Phytoplankton

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Phytoplankton is defined as free-floating unicells and colonies that grow photoautotrophically in aquatic environments. Phytoplankton plays a key role in the primary production and global elemental cycles of the Earth.

Introduction

Phytoplankton is composed of both eukaryotic and prokaryotic species. It colonizes the upper part of the water column, down to the limit of penetration of light. The structure and abundance of the phytoplankton populations are mainly controlled by inorganic nutrients such as nitrogen, phosphorus, silica and iron. Phytoplankton usually undergoes a fairly predictable annual cycle, but some species may develop explosively and form blooms. On smaller time scales, phytoplankton growth and division is tightly linked to the diel cycle. Phytoplankton is present in both fresh and marine waters, but here we focus on marine phytoplankton.

Methods of Study

For over a century light microscopy was the basic tool to observe phytoplankton. It is still invaluable to determine species composition. Since the late 1960s, both transmission and scanning electron microscopes have proved extremely useful in establishing accurate phytoplankton taxonomy using ultrastructural features such as ornamentation of body scales or architecture of flagellar roots. Later, fluorescence techniques such as epifluorescence microscopy and flow cytometry played a key role in the discovery of picoplankton (Waterbury *et al.*, 1979; Chisholm *et al.*, 1988). Flow cytometry, initially borrowed from the biomedical field, has become the method of choice to estimate cell abundance in the field, since it permits the counting and classification of several thousand cells per minute.

Chlorophyll is a key diagnostic marker of phytoplankton. The advent of rapid spectrophotometric and fluorometric methods in the 1960s made it possible to map phytoplankton distribution in the ocean. These methods are now superseded by optical measurements of *in vivo* fluorescence that allow real-time continuous monitoring of phytoplankton distribution. Pigment discrimination and quantification by high precision liquid chromatography (HPLC) directly from seawater samples provide estimates of the oceanic distribution of specific algal classes, based on diagnostic carotenoids such as zeaxanthin (specific for



cyanobacteria) and prasinoxanthin (specific for Prasinophyceae). Optical properties of chlorophyll are important for remote sensing from space. The initial coastal zone colour scanner (CZCS) has made it possible to establish the key features of phytoplankton distribution throughout the world oceans, revealing the true extent of features such as coastal upwellings or equatorial enrichment (Figure 1a). More recent sensors such as SeaWifs can provide chlorophyll ocean images over a specific region in near real time, allowing scientists to monitor phytoplankton dynamics (Figure 1b).

Although determining the composition and abundance of phytoplankton is possible with little bias, accurately measuring physiological rates in the field is much more difficult. Most of the methods used until recently relied on enclosing natural communities in bottles and following changes in key parameters over time (or more simply measuring endpoint values). Carbon-14 uptake introduced in 1951 by Steeman Nielsen (Steeman Nielsen, 1951) has been by far the most popular method to measure photosynthesis in the sea. Uptake rates for major nutrients, in particular nitrogen, are measured similarly by isotope incorporation methods. Phytoplankton mortality due to grazing can be assessed by the 'dilution' method (Landry and Hassett, 1982). In many respects, such incubation methods are prone to artefacts and uncertainties: for example, following bottle-enclosure the cells are maintained at a constant depth, corresponding to a constant light level; however, in the field, mixing processes constantly vary the light that cells experience; in bottles, nutrients are quickly depleted in the absence of diffusion etc. Therefore, oceanographers have tried to develop instantaneous measurements of physiological processes. For example, photosynthetic capacity can be estimated by measuring the dynamic response of chlorophyll fluorescence (Kolber and Falkowski, 1993) or the field population division rate can be assessed from changes in the proportion of cells in the different cell cycle phases throughout the day (Carpenter and Chang, 1988). To eliminate bottle artefacts, oceanographers have also performed 'mesocosm' experiments in which several cubic metres of seawater are enclosed in bags and manipulated, for example by adding nutrient or screening out ultraviolet



0.1 0.2 0.4 0.6 0.8 1 10

Phytoplankton pigment concentration (mg/m³)



Figure 1 Phytoplankton distribution as seen from satellites. (a) Global map of chlorophyll concentration obtained by the coastal zone colour scanner (CSZS) satellite. (b) Coccolithophorids bloom (arrows) in the English Channel as seen by the SeaWifs satellite. Both images are available from the NASA website: http://seawifs.gsfc.nasa.gov/scripts/SEAWIFS.html.

(UV) radiation. However, for logistical reasons, this approach is only feasible in coastal waters. In oceanic waters, the famous 'IronEx' experiment (Martin *et al.*, 1994) has opened a new area of whole ecosystem fertilization manipulations.

In the coming years, one may predict widespread use of molecular biology techniques both to characterize the composition of communities (Moon-van der Staay *et al.*, 2001) and to determine their physiological status, for example to assess which nutrient is limiting for a specific group (La Roche *et al.*, 1996).

Phytoplankton Diversity

Phytoplankton can be separated on the basis of cell size into micro-phytoplankton $(200-20 \,\mu\text{m})$, nano- $(20-2 \,\mu\text{m})$ and picophytoplankton $(2-0.2 \,\mu\text{m})$. Although the first two size classes are easily identified with long-established techniques such as optical microscopy, the last one can only be detected by fluorescence techniques (see above). Therefore, the existence and significance of picoplankton was only established in the late 1970s, when such techniques became widespread.

More than 4000 species and 500 genera of marine phytoplankton have been described, a rather small number in comparison to freshwater taxa, which total around 15 000 species (Sournia *et al.*, 1991). This number may be a gross underestimate since smaller eukaryotic forms remain little studied to date. However, many species and genera have ubiquitous distributions over the world oceans and therefore diversification of marine phytoplankton may be limited.

Diatoms (Bacillariophyceae) constitute one of the best known groups of phytoplankton. Cells are encased within a rigid cell wall made of silica, called the frustule. The frustule displays very intricate ornamentation that permits species identification. Both pennate and centric diatoms are present in marine waters. Some species such as *Skeletonema costatum* (Figure 2a) occur very frequently at high abundance in coastal areas. Others are more typical of the open ocean such as the giant diatom *Ethmodiscus rex*. Few diatom species are toxic, with the noticeable exception of those belonging to the genus.

Dinoflagellates (Dinophyceae) possess two perpendicular flagella and their body can be either naked or covered with cellulosic plates. Under specific hydrological conditions, such as after a prolonged period of calm weather, they have the capacity to form localized blooms, which can colour the water ('red tides'). Several species harbour toxins that are transferred up the food chain to shellfish and then to humans, causing diarrhetic shellfish poisoning (DSP; caused by *Dinophysis* species) or parasitic shellfish poisoning (PSP; caused by *Alexandrium*; Figure 2b).

Another major group of eukaryotic marine phytoplankton is constituted by the Prymnesiophyceae, characterized by two flagella and a thin filamentous appendage, called the haptonema. In contrast to dinoflagellates and diatoms, which are common in freshwaters, this class is mostly restricted to marine waters. Cells are covered by organic scales that can be calcified (coccoliths). A few species of Prymnesiophyceae are ubiquitous. This is the case with the coccolithophorid *Emiliania huxleyi* (Figure 2c), the blooms of which can be seen by satellite (Figure 1b) from the reflectance of coccoliths released by individual cells (Holligan *et al.*, 1983). Another genus, *Phaeocystis*, produces colonies whose polysaccharide matrix induces foam on the beaches of the North Sea (Lancelot *et al.*,



Figure 2 A few phytoplankton species. (a) The diatom *Skeletonema costatum* under bright field (left) and epifluorescence microscopy (right), revealing the red fluorescence of chlorophyll. (b) The dinoflagellate *Alexandrium tamarense*. (c) The prymnesiophyte *Emiliania huxleyi*. The picoplanktonic cyanobacteria *Synechococcus* (d) and *Prochlorococcus* (e). Scale bars: (a, b) 10 μm, (c) 1 μm (d, e) 0.5 μm. Panels (c) reproduced from Riebesell *et al.* (2000) *Nature* **407**: 364–367, (d)–(e) from Chisholm *et al.* (1988) *Nature* **334**: 340–343.

1987). Toxic species, such as *Chrysochromulina polylepis*, may also bloom sporadically, as was the case in 1988 off Norway.

The smaller-sized members of the eukaryotic phytoplankton are often difficult to classify because of the lack of distinct morphological features. Species diversity is apparently much more limited than for other classes, such as diatoms or dinoflagellates, since fewer than 40 picoplanktonic species have been described so far. However, this size range is a reservoir of novel groups and since the discovery of picoplankton species new classes such as the Pelagophyceae or the Bolidophyceae have been established. Among green algae, most picoplanktonic species belong to the Prasinophyceae, a primitive and probably polyphyletic group. Many groups are not represented in culture collections (such as the Center for Culture of Marine Phytoplankton, Bigelow, Maine [http://ccmp.bigelow.org]) and the development of novel culture media and isolation procedures constitute a high research priority.

Considered as insignificant in oceanic systems 20 years ago, cyanobacteria are now recognized as the most ubiquitous group of the marine phytoplankton. The small coccoid genus *Synechococcus* (Figure 2d) is present in all marine systems from the tropics to the poles, from eutrophic to oligotrophic waters, while the atypical genus *Prochlorococcus* (Figure 2e), which uses a modified chlorophyll *b* as its main light-collecting pigment instead of phycoerythrin, is probably the most numerous photosynthetic organism on Earth (Partensky *et al.*, 1999), colonizing at high densities $(10^5 \text{ cells mL}^{-1})$ the entire euphotic zone between 40°N and 40°S. *Trichodesmium* is a colonial filamentous species that fixes nitrogen and it can form extensive blooms in vast areas of the tropical oceans (Carpenter and Romans, 1991).

Still some of the players might be unknown. For example, evidence for the existence of photosynthetic aerobic bacteria has been very recently gathered in the Pacific Ocean (Kolber *et al.*, 2000).

Major Controlling Factors in the Ocean

For its growth, phytoplankton relies on two key environmental factors that can become limiting under natural conditions: light and nutrients. The latter include mainly carbon, nitrogen, phosphorus, silica, trace metals, such as iron, and a few vitamins. In healthy algae, carbon, nitrogen and phosphorus follow more or less closely a stocchiometric relationship of 106 : 16 : 1 (by atoms), the Redfield ratio. When the N:P ratio of the seawater departs from the 16:1 value, then either N or P becomes limiting. Temperature is more important for selecting species than for controlling biomass. Finally, physical processes, in particular vertical mixing (for which the wind is instrumental), play a major role as they determine the spatial distribution of these resources.

Light

Light decreases exponentially with depth at a rate that depends on the particle content of the water. The euphotic zone, a key concept in biological oceanography, is defined as the depth reached by 1% of the surface irradiance (Figure 3a). It ranges from a few metres in coastal and estuarine waters to one hundred metres in the eastern Mediterranean Sea or the Pacific gyre. Traditionally, this depth was considered as the lower limit beyond which phytoplankton could not photosynthesize. However, some picoplankton such as *Prochlorococcus* seem to be able to photosynthesize at lower levels down to 0.1% of the surface irradiance (Goericke *et al.*, 2000). As light decreases with depth, its spectral range also narrows in



(b)

Figure 3 (a) Schematic vertical distribution of phytoplankton in the open ocean. (b) Some of the key phytoplankton types and some of the factors involved in their control. No attempt has been made to link a given type of phytoplankton to a given factor as many of the relationships are still not clear.

the blue region, as this wavelength is the least attenuated. Therefore, phytoplankton cells acclimate to depth both by increasing their pigment content and by shifting their pigment composition (for example, *Prochlorococcus* increases its chlorophyll b/a ratio since chlorophyll b absorbs optimally blue wavelengths).

At high irradiances (typically in the top 20 m in clear oceanic waters), phytoplankton photosynthesis becomes photoinhibited, in particular because of damage to proteins of the photosystem core by UV wavelengths. Cells counteract these detrimental effects by increasing the amount of photoprotective pigments such as zeaxanthin or diatoxanthin.

Under nutrient-replete conditions, the vertical extent of the phytoplankton layer is dictated by the relative limits of the surface mixed layer delimited by the pycnocline and the euphotic zone. If the former is much deeper than the latter, then algae are unable to grow because they are constantly mixed down to light levels where they cannot grow. In temperate latitudes, it is often the shallowing of the pycnocline, due to the warming effect of increasing irradiance, that triggers the spring bloom (Sverdrup, 1953).

Another complicating factor is the diel cycling of light as well as its modulation over the course of the day. Although recognized for over a century, the importance of light:dark alternation has only recently been taken into account. In particular, it leads to a synchronization of the growth and division of phytoplankton populations (Smayda, 1975). Cells only begin to grow after sunrise and divide in general around dusk. Such phasing is clearly observed in phytoplankton-related optical parameters such as scattering and chlorophyll fluorescence and can then be used to estimate phytoplankton growth rates (Siegel *et al.*, 1989). Moreover this phasing probably propagates throughout the food web to other groups such as bacteria, although this has proved difficult to demonstrate.

Nutrients

Nutrient limitation is much less straightforward than light limitation because of the multiple interactions between different nutrients, these interactions being different depending on the scale that is being considered. At the single cell level, the limiting nutrient is *a priori* easily identifiable as the one which, if added, will induce an increase in growth rate. However, at the community level, things become already more complex because different species may be limited by different nutrients (e.g. diatoms by silica and other phytoplankton by nitrogen). If one considers longer time scales, nutrient depletion often induces shift in community structure (e.g. from diatomdominated systems to picoplankton), favouring species that are more efficient at scavenging the limiting nutrient or do not require it.

Until recently, nitrogen was assumed to be the universal limiting nutrient in marine waters. Throughout temperate latitudes, winter mixing brings up nitrogen from the deep ocean mainly as nitrate. In the spring, as the water stratifies and as phytoplankton grows, it depletes the surface layer of nitrate, which drops to nanomolar levels. Nitrogen is recycled mainly as ammonia and nitrate is only supplied by diffusion through the thermocline. In the early phase of stratification, chlorophyll concentration is uniform throughout the mixed layer, and then a maximum develops at depth near the nitracline (Figure 3a). This simple model leads to the key concept of new and regenerated production, the former being based on nitrate and the latter on ammonia (Dugdale and Goering, 1967). The so-called fratio is defined as the ratio of new to total production increases from oligotrophic to eutrophic areas. When f is high, the community is dominated by diatoms, and when f is low by nano-or picoplankton.

In the last decade, a much more complex picture has emerged. One key issue that helped change our whole view of oceanic pelagic ecosystems has been that of the high nutrient low chlorophyll (HNLC) regions, such as the equatorial or the northeast subarctic (see Figure 1a). In these regions, despite the presence of micromolar levels of nitrate, chlorophyll concentration remains low. To account for this anomaly, two hypotheses were put forward. One possibility was that phytoplankton biomass was kept under control by a very efficient grazer community. Alternatively, the late J. H. Martin proposed that iron, which is supplied primarily by atmospheric dust, becomes limiting in areas far away from the continents. The latter theory was very dramatically proved in the equatorial Pacific during a whole ecosystem iron fertilization experiment (Martin et al., 1994) as well as in Antarctic waters more recently (Boyd et al., 2000). Phosphorus also now appears as a key limiting nutrient in some areas such as the eastern Mediterranean Sea (Krom et al., 1991) or even more significantly in the Atlantic and Pacific gyres (Karl, 1999). In the latter regions, new nitrogen appears to be provided by nitrogen fixation, a phenomenon completely underestimated previously (Carpenter and Romans, 1991). The picture becomes even more complex as nitrogenase, the key enzyme involved in di-nitrogen fixation, requires iron as a cofactor. Silica can be limiting for diatom growth in coastal waters, especially off large rivers such as the Mississippi. The possibility for carbon dioxide to become limiting in the ocean has been raised (Riebesell et al., 1993) but the question is still a matter of debate, and could be restricted to specific groups, such as diatoms.

Food Web Structure and Dynamics

In the classic view of marine food webs (Steele, 1974), phytoplankton (diatoms) lies at the base of the pyramid

leading to fish and whales, through the mediation of crustacean herbivores (copepods). The discovery of picoplankton and of the importance of bacteria in the ocean led to the alternative concept of the 'microbial loop' (Azam *et al.*, 1983). In this situation, primary production is mainly achieved by very small picoplanktonic species that undergo predation by 'microzooplankton' (typically nanoflagellates and ciliates). Dead cells release organic matter that is quickly recycled by bacteria, providing nutrients to fuel phytoplankton growth again. It is now clear that these two types of oceanic food webs are not antagonistic but correspond to different situations. While the former is typical of the spring bloom situation, the latter ('microbial loop') is extremely widespread as it dominates in most oligotrophic and mesotrophic situations, such as those prevailing throughout the year in the large oceanic gyres (Figure 1a), but also in temperate areas, in winter or summer, i.e. outside of bloom periods.

Diatom-based food webs are fairly unstable and their dynamics resemble that of a batch culture. Cell abundance and chlorophyll increase rapidly and then drop back, once zooplankton has developed enough to graze efficiently. Such a food web structure leads to rapid export of photosynthetic carbon to the deep ocean because considerable losses occur at each step, for example through sedimentation of copepod faecal pellets. Despite our reasonable understanding of diatom-based food webs, novel processes that may play a role in their regulation have been discovered recently: for example, diatoms seem to be able to inhibit egg development in their main predator, copepods (Miralto *et al.*, 1999).

Microbial food webs function much like chemostat cultures and are more stable than the diatom-based food webs. For example, in oligotrophic gyres, the abundance of typical picoplankton species, such as Prochlorococcus, varies within a 2-fold range throughout the year. Since cells divide up to once a day, this stability is most likely due to very tight control by microzooplankton. The latter have very short generation times (even shorter than their prey, in contrast to copepods, which have generation times an order of magnitude longer than that of their prey), and can adapt very quickly to any change in phytoplankton biomass. Many of the processes at play in microbial food webs remain to be uncovered. Mixotrophy, common among dinoflagellates and prymnesiophytes, kleptoplastidy as achieved by the ciliate Mesodinium rubrum, symbiosis such as that occurring between nitrogen-fixing cyanobacteria (Richelia) and diatoms (Rhizosolenia), and parasitism, as between chytrids and diatoms or *Parvilucifera* and dinoflagellates, are probably much more prevalent than presently assumed. Finally, viruses are now known to be ubiquitous in marine waters and appear to be as important as top-down predators in controlling marine phytoplankton populations (Fuhrman, 1999).

Under specific favourable circumstances, a given species may develop very quickly and form very dense blooms.

This is the case with some prymnesiophytes and dinoflagellates. Prymnesiophyte blooms of E. huxleyi or Phaeocystis occur over vast areas in the North Atlantic (Figure 1b), as well as around Antarctica, and play a key role in global geochemical cycles, through the release of dimethyl-sulfonio-propionate (DMSP), a precursor for dimethyl sulfide (DMS), itself a gas involved in cloud nucleation (Westbroek et al., 1994). Dinoflagellate blooms are usually more restricted, but are of concern because of their potential impact on human health. Whether such blooms have recently increased in response to eutrophication and foreign species introduction, for example from ship ballast water, is a matter of debate (Richardson, 1997). Our view of marine phytoplankton has radically changed in the past two decades with the discovery of picoplankton and the realization that nitrogen is not the universal limiting nutrient. Many research challenges still lie ahead, in particular concerning how phytoplankton controls key elemental cycles and how it will respond to the forecasted global environmental changes. Novel approaches, in particular those originating from molecular biology, will certainly be critical to address these questions.

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