantified as the cell-volume-specific clearance rate) and predation risk (cell-volume-specific predator encounter cross section) for flagellates with different flow architectures. Figure adapted from Nielsen & Kiørboe (2021).

swimming flagellate that is pulled through the water by a beating flagellum, and a flagellate with an equatorial position of the forces. The models that describe these three situations are, respectively, a force acting in a point in the water, representing the force produced by the flagellum of the tethered flagellate (Stokeslet); two point forces of equal magnitude but of opposite direction (stresslet), representing the force produced by the flagellum and the oppositely directed drag force due the moving cell; and three point forces in a row (quadrupole), representing the two forces due to the beating flagella of a right–left arrangement and an oppositely directed force due to the moving cell and of a magnitude equal to the sum of the two propulsion forces. Despite their extreme simplicity, these three models provide good qualitative descriptions of observed flow fields (**Figure 5**).

The spatial attenuations of the three types of flow are very different: The flow generated by the equatorially arranged forces (quadrupole) dies out rapidly as it attenuates with one over the distance cubed (r^{-3}), while the flow of the attached flagellate extends much farther (attenuates with r^{-1}), and the swimming puller is intermediate (r^{-2}) (Andersen et al. 2015). Elaborate CFD models of more realistic swimming protists confirm the result of the simple analytical models (Jiang & Paffenhöfer 2008), and the observed flow attenuations for a range of zooplankters (from flagellates to copepods) with different swimming gaits also match the predictions well (Kiørboe et al. 2014).

Everything else being equal, the above would suggest that an equatorial arrangement of the propulsion forces, as found in many left–right-symmetric biflagellates, would be the preferred motility mode, and it is indeed very common. However, everything is not equal, and when traded off against foraging efficiency, the risk–gain ratio for this motility mode appears to be disadvantageous compared with the puller mode of stramenopile flagellates and the very special propulsion mode of dinoflagellates (**Figure 4**). However, both overall and within the different types, there is a trade-off between foraging efficiency and predation risk: The more efficient foragers also run a higher predation risk.

When comparing the stresslet and Stokeslet foragers, the latter are expected to have lower clearance rates than the former (Andersen & Kiørboe 2020) and disturb a larger area (see above),

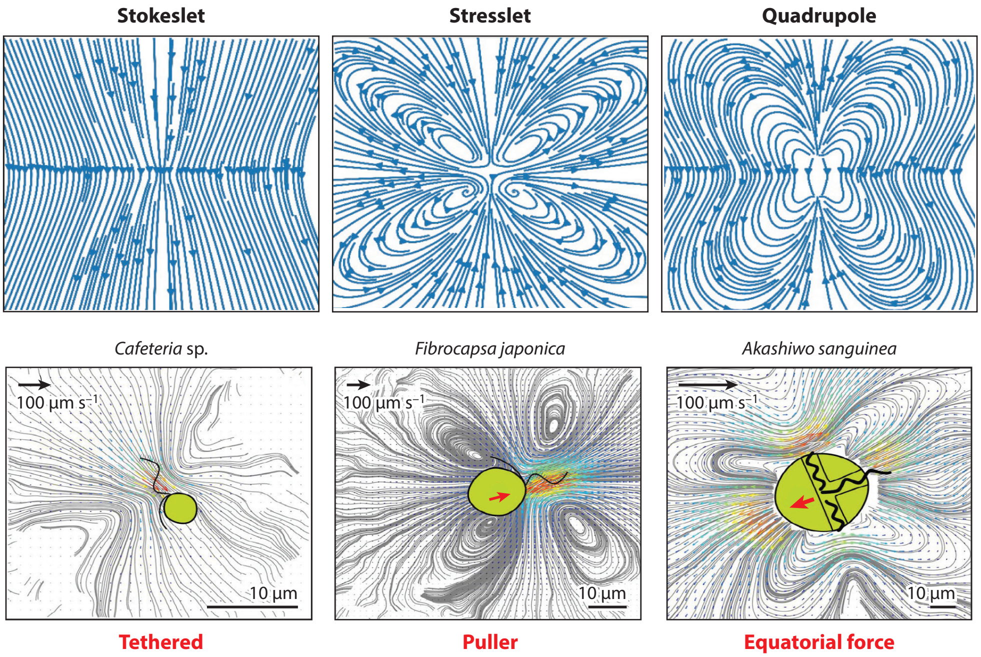

Figure 5

Architecture of flagellate feeding flows as revealed by particle image velocimetry (*bottom*) and the corresponding simple point force models that qualitatively describe the architectures (*top*). Figure modified from Nielsen & Kiørboe (2021).

and thus have a disadvantageous risk–gain ratio, as observed (**Figure 4**). This ignores that Stokeslet flow requires that the flagellate be tethered, meaning that it does not move (tethering to a surface may itself provide protection). Predator encounter rates depend not only on the distance at which the predator can perceive a prey (R) but also on the velocity difference between the predator and the prey (v) as well as the density of predators (N), $\pi R^2 vN$. If the predators are nonmotile ambush feeders (such as many copepod nauplii), the velocity difference is equal to the swimming speed of the prey, while the velocity difference between the prey and a cruising predator is governed mainly by the speed of the latter. This illustrates that the foraging trade-off and the different risk–gain ratios quantified in **Figure 4** may be modified by the environment, both by food availability (with more food available, foraging capability becomes less important) and the concentration and types of predators. In other words, the environment will select which foraging mode is the most advantageous (highest fitness), modifying the composition and function of the microbial food web accordingly. While it will likely be very difficult to demonstrate this in nature, it illustrates how organismal trade-offs and environmental conditions structure (microbial) communities. And such environmentally dependent trade-offs form the backbone of trait-based models of microbial food webs, which, in turn, may be compared with observations (see the section titled Defense Trade-Offs in the Prey of Flagellates, below).

Other Defenses

The second line of defense is to avoid being captured once perceived. Some species of haptophytes can perform powerful escape jumps when entrained in the feeding current of, for example, a ciliate (Jakobsen 2001). It is unclear how the flagellate perceives the fluid signal, but the haptonema is likely involved. An escape jump is initiated by the fast (few milliseconds) retraction of the haptonema, which may pull the cell body a few cell lengths toward the danger, followed by a reversal of the swimming direction and swimming at high speed away from the danger. Escape jumps have been demonstrated in just a few other protists but may be widespread. The instantaneous power requirements for such escape jumps may be high, but the metabolic cost of an actual jump is low due its short duration.

The final line of defense is to avoid being eaten when captured. Several flagellates, mainly among dinoflagellates and haptophytes, produce substances that are toxic to grazers and other metazoans (Granéli & Turner 2006). In many dinoflagellates, these toxins function as grazer deterrents in copepods: Once a cell is captured, the copepod examines the cell and rejects it if it contains toxins (Xu & Kiørboe 2018). Theoretical studies suggest that the cost of toxin production may be substantial when the cells are nitrogen starved due to the high nitrogen content of many toxins (Chakraborty et al. 2019), but experimental evidence for the costs has been difficult to establish and is equivocal (Park & Dam 2021, Ryderheim et al. 2021, Selander et al. 2006).

Haptophytes produce toxins that leak into the environment and have allelochemical effects. During blooms, leaked toxins may kill fish and wipe out competitors and grazers (Nielsen et al. 1990), but it is questionable whether such a public good can be considered a defense, as it works only at high cell densities and does not specifically benefit the individual cell that produces the toxin (Driscoll et al. 2016, Jonsson et al. 2009). Allelochemicals can be considered a private good only if the bloom is monoclonal, which typically appears not to be the case (Rengefors et al. 2017).

Other Costs of Advection-Based Foraging

Other costs of advection-based foraging include the construction and operating costs of the flagella. These typically constitute only a minor fraction (up to a few percent) of the construction and operation costs of the entire cell (Schavemaker & Lynch 2022). The construction costs alone, however, may account for a significant part of whole-cell construction costs, and increasingly so with decreasing cell sizes (e.g., up to ~50% in pico-sized flagellates). For such small cells, the advantage of a feeding current is small because the relative contribution of prey encounter due to advection over Brownian diffusion is small. For these small sizes, propulsion and the ability to find resources through chemotactic behavior may be more important than establishing a feeding current.

DEFENSE TRADE-OFFS IN THE PREY OF FLAGELLATES

Just as flagellates have evolved costly defenses to reduce predation mortality, their own prey may similarly harness a suite of defenses in the presence of flagellate grazers, or the bacterial community composition may change toward grazing-resistant forms [see reviews by Jousset (2012) and Matz & Kjelleberg (2005)]. Thus, predator (protist) cues cause bacteria to change to a filamentous shape (Corno & Jürgens 2006, Justice et al. 2008, Salcher et al. 2005, Tophøj et al. 2018), form microcolonies (Blom et al. 2010), or increase production of secondary metabolites (Mazzola et al. 2009), many of which are supposed to play a role in predator defense (Klapper et al. 2018). In all of these cases, however, the bacteria are attached, not freely swimming, which may also be necessary for leaked metabolites to evolve as private (selfish) defense goods (microcolonies are normally clonal).

There is less evidence of defenses in suspended bacteria, which are the main food for freely swimming flagellates. Flagellates are known to be highly selective in their feeding, discarding a large fraction of captured cells, but attempts to determine which prey cell-surface properties are responsible have been largely unsuccessful (Matz & Jürgens 2001). SAR11 bacteria appear to be able to evade retention by filter-feeding ascidians, possibly owing to a cell surface that is less hydrophobic than those of other similarly sized planktonic bacteria (Dadon-Pilosof et al. 2017).

Highly motile bacteria have a higher encounter risk with flagellates than slow bacteria, but they also have a much greater ability to escape after contact, overall leading to a lower predation risk (Matz & Jürgens 2005).

In contrast to the costs of viral defenses in bacteria, the costs of defenses against flagellate predators have rarely been identified or quantified. However, trade-offs must exist, or else all bacteria would be equally well defended. A rare example is the marine bacterium *Phaeobacter*: Phosphorus-replete cells are readily ingested but not digested by flagellates, while phosphorus-depleted cells are digested, demonstrating a trade-off between nutrient uptake and defense (Guillonneau et al. 2022).

ORGANISMAL TRADE-OFFS, TRAIT CASCADES, AND MODELING OF MICROBIAL COMMUNITIES

Organismal trade-offs are the main source of diversity and, together with environmental constraints, govern the structure and function of ecosystems. The most fundamental trade-offs are likely to be related to resource acquisition, and for phagotrophic protists an important trade-off is related to predation risk, as considered here.

Phagotrophic protists—like most heterotrophs—are predators and prey at the same time. That is, their optimal foraging strategy depends on both the prey and the predator landscapes. And their prey in turn must adapt to predation by harnessing defenses or be eaten, thereby allowing room for species with a higher level of grazing resistance. Whether the community changes occur through behavioral plasticity or replacement of species does not matter from a functional, trait-based perspective. That community composition can be altered through such trait cascades or trait-mediated nonconsumptive indirect interactions and can be as important as—or more important than—trophic interactions and cascades in organizing ecosystems has long been recognized in terrestrial systems and for larger organisms, where it is easier to demonstrate (Schmitz & Suttle 2001, Suraci et al. 2016). Trait cascades and trait-mediated indirect effects have recently been demonstrated experimentally in plankton ecosystems (van Someren Gréve et al. 2019) and deduced from field observations (Kenitz et al. 2017), but there is no direct, observational evidence of such effects among the microbes in these systems. However, they emerge in trait-based models of microbial ecosystems (Prowe et al. 2019).

Trait-based approaches have become a rapidly developing and highly successful branch of marine ecosystem modeling (Kiørboe et al. 2018). Rather than modeling species and how they interact, trait-based models consider individuals that are characterized by a few key traits that are interrelated through trade-offs. Thus, the quantification of trade-offs is key to trait-based modeling. Until recently, most trait-based models of plankton systems have emphasized competition within one trophic level, mainly phytoplankton or microbial communities (Bruggeman & Kooijman 2007, Coles et al. 2017, Follows & Dutkiewicz 2011), but trophic models that explicitly consider predation are now emerging (Cadier et al. 2019, Prowe et al. 2019, Serra-Pompei et al. 2020, Ward et al. 2012). Trade-offs have often been derived from heuristic descriptions and correlations between traits (Edwards et al. 2011, 2013), but ideally, they should be based on a mechanistic understanding of the underlying mechanisms. This allows extrapolation outside the calibration envelope and makes the predictive capability of the models more robust, and is what I attempted above (see the section titled Foraging Trade-Offs). Obviously, one cannot implement in ecosystem models the detailed fluid dynamical models on which the above trade-offs are based, but the mechanistic understanding allows one to simplify in a meaningful way. Thus, the trade-off described in **Figure 4** can to a large extent be rationalized from fluid mechanical considerations, and this simple relation can be directly implemented in trait-based models without considering the underlying details.

The different risk–gain ratios of flagellates with different flow architectures remain to be fully understood, and the foraging and risk implications of different flagellum beat patterns and kinematics are still largely unexplored. Consequently, there is still considerable room for improvement. Also, the foraging mechanisms and consequent trade-offs of widespread and often quantitatively important microeukaryotic flagellate predators (eukaryotrophs), such as *Telonema* and the species in the newly defined supergroup Provora (Tikhonenkov et al. 2022), must be established as an increasing number of these species become available in culture.

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