Biology 3102 – Microbial Eukaryotes
Supplementary Course Material #2, Chapter 5
Fall 2020

FEEDING

Food particles
In most environments, most heterotrophic eukaryotic microbes obtain their energy, and organic carbon for biosynthesis, by consuming *particles* rich in organic matter. In most cases these particles are other living microorganisms. The number of small particles in the world that might constitute food is huge. In the upper regions (photic zone) of the ocean there are usually 10^5-10^6 prokaryotic cells per millilitre, and 'bacterivorous' (prokaryote-eating) protists are the major consumers of this huge biomass. In addition there are 100s to 1000s of protists per millilitre, many of which are also eaten by other protists. There are also around 10^6-10^8 virus particles per millilitre in these environments, although these are very small (most are less than 200 nm across) and thus are challenging to consume efficiently as food (see below). Abundances of all of these lifeforms are typically 100-1000 times *higher* in surface sediments and similar habitats, although a greater proportion are attached to surfaces, rather than moving or floating freely.

Suspension Feeding
To start with, we will consider how eukaryotes capture other organisms in the water column. One class of strategies - 'suspension feeding' - covers various ways that organisms acquire particles of food within a water mass without 'hunting down' individual particles. The alternative – raptorial feeding – refers to the active capture of proportionately large individual food particles (see below).

Suspension feeding can be subdivided into three contrasting strategies:

1) Direct intercept feeding
2) Filter feeding
3) Diffusion feeding

Direct intercept feeding

Classic direct intercept feeding involves the predator producing a 'feeding current' by being attached to a surface and moving fluid past itself. The production of a feeding current brings particles into contact with part of the cell specialized for food capture (though see 'filter-feeding' below). Direct intercept feeding is a common strategy for small flagellates to employ. These produce the feeding current with their flagella, and usually capture suspended bacteria, and sometimes small eukaryotes, using a specialized 'cytostome' or other feeding apparatus. Sometimes the flagella themselves are involved in the initial contact with the prey, and then manipulating it for capture.

Cells that are swimming through a fluid, rather than being stationary and generating a feeding current, are often effectively performing direct intercept feeding as well. Interestingly, this feeding while swimming turns out to be less efficient than classic direct intercept feeding, all other things being equal (see below). As prey size becomes larger relative to predator size, this 'unattached direct intercept' feeding grades into 'raptorial' feeding (see below).

Classic examples of direct intercept feeders include many bicosoecids and chrysophyceans (two groups of stramenopiles). These generate a feeding current with their hair-bearing flagellum, and capture individual food particles that collide with the flagella or the anterior part of cell, and then use a hoop-like feeding apparatus for actual phagocytosis. Many 'typical excavates' are also essentially direct intercept feeders.

Clearance and Handling

In understanding direct intercept feeding, and comparing to other feeding modes, there are two concepts we need to consider here: 'Clearance' and 'handling time'.

Clearance refers to the amount of fluid that is 'sampled' by the feeding cell per unit time (i.e. that would be cleared of suitable particles, if food capture were 100% efficient). Clearance is a primarily function of the effective size of the predator's capture area, and speed of fluid flow in the feeding current, although prey size is a factor as well (large prey will collide with the capture area more often than small prey). Clearance is often expressed normalized to the size of the predator. Small flagellates feeding on prokaryotes are able to clear up to ~10^5 predator cell volumes per hour.

Handling time refers to the time taken for the cell to capture and phagocytose each particle of food. Most direct intercept feeders capture particles individually, and thus must finish handling one particle of food before they can handle another. Measured handling times for small flagellates feeding on bacteria range from a few seconds to several tens of seconds. For example, in one study, handling time averaged ~4 seconds in the case of the chrysophycean stramenopile *Ochromonas* sp., but 95 seconds in the bicosoecid stramenopile *Cafeteria roenbergensis* (Boenigk & Arndt, 2000a).

The maximum ingestion rates observed for small heterotrophic flagellates are usually around 50-100 prokaryotic cells per hour. This suggests that handling time could be significantly affecting the rate of ingestion. However, these maximum ingestion rates are observed in cultures in which prey abundances that are 10 times higher (or more) than those normally encountered in nature. In most of the ocean most of the time, small

flagellates ingest <10 prokaryote cells per hour, and are clearly limited mostly by clearance rate, not handling time (i.e. by the number of prokaryotes encountered, not by how quickly each captured food particle is eaten).

Filter feeding

Filter feeding is distinguished from direct intercept feeding because the feeding current moves *through* an array-like capture device - the filter. In principle, particles of food are captured when they are larger than the pore diameter of the filter (i.e. the distance between adjacent components of the filter) and so are trapped.

Some small flagellates are filter feeders that employ radiating filopodia or axopodia to act as the filter – one example is a group of stramenopiles called pedinellids. Filter feeding is also common in ciliates. In many ciliates, large arrays of membranelles (i.e. elongate blocks of cilia) form the filter, with the spaces between adjacent membranelles representing the pores. The situation is complicated somewhat by the fact that these membranelles usually also function to *generate* the feeding current by beating. In a few ciliates, however, there are separate sets of beating 'current-generating' membranelles, and passive filtering cilia.

One might expect that filter feeders would have much higher clearances than direct intercept feeders, since the filter greatly increases the effective capture area of the predator. However, there is substantial resistance to flow through a filter, due to viscous drag on the elements that make up the filter. Resistance increases as the pore size of the filter gets smaller. This resistance means that the velocity of the fluid passing through the filter is lowered, and thus clearance is reduced. In practice, these two effects roughly cancel each other out, and the clearance of a typical filter feeder ends up being similar to that of a direct intercept feeder of the same size (volume). However, filter feeders are usually able to handle multiple items of food quickly (e.g., by performing phagocytosis at several different points of the cell simultaneously) and thus handling time presumably has little influence on feeding rate at normal prey densities.

Choanoflagellates are small flagellates that have a collar of microvilli surrounding the single flagellum. Particles of food adhere to the microvilli and are then phaogcytosed. Choanoflagellates are often thought of as the quintessential 'filter feeders' amongst small eukaryotes, however they are actually an unusual case. Viewed as a filter, the collar has exceptionally tiny pores, since the gaps between adjacent microvilli are very small - of the order of 0.1-0.3 micrometres (i.e. 100-300 nm). This means that the resistance to flow through the filter is very high, and the velocity of the fluid actually passing through the filter (and thus volume of fluid per unit time) is correspondingly low. Measured fluid velocity measurements for choanoflagellates are around 10-20 micrometres per second, compared to ~100 micrometres per second for the fluid around a typical small flagellate that undergoes direct intercept feeding (Boenigk & Arndt, 2000b), or that filter-feeds with a very coarse filter.

Recent work on choanoflagellates suggests that the extracellular flagellar vane that has been observed in some species has an important function in compensating for the high resistance to flow through the filter, and thus making the typical choanoflagellate filter system function efficiently. Without the vane, computational modelling indicates

that fluid will not pushed out of the top of the collar quickly enough (it tends to instead move side-to-side too much – like the flagellum is 'stirring' the fluid lying within the collar), and thus is not replaced by fluid flowing through the filter. A flagellar vane that interacts very closely with the inner side of the collar is predicted act like a pump, pushing much more fluid up and out of the collar, and thus creating much more suction to force new fluid through the filter (Nielsen et al. 2017). Alternatively, choanoflagellates with the narrowest distances between microvilli might actually experience very little flow through the filter at all, and be effectively operating as 'direct intercept' feeders (see above), with the outer side of the collar representing the contact area for prey passed close to the cell by fluid flow *around* the cell and collar, not *through* the collar.

On the other hand, one consequence of the unusually fine filters seen in choanoflagellates is that they may be one of the few types of cells that can make significant use of viral particles as prey items (see page 1; for a recent treatment see Brown et al. 2020).

One important difference between filter feeding and direct intercept feeding concerns the size of prey. Direct intercept feeders and raptorial feeders (see below) usually cannot feed efficiently on particles of food much smaller than 1/10$_{th}$ their own size in linear dimensions. Reasons for the reduced efficiencies include; (i) the reduced chance of collisions with small particles, and (ii) the possibility of handling time becoming limiting, if each particle must be handled individually. Filter feeding is a way of accessing proportionately small particles efficiently. For example, almost all ciliates that feed on particles smaller than 10 times their own size use a filter-feeding strategy, while those that feed on larger prey tend to be raptorial feeders (see below).

Diffusion feeding

Diffusion feeding is similar to filter feeding in that a food-collection array is usually employed (typically based on filopodia or axopodia), but differs in that the predator does *not* produce a feeding current. The food collection device generally bears extrusomes (see supplementary notes chapter on extrusomes) or some other device to adhere to prey cells that collide with the food collection array. Diffusion feeding is probably *not* a competitive way of collecting non-motile prey – relying solely on Brownian motion to bring particles into contact with the predator would result in clearance that was at least 100 times lower than for filter feeding. However, diffusion feeding can be an effective method of capturing *motile* prey that run into the predator. Many prokaryotic cells and protists are highly motile. Microbial eukaryotes that use diffusion feeding in planktonic habitats include various 'heliozoa', radiolaria, planktonic foraminifera, and others.

Suspension feeding while attached to surfaces

Many organisms attach to surfaces during feeding. Even in the plankton, a large proportion of suspension feeders are associated with suspended detritus particles, moribund algae, etc. One obvious reason why being tethered to a detrital particle might be advantageous is prey availability. There is usually a much higher prey concentration immediately around particles, compared with the water column; for example, motile prokaryotic cells are often attracted to the higher concentrations of dissolved organic matter in the region around a piece of detritus or around a dying algal cell.

In addition, for most filter feeders and direct intercept feeders, there are intrinsic hydrodynamic benefits that arise from being attached to a particle rather than swimming freely through the water column. In brief, it turns out that the fluid velocity very close to a beating flagellum of an attached cell can be much higher than the relative speed with which the same cell could swim through the same fluid, if it were not attached. One consequence is that a small filter or food-capture structure that is located close to the flagellum could experience a much higher flow rate than the same structure on a swimming cell, and hence achieve a higher clearance, all other things being equal. Experiments on small stramenopiles estimate an increase in clearance of ~35% in one species when the cells are attached, and an increase of around 70% in a second species (Christensen-Dalsgaard & Fenchel, 2003).

However, a cell that is located *very close* to the surface to which it is tethered tends to experience *reduced* hydrodynamic efficiency due to viscous drag associated with that surface (i.e. its no-slip zone, or 'boundary layer'). To reduce this negative effect, tethered cells will generally be raised from the surface. The influence of the surface is smaller for a feeding current directed parallel to the surface, and larger for flow directed perpendicular to the surface. Thus, for example, filter-feeding ciliates that direct a feeding current parallel to the surface that they attached (e.g. 'walking' ciliates like *Euplotes*) can stand on elongate cirri that raise the current-producing membranelles roughly half a cell length from the surface. Meanwhile, ciliates and flagellates that produce feeding currents approximately perpendicular to the surface are generally highly elongate, with the oral apparatus at the one end of the cell, and attachment at the other (e.g. most species of the famous ciliate *Stentor*), or are poised on stalks that are several times longer than the diameter of the filter or other feeding structures (e.g. vorticellid ciliates). It has been noted, however, that stalked cells often preferentially take a posture where the long axis of the cell is at a marked angle to the stalk. This means that the feeding current produced by the cell tends to be at an oblique angle to the substrate, rather than perpendicular to it. This further reduces the influence of the boundary layer and increases velocity of the feeding current, albeit modestly when measured in the ciliate *Vorticella* (Pepper et al., 2013). It is also inferred to markedly reduce the 'recirculation problem' as discussed below.

So far we have discussed suspension feeding as if the feeding organism is constantly processing 'fresh' fluid. However, feeding currents in the real world are not endless linear streams, but frequently generate circular flow patterns, meaning that the same fluid may pass through the feeding system repeatedly over a short interval of time. These patterns are particularly strong for cells attached perpendicularly to a surface. Repeated recirculation is an obvious problem for the feeding cell because it will gradually strip the recirculating fluid of (immobile) suitable food particles, and yield less and less nutrition for the same flow-production effort. Consequently, attached cells exhibit various adaptations to avoid excessive recirculation. Temporarily attached cells can simply move periodically from one location to another. More permanently attached cells often have some sort of 'shrugging' behavior where they will spontaneously contract the cell or attachment stalk (especially in ciliates), or flex the flagellum with which they are attached to the substrate (some flagellates) – These behaviors act to periodically disrupt recirculation flow patterns (though could be involved avoidance of predation as well).

Meanwhile, the angled posture taken by the stalked ciliate *Vorticella* (see above) has been inferred by computational modelling to dramatically reduce recirculation relative to a cell oriented perpendicularly to a flat surface (Pepper et al. 2013). This might well be the main reason why the cell adopts this angled posture.

Raptorial feeding

Raptorial feeding has much in common with direct intercept feeding by *unattached* cells and, in fact, some researchers have used the terms more-or-less synonymously. We prefer here to restrict the term 'raptorial feeding' to situations where the predator actively moves to encounter its prey, either by swimming or by locomoting along a surface (following, e.g. Boenigk and Arndt, 2000b), thus there is no feeding current produced. Many raptorial feeders consume proportionately large prey items. In extreme cases the cell will consume just one proportionately huge prey item before undergoing reproduction. This is seen in some species of colpodellids (predatory flagellates related to apicomplexan parasites) in which the predator may consume only one prey cell larger than itself before forming a reproductive cyst in which it divides into four daughter cells. The prey of raptorial predators is frequently immobilised or killed outright using extrusomes, especially if the prey is motile. Raptorial feeding has been studied most in ciliates, particularly those that feed on other ciliates (e.g. *Didinium,* which feeds on *Paramecium*). Dinoflagellates, as a group, also excel at raptorial feeding on large prey.

Many raptorial feeders associate with surfaces. Surfaces represent locations with relatively high concentrations of potential prey items, that can be exploited by suitably adapted raptorial feeders. Certain flagellates and small amoebae are able to feed raptorially on attached prokaryotes – picking them individually from surfaces. Concentrations of prokaryotes (e.g. small colonies) may also be viable food for large amoebae, which can engulf multiple cells simultaneously. Raptorial predators that move by gliding, skidding, or walking/crawling also eat attached eukaryotic prey. For example, some gliding heterotrophic euglenids and 'walking' ciliates specialize in eating pennate diatoms, which are generally attached to surfaces.

Dealing with large prey

Some cells, especially many raptorial feeders and diffusion feeders, will feed on prey that are close to their own size, or even larger than themselves. Some cells have a remarkable capacity for ingesting large objects by simple distension of the cell. Another strategy is to extend an extremely broad but very thin pseudopod that is 'wrapped around' the prey item to enclose it in a digestive vacuole. The dinoflagellate *Protoperidinium* produces a structure of this sort called a 'pallium' that allows it to digest entire colonies of centric diatoms with a total volume several times larger than that of the predator. Conversely, the ciliate *Pseudomicrothorax* is a famous case where a cell has adaptations to 'ball-up' its elongate prey (large filamentous cyanobacteria) into a more ingestible shape (see below), thereby avoiding the need for a dramatic change in cell shape by the predator.

Another strategy is to pierce the prey cell membrane and transport some or all of the cytoplasm and organelles directly into a food vacuole - this is called '*myzocytosis*'. This allows the predator to control the volume and shape of the prey item. Myzocytosis is quite widespread in dinoflagellates, many of which have an extendable tubular feeding structure called a 'peduncle' that is supported by a telescoping array of microtubules.

Myzocytosis is also seen in Suctoria (strange sessile ciliates that lack cilia in the feeding phase and operate as diffusion feeders). Some heterotrophic euglenids can perform either myzocytosis or conventional phagocytosis, depending on the size of the prey.

Feeding specialization

Traditionally it had been supposed that direct intercept feeders and filter feeders are quite indiscriminate feeders, consuming any captured particles that they are able to enclose with the feeding apparatus. One of the reasons for this presumption was the observation that many such cells will readily ingest items such as inorganic dye particles and latex beads in laboratory experiments. It has been shown that direct intercept feeders preferentially consume the larger prokaryotes in their environment, but this is sometimes explained as a simple consequence of the increased chance of contact between the cell and the larger prey (see notes on 'clearance' above). On the other hand, even filter feeders tend to show higher rates of ingestion of real prokaryotic cells than of latex beads of the same size, and tend to ingest live prokaryotic cells at higher rates than killed prokaryotes. They also tend to egest (exocytose) indigestible material more rapidly than actual food. Experiments where the same microbial eukaryote is fed with different species of bacteria tend to show a wide range of growth rates, strongly suggesting selectivity by the predator (and/or different 'evasion' abilities by the prey). Consequently, it is reasonable to assume that considerable prey selectivity is routinely in operation in direct intercept and filter feeders.

By contrast, many raptorial feeders are obviously and demonstrably specialized for particular prey, and it has never been in doubt that they are highly selective. For example, the ciliate *Didinium* feeds only on *Paramecium*. Part of the selectivity mechanism involves the fact that the 'offensive' extrusomes (toxicysts) that it uses to subdue its prey are triggered by contact with *Paramecium*, and not with other ciliates (see also the chapter on extrusomes). *Pseudomicrothorax* is a ciliate that is specialized for the consumption of large filamentous cyanobacteria. Adaptations include a round-bore feeding apparatus that efficiently moves the cyanobacterial filament into the *Pseudomicrothorax* cell, and a very fast-acting (enzymatic?) mechanism for destroying the rigidity of the cyanobacterial cell wall during ingestion, even before complete enclosure of the food vacuole. This latter mechanism allows the straight, rigid filament to be curled up into a ball as it is ingested, permitting *Pseudomicrothorax* to engulf filaments that are longer than itself.

Phagocytosis

Note: the notes below on phagocytosis and exocytosis are mostly abstracted from Hausmann et al, (2003) p226-238, which is recommended reading.

Once a cell has captured and recognized a food particle (or 'prey'), the particle will usually be phagocytosed. There are two things the cell has to achieve in order to perform the act of enclosing a particle in a food vacuole. One is to generate the membrane that can be used as the food vacuole membrane. The other is to physically maneuver the vacuole membrane around the prey item. There are two basic strategies for doing this: one uses pseudopodia, the other employs some sort of specialised feeding apparatus – a cytopharynx, or similar structure (note – there are certainly intermediate strategies that

have characteristics of both approaches – see below).

In the simplest approach using pseudopodia, the cell extends itself around the prey item until it is completely enclosed. In other words, the cell uses its own cell membrane as the food vacuole membrane. The work done in the extension of pseudopodia is achieved by actin and myosin interactions and/or by polymersation of actin microfilaments. Pseudopodial engulfment is used by amoebae (quelle surprise…) but also by many small flagellates, especially those that have plastic (rather than rigid) cell surfaces. Obvious users of pseudopodial engulfment include small flagellates in the taxon Rhizaria, however, pseudopodial-type phagocytosis is also seen in some cells that have separate food capture devices. Choanoflagellates, for example, phagocytose captured particles using tiny pseudopodia that 'climb' up a microvillus (within the collar) and surround a captured food particle that is in contact with that particular microvillus.

Specialised permanent feeding apparatuses tend to be more common in larger cells, especially those with strongly reinforced surfaces that make much of the cell membrane unavailable for endo- and exocytotic activity (e.g. ciliates, euglenids). Nonetheless, some small cells also have specialized feeding apparatuses (e.g. many kinetoplastids). While there are exceptions (e.g. stramenopiles), many specialised feeding apparatuses include arrays of microtubules that run *parallel* to the path that the prey takes as it is ingested. This pattern is seen in organisms as distantly related as euglenids and ciliates (including the tentacles of suctorians) and dinoflagellates (peduncle). In many taxa the feeding apparatus includes vane-like structures, typically composed of microtubules. In several cases it is documented that these vanes reorient themselves during phagocytosis such that they very closely surround the forming food vacuole, broadly defined.

While pseudopodia, in a sense, 'push out' the predator cell to surround the prey item, most cytopharynxes work by pulling the prey into the predator cell. The close connection between the forming food vacuole and longitudinal microtubules in many cases suggests that the motive force might be provided by molecular motors like dynein or kinesin interacting with the longitudinal microtubules of the feeding apparatus. Alternatively the microtubules might be an anchor for motor proteins. Thus, there are obvious conceptual parallels between ingestion by cytopharynxes and cell gliding (see the course notes on 'locomotion'), but this has not been studied in great detail. Even in cells with complex cytopharynxes, however, phagocytosis still involves the actin cytoskeleton as well.

Under optimal conditions microbial eukaryotes can feed so quickly that supply of membrane for food vacuole formation becomes a major logistical problem – Calculations from several species indicate that a cell could require an area of membrane equivalent to the entire cell membrane every 5 minutes or so. If the cell has a robust and highly structured surface (e.g. ciliates) there is a limit to the amount of cell membrane that is free for use as food vacuole membrane. Instead, such cells generally 'build' the food vacuole largely from a pool of endomembrane, using pre-made vesicles within the cell to supply large amounts of additional membrane by fusion with the forming food vacuole. Many ciliates contain flattened vesicles or lipid-rich bodies that are clustered around the cytopharynx region, ready for such deployment. The strategy of building the food vacuole from endomembrane vesicles is also seen in some pseudopodial systems as well,

however, especially in amoebae that capture extremely large prey relative to their body size, and therefore have a peak need for very large amounts of food vacuole membrane.

An interesting case of the integration of prey capture and recognition with phagocytosis is provided by the 'heliozoan' *Actinophrys sol*, which is a diffusion feeder. *Actinophrys* captures motile microbial eukaryotes that collide with its axopodia by the triggering of extrusomes that release adhesive material. This material binds to the prey, especially to flagella/cilia. A major component of this adhesive material is a particular glycoprotein. This glycoprotein, however, *also* binds to the cell surface of *Actinophrys*, and can trigger phagocytosis when present at high local concentrations, which are brought about by the glycoprotein being attached to the surface of the prey (Sakaguchi et al. 2001). Thus, the same secretion from the extrusomes serves both to immobilise a prey item, and to 'mark' it as an object to be phagocytosed.

Exocytosis

Following digestion, residual material in the old food vacuole is released to the environment. This 'cell defecation' is a type of exocytosis and involves the fusion of the old food vacuole membrane with the cell membrane. In some cases, food vacuole exocytosis is confined to particular areas of the cell – Ciliates provide the best-known example, as they typically use a dedicated site on the cell called a 'cytoproct'.

Exocytosis is not only the time that the cell expels waste - it is also an opportunity to recycle the old vacuole membrane. Since the vacuole membrane fuses with the cell membrane, it would, by default, become cell membrane. If the food vacuole membrane were originally largely contributed by the endomembrane system rather than the cell membrane (as in ciliates - see above), there would be the potential for excess cell membrane to accumulate over time. However, the cytoproct of ciliates is also a site where there is extensive vesiculation of the cell membrane, that is, the conversion of cell membrane back into endomembrane. In the model ciliate *Tetrahymena* it is possible to image the continuous transport of endomembrane from the cytoproct region back to the oral region, ultimately to replenish the supply of endomembrane available for food vacuole formation.

Why Phagotrophy rather than Osmotrophy?

Osmotrophy refers to the consumption of dissolved organic material (DOM) rather than particulate organic material. Many microbial eukaryotes are able to take up DOM by pinocytosis, or directly across the cell membrane without forming vacuoles/vesicles.

Most parasites, and 'decomposer' organisms (e.g. Fungi and fungus-like groups such as Oomycetes) live in environments with very high concentrations of DOM. Osmotrophy is an important mode of obtaining organic carbon in many parasites and decomposers, and many are actually incapable of phagotrophy.

By contrast, heterotrophic microbial eukaryotes that live in environments with moderate-to-low levels of dissolved organic matter (e.g. the ocean, freshwater, many sediments) generally derive very little of their energy from DOM. Most eukaryotes are not particularly well suited to efficiently using dilute DOM, in fact. For example, the model ciliate *Tetrahymena* grow reasonably well as a pure culture on dissolved organic matter alone, however it requires *very* high relative concentrations of DOM to do this. If

the same *Tetrahymena* strain is fed particulate food that it can phagocytose (i.e. live prokaryotes), it can achieve the same rate of growth with about *50,000 times less* organic material per unit volume of culture.

The uptake of DOM is heavily dependent on the surface area of the organism. Prokaryotes, being smaller than most eukaryotes, tend to have much larger ratios of surface area to volume. Consequently, they would be expected thrive at lower DOM concentrations than microbial eukaryotes, and also to out-compete 'typical' microbial eukaryotes for DOM, all things being equal. Simplistically put, a microbial eukaryote that is faced with the prospect of competing with prokaryotes for scarce DOM, is better off eating the prokaryotes that efficiently sequester DOM and thus, in effect, convert some of it into concentrated, particulate form.

Phagotrophy in Mixotrophs

Mixotrophy, as the term is used in this course, refers to organisms that are capable of both phototrophy and *phagotrophy* (warning: sometimes 'mixotrophy' is used to also refer to algae that are phototrophic and *osmotrophic*). There are several kinds of mixotrophic eukaryotes. Many microbial eukaryotic 'algae' have retained the ancestral ability to phagocytose particles. Meanwhile some 'protozoan' organisms enter into symbiotic associations with algae, or retain for a time photosynthetically active plastids from algal prey - a phenomenon called 'kleptoplasty' or 'kleptochloroplasty' (Mitra et al. 2016). We may look at these associations in a more detail later in the course. At this point, it is enough to say that mixotrophic organisms are extremely common in environments such as the open ocean: for example, it appears that many or most photosynthetic dinoflagellates are also phagotrophs (often they eat ciliates), and mixotrophic haptophytes appear to be abundant in ocean water (Unrien et al. 2014). There is even some evidence of phagotrophy by certain marine 'prasinophyte' green algae (by contrast, no diatoms are known to be capable of phagotrophy). Conversely a large fraction of the planktonic ciliates, foraminifera and radiolaria in these environments either support symbiotic algae or undertake kletoplasty (Mitra et al. 2016). In some ocean water bodies, especially the photic zones of oligotrophic regions, mixotrophic organisms are estimated to be responsible for half, or even the *majority,* of the bacterivory (i.e. phagotrophy of prokaryotes), outweighing purely phagotrophic protists (The mixotrophic cells generally consume prokaryotes at a much lower rate than purely heterotrophic cells of equivalent size, but their abundances are much higher, leading to a comparable or greater total effort; e.g. Unrien et al. 2007). The most important mixotrophic bacterivores in these systems are small algae such as haptophytes (Unrien et al. 2014).

One important issue concerning mixotrophs is understanding the primary purpose of phagocytosis by these organisms - after all, photosynthetic organisms are already able to obtain energy from sunlight, and to fix inorganic carbon, and thus synthesise *de novo* organic molecules such as carbohydrates. The primary role of phagocytosis in mixotrophs varies widely (Mitra et al. 2016): Some mixotrophs really do obtain significant amounts of their total energy/organic carbon requirement from both photosynthesis and from phagotrophy, with the balance between the two potentially shifting depending on the amount of light, and the availability of suitable prey. Other mixotrophs rely almost entirely on photosynthesis for energy and organic carbon - they

use phagotrophy primarily as a method of increasing access to scarce nutrients, such as phosphorous, or biologically available nitrogen (at times of nutrient scarcity, it can easily be the case that the great bulk of a scarce nutrient in a system is incorporated in living microbial biomass). This nutrient-acquisition role for mixotrophy in many species is strongly suggested by observations of increased feeding rates in response to absolute and/or relative nutrient scarcity, irrespective of how much light is available. Mixotrophs of this second kind could have an advantage over non-phagotrophic algae when nutrients such as nitrogen or phosphorus are limiting.

Recommended readings:
Hausmann et al., 2003: p222-238

Important Sources:

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