Ecophysiology of mixotrophs

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January 19, 2000

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Chapter 1

Introduction

1.1 Definition of the mixotroph

Mixotrophy is defined as the capability of one organism to be autotrophic and heterotrophic at the same time. Therefor an organism that can survive in water as a chemolitho-autotroph and, at the same time, is capable of ingestion and digestion of organic compounds from human blood, is called a mixotroph. Although many of infectious micro-organisms are mixotrophic in a similar way as mentioned above, they form just a small group in the total of mixotrophic organisms. The term mixotrophy is primarily used in the ecological literature for protists that are both phototrophic and phagotrophic (Stoecker, 1998). These organisms combine heterotrophy with photo-autotrophy. In studies of life in the oceans this phenomena was discovered in ciliates and flagellates, but soon it appeared to be very common (Stoecker et al., 1988). Mixotrophy is prevalent among planktonic chrysophytes, prymnesiophytes, perhaps cryptophytes, dinoflagellates, ciliates, and among the sarcodines. But also in multicellular organisms there is a form of symbiosis that resembles unicellular mixotrophy. Many sponges, flatworms and other multicellular organisms also combine phototrophy with phagotrophy or osmotrophy, by harboring algal endosymbionts. Mixotrophs include phagotrophic ‘algae’ that are primarily phototrophic, as well as photosynthetic ‘protozoa’ that are primarily phagotrophic. These photosynthetic ‘protozoa’ often have photosynthetic abilities due to algal endosymbionts or sequestered plastids, which are also present in many species of coral, some flatworms etc. These mixotrophic organisms can be abundant in the euphotic zone in fresh, estuarine, coastal and open ocean waters at latitudes ranging from equatorial to polar. Although in some taxa mixotrophy appears to be associated with oligotrophic environments, mixotrophic protists can be abundant in eutrophic and mesotrophic waters as well as oligotrophic regions (Stoecker, 1998). The costs and benefits of mixotrophy in different taxa and environments are still largely a subject of speculation. Cost and benefits seem largely dependant on the strategy within the mixotrophic organisms itself. Investigation of the great diversity of different strategies in mixotrophy is necessary.

All living organisms on earth have only a limited number of strategies to obtain energy and matter to grow and maintain structure. Organisms can use photosynthesis in combination with inorganic nutrients and be phototrophic or they can use food of organic or inorganic origin, and be heterotrophic or chemolitho-autotrophic respectively. A sharp definition of all these strategies is necessary to categorise and understand the many different mechanisms. For example a prototroph can also catabolise organic molecules, because it possesses the enzymes of the Krebs-cycle. The difference between a phototroph that catabolises organic molecules, and a mixotroph is made by the origin of the organic molecules. It’s a very well known principle that a phototrophic organism like a plant can use its reserves to obtain the energy necessary to grow and maintain its structure. In this case the organic material that is used has not been part of any other organism. Thus a plant is called a ‘normal’ photo-autotroph and not a mixotroph. The reason why a ‘normal’ phototroph can not be heterotrophic is because it lacks the capability, necessary to transform prey organisms, or large organic molecules from the environment into energy. It also lacks the mechanisms to ingest prey or other organic food. So phototrophy will be the capability to obtain energy from light and material for growth from inorganic nutrients. Heterotrophy would then be the capability to ingest and use organic matter as energy source and source of material for growth. Chemolitho-autotrophy is the capability to ingest and use molecules of inorganic origin as energy source and source of structural material. The different strategies in living organisms are summarised in Table 1.1. In this table the definitions of the different strategies are posed: An organism is assumed to be chemolitho-autotrophic when it is neither heterotrophic nor phototrophic. A normal photo-autotroph, like a plant, is defined as not heterotrophic and obligate phototrophic. Likewise a normal heterotroph, like a mammal, is an organism that is not photoautotrophic and obligate heterotrophic. A mixotroph here is an organism that is able to combine two strategies.

The prevalence of the mixotroph combining phototrophy and heterotrophy poses questions at the ecosystem level. Classic trophodynamic models rely on plant/animal dichotomy which segregates primary producers from consumers by trophic level. When mixotrophy is being incorporated in these classic trophodynamic models no segregation can be made by trophic level and questions remain about the effects of mixotrophy on production and consumption within the microbial and other foodwebs. These effects can be quite great, as this form of mixotrophy is found to take a very important, and maybe even dominant place in the microbial foodweb of aquatic ecosystems (Stoecker, 1998, Bouvier et al., 1997, Gowing and Garrison, 1992, Stoecker et al., 1989). This may be due to its capability to use more resources than either a phototroph or a heterotroph: When there is not enough organic food, a facultative mixotroph can switch to using light as an energy source. And when light is limiting it can switch back to feeding heterotrophically on organic substances, or even to feeding on its competitor (Skovgaard, 1996). In a dynamic system like an aquatic microbial community this might be the factor by which an organism can survive and reproduce so effectively that it can form blooms. Bloom-forming flagellates are often mixotrophic (Stoecker, 1998).

In this literature-study I will restrict myself to the ecophysiology of this form of mixotrophy. These organisms are the smallest complete systems on earth with consumption as well as production. And they are of great importance in studies of the ecosystems of the oceans, especially when an attempt is made to develop models to describe these ecosystems. The photo-hetero-mixotroph might be a crucial factor in understanding energy and mass flows through these systems. So in this study the word mixotrophy will be used to address the combination between photo-autotrophy and heterotrophy. The mixotrophs that form other combinations: the chemolitho-hetero-mixotroph and the chemolitho-photo-mixotroph will be discussed just briefly.

An example of an organism that is chemolitho-autotrophic and heterotrophic at the same time, can be given by *Pseudomonas sp.*, *Acinetobacter sp.* and *Aeromonas sp.*. These microorganisms are able to reduce nitrate to nitrite, but their growth is enhanced by heterotrophic metabolisation of organic growth factors from microbial (or other) origin (Liessens et al., 1992). *Xanthobacter tagetidis* is a bacterium that can grow simultaneously as a chemolitho-autotroph on thiosulfate and as a heterotroph on thiophene-2-carboxylic acid and other acids like acetic acid (Padden et al., 1998). This kind of mixotrophy is often observed in bacteria, but there were no data available on other organisms with this strategy.

A chemolitho-photo-mixotroph would be an organism that is capable of phototrophy and chemolitho-autotrophy. No examples could be found of organisms that have this strategy, but the strategy might have been the link between chemolitho-autotrophic and photosynthesising organisms. On the other hand phototrophs are more likely to have originated from heterotrophic organisms, posessing all the enzymes of the Krebs-cycle. Heterotrophic organisms being the link between photo-autotrophy and chemolitho-autotrophy.

Table 1.1: Strategies in mixotrophic and not mixotrophic organisms

			Heterotrophy	
		obligate	*facultative*	*not*
Phototrophy	*obligate*	**obligate mixotroph (1)**	**facultative mixotroph (2)**	'normal' phototroph
	facultative	**facultative mixotroph (3)**	**'true' facultative mixotroph (4)**	chemolitho-photo mixotroph
	not	'normal' heterotroph	chemolitho-hetero mixotroph	chemolitho autotroph

Chapter 2

Different strategies in mixotrophy

It is difficult to categorise mixotrophic strategies because of the great diversity of mixotrophic protists and because data on the functional responses of most mixotrophs are incomplete or lacking (Stoecker, 1998). Nevertheless it is useful to attempt categorisation in order to develop a general understanding of mixotrophy as an evolutionary strategy as well as an important factor in planktonic ecosystem dynamics.

The division between obligate and facultative as made in Table 1.1 is useful for dividing different strategies within mixotrophs into well formulated groups. Every combination between autotrophy and heterotrophy that is given in Table 1.1 can be supported by many examples. Based upon the combinations possible, mixotrophic organisms can also be divided in obligate and facultative. Facultative mixotrophic organisms can be partly facultative or fully facultative. The obligate mixotroph needs both of its strategies, the partly facultative mixotrophic organism can use another strategy to supplement or backup its main strategy, and a fully facultative mixotroph can live from either one of its strategies. The following types of mixotrophs can now be distinguished (Stoecker, 1998):

1. Mixotrophy is obligate: Both light and particulate food is necessary for sustaining growth and maintenance. Probably due to the need of derived growth factors (Skovgaard, 1996, Keller et al., 1994)
2. Phototrophy is obligate but heterotrophy is facultative: Only photosynthesis is essential for growth and maintenance, heterotrophy can be used to backup the photosynthetic apparatus in times of low light intensity. Probably a very great advantage in competition for light-energy (Combres et al., 1994).
3. Heterotrophy is obligate but phototrophy is facultative: Only food is necessary for sustaining growth and maintenance, but photosynthesis can be used to backup heterotrophy in times of low food concentrations. Probably due to need of constant replacement of chloroplasts by chloroplasts from prey (Skovgaard, 1998).
4. Mixotrophy is facultative: Ability to grow exclusively by either photosynthesis or phagotrophy/uptake of organic compounds (Skovgaard, 1996).

Obligate mixotrophy was found in *Laboea strobila* by Stoecker et al., 1988, but in Stoecker, 1998, the same species was suggested to be a plastid retaining phagotroph, or a facultative phototroph and a obligate heterotroph. It was concluded that the organism could do without photosynthesis. It was even suggested that completely obligate mixotrophy did not exist (Stoecker, 1998. In this study *Laboea strobila* is still assumed to be an obligate mixotroph in reality. Obligate mixotrophy may take an important place in oceanic life, especially when it is taken into account that food-supplies are mostly limited. There are still many doubts about the mixotrophic mechanisms in diverse organisms and many may turn out to be obligate mixotrophs.

Mixotrophic organisms can also be divided into two groups by the way they obtain their chloroplasts. One type of organisms have ‘true’ chloroplasts; permanent endosymbionts that will stay intact during the entire lifecycle of the host. It has not been proven that organisms that use this strategy exist, but it is strongly assumed in *Fragilidium subglobosum* (Skovgaard, 1996). The

Table 2.1: Subdivisions made in mixotrophic organisms

obligate mixotroph (1)	
facultative mixotroph	
obligate phototrophy (2)	
heterotrophic feeding when:	**a.** organic nutrients are limiting
	b. trace organic growth factor is limiting
	c. light is limiting (to get carbon)
	(**d.** to supplement carbon nutrition)
obligate heterotrophy (3)	
phototrophic activity when:	**a.** prey is limiting
	b. to supplement carbon nutrition
'true' facultative mixotroph (4)	

other group consists of the organisms that obtain their chloroplasts from their algal prey through phagocytosis and separation of the chloroplasts from other algal organelles in digestive vacuoles, these are called klepto-chloroplasts. These klepto-chloroplasts will degrade after a certain amount of time and will be digested when they cease to be functional. An example of an organisms that uses this kind of strategy is *Gymnodium 'gracilentum'*. This dinoflagellate will lose its chloroplasts, when incubated without prey, after 4-5 days (Skovgaard, 1998). Klepto-chloroplasts are very common in the facultative mixotroph that is obligately heterotrophic. A few examples exist from the obligate mixotroph, but they have not been found in the 'true' facultative mixotroph and the obligately phototrophic mixotroph.

The facultative mixotroph has been divided in an obligate phototrophic mixotroph, an obligate heterotrophic mixotroph and a 'true' facultative mixotroph. The facultative mixotroph that is obligately phototrophic can be divided in organisms that start heterotrophic feeding when dissolved inorganic nutrients are limiting, organisms that feed when a trace organic growth factor is limiting and organisms that feed when light is limiting (to get carbon). The facultative mixotroph that is obligately heterotrophic can be divided in organisms that photosynthesise when prey is limiting and organisms that photosynthesise to supplement carbon nutrition (Stoecker, 1998). This is summarised in Table 2.1. Further subdivisions in all mixotrophic organisms can be made by the kind of chloroplasts they use, kleptochloroplasts or permanent endosymbionts, and by the way they feed, phagotrophic or osmotrophic. Osmotrophy meaning uptake of dissolved organic carbon (DOC). Examples of organisms that use the strategies and substrategies mentioned above will be given in the next chapter.

Chapter 3

Mixotrophic organisms and their ways of mixotrophic feeding

3.1 Mixotrophic organisms

3.1.1 Phytoplankton

In freshwater lakes, phagotrophic phytoflagellates have been reported to contribute from $\leq$10% to over 41% of the phytoplankton, with the average contribution lowest in a eutrophic lake and highest in a humic, dystrophic lake. In a euphotic lake, phagotrophic phytoflagellates have been estimated to contribute, depending on the season, 2-45% of the total bacterivory while in an oligotrophic lake phagotrophic phytoflagellates were found to account for >50% of the bacterivory in summer (Stoecker, 1998).

Phagotrophy is also common among marine phytoflagellates, with both bacterivory and grazing/predation on protists reported. In estuaries and coastal waters, phytoflagellates may account for >50% of the flagellate bacterivory in summer and probably somewhat less in winter. Phytoflagellates can also be important grazers on other protistan plankton, ranging from picophytoplankton to microplankton. In oligotrophic, subtropical surface waters, <5-50% of the pigmented nanoplankton have been observed to be phagotrophic. Flagellates such as chrysomonads, haptophytes and dinoflagellates are a large group of phytoplankton organisms in which mixotrophy is very wide-spread. Bloom-forming mixotrophic dinoflagellates may be particularly important as grazers on relatively large-sized prey including other phytoplankton and oligotrichous ciliates in coastal waters (Stoecker, 1998). Three different feeding mechanisms have been described among dinoflagellates, all allowing the ingestion of relatively large prey (Hansen and Nielsen, 1997).

In many cases in both fresh and marine waters, phagotrophy by phytoflagellates has been associated with inorganic nutrient limitation although in lakes phagotrophy has also been associated with low light.

If mixotrophy is defined so as to include osmotrophy by algae, the majority of phytoplankton might be considered mixotrophic. Many phytoplankters, like *Cryptomonas sp.*, can take up dissolved organic carbon, or, under inorganic nutrient stress, use dissolved amino acids or other organic sources of nitrogen (Stoecker, 1998, Vincent and Goldman, 1980).

3.1.2 Planktonic ciliates

Mixotrophic ciliates either sequester plastids derived from their prey or harbor algal endosymbionts. Plastid-retaining species are usually in the family Strombidiidae and are often an important component of the mixed layer plankton in fresh and marine waters under stratified conditions. One rather unusual non-strombidiid species, *Perispira ovum*, retains a variety of organelles from its euglenid prey and is associated with micro-oxic layers in estuaries. Mixotrophic ciliates with algal endosymbionts are common in freshwater ponds and lakes, but rare in the ocean. They are often associated with the pycnocline or micro-oxic layers.

Mixotrophic ciliates are found in freshwaters ranging from polar to tropical environments. In an oligotrophic, Antarctic lake, mixotrophic ciliates accounted for up to 90% of ciliate numbers.

In a temperate, mesotrophic lake, mixotrophic ciliates seasonally comprised <1-51% of the ciliate numbers and <1-13% of the ciliate biomass. In subtropical, oligotrophic lakes, mixotrophic ciliates can comprise 27-59% of the ciliate biomass and 3-20% of the total photosynthetic biomass. In tropical, oligotrophic Lake Tanganyika, mixotrophic ciliates seasonally accounted for <1 to >90% of the microprotozoan numbers and ~32% of the average autotrophic biomass.

In marine waters, mixotrophic ciliates can also be seasonally important. In coastal Antarctic waters, mixotrophic ciliates can comprise 25-47% of the ciliate assemblage in the mixed layer during the spring bloom and post bloom period. In the Nordic Seas in summer, mixotrophs were found to make up 40-52% of the ciliate assemblage in surface waters. During the spring bloom in the north Atlantic, 50-73% of the ciliates have been reported to be mixotrophic. In the more oligotrophic waters of the Mediterranean Sea, ≥50% of the ciliate biomass or biovolume is accounted for by mixotrophs. In the equatorial Pacific upwelling, mixotrophic ciliates are relatively rare, <10% of the oligotrichous ciliates were plastidic.

3.1.3 Cyanobacteria

Cyanobacteria are also known to be mixotrophic. Many cyanobacteria can take up organic substances through pinocytosis to use as a supplemental carbon nutrition. *Cyanothece sp.* is an example of a cyanobacterium that can use glycerol as a substrate to grow efficiently mixotrophic and even heterotrophic in the dark. It was proven by Schneegurt et al., 1997 that heterotrophic cultures even grew faster in the dark and to higher densities than purely phototrophic cultures could in light.

3.1.4 Zooplankton, multicellular organisms

Small (< 1mm) acoel flatworms (class Turbellaria, order Acoela) with algal endosymbionts are a common, though sporadic, component of the open-ocean plankton in warm waters. Although these acoels are photosynthetic, they are also predatory on other plankton organisms and thus can be called mixotrophic in a strict sense of the word. Species that are known to harbor algal endosymbionts are many species of *Convoluta* for example. The origin of these algal endosymbionts can vary, but there are species known to harbor dinophytes and prasinophytes (Stoecker et al., 1989).

Endosymbiosis is a very common phenomena in multicellular organisms of plankton and coral. Many species of coral are able to live in symbiosis with algae. And contrary to the earlier, widely accepted belief that corals harbor only one symbiont, it was even found that the ecologically dominant Caribbean corals *Montastraea annularis* and *M. faveolata* can act as hosts to dynamic, multi-species communities of *Symbiodinium sp.* (Rowan et al., 1997). In sponges, one form, which resembled *Cymbastela notiana*, was found in the gulf waters of South Australia. A second form was found on the protected sides of islands along the oceanic coast of Victor Harbour in South Australia. These sponges are rare but when present occur at depths of 5-15 m in association with foliaceous red and brown macroalgae. This form of Cymbastela was capable of photosynthetically compensating on a net 24-h basis at 4.5 m depth in the winter, whereas it was a net producer at that depth during the summer (Cheshire et al., 1995).

Mixotrophy is also know from open ocean planktonic foraminifera, plyostine radiolaria and acantharia for instance. These large sarcodines live in the euphotic zone and prey on phytoplankton, protozoa and crustacean zooplankton. They can be dominant predators on zooplankton in the open oligotrophic ocean, but they can also be significantly phototrophic. For example ≥90% of the foraminifera have been reported to harbor algal endosymbionts and also in other types of organisms endosymbionts are frequently found (Stoecker, 1998).

3.2 Mixotrophic feeding

3.2.1 Obligate mixotrophs

An obligate mixotroph may be obligate because it needs photosynthesis for carbon nutrition, but cannot take up inorganic nutrients directly from its environment, so it will need to feed to get its fosforus and nitrogen. It may lack enzymes to use its prey as only carbon nutrition source, so it must be phototrophic for carbon and heterotrophic for dissolved inorganic nutrients.

An obligate mixotroph may be obligate due to its need of some trace organic growth-factor which it cannot synthesise and is only present in its prey. In cultures it was not possible to grow some phytoplankton species without bacteria, so these species may be obligate mixotrophic in cultures due to the need for a trace organic growth factor that may be present in their environment, but not in these cultures (*Ochromonas sp.*Keller et al., 1994).

An obligate mixotroph may be obligate due to the way it obtains its chloroplasts. If it uses klepto-chloroplasts, but cannot do without phototrophy, it is also obligately phagotrophic. Because its chloroplasts will cease to be functional after a certain amount of time, if it does not replace them it cannot photosynthesise. *Laboea strobila*, an abundant planktonic ciliate from the group of the oligotrichs, is phagocytic, digestive vacuoles containing algal cells were proven in the cell (Stoecker et al., 1988). It sequesteres its chloroplasts free from algal cytoplasm from several algal taxa. These chloroplasts can remain functional for at least two weeks, and when cells divide, chloroplasts are divided between daughter cells (Stoecker et al., 1988). When they become dysfunctional they are re-incorporated into food-vacuoles and digested. In temperate coastal areas, this ciliate can account for about 2 to 3% of total chlorofyll α in the water column (Stoecker et al., 1988). In *L. strobila* the amount of inorganic carbon fixed h^{-1} at saturating irradiance equals 12.6% of the body carbon of the ciliate (assuming a volume to carbon conversion factor of $0.1 \mathrm{pgC}\mu\mathrm{m}^{-3}$ for the ciliate (Stoecker et al. 1987[1])) and its assimilation efficiency lies well within the range of phytoplankton. Stoecker et al., 1988 calculated that 37% of the total carbon budget could be accounted for by photosynthesis under saturating light intensities and saturating food concentrations. In dark circumstances the cells of *L. strobila* change shape and become smaller. So it was concluded that the species is partially dependant on the carbon fixed by photosynthesis, chloroplasts play an important role in their metabolism, but for its chloroplasts and organic growth factors it is dependant on phagotrophy, so this organism may be a good example of an obligate mixotrophic organism (Stoecker et al., 1988). Many ciliate species retain klepto-chloroplasts and they always have them, although number and type can vary. When starved, cells gradually lose their chloroplasts and their specific shape and size, but as soon as algae are present they start feeding and when the chloroplasts reappear in the cytoplasm, the specific size and shape returns as well.

3.2.2 Facultative mixotrophs

The strategy of obligate phototrophic algae to supplement their carbon metabolism with osmotrophic derived carbon and the strategy of obligate phototrophs that supplement their photosynthesis with phagotrophic derived carbon seem to be separate strategies that do not occur in the same organism. In *Cryptomonas sp.* for example, which can use glucose from its environment, there was no phagotrophy found (Gervais, 1997). Obligate phototrophic mixotrophs mostly are algae, and osmotrophy is found very often, but phagotrophy seems a strategy the is not frequently used by obligate phototrophic mixotrophs, the majority of mixotrophic algae uses osmotrophy. Certain photosynthetic dinoflagellates however are known to be capable of phagocytosis of other dinoflagellates. Connected with the relatively infrequent use of phagotrophy in obligate phototrophs, is the use of permanent endosymbionts. Klepto-chloroplasts have not been found in obligate phototrophic mixotrophs.

Obligate phototrophy, facultative mixotrophic phototrophs

Many algal species have been found to take up dissolved organic carbon in experiments with high concentration of a few different kinds of organic carbon like glucose, malate and acetate (Combres et al., 1994, Vincent and Goldman, 1980, Gervais, 1997), but even cyanobacteria have been found to be capable to utilise organic molecules like glycerol (Schneegurt et al., 1997). But the low uptake rates of these organic nutrients have led to the conclusion that in most planktonic communities it is unlikely that algae can effectively compete with bacteria. Algal heterotrophy has been considered a laboratory artifact of axinic culture-conditions and artificially high concentrations of organic

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[1] Stoecker, Silver, 1987, Chloroplast retention by marine planktonic ciliates. Annales of the N.Y. Academy of Sciences 503:562-565

compounds. But it has been showed that some species of ultraplanktonic flagellates have active transport systems for glucose and acetate which permits them to be competitive. Deep living populations of *Oscillatoria agardhii* var. *isothrix* have been found to be capable of organic nutrient transport at ambient substrate concentrations (Vincent and Goldman, 1980). In Lake Tahoe it was shown by Vincent and Goldman, 1980 that the two species *Monoraphidium contortum* and *Friedmannia sp.* were able to take up acetate from their environment and were also physiological equipped to utilise acetate for growth in both light and dark. These two species achieved their maximum population densities at the boundary of the euphotic zone. Vincent and Goldman, 1980 estimated that in terms of total carbon flow the contribution of acetate-carbon to overall algal nutrition might be very low, but the organic carbon uptake did seem significant when compared to estimated photosynthetic rates for these two species, which were responsible for acetate photo-assimilation.

So algal populations living at te lower boundary of the euphotic zone are shown to be able to meet a part of their carbon requirement by osmotrophy (pinocytosis) of dissolved organic carbon. These algae exhibit a trophic strategy that is facultative mixotrophic, but obligate phototrophic. The osmotrophy in this case is fully facultative. Many planktonic algae can use diverse organic nutrients as acetate and glucose, even dextran (of up to 2000 kDa, in *Alexandrium catenella*(Legrand and Carlsson, 1998)) and maybe many more to supplement their phototrophic carbon metabolism to survive a period of darkness, or to sustain growth when light is limiting. Vincent and Goldman, 1980 found in Lake Tahoe that in long periods of insufficient light it is likely that the biochemical costs of cellular maintenance of many algae are at least partly met by heterotrophic nutrition. It was even discovered that certain algae in the deep euphotic zone ‘remain viable and retain a fully operational photosynthetic apparatus for periods in excess of 12 months’ (Vincent and Goldman, 1980)[2]. These organisms thus can have a great advantage with this supplementary trophic system, especially when living at the lower boundary of the euphotic zone, where the input from solar radiation is extremely low but inorganic and organic nutrients are in relatively plentiful supply, because of the low competition with bacteria and other photosynthetic algae. Light stimulated organic nutrient uptake was also recorded in this narrow region near the bottom of the euphotic zone, this may be an extra advantage.

An example of species of algae that are well adapted to life conditions of limited light is given in Gervais, 1997. Here it is posted that the species that dominate the maxima in algal biomass, found in mesotrophic stratified lakes at the oxic/anoxic boundary layer (the summer chemocline), often belong to the Cryptophycea. Species like *Cryptomonas phaseolus* and *Cryptomonas undulata* that often dominate these chlorofyll maxima, are already adapted to low light intensities and have maximum growth rates at lower light intensities as other species from the Cryptophycea, like *Cryptomonas cf. ovata*. Photo-inhibition occurs in *C. phaseolus* at the light intensities where *C. cf. ovata* grows at maximum rate. *C. phaseolus* showed the fastest growth between photon flux densities of ~ 7 and ~ 85 μ molm^{-2}s^{-1}. As also in *C. undulata*, growth is supported at very low light intensities. The species increased their internal chlorofyll concentration when light intensity dropped. But another adaptation to low light intensities was found: When glucose was added to the medium, the uptake of glucose by *C. phaseolus* and *C. undulata* was proven. Thus these species are examples of obligate phototrophic mixotrophs that feed when light intensity is low (strategy 2, substrategy c). Growth was enhanced by glucose uptake. Unlike other species (*C. rostratiformis*) that improve their light energy supply by vertical migration, these species are able to improve their energy supply by supplementing photosynthesis with osmotrophy (Gervais, 1997). This strategy may not be very important in natural stratified mesotrophic lakes, because the glucose concentration might be too low to have a significant effect on growth. But Vincent and Goldman, 1980 have theorised that the uptake of DOC in general could be sufficient to sustain algal populations in the dark for prolonged periods.

Acetate seems to play a more crucial role as supplementary energy source for algae then glucose. This may be due to uptake mechanics and the relatively high concentrations in most natural lakes. Utilisation of acetate in microalgae proceeds via the glyoxylate cycle.

The species *Uroglena americana* is an example of a phototrophic organism that feeds to obtain a trace organic growth factor (strategy 2, substrategy b). It is thought to feed in order to

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[2] Vincent, 1978, Survival of aphotic phytoplankton in Lake Tahoe throughout prolonged stratification. Int. Ver. Theor. Angew. Limnol. Verh. 20

obtain phospholipids. An example of a phytoflagellate that feeds in respons to inorganic nutrient limitation, but not in respons to low light intensities is *Prorocentrum minimum* (Stoecker et al., 1997)(strategy 2, substrategy a). For this species photosynthesis is directly related to food concentration and feeding to irradiance, when DIN concentrations are low. Rather nutrient ratios than absolute concentrations of individual DIN may be important in inducing phagotrophy.

Other mixotrophic algae do not use osmotrophy but phagotrophy as a strategy to obtain organic substrate for heterotrophic growth. The classes Prymnesiophyceae and Dinophyceae have many phagotrophic members. From the Prymnesiophyceae species of *Chrysochromulina* are capable of ingestion of small green flagellates (Jones et al., 1993) and *Prymnesium patelliferum* is capable of ingestion of prey that can be even larger than the cells of *P. patelliferum.* It releases toxin to immobilise the prey by puncturing the cell membranes. It only attacks cells that have lost their motility, by forming chemically stimulated pseudopodia prior to the phagocytosis of the entire prey cell. It can attack prey with several cells simultaneously, a form of merging between cells is seen to digest the prey (Tillmann, 1998).

From the *Dinophyceae* species of *Dinophysis* have been shown to be capable of ingestion of related phagotrophic flagellate species and ciliates. Food-vacuoles were discovered in *Dinophysis norvegica* and *Dinophysis acuminata*, in *D. acuminata* up to 36% of its population contained food-vacuoles. In the true phagotrophic *D. rotundata* a similar frequency of its population was found to contain these vacuoles (50%), which proves the importance of phagotrophy for the mainly photosynthetic *Dinophysis acuminata* (Jacobson and Andersen, 1994). Mixotrophy in these mainly phototrophic species is only induced when photosynthesis is not able to meet the cells needs or to provide some special compound found only in organic material. The *Dinophysis* species are with this ability to take up organic compounds, capable of maintaining high numbers, even under light limiting conditions (Granéli et al., 1997).

From the class of the Chrysophyceae, a flagellate from the genus *Ochromonas* is capable of phagotrophic feeding on specific different types of bacteria (Keller et al., 1994). The phagotrophy in this species of *Ochromonas* occurs under conditions of limited inorganic nutrients or limited light. In the *Dinobryon* genus there are also many species known to have a phagocytic strategy next to photosynthesis. *Dinobryon divergens* and *Dinobryon sertularia* feed on bacteria and use the carbon as a supplement to phototrophically derived carbon or to obtain certain micronutrients. These species die in the dark and cannot use the organic carbon to survive under light limiting conditions, phagotrophy does not seem to be dependent on metabolic energy supply in this species, it is rather dependent on the physiological state of the cell (Jones and Rees, 1994). Ingested ciliates, dinoflagellates and diatoms were also identified inside the cells of *Ceratium furca*, *Gymnodium sanguineum* and *Gyrodineum uncatenatum* by Bockstahler and Coats, 1993. And Jacobson and Andersen, 1994 showed the presence of food vacuoles in some photosynthetic dinoflagellates like *Dinophysis acuminata* and *D. norvegica*, but it may be common for many *Dinophysis* species. *Heterocapsa triquetra* is also capable of phagocytosis. In this case ingestion of prey is dependent on light limitation. Phagotrophy is stimulated by low light intensity and low nutrient conditions and may be important in maintaining the population under these conditions (Legrand et al., 1998).

Most algae are capable of osmotrophy of dissolved organic carbon from their environment, but the environments on earth, especially oceans, are mostly oligotrophic, so still, the effect of osmotrophy on complete systems is estimated very small. The effect of phagotrophy is highly variable. Phagocytosis of prey can contribute approximately 3 to 60% of carbon compared to photosynthesis (Bird and Kalff, 1987) In general this mechanism may, like osmotrophy, be just of little importance to the algal community as a whole, but be the solution to survival under temporal light limiting conditions for many algal species.

Another effect of limiting energy lies in the necessity of carbon and energy for the assimilation of nitrogen from nitrate and ammonium. Nitrogen uptake may be influenced by carbon uptake. Normally cells depend on photosynthesis for nitrogenassimilation, because assimilation of nitrogen requires carbon-skeletons and energy in ATP. Nitrogen assimilation stops when light, and thus energy, becomes limited. Growth might be able to continue if nitrogen would not be the restricting factor. If nitrogen is limiting then phototrophy would build up carbon reserves and nitrogen uptake would be possible in the dark. But if light is limiting an external source of carbon could be stimulating growth by stimulating nitrogenassimilation. This mechanism has been shown to be important for *Scenedesmus obliquus*, 'true' facultative mixotroph (Combres et al., 1994), but it is suggested to be a common mechanism for planktonic mixotrophic algae in general, mostly for the obligate phototrophic algae. Furthermore not only inorganic nitrogen (mostly nitrate), but also nitrogenous organic compounds can be sources of nitrogen for phytoplankton nitrogen-assimilation. Acetamide and formamide (but no longer chain aliphatic amides) were found to be used effectively by *Emiliana huxleyi* for growth. Also other phytoplanktonic organisms, like the dinoflagellate *Prorocentrum minimum* and the diatom *Thalassiosira pseudonana* were found to utilise acetamide very effectively. In the presence of nickel, Hypoxanthine and urea also stimulated growth in *Emiliana huxleyi* (Palenik and Henson, 1997).

If very high concentrations of dissolved organic carbon are offered, some species of algae and cyanobacteria that are in general obligate phototrophic may be seen as 'true' facultative mixotrophs, because they can survive and even grow (with growth rates that approximate phototrophic rates) in complete darkness, if supplied with plenty organic 'food' (Combres et al., 1994, Jones et al., 1993, Schneegurt et al., 1997).

Obligate heterotrophy, facultative mixotrophic heterotrophs

Obligate heterotrophic organisms are known to harbor chloroplasts, derived from algae. Although their efficiency decreases (Stoecker et al., 1988), these algal plastids are proven to remain functional in many species of ciliates (Stoecker et al., 1988, Stoecker et al., 1987), but are also thought to be present and functional in dinoflagellates (Bouvier et al., 1997, Skovgaard, 1998). The chlorofyll a concentration in cells that are obligate heterotrophic and facultative mixotrophic rises with the concentration of prey organisms, rather than with light intensity (Jones et al., 1995), as a specific characteristic of this kind of mixotrophs.

Many oligotrich ciliates of marine microplankton are in this way mixotrophic. In the Black Sea the biomass of phagotrophic mixotrophs was recorded to account for 1 to 15% of the total phagotrophic protozoan biomass and the feeding activity ranged from 14 to 24% of total ingestion rates of bacteria and nanoplankton by this phagotrophic community. At Great Harbor, Woods Hole, Massachusetts, 50% of all ciliates contained chloroplasts (Stoecker et al., 1987). Mixotrophic dinoflagellates preferentially feed on nanoplankton and do not feed substantially on bacteria. Mixotrophic ciliates feed effectively on both bacteria and nanoplankton. Microsized mixotrophic ciliates in the Black Sea have a specific ingestion rate for bacteria that is higher than that of pure phagotrophic ciliates, though the biomass of prey organisms is of no influence on ingestion rates, suggesting only dependence of ingestion rates on light and nutrient availability (Bouvier et al., 1997).

Mixotrophic ciliates represent a significant part of the ciliate population in environments ranging from the Pacific Ocean and Atlantic Ocean to the Mediterranean Sea (Laval-Peuto and Rassoulzadegan, 1988). These ciliates are mostly oligotrichs and tintinnids, which keep their chloroplasts even when starving or in the dark. Of all oligotrichs at Great Harbor 69% contained chloroplasts, which accounts for 45±27% of all ciliates with chloroplasts (Stoecker et al., 1987). Of the oligotrichs *Strombidium sp.* and *Tontonia sp.* contain functional chloroplasts and can photosynthesise at 42 and 41 pgcell^{-1}h^{-1} with cell volumes of 7.6 and 1.4 x 10^4 μm^3 respectively. Another oligotrich is *Laboea strobila* which has a much higher photosynthesis, but which is next to obligately heterotrophic also obligately phototrophic. Ciliates with obligate heterotrophy and facultative mixotrophy do not have the same photosynthetic capabilities of photosynthetic algae, but heterotrophically they can be very efficient, which may also be caused by partly photostimulated ingestion. The species mentioned here are examples of obligate heterotrophic mixotrophs that start photosynthesis when prey is limited and they keep their chloroplasts even in the dark for this situation (strategy 3, substrategy a).

Of the *Strombidium* species *Strombidium capitatum* has been investigated for its heterotrophic capabilities, next to its photosynthetic ones (Legrand et al., 1998, Crawford and Stoecker, 1996, Stoecker and M.W., 1990). A large variation in cell size and respiration rate was observed. Volume specific growth rates were calculated as $\simeq$ 2.5 to 20.1 x 10^{-6}nlO$_2$ μm^{-3}h^{-1} for cells between 2.4 and 14.9 x 10^4 μm^3. For the smaller cells the mean carbon specific respiration rate lies between 3.8 and 4.1% cellCh^{-1} over 8h of their survival and for large cells between 1.1 and 1.4% cellCh^{-1}. This higher respiration rate may be explained due to high motility of smaller cells. Under starvation there is an inverse relationship between weight specific respiration rate and survivorship. The specific respiration rate is not lowered by the cells under starvation and this may be due to the digestion of at least a part of the sequestered plastids (Legrand et al., 1998).

Mixotrophy by using klepto-chloroplasts is also known from dinoflagellates, where many species that lack chloroplasts of themselves are known to host them, retained from cryptophyte prey. Klepto-chloroplasts were found in a number of species like *Poterioochromonas malhamensis*, *Fragilidium* cf. *mexicanum* (Jeong et al., 1999) and *Gymnodium 'gracilentum'* (Skovgaard, 1998). *Poterioochromonas malhamensis* is a nice example of obligate heterotrophic mixotrophs that start feeding when prey is limited (strategy 3, substrategy a). *Gymnodium 'gracilentum'* on the other hand is a mixotroph that uses its photosynthesis supplementary to its obligate heterotrophic strategy (strategy 3, substrategy b). Without prey *G. 'gracilentum'* will lose its 2-4 twolobed chloroplasts after 4-5 days, the cells become smaller and change shape, though they appear to remain healthy because they still possess viable lamellae. Amorphous food-vacuoles can be seen in the posterior end of the cells of *G. 'gracilentum'*. Under experimental, light conditions this species was able to grow in the absence of prey organisms. At a light intensity of 90 μmolm^{-2}s^{-1} the species was able to fix 187% of its own cell carbon cell^{-1}d^{-1} by photosynthesis, after 12-48 hours of starvation this rate dropped to 81 and 3%cell^{-1}d^{-1} respectively. Under the same light conditions and in the presence of food it was able to take up 51% of its total carbon uptake through photosynthesis (Skovgaard, 1998). The photosynthetic capabilities in this species are fully supplemental to its phagotrophic capabilities, it will feed to supplement its carbon nutrition, but it still needs prey to get nitrogen, phosforus and other nutrients needed for growth and maintenance. But as the prey species is of great importance to feeding rates and feeding efficiency, as has been shown in *Fragilidium* cf. *mexicanum* (Jeong et al., 1999), the ability to wait for suitable prey under food limiting conditions can be of great significance to survival of these dinoflagellates.

So the use of klepto-chloroplasts by ciliates and dinoflagellates can give these organisms a great advantage over other pure phagotrophic and pure phototrophic organisms in for example the oligotrophic oceanic waters. The effects of this advantage may be particularly significant in dense algal blooms and in periods of too high grazing pressure by other phagotrophs.

3.2.3 'True' facultative mixotrophs

The 'true' facultative mixotroph is the 'ideal' mixotroph that can grow equally well as a phototroph, utilising light as an energy source and inorganic nutrients as a source of materials for growth, and as a heterotroph, utilising organic molecules as a source for energy and materials for growth. The inorganic nutrients are its only source of P and N if growing phototrophically. And when it is growing heterotrophically the organic molecules are its only source for P and N. The same maximum growth rate can be achieved phototrophically, heterotrophically, or using a combination of these strategies.

One of the very few examples of a protists that can grow independently both phototrophically as heterotrophically, is the thecate dinoflagellate *Fragilidium subglobosum*, a mixotroph that apparently preys exclusively on species of another dinoflagellate genus, *Ceratium* (Skovgaard, 1996). Unusually for a thecate dinoflagellate, this species consumes its prey by direct engulfment, even though the prey organism may be up to 2 times its own size. The prey cell is transformed into a roundish food vacuole located in the centre of *Fragilidium subglobosum*. When feeding on the small *C. lineatum*, it ingests up to 13 cells prior to encystment and cell division. However, when feeding on the large *C. tripos*, it only ingests a single cell before it divides (Skovgaard, 1996, Skovgaard, 1998).

The maximum photosynthetic growth rate of *Fragilidium subglobosum*, 0.31 ± 0.02 d^{-1}, is fully comparable to volume specific growth rates of phototrophic dinoflagellates, just as the light intensity at which maximum growth is achieved, 130 μmolm^{-2}s^{-1}) is close to the mean values of 16 phototrophic dinoflagellate species given by Richardson et al. (1983)[3] (Skovgaard, 1996). *F. subglobosum* begins photosynthetic growth above 7 μmolm^{-2}s^{-1} and growth remains constant from $130 - 395$ μmolm^{-2}s^{-1}. Photoinhibition may occur with light-intensities higher than 400 μmolm^{-2}s^{-1} (Skovgaard, 1996). Also the heterotrophic capabilities of *Fragilidium subglobosum*, growth rate of 0.23 ± 0.03 d^{-1} and a ingestion rate of 4.2 ± 0.2 *C.lineatum*d^{-1} are comparable to heterotrophic dinoflagellates in general (Skovgaard, 1996). There is a relatively small difference between the growth rate for independent photosynthetic growth and the growth rate for independent phagotrophic growth. The difference between phagotrophic or phototrophic growth and

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[3] Richardson, Beardall, Raven, 1983, Adaptation of unicellular algae to irradiance: an analysis of strategies. New Phytol. 93:157-191

mixotrophic growth is much greater. *Fragilidium subglobosum* grows slower as a phototroph or a phagotroph than as a mixotroph. In food-satiated cultures, mixotrophic growth rates ranged from 0.31 ± 0.04 d^{-1} (with an ingestion rate of 2.1 ± 0.3 $C.lineatum \mathrm{d}^{-1}$) at 11 $\mu\mathrm{molm}^{-2}\mathrm{s}^{-1}$ to 0.47 ± 0.03 d^{-1} (with an ingestion rate of 5.8 ± 0.4 $C.lineatum \mathrm{d}^{-1}$) at 160 $\mu\mathrm{molm}^{-2}\mathrm{s}^{-1}$ (Skovgaard, 1996).

But many dinoflagellates have been observed to obtain both permanent algal endosymbionts and structures that resemble food vacuoles (Giacobbe, 1995). These algae seem to be able to feed effectively enough on other algal species to live independently heterotrophic or independently phototrophic, like *Fragilidium subglobosum*. So many of the dinoflagellate species might be ‘true’ mixotrophic organisms.

The organic molecule acetate is used by organisms at the surface of eutrophic ponds, like *Scenedesmus* species. The mixotrophic growth rate of this species is the sum of the rates of growth in phototrophy and in heterotrophy, so in this species the strategies are completely supplemental and it can grow on high acetate concentrations in light and dark (Combres et al., 1994). If it can survive by heterotrophic feeding as well as by phototrophic metabolism it will be called a ‘true’ mixotroph. Although *Scenedesmus sp.* is a typical species for very eutrophic environments it may not be ‘true’ facultative mixotrophic when food is not offered in high concentrations.

Chapter 4

Basics of feeding

An estimate of the costs of either strategy in a mixotroph should help in understanding the dynamics behind switching between these strategies in mixotrophic organisms. It will also give an idea as to how mixotrophic organisms are able to compete with strict phago-, and phototrophic organisms.

Phototrophy requires synthesis and maintenance of photosynthetic apparatus, that, in phototrophic algae constitutes for up to 50% of cell biomass. This apparatus is costly and will, in mixotrophic protists, take up to 50% of the energy, carbon, nitrogen, phosphorous and iron budget of the cell. The cost and maintenance of a feeding apparatus are estimated as 10% of all budgets. As a consequence, the maximum growth rates of heterotrophs are generally higher than that of strict phototrophs (**?**).

So it is to be expected that a mixotrophic organism has a much lower maximum growth rate than a strict phagotroph and a somewhat lower growth rate than the strict phototroph. This would suggest that a mixotroph is not able to compete with strict phagotrophs and phototrophs under not growth limiting conditions. When light and inorganic nutrients are highly available, but when food concentrations are low, the strict phototroph has an advantage over the mixotroph. Same as when food is abundant, but light intensity and nutrient concentrations are low, the strict phagotroph has an advantage over the mixotroph.

But under limiting conditions for food, light and dissolved inorganic nutrients, the mixotroph seems to have a great advantage over strict phagotrophic and phototrophic organisms of the same size. Use of both strategies occurs at limiting conditions, the switches from one strategy to the other are made under limiting conditions. At limiting conditions growth can even be enhanced as compared to favouring conditions for either strategy.

4.1 Usage of both strategies in a obligate mixotroph

For the obligate mixotroph there is no choice between strategies, it must photosynthesise to obtain its carbon and it must be phagotrophic to obtain nutrients, organic growth factors or merely the chloroplasts it needs to photosynthesise. Little is known about the dynamics of the relationship between the two strategies. The reason for the obligate relationship lies in the necessity of obtaining chloroplasts from prey to photosynthesise and light may be a necessity for maintaining heterotrophic activity or merely to obtain enough energy for maintenance and growth (Stoecker et al., 1987, Stoecker et al., 1988, Keller et al., 1994).

It can be assumed to rely on photosynthesis at high light intensity and low food concentration. When food concentration is high and light intensity is low, the organism will rely mainly on its heterotrophic capabilities. When no light or no food is available, the period for which it is able to survive will be short, relative to a facultative mixotroph.

4.2 Switching in a facultative mixotroph

The dynamics of feeding in a facultative mixotroph are less complicated then those in the obligate mixotroph. The organism that is facultative mixotrophic uses the strategy of which it is not obligately dependent just as a supplement of its main nutritional strategy. When the obligate phototrophic organism suffers from nutrient or light limitation it begins phagotrophy or osmotrophy, so there is a direct relationship between their obligate phototrophic mode and their heterotrophic feeding. The same accounts for many of the obligate heterotrophs. Most begin photosynthesis when prey gets limited. Some of the facultative mixotrophs use their facultative mixotrophic capabilities to supplement their main nutrition even when their main nutrition is not limited. This supplemental carbon nutrition serves as a great benefit in competition. Again, little is known about the dynamics of these relationships, but many investigators have started research to obtain data that will provide a better understanding of facultative mixotrophs.

4.2.1 Facultative mixotroph that is obligate phototrophic

The obligate phototrophic mixotrophs have been divided in the ones that start heterotrophic feeding when light is limiting, the ones that start feeding when an organic growth factor is limiting and the ones that feed when inorganic nutrients are limiting. Combinations of these may be possible and organisms that use phagotrophy or osmotrophy as a completely supplemental strategy to photosynthesis also exist (strategy 2, substrategy d, *Dinobryon divergens* and *Dinobryon sertularia* (Jones and Rees, 1994)). Less attention will be paid to this form because the dynamics and basics are more or less the same as for the obligate heterotrophic mixotroph that uses phototrophy in a supplemental way. The obligate phototrophic mixotroph seems to be most abundant in the region of marine and other waters where light- and nutrient-limiting conditions occur most. The algae that are best capable of heterotrophy can be found mainly at the edge of the euphotic zone, where light conditions are highly variable, and where the ability to temporarily grow under highly light limiting conditions can be of crucial importance to survival. In these boundary layers of water, the effect of temperature can also be significant. A response of phagotrophy to temperature has been shown in *Dinobryon sp.* for example (Jones and Rees, 1994).

Ochromonas sp., an oceanic phytoplankter, is capable of phagocitosis of bacteria. It starts using phagocitosis to obtain energy when light or inorganic nutrients become limiting, but it is still obligate phototrophic. This species is not able to sustain phagocitotic feeding when no light energy is available. After 24 hours of darkness, no feeding occurs anymore. This suggests that the species uses phagotrophy only for maintenance when light becomes limited and just for obtaining nutrients when inorganic nutrients are limiting. It cannot survive in the dark, nor does its chlorofyll level drop when prey is offered (Keller et al., 1994). In this case the ability to feed heterotrophically is of less importance to survival in dark periods, but more as a supplemental way of obtaining energy. In these cases it is often observed that light may even be a necessity for maintaining heterotrophic activity (Keller et al., 1994).

An example of a phytoflagellate that eats only in response to inorganic nutrient limitation but not in response to light limitation is *Prorocentrum minimum* (Stoecker et al., 1997)(strategy 2, substrategy a). Examples of phytoflagellates that eat in response to light limitation are much more common however. A very clear example (of strategy 2, substrategy c) being *Chrysochromulina brevifilum* (Jones et al., 1995), which reacts to rapid changes in light intensity by dropping or rising ingestion just as rapidly. And, as mentioned, *Uroglena americana* is an example of a phototrophic organism that feeds to obtain a trace organic growth factor (strategy 2, substrategy b).

4.2.2 Facultative mixotroph that is obligate heterotrophic

The obligate heterotrophic mixotrophs have been divided in the ones that start photosynthesis when prey is limiting and the ones that start photosynthesis to supplement their carbon nutrition (to be stronger in competition with other heterotrophs).

The rate of feeding for obligate heterotrophic mixotrophs is not affected by concentration of DIN nor by light intensity. The rate of feeding for these mixotrophs will only be regulated by prey concentration and by prey species. The percentage of cells of *Fragilidium cf. mexicanum* that contains a prey cell is significantly affected by the prey species. When different prey species are offered *F. cf. mexicanum* selects for certain species. The frequency of feeding that can be calculated from this percentage over a certain amount of time is not affected by prey concentration when the species of prey is not a ‘preferred’ one (Jeong et al., 1999). Rate of photosynthesis is also mainly regulated by the abundance of prey. No prey, no photosynthesis in cases where the organism sequesteres plastids from its prey.

These sequestered chloroplasts are not lost under starvation, though decreasing in number. And when light is offered, the ciliate can obtain energy to sustain at least part of its structural biomass by maintenance for short periods of time. Their high affinity for food and their potential to survive short periods of very low food abundance gives these organisms a great advantage over other marine phagotrophic organisms, which can be seen by the fact that many of the ciliates that are facultative mixotrophic heterotrophs are able to account for a large portion of the total phagotrophic biomass and total phagotrophic feeding.

The two substrategies in obligate heterotrophic mixotrophs differ only in their dependance on light and food. The organism that starts photosynthesis when food is limited is controlled only by food concentration and does not respond to light intensity. The rate of photosynthesis of one that feeds to supplement its carbon requirements is dependent on light intensity rather than on food concentration. Combinations in which an organism can supplement its energy budget when light intensity is high and use its photosynthesis in time of organic nutrient-stress are known. (Skovgaard, 1998, Jeong et al., 1999, Stoecker and M.W., 1990, Laval-Peuto and Rassoulzadegan, 1988)

More examples of dinoflagellate with klepto-chloroplasts are presented in Skovgaard, 1998. Mentioned here are *Amphidium poecilochroum*, *Pfiesteria piscicida* and *Gymnodium acidotum*) as organisms that facultative mixotrophic heterotrophs.

For mixotrophic ciliates the benefit from photosynthesis has been shown to be underestimated because of assimilation by the plastids of unlabelled respired ciliate CO_2 and because of preferential respiration of recently fixed carbon stored as polysacharide.

4.3 Switching in the 'true' facultative mixotroph

A 'true' facultative mixotroph can maintain itself exclusively by either photosynthesis or phagocitosis/carrier mediated transport of organic material. So photosynthesis and heterotrophic feeding of an ideal facultative mixotroph would be directly dependent on light intensity, concentration of dissolved inorganic nutrients and the presence of prey organisms or organic matter.

This dependency can express itself in two different ways. The mechanisms could be coupled, so feeding would decrease with the increase of light intensity and concentration of inorganic nutrients while feeding would increase with prey concentration while photosynthesis would decrease. The two mechanisms could also be completely supplementary. The organisms would photosynthesise as soon as inorganic nutrients and light would be available. Phagotrophy or osmotrophy would start directly in the presence of prey or organic nutrients respectively. In most organisms that exhibit 'true' mixotrophy a combination would be expected, where at a time of low light intensity a species would try to compensate by elevating ingestion rate and at time of low prey abundance by increasing chlorofyll levels or swimming upwards in the water column. At times of high abundance of prey and light, it may use both strategies at the same time, completely supplementary to eachother.

The best example that can be found presently of an organism that appears to be fully facultative mixotrophic is *Fragilidium subglobosum* (Stoecker, 1998). This species was mentioned before to be fully capable to grow exclusively phototrophic as well as phagotrophic. And above a light intensity of 63 μmolm^{-2}s^{-1}, *Fragilidium subglobosum* is able to increase its ingestion rate in response to decreasing light intensities (Figure 4.1), thereby acquiring more energy from phagotrophy when light energy decreases. Beneath 63 μmolm^{-2}s^{-1} the species has a relatively low ingestion rate. This may be caused by a increased efficiency of digestive system in conditions of sufficient light. When both light and food are present, *Fragilidium subglobosum* will prefer phagotrophy. The decrease in cellular chlorofyll α content in food-supplied cultures as compared with monocultures supports this suggestion. Another support is given by the growth enhancement relative to phototrophic growth in food-supplied cultures. The growth enhancement at the intermediate light intensities which are associated with maximum ingestion rates (63 to 107 μmolm^{-2}s^{-1}) is low relative to the growth enhancement at the low light intensities (0 to 10 μmolm^{-2}s^{-1}). Assuming a phagotrophic yield of 41%, photosynthesis must be depressed by the high phagotrophic activity. Assuming this phagotrophic yield the estimated phagotrophic percentage of total mixotrophic

Figure 4.1: Effect of light intensity on phototrophic growth rate of *Fragi lidium subglobosum* in monocultures (open rounds) and on mixotrophic growth rate (closed rounds) and per capita ingestion rate (closed triangles) of *F. subglobosum* in food-satiated cultures ($\sim$ 800 *Ceratium lineatum* ml^{-1}). Error bars indicate ± 1 SE, n=4.

Figure 4.2: Estimated percentage of *Fragilidium subglobosum* growth acquired through phagotrophy at various light intensities in food satiated cultures; phagotrophic yield of 41%, calculated on the basis of cultures growing in the dark, was applied to all light intensities

growth as a function of light intensity shows that *Fragilidium subglobosum* acquires between 34 and 100% of its energy need from phagotrophy in mixotrophic cultures. Highest dependence exists in darkness and the lowest dependence exists at the light-intensity where photosynthesis is at its maximum and therefor will be the main source of nutrition (Figure 4.2). Food uptake by *Fragilidium subglobosum* was more pronounced in phagotrophically or mixotrophically adapted cultures than in cultures which were not previously adapted to phagotrophy. This made clear that *Fragilidium subglobosum* needs 24 hours to adapt to phagotrophic metabolism. The reason for this delay may be the development of cell functions involved in food uptake and/or digestion. This may be necessary after a long period of growth under pure phototrophic conditions (Skovgaard, 1996).

It can be concluded that *Fragilidium subglobosum* uses both strategies in a supplementary way when it in intermediate nutrition (e.g. intermediate light intensity ±150 μmolm^{-2}s^{-1}, intermediate DIN concentration and intermediate prey density). The boundaries at which switches occur in *Fragilidium subglobosum* are not known, but are estimated as: Pure phagotrophy occurs at light intensities beneath 10 μmolm^{-2}s^{-1}, pure photosynthesis occurs at very low prey concentration at light intensities higher then 107 μmolm^{-2}s^{-1}. Particularly fast growth, compared to phototrophic and heterotrophic growth occurs at intermediate light intensities (63 to 107 μmolm^{-2}s^{-1}) at food saturation, where phagotrophy is stimulated and phototrophy is still significant.

Complex mechanisms are involved and much is unknown about the physiology of organisms that can switch between phototrophy and phagotrophy facultatively. But the strategies seem to interfere with one another for some reason. Only at intermediate light intensities and food concentration both strategies can supplement each-other and give the organism enhancement in growth. At high food concentration and low light intensity, the heterotrophic mechanism takes over and suppresses photosynthesis. At high light intensity and low food concentration, heterotrophic feeding should be suppressed, but in *Fragilidium subglobosum* phagotrophic digestion seems to be stimulated by light.

When the estimation of metabolic costs of both mechanisms is included, it seems strange that the growth rate of a 'true' facultative mixotroph like *Fragilidium subglobosum*, growing purely phototrophic, is so close to the growth rates of strict phototrophic organisms, and when growing purely heterotrophic, is so close to growth rates of strict heterotrophs. Especially the last is surprising, because the cost of having a photosynthetic apparatus is estimated at 50% of total energy and nutrient budgets.

Chapter 5

Conceptual dynamics of feeding

An attempt is made to give an approximation of the dynamics in mixotrophic feeding. Assumptions that have been made in Stoecker, 1998 about the dynamics of feeding in mixotrophs were together with the data presented in Skovgaard, 1996, Jones et al., 1993, Granéli et al., 1997, Jeong et al., 1999, Combres et al., 1994, Gervais, 1997, Vincent and Goldman, 1980 and Jones et al., 1995 translated in a number of graphs that give a qualitative image of the relationships between heterotrophy and autotrophy in mixotrophic feeding.

Every graph treats the response of a mixotrophic organism to changes in one parameter of its nutrition: concentration of food, concentration of DIN or light intensity. In every graph there are a few rather general features that return in all graphs. Those will be treated here.

Of each four subdivisions in mixotrophic organisms, one 'ideal' organism of that group is taken. This organism can perform each substrategy of photosynthesis and each of feeding that is discovered in the group of organisms that forms that subdivision. A substrategy being a different way of feeding or photosynthesising as pointed out in Table 2.1.

All the 'ideal' mixotrophic organisms are assumed to grow at their maximum rate under the given conditions. They can only grow at an other rate then maximum rate when concentrations are too low or when suppressed or stimulated by an other substrategy or mechanism. For example the true facultative mixotroph that will grow at a higher rate that the maximum for both strategies when it combines the strategies. Rate of feeding will be shown as the rate of growth that is reached, by the rate of feeding under the given conditions. In every graph only one parameter is changed, the other parameters will stay at the value that is sufficient for the organism to use the substrategy that is being explained. So in the case of response to food concentration, light intensity and concentration of DIN will be sufficient to grow at a maximum rate under solely photosynthetic conditions. When a concentration or light intensity reaches the magic zero it can naturally not be any less. Still the condition of the organism at concentration or intensity below zero is given to point out the possibility of survival in case there is no food, light or DIN available.

5.1 Usage of both strategies in the obligate mixotroph

An obligate mixotroph needs photosynthesis as well as heterotrophic metabolism to survive, so when either light or organic food runs out, the mixotroph will die. This aspect of the obligate mixotroph is shown in Figure 5.1 and Figure 5.3 by a cross. Another aspect of the obligate mixotroph is that the organism will not be capable to reach maximum rates for either strategy of feeding, photosynthesis or feeding. When the strategies are used simultaneously, they cannot give the organism a growth rate that is twice as high as organisms that have only one strategy. This was concluded from data on organisms that are assumed to be obligate mixotrophic (Stoecker et al., 1988, Keller et al., 1994). This in contrast to the facultative mixotroph that can have a enhanced growth rate as a mixotroph compared to it's growth rate as a phototroph or heterotroph. Somehow, when using both strategies, the rate of each is limited by the other and an equilibrium between the two is reached.

The response of an obligate mixotrophic organism to changes in light intensity are approximated in Figure 5.1. Here it is shown that the organism will die when there is no light, that the

Figure 5.1: Dynamics of feeding and photosynthesis of the Obligate Mixotroph in reaction to Light intensity. The Y-axis presents the growth-rate of the organism that is reached due to Photosynthesis/Feeding. The X-axis shows light intensity. The symbol † stands for death of the organism. Max stands for the rate of Photosynthesis/Feeding at which growth rate of the organism is at its maximum. The numbers of the different strategies in feeding and photosynthesis correspond with the numbers in the explaining text.

Figure 5.2: Dynamics of feeding and photosynthesis of the Obligate Mixotroph in reaction to concentration of Dissolved Organic Nutrients (DIN). The X-axis shows the concentration of DIN. The symbol † stands for death of the organism.

rate of feeding will only approach maximum rate of feeding, hence a rate of feeding that will make the organism grow at its maximum rate, when photosynthesis reaches its minimum. When photosynthesis becomes too low, the organism collapses and so feeding will stop. Photoinhibition is shown at the higher light intensities. From Figure 5.1 it can be concluded that the photosynthetic rate will increase to a maximum when light intensity increases. When light intensity becomes too high for the pigments in the organisms, the rate will decrease again. Feeding responds to light in the reversed way; it will decrease with the rising of the light intensity, because the rate of feeding is assumed to be inhibited by photosynthesis.

In Figure 5.2 the reaction of the obligate mixotroph to changes in concentration of Dissolved Organic Nutrients (DIN) are shown. There are two possible reactions of photosynthesis to changes in DIN: substrategy Photosynthesis (1) and Photosynthesis (2), both shown in Figure 5.2. In Photosynthesis (1) the photosynthetic rate decreases when DIN decrease, in Photosynthesis (2) it remains the same when it uses organic matter via the heterotrophic mechanism to create the inorganic nutrients it needs. For the response of feeding to DIN there are also two possibilities: Feeding (1) and Feeding (2). In Feeding (1) the rate of feeding will change in response to changes in rate of photosynthesis, thus it will rise to its maximum until the organism collapses, as photosynthesis decreases to its minimum. In Feeding (2) it will not respond to changes in DIN concentration, because the rate of photosynthesis stays the same as well and it feeding will not be suppressed nor stimulated.

Figure 5.3 shows the response of the organism to food concentration. Here the same kind of response can be seen as the response to light intensity. The rate of feeding increases with the food concentration to a maximum and the rate of photosynthesis decreases with the food concentration because of inhibition by the feeding process, making the total of feeding and photosynthesis the maximum growth rate at high food concentrations.

5.2 Switching in the facultative mixotroph

Switching in a facultative mixotroph is naturally more complicated than switching in an obligate mixotroph. Facultative mixotrophs have been divided into two subgroups, which will be discussed separately below.

Figure 5.3: Dynamics of feeding and photosynthesis of the Obligate Mixotroph in reaction to Food concentration. The X-axis shows the concentration of usable organic molecules. The symbol † stands for death of the organism.

Figure 5.4: Dynamics of feeding and photosynthesis of the Obligate Phototrophic Mixotroph in reaction to Light intensity. The X-axis shows the light intensity. The symbol † stands for death of the organism. The numbers of the different strategies in feeding and photosynthesis correspond with the numbers in the explaining text.

5.2.1 Facultative mixotroph that is obligate phototrophic

In Figure 5.4, Figure 5.5 and Figure 5.6 the concept of the dynamics of feeding and photosynthesis of an obligate phototrophic mixotroph are shown. In Figure 5.4 can be seen that there are two substrategies for photosynthesis and three substrategies for feeding when the factor light intensity is varied. The differences between these substrategies lies in the motivation to start feeding. An obligate photosynthetic organism can face a number of different limitations in its environment that it can overcome by being mixotrophic. Some mixotrophs that are obligate photosynthetic start feeding when DIN concentration becomes too low, some begin feeding when light intensity becomes low and some must always feed to obtain a trace organic growth factor. Some react to light limitation by increasing their photosynthesis at low light intensities.

Substrategy Feeding (1) in Figure 5.4 is feeding in case of low DIN concentration, this substrategy of feeding does not respond to low light intensities, but solely to low DIN. The feeding decreases when light intensity drops, because the organism is not able to perform feeding to obtain DIN when there is no energy available from the photosynthetic apparatus. The mechanism to obtain DIN when no DIN is available from the environment can be a great advantage over plain phototrophs. The line Feeding (2) in the figure represents the feeding that is started at low light intensities to survive in periods of relative darkness. This strategy poses a possibility of survival as can be seen in Figure 5.4, where the rate of Feeding (2) is still above zero when there is no light. This feeding is supplemental to photosynthesis. The third strategy of feeding for the obligate phototrophic mixotroph (Feeding (3)) is to feed for a trace organic growth factor that it can not make for itself and that is present in the environment. This saves the cost of making it, but it poses a danger when the growth factor is not present anymore in the environment. Rate of photosynthesis increases to a maximum with light intensity until photoinhibition slowly makes it decrease again (substrategies Photosynthesis 1-4). There are organisms that can enhance their photosynthesis under light limiting conditions, they are represented by the line Photosynthesis 2.

The reaction to changes in the concentration of DIN can also be predicted from the strategies that were shown in reaction to light intensity (Figure 5.5). The first strategy (Photosynthesis (1) and Feeding (1)) is to feed to obtain DIN, so organisms that have this strategy start feeding as soon as DIN are present and will increase with photosynthesis to a maximum that lies far below the maximum for photosynthesis. Another strategy of feeding is not influenced by the concentration of DIN, because it is a response to low light intensities and has the purpose to supplement carbon nutrition. Feeding (3) is also not influenced by DIN, this type of feeding will only be stopped

Figure 5.5: Dynamics of feeding and photosynthesis of the Obligate Photosynthetic Mixotroph in reaction to concentration of Dissolved Organic Nutrients (DIN). The X-axis shows the concentration of DIN.

Figure 5.6: Dynamics of feeding and photosynthesis of the Obligate Photosynthetic Mixotroph in reaction to Food concentration.

when the trace organic growth factor runs out.

Changes in food concentrations will result in the increase to a maximum of the rate of feeding when the concentrations increase. This accounts for all three types of feeding in obligate phototrophic organisms. See Figure 5.6. Only type (3) will reach its maximum much sooner, because the rate of feeding (3) has no need to get high and cannot due to the low concentrations of the trace organic growth factor. Photosynthesis is not very likely to be influenced by the food concentration, nor by the rate of feeding, because feeding is supplemental to photosynthesis or has no purpose in carbon metabolism, and will not influence it. Only in the case where the organism is feeding to get a growth factor. In this case the organism will die when the concentration of the growth factor gets too low, and thus photosynthesis will drop.

5.2.2 Facultative mixotroph that is obligate heterotrophic

In the case of the facultative mixotroph that is obligate heterotrophic the situation is reversed. The heterotrophic feeding rates are always higher than the photosynthetic rates, in contrast to the facultative mixotroph that is obligate phototrophic, where the photosynthetic rates are always higher (Figure 5.7). This difference is due to the difference in dependence upon one strategy or the other. Strategies within photosynthesis and heterotrophy are shown also in this figure by the numbers. Feeding (1) and Photosynthesis (1) represent the organism that photosynthesises to obtain carbon when prey or organic nutrients are limiting. Feeding (2) and Photosynthesis (2) represent an organism that feeds to supplement its carbon nutrition under light circumstances. Organisms that photosynthesise for these purposes do so mostly by obtaining plastids from algae or other microorganisms that have chloroplasts.

In heterotrophic mixotrophs, feeding is not dependent on light intensity as is shown in Figure 5.7. Light intensity therefor has no effect on the Feeding (1) and Feeding (2) strategies. It has an effect on the Feeding (3) strategy. Here feeding is stimulated by high light intensities and can reach a higher rate compared to its normal maximum. This strategy is both used in organisms that start photosynthesis in food limiting conditions as well as in organisms that use it supplemental to their carbon nutrition. The dependence of photosynthesis on light is also for both known strategies in this type of mixotrophs the same. An increase in light intensity is accompanied by an increase in photosynthesis to a maximum. The decrease at high light intensities again is caused by photoinhibition.

Feeding is also not influenced by the concentration of DIN in any strategy (Feeding (1-3)) of heterotrophic mixotrophy. Only the photosynthesis is influenced by this as can be seen in Figure 5.8. Photosynthesis (1) will decrease when the concentration of DIN decreases, because

Figure 5.7: Dynamics of feeding and photosynthesis of the Obligate Heterotrophic Mixotroph in reaction to Light intensity. On the X-axis the light intensity is shown. The numbers of feeding and photosynthesis strategies correspond with the numbers in the text

Figure 5.8: Dynamics of feeding and photosynthesis of the Obligate Heterotrophic Mixotroph in reaction to concentration of Dissolved Organic Nutrients (DIN). The X-axis shows the concentration of DIN.

photosynthesis will only be used in case of limited prey. When prey is limited, the DIN concentration in the environment will be significant due to the loss of the mechanism to make its own DIN out of food. In the second strategy, Photosynthesis (2), the organism feeds to supplement its carbon budget, so it will feed as soon as prey is present. Then the organism has sufficient DIN.

In Figure 5.9 it is shown that the organism dies when no food in the form of prey, organic molecules or particles of organic material, is available. Also can be seen that Feeding (1) and Feeding (2) react the same to food concentration. Photosynthesis (2) is dependent on the feeding of the organism due to its supplemental character and will therefor, like feeding, decrease when food concentration decreases. Photosynthesis (1) will be used to obtain carbon when food is limiting, so when the food concentration drops, Photosynthesis (1) will increase to a maximum that can be sustained, for a short period, in the absence of food.

5.3 Dynamics of feeding in the ‘true’ facultative mixotroph

A ‘true’ facultative mixotroph has more possibilities available to adapt to changing environments then any other organism. If all different substrategies that ‘true’ facultative organisms have, could be united into one organism, the ‘ideal’ organism would be born: An organism that can survive under almost any possible trophic circumstances.

In Figure 5.10 to 5.12, four different substrategies in feeding and three different substrategies of photosynthesis are shown for the ‘true’ facultative. The substrategy indicated by Feeding (1) is a substrategy in which feeding is suppressed by photosynthesis and by concentration of DIN in the environment. The basic function of Feeding (1) is to complement carbon metabolism in

Figure 5.9: Dynamics of feeding and photosynthesis of the Obligate Heterotrophic Mixotroph in reaction to Food concentration. The X-axis represents the concentration of food (molecules, particles or prey). The symbol † stands for death of the organism.

Figure 5.10: Dynamics of feeding and photosynthesis of the 'True' Facultative Mixotroph in reaction to Light intensity.

Figure 5.11: Dynamics of feeding and photosynthesis of the 'True' Facultative Mixotroph in reaction to concentration of Dissolved Inorganic Nutrients (DIN).

case of a low photosynthetic rate or to require DIN when environmental concentrations are low. Strategy Feeding (1) is controlled by rate of photosynthesis. This can also be seen in Figure 5.12 where Photosynthesis (1) is indifferent to food concentration. Photosynthesis (1) only reacts to light intensity and DIN concentration. Feeding (3) is a substrategy in which rate of feeding is controlled by food concentration, rather then by photosynthesis. In this case the metabolism of organism is controlled by feeding rather then by photosynthesis. Photosynthesis is suppressed by feeding when food concentrations are high. This can be seen in Figure 5.12, where Photosynthesis (3) decreases with food concentration.

Substrategy Feeding (2) is a strategy where feeding is extra stimulated by lower light intensities or low DIN concentration, compared to other mixotrophs, to have an extra advantage over most other organisms in zones where light, DIN and food are at intermediate intensity/concentrations. Feeding will continue without being suppressed in a wider range of concentrations of DIN and in a wider range of light intensities, giving the organism a higher growth rate in these intermediate concentrations, because photosynthesis is already active while feeding is not yet suppressed. Substrategy Photosynthesis (2) is like Feeding (2), but extra stimulated by lower food concentrations, when compared to other mixotrophs. Feeding (4) is extra stimulated by high light concentration: photoactivation. Assumed in this case is that the process of digestion is accelerated by light.

In Figure 5.10 the response to change in light concentration of the 'true' facultative is shown. Feeding (4) is a substrategy that can be combined with Feeding (1) and with Feeding (3). It is not seen combined with Feeding (2). The extra stimulation of digestion by high light intensities, compared to other 'true' mixotrophs can be seen by elevation of the line of Feeding (4) compared to Feeding (3) and Feeding (1). All photosynthetic strategies are the same in their reaction to light intensity: When light intensity increases, the rate of photosynthesis goes up. Feeding (3) is indifferent to light intensity and in substrategy Feeding (2), feeding is extra stimulated by low light intensities, as can be seen by the difference between the line of Feeding (1) and Feeding (2) in Figure 5.10. Photoinhibition is taken into account by the decrease of all photosynthetic substrategies at very high light intensities.

Figure 5.11 shows the response of the different substrategies in photosynthesis and feeding to changes in DIN concentration. When DIN concentration increases it shows that strategy Feeding (3) is indifferent to changes in DIN concentration and feeding rate in Feeding (1) will be suppressed, causes by the increase of photosynthetic rate. Feeding in Feeding (2) will be suppressed later by photosynthesis than in Feeding (1), giving the organism a higher growth rate in intermediate concentration ranges.

In Figure 5.12 the response of the 'true' facultative to changes in food concentration are shown. Feeding will increase with food concentration at all times. Photosynthesis will be suppressed by higher rates of feeding, in substrategy Photosynthesis (2) this will happen at a higher rate of

Figure 5.12: Dynamics of feeding and photosynthesis of the ‘True’ Facultative Mixotroph in reaction to Food concentration.

feeding then in Photosynthesis (3). Photosynthesis (1) is indifferent to food concentration.

Chapter 6

Models for mixotrophy

Information on the physiological ecology of mixotrophic protists is crucial to understanding their role in planktonic food webs and the impact of mixotrophy on trophodynamics and food web structure. Once mixotrophs are compartmentalised by functional type it may be possible to incorporate them into general food web models for aquatic ecosystems and thus to explore the impact of mixotrophy on ecosystem dynamics. Understanding ecophysiological aspects of mixotrophic organisms can help to understand ecosystems in whole.

Few models exist in which mixotrophs have been incorporated. Examples of existing models can be found in Taylor and Joint, 1990. Here steady state solutions are presented for a model of the surface mixed layer. This model incorporates phytoplankton, picophytoplankton, bacterioplankton, microzooplankton, dissolved organic carbon, detritus, nitrate and ammonia. The models allows for mixotrophy of the microzooplankton, but not for other forms of mixotrophic feeding. The model seems to agree with summer observations in some aquatic systems, but is only applicable to the top layers of the water. Mixing in these layers was investigated, but the lower layers can be of great importance as well for ecology in oceanic systems. Mixotrophs can be found in layers where no normal photo-autotroph can survive, and can be net producers at those depths.

Other models on mixotrophy try to deal with the phenomena itself. In Thingstad et al., 1996 a model is presented that gives an analysis of algal mixotrophy in a chemostat scenario. The mixotroph was assumed to be a ‘true’ facultative mixotroph. A two species situation, with a mixotroph and prey bacterium, and a three species situation, with a mixotroph, a prey bacterium and a ‘true’ phototroph/heterotroph, were analysed. In the two species situation the conclusion was that high affinity for prey resulted in a low quantitative importance of heterotrophy in nutrition of the mixotroph, due to inhibition of the growth of prey by predation. In the three species situation with phagotrophs it was concluded that the mixotroph could compensate for its low affinity for prey by a high affinity for DIN. When competing with a ‘true’ phototroph they found an optimum value for the mixotrophs phagotrophic ability at which mixotroph biomass is maximized. With mixotrophs, bacteria, phototrophs and heterotrophs present no equilibrium was predicted by this model. This model only deals with a few aspects in mixotrophy and just one of many strategies is analysed. The strategy that was analyzed is the most effective for modelling but in reality it is not very abundant. The strategy of phototrophs to use osmotrophy to supplement their energy and carbon budgets seems far more important for instance.

These models are in general too specific to be useful for creating a general model on the ecological relevance of mixotrophic organisms or a general model on a one-species community (a complete system in one organism). In Kooijman and Zonneveld, ???? a simple model is presented in which it is attempted to use a mixotrophic organism to create a basic system with predation, consumtion and degradation. The model is based upon the assumptions that a mixotroph consists and can be desribed by two generalised compounds: structural biomass and reserves. All processes in the organism can be described as conversions between structural biomass and reserves in dead or life form. This model is an analysis of a mixotroph that uses both strategies supplemental and simultaneously. An example of an organism that is almost fully facultative and uses both strategies almost completely supplemental to eachother is *Fragilidium subglobosum* (Skovgaard, 1996). This model may be of importance as a step between understanding mixotrophs and understanding ecosystems.

To model an ecosystem the ‘ideal’ mixotroph: the ‘true’ facultative mixotroph can be of great importance. Such a mixotrophic organism has the complete package of important metabolic groups and trophic levels: It is a producer and a predator, even a decomposer, united in one system with a fixed size and with measurable inputs and outputs.

6.0.1 A list of data on the ecophysiology of mixotrophs

Data on the uptake of organic nutrients (acetate) in algae that live near the boundary of the euphotic zone, like *Monoraphidium contortum* and *Friedmannia sp.*, are given in Vincent and Goldman, 1980. Data on growth in light and dark of 5 Cryptomonas species under added glucose mixotrophic conditions were presented in Gervais, 1997. Data on phototrophic, heterotrophic and mixotrophic growth (responses) of *Fragilidium subglobosum* were given in Skovgaard, 1996 and Skovgaard, 1998. The data presented on switching between phototrophic and mixotrophic and between phototrophic and phagotrophic growth can be very useful for the creation of a realistic explanatory model. Data on mixotrophic, autotrophic and heterotrophic growth in light and dark on acetate are presented in Combres et al., 1994. Specific attention is paid to the ammonium uptake as function of acetate concentration in the medium. Data on the heterotrophic capabilities and mortality in starvation of the mixotrophic dinoflagellate *Strombidium capitatum* are presented in Crawford and Stoecker, 1996. Data on the heterotrophic capabilities, prey selectivity, growth and grazing rates and the effect of light and nutrients on feeding of the dinoflagellate *Fragilidium* cf. *mexicanus* on other red-tide and toxic dinoflagellates is given in Jeong et al., 1999.

Chapter 7

Symbiosis and mixotrophy, the same thing?

In this study I have only briefly mentioned the many different multicellular lifeforms the are known to combine photosynthesis and heterotrophy within one organism. I had to restrict the subject to get some grip on the phenomena mixotrophy. The simplification made by excluding multicellular forms of symbiosis, resembling mixotrophy was made because the diversity of symbiotic forms is so great that it would not be possible to give a good categorisation within this study. Thereby, since the phenomena mixotrophy has just relatively recently been discovered as being of great importance to planktonic communities and to microbial life in general, not many researchers have tried to capture dynamics and mechanisms in multicellular lifeforms resembling mixotrophy in planktonic algae.

This was partly because these multicellular lifeforms are often not seen as mixotrophs but merely as the symbiosis of two different organisms and therefor they are not investigated the way unicellular mixotrophs are. Partly it is because relationships between multicellular organisms and algae are a lot more complicated and diverse and therefor a good understanding and categorisation takes much more research and time.

However, the diversity and abundance of symbiotic relationships between photosynthetic microorganisms and heterotrophic multicellular lifeforms is great and resembles in many ways the mixotrophy found within planktonic algae. So many forms of life use endosymbionts to have a mixotrophic lifestyle, is this the same as real mixotrophy within one cell? The way corals are able to harbor complete multispecies communities of algae within their structures and the way they use the materials and energy that they deliver, resembles in many ways the use of klepto-chloroplasts and the use of permanent endosymbionts in ciliates and dinoflagellates. Reef building corals use the algae they acquired through feeding, to supplement their carbon nutrition. They have an obligate, mutualistic symbiosis with phototrophic dinoflagellates (*Symbiodinium spp.*) (Rowan et al., 1997). This can be summarised as one organism that is capable of photosynthesis and heterotrophic feeding, much like the unicellular ciliate *Laboea strobila* that is dependant on photosynthesis for a large part of its carbon budget, and dependant on phagotrophy of algae for its capability to photosynthesise (its chloroplasts) and its inorganic (and organic) nutrients.

The sponges that were found by Cheshire et al., 1995 in the gulf waters of South Australia and on the protected sides of islands along the oceanic coast, that are living in association with red and brown macroalgae, are capable of compensating, or even producing through photosynthesis. The algae have been derived by ingestion and are used in the organism as permanent endosymbionts, capable of photosynthesis. The sponges get their inorganic nutrients and organic nutrients from their algal prey, but can use their endosymbionts to let photosynthesis take part in their carbon requirement.

Physiologically, off coarse, these symbiotic life-forms are not the same as mixotrophic unicellular organisms, but when looked upon as an entity the multicellular symbiotic relationship with algae resembles the mixotrophic relationship within a heterotrophic unicellular organism with permanent algal endosymbionts. Just as a parasitic plant like *Viscum album* resembles the osmotrophic algae. These resemblances support the endosymbiotic theory, which declares that the chloroplasts of the ‘true’ phototrophic organisms were evolved from endosymbiotic algae. And indeed, even the ‘true’ phototrophic organism bears resemblances with unicellular mixotrophs when it comes to chloroplasts and use of organic and inorganic nutrients. The only difference is that for the ‘true’ phototroph, it has never been proven that the uptake of organic matter from its environment can take an important part in its metabolism. Maybe this is still to be discovered.

Due to the many doubt about the boundaries between endosymbiosis and mixotrophy, it can best be seen as the same thing. When an organism with an algal or other photosynthetic endosymbiont is treated as a unit in an environment, instead of dividing the organism in a photosynthetic- and a heterotrophic unit, it can be seen as a mixotroph. Since it ingests prey and takes up light to have them both take an important, or equal part in their metabolism. There are even organisms (like sponges and flatworms) that can grow independently on photosynthesis as well as on heterotrophic feeding. They would be ‘true’ facultative mixotrophs. The fact that these organisms can use both strategies to survive gives the strong suggestion that all autotrophic organisms originated from mixotrophic heterotrophs. The endosymbiosis theory theorises that all chloroplasts in phototrophs are in fact endosymbionts that are extremely specialised and can no longer live outside of their host, while the host is dependant on their presence. The endosymbiotic theory and other arguments create a very thin line between endosymbiosis and mixotrophy. So it can very likely be assumed to be just the same thing in the end.

The implications of counting multicellular, symbiotic, organims with the mixotrophs are great. It will mean that there are just few organisms that are not in some way mixotrophic. Since there are just three strategies to obtain energy to sustain life, but so many different lifeforms, this is not hard to believe. It is just hard to prove.

Chapter 8

Conclusions

The ‘true’ facultative mixotroph seems to be very rare, the strategies that are very wide-spread are the ones that supplement one strategy by another one. The ability to completely replace a strategy with the other makes an organisms very versatile, but it may also be restraining to the speed of switching between strategies and the total maintenance and structural cost of the mechanistics necessary for both strategies. No estimations have been made about these costs and many different numbers are given for the amount of total weight or carbon that is being reserved for photosynthetic or heterotrophic apparatus.

Facultative mixotrophs that are either obligate phototrophic or heterotrophic seem to be most common. Especially because these are just supplemental strategies to sustain growth under limiting conditions or to enhance growth to have a better position in competition, these strategies form a great advantage for many planktonic organisms. Blooms are often caused by mixotrophic organisms with strategies like these.

A summary of strategies can be given by the following enumeration. Withing these strategies there are different substrategies that mostly deal with the cause and timing of switching between strategies. Table 2.1 gives a summary of the different strategies and substrategies in mixotrophic organisms.

1. Mixotrophy is obligate: Both light and particulate food is necessary for sustaining growth and maintenance. Probably due to the need of derived growth factors (Skovgaard, 1996, Keller et al., 1994)

2. Phototrophy is obligate but heterotrophy is facultative: Only photosynthesis is essential for growth and maintenance, heterotrophy can be used to backup the photosynthetic apparatus in times of low light intensity. Probably a very great advantage in competition for light-energy (Combres et al., 1994).

3. Heterotrophy is obligate but phototrophy is facultative: Only food is necessary for sustaining growth and maintenance, but photosynthesis can be used to backup heterotrophy in times of low food concentrations. Probably due to need of constant replacement of chloroplasts by chloroplasts from prey (Skovgaard, 1998).

4. Mixotrophy is facultative: Ability to grow exclusively by either photosynthesis or phagotrophy/uptake of organic compounds (Skovgaard, 1996).

Worldwide, mixotrophy is very widespread in the top layer of oceanic water (up to 90% of organisms can be mixotrophic) and in the layer beneath mixotrophs would account for the greatest part of the organisms present there. It is a phenomena that has been very important in evolution and is very important for the continuing of life in the oceans, with their rapidly changing environmental conditions and highly variable food and anorganic nutrients concentrations and light intensities. Symbiosis between a heterotrophic multicellular organism and phototrophic unicellulars can also be seen as a form of mixotrophy. When these forms are also taken into account, mixotrophy may well be the most abundant strategy in oceanic lifeforms.

To model an ecosystem the ‘ideal’ mixotroph: the ‘true’ facultative mixotroph can be of great importance. Such a mixotrophic organism has the complete package of important metabolic groups and trophic levels: It is a producer and a predator, even a decomposer, united in one system with a fixed size and with measurable inputs and outputs.

Bibliography

Berland B., Maestrini S., Grzebyk D. and Thomas P. Recent aspects of nutrition in the dinoflagellate *Dinophysis cf. acuminata. Aquatic Microbial Ecology*, 9(2):191–198, 1995.

Bird D. and Kalff J. Algal phagotrophy: Regulating factors and importance relative to photosynthesis in *Dinobryon* (chrysophyceae). *Limnology and Oceanography*, 32(2):277–284, 1987.

Bockstahler K.R. and Coats D.W. Spatial and temporal aspects of mixotrophy in chesapeake bay dinoflagellates. *J. Euk. Microbiol.*, 40(1):49–60, 1993.

Bouvier T., Becquevort S. and Lancelot C. Biomass and feeding activity of phagotrophic mixotrophs in the northwestern black sea during the summer 1995. *Hydrobiologia*, 363(0):289–301, 1997.

Cheshire A., Butler A., Westphalen G., Rowland B. and Stevenson J. Preliminary study of the distribution and photophysiology of the temperate phototrophic sponge *Cymbastela sp.* from south australia. *Marine and Freshwater Research*, 46(8):1211–1216, 1995.

Combres C., Laliberté G., Reyssac J. and Noüe J.d.l. Effect of acetate on growth and ammonium uptake in the microalga *Scenedesmus obliquus. Physiologia plantarum*, 91:729–734, 1994.

Crawford D. and Stoecker D. Carbon content, dark respiration and mortality of the mixotrophic planktonic ciliate *Strombidium capitatum. Marine Biology*, 126:415–422, 1996.

Gervais F. Light-dependent growth, dark survival, and glucose uptake by cryptophytes isolated from a freshwater chemocline. *Journal of Phycology*, 33:18–25, 1997.

Giacobbe M. Morphological observations on *Dinophysis* species (dinophyceae) from mediteranean coastal waters. *Crypyogamie, Algologie*, 16(4):233–245, 1995.

Gowing M. and Garrison D. Abundance and feeding ecology of larger protozooplankton in the ico edge zone of the weddel and scotia seas during the austral winter. *Deep-Sea Research*, 39(5):893–919, 1992.

Granéli E., Anderson D., Carlsson P. and Maestrini S. Light and dark carbon uptake by *Dinophysis* species in comparison to other photosynthetic and heterotrophic dinoflagellates. *Aquatic Microbial Ecology*, 13(2):177–186, 1997.

Hansen P. and Nielsen T. Mixotrophic feeding of *Fragilidium subglobosum* (dinophyceae) on three species of *Ceratium*: effects of prey concentration, prey species and light intensity. *Marine Ecology Progress Series*, 147:187–196, 1997.

Jacobson D. and Andersen R. The discovery of mixotrophy in photosynthetic species of *Dinophysis* (dinophyceae): light and electron microscopial observations of food vacuoles in *Dinophysis acuminata*, *D. norvegica* and two heterotrophic dinophysoid dinoflagellates. *Phycologia*, 33(2):97–110, 1994.

Jeong H., Shim J., Kim J., J.Y. P., Lee C. and Lee Y. Feeding by the mixotrophic thecate dinoflagellate *Fragilidium* cf. *mexicanum* on red-tide and toxic dinoflagellates. *Marine Ecology Progress Series*, 176:263–277, 1999.

Jones H., Durjun P., Leadbeater B. and Green J. The relationship between photoacclimation and phagotrophy with respect to chlorophyll a, carbon and nitrogen content, and cell size of *Chrysochromulina brevifilum* (prymnesiophyceae). *Phycologia*, 34(2):128–134, 1995.

Jones H., Leadbeater B. and Green J. Mixotrophy in marine species of *Chrysochromulina* (prymnesiophyceae): ingestion and digestion of a small green flagellate. *Journal of Marine Biology*, 73:283–296, 1993.

Jones R. and Rees S. Influence of temperature and light on particle ingestion by the freshwater phytoflagellate *Dinobryon*. *Arch. Hydrobiol.*, 132(2):203–211, 1994.

Keller M., Shapiro L., Haugen E., Cucci T., Sherr E. and Sherr B. Phagotrophy of fluorescently labelled bacteria by an oceanic phytoplancter. *Microbial Ecology*, 28:39–52, 1994.

Kooijman S. and Zonneveld C. Light-induced mass turnover in a mono-species community of mixotrophs. *in press*, ????

Laval-Peuto M. and Rassoulzadegan F. Autofluorescens of marine planktonic oligotrichina and other ciliates. *Hydrobiologia*, 159:99–110, 1988.

Laybourn-Parry J., Rogerson A. and Crawford D. Temporal patterns of protozooplankton abundance in the clyde and loch striven. *Estuarine, Coastal and Shelf Science*, 35(5):533–543, 1992.

Legrand C. and Carlsson P. Uptake of high molecular weight dextran by the dinoflagellate *Alexandrium catanella*. *Aquatic Microbial Ecology*, 16:81–86, 1998.

Legrand C., Graneli E. and Carlsson P. Induced phagotrophy in the photosynthetic dinoflagellate *Heterocapsa*. *Aquatic Microbial Ecology*, 15(1):65–75, 1998.

Liessens J., Vanbrabant J., De Vos P., Kersters K. and Verstraete W. Mixed culture hydrogenotrophic nitrate reduction in drinking water. *Microbial Ecology*, 24:271–290, 1992.

Padden A.N., Kelly D.P. and Wood A. Chemolithoautotrophy and mixotrophy in the thiophene-2-carboxylic acid-utilizing *Xanthobacter tagetidis*. *Archive of Microbiology*, 169:249–256, 1998.

Palenik B. and Henson S. The use of amides and other organic nitrogen sources by the phytoplankton *Emiliania huxleyi*. *Limnology and Oceanography*, 42(7):1544–1551, 1997.

Riegman R. On the ecology of the mixotrophic harmful algal species *Chrysochromulina polylepis*. *Nederlands Instituut voor Onderzoek der Zee (NIOZ)*, 1995.

Rowan R., Knowlton N., Baker A. and Jara J. Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature (London)*, 388(6639):265–269, 1997.

Sanders R. Seasonal distributions of the photosynthesizing ciliates *Laboea strobila* and *Myrionecta rubra* (=*Mesodinium rubrum*) in an estuary of the gulf of maine. *Aquatic Microbial Ecology*, 9(3):237–242, 1995.

Schneegurt M., Sherman D. and Sherman L. Growth, physiology, and ultrastructure of a diazotrophic cyanobacterium, cyanothece sp. strain atcc 51142, in mixotrophic and chemoheterotrophic cultures. *Journal of Phycology*, 33:632–642, 1997.

Skovgaard A. Mixotrophy in *Fragilidium subglobosum* (dinophyceae): growth and grazing responses as functions of light intensity. *Marine Ecology Progress Series*, 143:247–253, 1996.

Skovgaard A. Role of chloroplast retention in a marine dinoflagellate. *Aquatic Microbial Ecology*, 15:293–301, 1998.

Stoecker D. Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. *Europ. J. Protistol.*, 34:281–290, 1998.

Stoecker D., Li A., Coats W., Gustafson D. and Nannen M. Mixotrophy in the dinoflagellate *Prorocentrum minimum*. *Marine Ecology Progress Series*, 152:1–12, 1997.

Stoecker D., Michaels A. and Davis L. Large proportion of marine plaktonic ciliates found to contain functional chloroplasts. *Nature*, 326(23):790–792, 1987.

Stoecker D. and M.W. S. Replacement and aging of chloroplasts in *Strombidium capitatum* (ciliophora: Oligaotrichida). *Marine Biology*, 107((3)):491–502, 1990.

Stoecker D., Swanberg N. and Tyler S. Oceanic mixotrophic flatworms. *Marine Ecology Progress Series*, 58:41–51, 1989.

Stoecker D.K., Silver M., Michaels A. and Davis L. Obligate mixotrophy in *Laboea strobila*, a ciliate which retains chloroplasts. *Marine Biology*, 99:415–423, 1988.

Taylor A. and Joint I. A steady-state analysis of the 'microbial loop' in stratified systems. *Marine Ecology Progress Series*, 59:1–17, 1990.

Thingstad T., Havskum H., Garde K. and Riemann B. On the strategy of "eating your competitor": a mathemmatical analysis of algal mixotrophy. *Ecology*, 77(7):2108–2118, 1996.

Tillmann U. Phagotrophy by a plastidic haptophyte, *Prymnesium patelliferum. Aquatic Microbial Ecology*, 14:155–160, 1998.

Vincent W. and Goldman C. Evidence for algal heterotrophy in lake tahoe, california-nevada. *Limnology and Oceanography*, 25(1):89–99, 1980.

Wetzel R. Modeling the microbial loop: An estuarine modeler's perspective. *Microb. Ecol.*, 28:331–334, 1994.