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CHARACTERISTICS AND IMPORTANCE OF PLANKTONIC FLAGELLATES IN MARINE COASTAL AND OCEANIC ECOSYSTEMS

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Resumo

Flagelados constituem um grupo morfologicamente e fisiologicamente diversificado do plâncton, com representantes de todas as divisões do Reino Protoctista. A grande importância destes organismos reside em sua diversidade metabólica, no pequeno tamanho e na capacidade de movimento, tornando-os mais competitivos em ambientes oligotróficos com baixa e contínua entrada de nutrientes. Nos últimos 25 anos, houve um significativo aumento no conhecimento sobre esses organismos, em conseqüência do avanço tecnológico e do interêsse crescente; entretanto é consenso, que o número de espécies ainda não descritas excede o número daquelas já conhecidas. As dificuldades no estudo de flagelados devem-se ao seu pequeno tamanho, à natureza delicada de seu revestimento, a deformação e/ou contração das células preservadas e à dificuldade em trabalhar com células vivas, essencial para observação da forma, tamanho e do seu movimento. A freqüente necessidade de microscópio eletrônico para a identificação de espécies, que depende de detalhes submicroscópicos, e a complexidade e desconhecimento dos ciclos de vida de muitas espécies também representam um obstáculo. Em águas costeiras e oceânicas brasileiras foram realizados poucos estudos sobre flagelados planctônicos até a presente data. A revisão dos dados existentes revela que a maioria das espécies registradas pertence ao grupo dos cocolitoforídeos (> 60%); os outros grupos são Chlorophyceae, Prasinophyceae Chrysophyceae, Raphidophyceae, Cryptophyta, Euglenophyta e Haptophyta. Os flagelados (10^5- 10^6 células l^{-1}) são organismos predominantes em águas oceânicas, mas também em áreas costeiras como no estuário da Lagoa dos Patos, eles são proporcionalmente mais importantes do que o microplâncton na maior parte do ano, contribuindo com 70% da clorofila *a* e da produção primária particulada total. A presença de flagelados potencialmente nocivos tem sido registrada em vários pontos da costa brasileira, sugerindo uma ampla ocorrência e um risco potencial para o ambiente marinho.

Palavras-chave: Fitoplâncton, biodiversidade, algas tóxicas e Brasil.

Abstract

Marine flagellates are morphologically and physiologically diverse planktonic organisms including several taxonomic Divisions of the Kingdom Protoctista. The ecological importance of flagellates comes from their diverse metabolic capacity, small size and motility, thus having great competitive advantage in environments with low but continued nutrient inputs. The knowledge of this group has improved over the last 25 years due to development of new techniques and increasing interest; however there is a consensus that the number of undescribed species still exceeds the number of known ones. Difficulties in flagellate studies concearned with their small size, delicate nature of cell covering, shrinking or deformation of preserved cells thus requiring examination of alive cells for cell size, shape, and motility observation. The reliable identification of many species depends on submicroscopic details, requiring electronic microscope thecniques; the complexity of their life cycles also impose difficulties. Marine flagellates in Brazilian coastal and oceanic waters are poorly known, with few studies conducted up to the present. Here we present a review of existing data on the ocurrence of flagellates over the Brazilian coast. Most reported flagellate species are calcified coccolithoforids (>60%) and the remaining groups are Chlorophyceae, Prasinophyceae, Chrysophyceae, Raphidophyceae, Cryptophyta, Euglenophyta and Haptophyta. Flagellates (10^5 and 10^6 cells l^{-1}) are the predominant organisms in oceanic waters, but also in inshore areas like Lagoa dos Patos estuary, they are relatively more important than microplankton during the year, contributing with 70% of chlorophyll *a* and particulate primary production. Potentially harmful flagellates have been recorded in diferent sites on the Brazilian coast, suggesting that they are widely spread and represent a potential risk to the coastal environment.

Key-words: Phytoplankton, biodiversity, harmful microalgae, Brazil.

Introduction

Flagellates are ubiquitous microscopic organisms inhabiting the most diverse terrestrial, freshwater and marine environments including the plankton, where they are important in coastal and oceanic waters, from the tropics to high latitude ecosystems. The numerical importance of flagellates becomes evident from phytoplankton countings on preserved material, which consistently withdraw a high component of unindentified flagellates (Throndsen, 1997). There is an increasing understanding about the importance of flagellates in most aquatic environments and it is clear now that they are important not only due to their numerical abundance, but mainly because of their role in the ecosystem. The interest in these organisms increased due to harmful effects of flagellate blooms that caused tremendous impact on marine ecosystems in the eighties, in addition to the influence on global climatic changes (Moestrup, 1994; Malin *et al.*, 1994; Throndsen, 1997), as outlined below. This paper will approach all flagellates except dinoflagellates.

The role of flagellates in the food web

Planktonic flagellates may be autotrophic or heterotrophic, but most of them are mixotrophic, i.e. even displaying chlorophyll *a* as the main photosynthetic pigment, they also ingest food particles to fullfill their nutritive requirements. This diverse metabolic capacity is one of the reasons for their success and abundance in marine environments. The other is their reduced cell size, making them more competitive than large organisms in environments of low but continued nutrient input as oligotrophic waters. The reduced nutrient storage capacity of small nanoplanktonic cells (<20 μm) is compensated by the high surface/volume ratio which enlarges nutrient exchange, enhancing metabolic processes as absorption, photosynthesis and respiration. In addition, the swimming capacity of flagellates favours a constant water flow around the cell microzone improving nutrient uptake of autotrophic species.

Photosynthetic flagellates usually predominate in stratified waters, where physical stability associated to optimal ilumination and the supply of nutrients by deep water enhance the growth of mobile organisms. They dominate phytoplankton when the levels of community biomass and primary production are low, and their relative importance increases with water depth contributing with up to 75% of autotrophic biomass and 80% of primary production in oceanic waters with small seasonal and spatial variability (Morris, 1980). However, this does not mean that flagellates are less abundant or important in more eutrophic or polluted waters, where the bulk of biomass and primary production is formed by larger cells, i.e. the microplankton (>20 μm). Cultural eutrophication is usually due the increase of N and/or P, but not of silicate (Smayda, 1990). Consequently, under eutrophic conditions, non-siliceous flagellates can grow and reach high biomass, whereas diatom growth remains limited by the silicate availability. Changes in the concentration of ammonium and humic acids, due to anthropogenic activities, also influence the speciation of available N in coastal areas, coinciding with species shifting. For example, the prymnesiophyte *Chrysochromulina polylepis* grows fast, relative to other species in the presence of ammonium and humic acids (Riegman, 1998).

The transition from a mainly autotrophic phase to increasing heterotrophic conditions, together with environmental stability, coincides with increased importance of flagellates, which frequently succeed diatoms during cyclic environmental variability (seasonal or other periodical events), and at random disturbances. Mixotrophic and heterotrophic flagellates may be osmotrophs, bacterivores, herbivores and/or carnivores. As bacterial consumers, they are an important component of the microbial food chain, being consumed by ciliates and other metazoan organisms. The existence of mixotrophic, heterotrophic and autotrophic species, implies that their identification is essential for the understanding of the trophic structure and the functioning of different aquatic ecosystems.

Biodiversity

Proper species identification is becoming more important nowadays as new environmental problems arise. Questions like species sucession and the production of toxins by some species depend also on the reliable identification of organisms.

There are just fewer than 4,000 described marine phytoplankton species, but the discovery rates of new taxa in all microbial groups indicate that their numbers will certainly increase in the near future. The consensus of phycologists is that the number of undescribed species exceeds the number of known ones (Norton *et al.*, 1996). There has been a tendency in the past to erect taxa on the basis of tiny, subtle differences in morphology – sometimes in individual specimens–without attempting to establish their stability or inheritability. This has led to inflated estimates species number and at this moment, a degree of uncertainty persists as to how many taxa really exist. The recent trends in molecular biology, genetic tools and life cycle studies when applied in combination with electron microscopy will reduce this gap. Phylogenetic reconstructions of flagellate taxa based on the sequence of the small subunit ribosomal RNA gene (18S ribosomal DNA) and morphological data are already available for Haptophytes (Edvardsen *et al.*, 2000).

The fossil records suggest that several flagellate taxa are very ancient and must therefore have survived immense climatic fluctuations and several catastrophic episodes. We have no idea of how many species of flagellates have become extinct, although some of them (coccolithophorids, silicoflagellates, dinoflagellates and prasinophytes) seem to have undergone significant extinctions as there are at least as many fossil species as living ones. Presumably most of the extinct flagellate species have not and may never be found.

Natural and anthropic impacts

In spite of the buffering effect of its huge volume, the ocean is fragile and its inhabitants vulnerable to irretrievable changes. The changing of chemical and physical water characteristics through human activities affects the contribution of flagellates to biomass and primary production, and its species composition (Moestrup, 1994). The final consequences are unexpected but they include community alterations from primary producers to man (Hallegraeff *et al.*, 1995). The massive increase in the world's population density and industrial activity raises public anxiety for imminent climate change as global warming. For at least 150 years, CO_2 concentration in the atmosphere has been rising as a result of man's activity of cutting down forests and burning fossil fuel. It is now expected that there will be a global rise in atmospheric temperature, the greenhouse effect. Over 99% of the CO_2 added to the earth's atmosphere throughout its history has been taken up by phytoplankton and sedimented to the sea floor to form the fossil fuels and calcareous rocks. Some flagellates like the coccolithophorid *Emiliania huxleyi*, are suspected to play an important role in carbon cycling transforming CO_2 into limestone. In all oceans, particularly at mid-latitudes, this species forms gigantic blooms and is by far the most abundant representative of coccolith-bearing organisms which are the major contributors to the ocean floor limestone sediments. This leads to the largest long-term sink of inorganic carbon on earth, the mechanism known as the biological pump. The concentration of other gases which contribute to warming the atmosphere (water vapour, methane, ozone, nitrous oxide, chlorofluorocarbons) are also increasing slowly. An increase of 1.5-4.5^0 C in mean global temperature of the oceans, will result in higher evaporation and hence a more vigorous hydrological cycle, with increased runoff and greater buoyancy in coastal waters. The growth advantage will shift from larger diatoms to smaller species like flagellates, with a lengthening of the food chain between the primary producers and the large consumers (Mann & Lazier, 1996).

In addition to coccoliths and organic carbon, *E. huxleyi* blooms release the gas dimethyl sulfide (DMS) - a degradation product of DMSP (dimethylsulfoniopropionate) - which produces sulfur (S) particles, that act as cloud condensation nuclei in the atmosphere (Westbroek *et al.*, 1994). Many other haptophytes (*Phaeocystis pouchetii*, *Prymnesium parvum*, *Chrysochromulina polylepis*, etc.) and flagellates like raphidophytes (*Heterosigma akashivo*), dinophytes (*Gymnodinium* sp., *Gyrodinium aureolum*, *Heterocapsa* sp., *Scripsiella trochoidea*) and chlorophytes (*Tetraselmis subcordiformis*) are also known to produce DMS (Malin *et al.*, 1994; Gabric *et al.* 1993; Turner *et al.*, 1988). The biological function of DMSP/DMS is still not fully understood, although there is evidence suggesting it accumulates in phytoplankton with increasing salinity, and that it acts as a cryoprotectant in some Antarctic seaweeds and ice algae (Malin *et al.*, 1994). Charlson *et al.*

(1987) put forward the hypothesis that the production of DMS by marine phytoplankton might represent a climate regulating mechanism. The increased seawater temperature and or light would lead to increased DMS emissions and consequently to increased cloud albedo which would counteract the initial temperature/light increase.

Antrophogenic perturbation through eutrophication can affect DMS and other gas emissions through the increase in nitrate and phosphorus levels whereas silicate concentration remains constant. For the period between 1959 and 1980, there is evidence for the North Sea increasing algal biomass, duration of blooms, and a species shift favouring flagellates rather than diatoms. Current efforts to limit nutrient imputs into areas such as the North Sea could reduce the prevalence and/or duration of coastal blooms of *Phaeocystis* and other flagellates, which would reduce DMS emissions. Stratospheric ozone depletion has led to a global increase in UV radiation and there is evidence showing that it is affecting marine phytoplankton, by decreasing primary productivity and changing species composition (Malin *et al.*, 1994). Diatoms generally contain low levels of DMSP and are more susceptible to UV-B damage than dinoflagellates. *Phaeocystis pouchetii*, for instance produces UV-B screening properties compounds. Therefore changes in community taxonomic structure favouring DMSP producing phytoplankton species might lead to increased DMS emission (Malin *et al.*, 1994).

The Organisms

Flagellates are present in all Divisions of the Kingdom Protoctista (eucaryotic algae, protozoa and some fungi, Margulis & Schwartz, 1988). Despite the morphological and physiological diversity of organisms, the presence of flagella (one or more) is the feature which determines the major life conditions and constraints of unicellular (monads) and colonial flagellates. Basic morphological characteristics of marine flagellates (except dinoflagellates) are depicted in Figure 1, including both photosynthetic (autotrophic, auxotrophic and mixotrophic) and strictly heterotrophic species. Photosynthetic flagellates (or phytoflagellates) and apochlorotic organisms are characterized following the classification presented by Van den Hoek *et al.* (1995) and Margulis & Schwartz (1988), respectively.

Division Heterokontophyta

The heterokont flagellate cells bear a long flagellum with stiff hairs called mastigonemes (or flimmers), which is directed onwards during swimming, and a shorter smooth flagellum pointing backwards. The chloroplast is enclosed by a double membrane (the envelope) and by a fold of endoplasmic reticulum, the so-called chloroplast ER. Within the chloroplast the thylacoids are grouped into stacks of three, called lamellae, and form a girdle lamella surrounding the periphery of the chloroplast beneath its envelope.

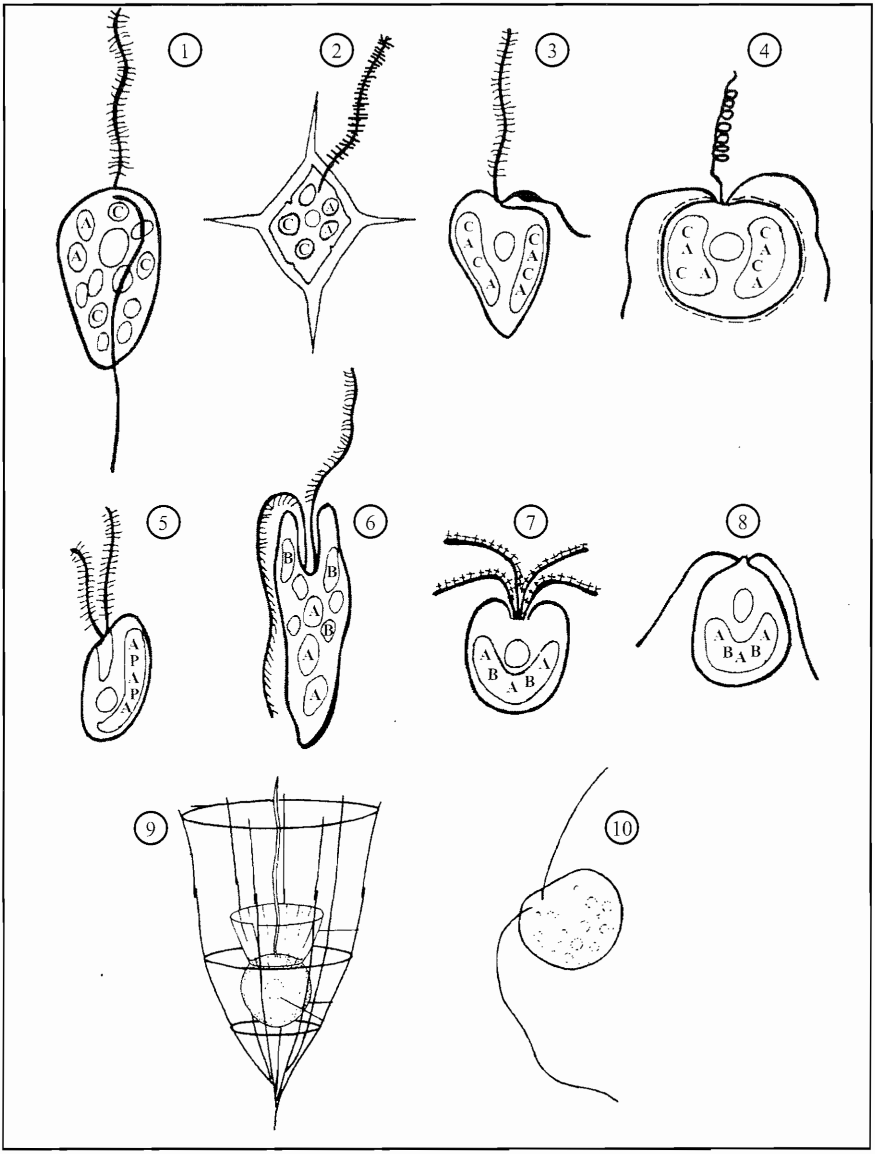

Figure 1: Schematic pictures of planktonic flagellate from different taxonomic groups: Raphidophyceae (1), Dictyochophyceae (2), Chrysophyceae (3), Haptophyceae (4), Cryptophyceae (5), Euglenophyceae (6), Prasinophyceae (7), Chlorophyceae (8), Choanoflagellidea (9) e Kinetoplastidea (10). Chlorophyll *a*, *b* and *c* and phycobilins are represented by characters A, B, C, and P respectively. The drawings from 1 to 8 were adapted from Chrétiennot-Dinet (1990), while the 9 and 10 are from Throndsen (1993).

Class Raphidophyceae (Fig.1- 1)

Raphidophytes cells are large (20- 100 μm), with a curved dorsal side and a flatter ventral side. Two flagella arise from a small funnel-shaped invagination on the ventral side of the cell, the backwards pointing flagellum lies in a shallow ventral longitudinal groove. Green, yellow-green or yellow-brown numerous ellipsoidal chloroplasts contain chlorophyll a, c_1, c_2, and accessory pigments. The nucleus lies in a capsule of relatively viscous cytoplasm, surrounded by an outer more fluid layer containing the chloroplasts. Organisms present trichocysts or spherical mucilage vesicles which discharge threads of mucilage upon stimulation. Marine species give rise to blooms in summer, mainly in bays and enclosed waters, and red tides cause severe disruption of fish farming in Japan. The class is observed worldwide and represents a potential hazard, including Brazilian coastal waters. The main genera are *Chattonella*, *Fibrocapsa* and *Heterosigma*.

Class Dictyochophyceae (silicoflagellates) (Fig. 1- 2)

Vegetative unicellular silicoflagellates present a single flagellum bearing mastigonemes, although, during life cycle, flagellate cells are produced bearing two flagella (a long one with mastigonemes, and a smooth vestigial). The vegetative cells are usually smaller than 50 μm, and present a siliceous skeleton composed of hollow robust tubes of silica forming a basket wich lies outside the plasmalemma. Chloroplastas are golden-brown, marine chlorophyll *a* and *c* being masked by accessory photosynthetic pigments. From the central cytoplasma, containing the nucleus and golgi bodies, numerous thin strands extend out radially, anastomosing with each other and connecting distally with the peripheral cytoplasm containing the chloroplasts. The extant species (*Dictyocha*, *Disptephanus*) are widely distributed throughout the world´s oceans.

Class Chrysophyceae (Fig 1- 3)

Chrysophytes owe their name to the golden-yellow to brown colour of the chloroplasts (in greek chrysos = gold), as the green colour of the chlorophylls is masked by accessory pigments. In several genera, siliceous body scales are present, lying against the surface of chloroplasts, and spherical silica-walled cysts are formed within the cells, the silica being deposited within a special vesicle. This class comprises mainly freshwater species, only a few being found in brackish or salt water. However, it has been appreciated recently that members of this class probably play an important role in the primary production of the oceans, as nano- and picoplankton. Flagellate species of *Ankylochrysis* and the tiny *Ochromonas* appear to be common in the open ocean and inshore waters.

Division Haptophyta (Fig. 1- 4)

Haptophytes are mainly small unicellular flagellates of nanoplankton (2- 20 μm) and picoplankton (< 2 μm), but the life cycle of some species have amoeboid, coccoid, palmelloid or filamentous stages. Flagellate cells bear two flagella without mastigonema, and a thin, short or long filamentous appendage called haptonema, with a different structure (6 or 7 microtubules in a crescent-shaped array) of the flagella (2 central microtubules and 9 peripheral doublets). The chloroplast envelope is enclosed by a fold of endoplasmic reticulum and the thylacoids are stacked in threes to form lamellae without girdle lamella. Chloroplasts are golden or yellow-brown, the green of the chlorophylls (chlorophyll a, c_1, and/or c_3 and c_2) being masked by accessory pigments. The surface of the cell may be covered with tiny scales, granules of organic material, or calcified scales (coccoliths) easily seen with the light microscope. The calcified scales usually have a characteristic structure arranged radially. Only a few species occur in freshwater, the great majority of the haptophytes are marine and almost all of the planktonic species are small (nanoplankton). Together with the picoplankton, they are responsible for most of the primary production in the open ocean, away from neritic and inshore waters. Nevertheless, haptophytes are also common in nearshore or inshore phytoplankton.

Division Cryptophyta (Fig. 1- 5)

Cryptophytes are nanoplanktonic organisms (2- 20 µm) bearing two flagella covered by organic scales, the longer with two rows of mastigonemes and the shorter with a single one. The cells, enclosed by a stiff periplast made up of rectangular or polygonal protein plates, present a dorsal convex and a ventral flat side, with a shallow longitudinal groove lined by prominent trichocysts at its anterior end. Chloroplasts (1-2 per cell) are surrounded by a fold of endoplasmic reticulum, the thylakoids are often arranged in pairs to form lamellae without girdle lamella, and the pyrenoid projects out from the inner side of the chloroplast. The pigments chlorophyll a and c_2 are masked by the blue, blue-green, reddish, red-brown, olive green, brown or yellow-brown accessory pigments. The function of the Maupas bodies immediately adjacent to the gullet is still unknown. Marine species are found in tidal pools or puddles of brackish water, but also in the nanoplankton of the open ocean. Sometimes, they dominate blooms and under calm weather some species migrate vertically within the water column adjusting their vertical position to optimum conditions. One species of cryptophyte is the endosymbiont of the autotrophic ciliate *Mesodinium rubrum*.

Division Euglenophyta (Fig. 1- 6)

Euglenophyte flagellates are usually smaller than 70 µm, their anterior flagella arising from the bottom of a flask-shaped invagination. There are almost always two flagella bearing a unilateral array of delicate hairs, and shorter hairs, although one of the flagella may lie entirely within the ampulla. The spiral construction of the cell is provided by a pellicle composed of protein strips within the cytoplasm, which wind helically around the cell. The chloroplast envelope, when present, consists of three membranes, and the thylacoids are usually grouped into three forming lamellae, without girdle lamella. The green chloroplasts (chlorophyll *a* and *b*) are not masked by accessory pigments; there is an orange-red eyespot free in the cytoplasm consisting of carotenoid droplets. Euglenophytes are mainly found in freshwater and marine inshore environments, and many species are heterotrophic. They form green films on estuarine mud- and sandflats, when myriads of euglenophytes move up to the surface becoming exposed by the tide, and back into the sediment before submergence by the tide. The vertical movements are steered by phototactic behaviour controlled by an endogenous, clock-like daily rhythm.

Division Chlorophyta

This division comprises mainly freshwater species, but a few species of the classes Chlorophyceae and Prasinophyceae are brackish or marine. The most striking feature is the green colour of chloroplasts (chlorophyll *a*, *b*; Class Prasinophyceae also chlorophyll *c*), not masked by accessory pigments. The chloroplast is enclosed only by the double membrane, and thylacoids (2-6 or more) are grouped to form lamellae, without girdle lamella.

Class Prasinophyceae (Fig. 1- 7)

Prasinophyte cell and flagella (1-8) are covered with minute organic scales, the flagella also being covered with simple hairs. Cell shape quadrangular or bilaterally compressed, often with a depression where the flagella originate. The minute marine flagellates (few micrometers) are important primary producers in the euphotic zone of the oceans. Other species like *Halosphera* and *Pterosperma* form large cyst-like stages (diameter 100-800 μm) resistant to microbial degradation and fossilize readily. Prasinophyte species also live as endosymbionts in radiolarians and the dinoflagellate *Noctiluca*.

Class Chlorophyceae (Fig.1- 8)

Flagellate chlorophytes are round, ovoid or lobed, naked or with cellulose wall. An eyespot is located in the chloroplast. There are 1, 2, 4 or 8 flagella a few species being important as marine nanoplankters (2- 20 μm) (e.g. *Brachiomonas*, *Carteria*, *Dunaliella*).

Division Zoomastigina (Zooflagellates)

Exclusively heterotrophic flagellates, osmotrophs or phagotrophs with one or more flagella, including free living and parasitic species. The two classes below are frequently found in marine plankton.

Class Choanoflagellidea (Fig.1- 9)

Oval cells distinguished by having a lorica (silica) from which the single smooth flagellum emerges at the rear and pushes the cell. At its base there is a collar of fine pseudopodia. They are coastal and oceanic, present from polar to tropical regions, colorless or containing green plastids. Most of them are smaller than 30µm.

Class Kinetoplastidea (Fig.1- 10)

Difficult to identify, the naked spherical, ovoid or flattened cells are usually small (< 20 µm). They present two heterodinamic flagella, one located forward pulling the cell, the other located backwards, and all contain a special large mitochondrion called the kinetoplast. This class includes many important pathogens and parasites on man and animals causing sleeping sickness and Chaga's disease. Marine species are known from coastal and litoral waters, being common in stagnant water. However, there is little information on oceanic species.

Methods and difficulties in studying flagellates

The progress in the knowledge of flagellates is a consequence of the development of new techniques enabling a more accurate identification and quantification of some groups. For example, intensive studies on haptophyte nanoflagellates carried out in the last 25 years improved the knowledge, specially of coccolitophorides, based on oceanographic and geological studies using the preserved fossil forms, as markers in stratigraphic correlations. Naked forms were initially described based on live culture material at optical microscope; however, the amazing diversity within this group was emphasized when freshly collected samples were prepared for transmission electron microscopy. The number of species already described for one genus, *Chrysochromulina*, is close to 50; however, the number of unidentifiable *Chrysochromulina*–like groups of scales that appear in almost any whole mount preparation from a marine environment, suggests that this number can probably be greater than 100 (Thomsen, Buck & Chavez, 1994).

Identification of organisms

The first important question to be answered in any biological study is which species are present. This is not a trivial question when working with protists, and up to now we have serious difficulties in answering this simple question. This happens mainly due to their small size and the delicate nature of the organisms. Cell size and form, number and size of flagella, type of movement, cell covering and other cell structures are the main characteristics used in the identification of flagellates. In addition, cell deformation and/or contraction occurs in preserved cells and examining living cells is required for reliable measurements. Observing the form and swimming behaviour are also important for the proper identification (Throndsen 1997; Billard & Chretiennot-Dinet, 1995). Very helpful microscopical tools in studying flagellates are the Phase contrast and Nomarski interference contrast. Phase contrast techniques are well suited in studying small organelles of the protoplasm, the former permitting visualization of the details especially of transparent living organisms, while Nomarski provides high resolution contrast which can be varied instrumentally giving cellular organelles a three-dimensional appearance of relief. However, the reliable identification of many species depends on submicroscopic details, perceptible only by electronic microscope techniques, described in Billard & Chretiennot-Dinet (1995). After the electronic microscope was introduced as a regular instrument, there was a dramatic increase in microalgae diversity knowledge and the recognition of new groups and species. Transmission electron microscope is preferred for haptophytes as the resolution of the scanning electron microscope is often insufficient to resolve the minute details of their scales (Moestrup & Thomsen, 1995). On the other hand, raphidophytes usually do not require electron microcopy due their large size, and for the identification of cryptophytes and euglenophytes both scanning and transmission microscopy may be used.

Another problem in identifying flagellates is the complex life cycle of many species, including diverse life forms, i.e. during the life a single species may present morphologically different stages which may be recognized as distinct species. This is also a common problem to other protists like dinoflagellates and amoeba, but not for diatoms. As the life cycle of most flagellate species is still unknown, this will remain an important task to be solved. Novel molecular and immunological approaches are promising tools in this matter.

Fixing, preservation and storage of natural samples

Most of our present knowledge of phytoplankton concerns "preservable" species presenting a rigid wall or cell protection like the cellulose of thecate dinoflagellates, calcium carbonate of coccolitophorids and silicate of diatoms. They preserve easily and can thus be studied long after their collection. Less is known about the importance of planktonic "naked" autotrophic, mixotrophic and heterotrophic flagellates, in part due to their small size, the absolute requirement of examining living material, as well as to the needed skill of familiarity with an high number of taxonomic taxa of microorganisms.

The main difficulty in working with delicate flagellates is the lack of adequate fixing and preserving agents, making storage difficult for most species. Concentration of 1-5% of the lugol's solution, formaldehyde, glutaraldehyde or their mixture with other substances are frequently used as fixing and storage agents for marine photosynthetic and heterotrophic flagellates, but none of them fixes and preserves well all organisms (Throndsen, 1978a; Sherr & Sherr, 1993). Thus, replicate sampling using at least two fixing/preserving agents is recommended to avoid losing a whole group of organisms. Storage time should be as short as possible, as loss occurs with storage time. It has been a common practice to store samples in the dark at room temperature for weeks to months, prior to inspection, but evidence has shown that storage of preserved flagellates in liquid samples can lead to variable, often significant cell loss in less than one day (Sherr & Sherr, 1993). Therefore, it is highly recommended that preserved samples be processed, filtered, mounted on slides, and stored frozen as soon as possible, and that control experiments to determine the potential magnitude of cell loss of preserved compared to live samples be made. Techniques like the Filter-Transfer-Freeze and glycerine mountings are recommended for this purpose (Sherr & Sherr, 1993).

Estimating cell concentration and biomass of photosynthetic and heterotrophic cells

Sophisticated and expensive methods like flow cytometry and high performance liquid chromatography have been introduced in the study of marine phytoplankton to estimate cell number and size and tentatively to identify the group of species presents . However, these are unusual routine methods and the light microscope (LM) is still in use to determine the community structure and the biomass of microorganisms in seawater samples. The traditional Utermöhl (1958) settling method does not allow for recognizing small cells (<5 µm), which represent an important fraction of flagellates. Thus, a combination of the Utermöhl method and epifluorescence microscopy (EFM) is recommended (Booth, 1993). The latter allows for the distinction of autotrophic and heterotrophic cells by the autofluorescence of pigments, or by diverse staining techniques (Sherr *et al.*, 1993). Due to the characteristic emission spectra of light under variable excitation, some taxonomic groups may be recognized through the EFM using specific combinations of excitation and emission filters, but in general species can't be identified through the EFM. The use of color image analysis can be combined with EFM to provide fast estimations of a large number of samples (Verity and Sieracki, 1993) and the trouble of the time-consuming cell counting and measuring and calculating biovolume may thus be surpassed when introducing computer-based analysis.

The biomass of flagellates (as carbon) can be estimated from cell concentrations and dimensions (Hilledbrand *et al.*, 1999), using standard conversions from cell volume to carbon (Verity *et al.*, 1992). This number, traditionally difficult to obtain, is most useful in ecological studies, as it can be used to compare the biomass of different trophic components of the community. For this purpose, a standard unit is needed and using cell concentration alone is cumbersome due to the high cell size variability of plankton.

Culture

Marine flagellates can be grown successfully in the laboratory by different approaches, the choice depending on the purpose of the study (Lee, 1993). The cultivation of single species can be critical in determining the systematic position of flagellates; other properties that can be studied include physicochemical, physiological and intraspecific biological aspects like pigment composition, ultrastructure, molecular biology and genetics, life cycles, growth rate, nutritional mode, feeding rate and behaviour, storage/toxin products. For some purposes, strains of cultured cells may be used but when any attempt is made to correlate the occurrence of a flagellate species with the ecology of its habitat, it is essential to obtain cultures of cells from local waters. The autoecological approach answers many questions but it is far from complete, falling short of giving a realistic understanding of small-scale biotic interactions. However, our understanding of the ecological role of flagellates in nature will undoubtedly improve as more species are brought into culture (Caron, 1993). Functional problems can be addressed with batch or continuous multispecies cultures.

Natural seawater contains many elements in abundance, but others are scarce for growing crops of autotrophic and auxotrophic flagellates, and various enrichments have been designed to supply the scarce materials in usable form, at levels that are neither toxic nor severely growth-rate limiting. The most frequently used media for flagellate cultures are the same as for dinoflagellates or diatoms (without silica), as described by Guillard & Ryther (1962), Guillard (1975, 1995), McLachlan (1973); Keller & Guillard (1985) and the "Erdschreiber" medium described in Throndsen (1978b). Incubation conditions of heterotrophic and mixotrophic flagellates should be similar as for photosynthetic species regarding illumination, photoperiod and temperature, with suitable prey made available (Caron, 1993). Methods for the establishment of cultures and maintenance of stocks and production, laboratory procedures, manipulations and different media for phototrophic and heterotrophic species are described by different authors (e.g. Guillard, 1975, 1995; Caron, 1993; Lee, 1993).

The first step to start a flagellate culture is to isolate a single specimen from nature by either single cell isolation by micropipette (Guillard & Keller, 1984) or the serial dilution technique (Throndsen, 1978b, 1995). The general enrichment of net concentrated samples (10-20μm pore size) provides adequate material for easier cell isolation. Afterwards, the population is allowed to increase until some factor, usually nutrients, stops cell multiplication. Well before the half-life of the culture is reached, a daughter culture is inoculated from it and the parent culture is kept as a "backup" culture and as inoculum source.

Flagellate studies in Brazil

Marine flagellates in Brazilian coastal and oceanic waters are poorly known, specially the naked forms, partially due to the necessity of routine working with electron microscope, not available in most research centers. However, from the available information on flagellates in some regions, it becomes clear that they are important and deserve detailed study. At present, a total of 114 flagellate species has been observed in diverse Brazilian coastal and oceanic waters (Table 1). Most of them are calcified coccolithophorids and only a minor fraction (35%) belong to fragile small organisms (Chlorophyceae, Chrysophyceae, Cryptophyceae, Euglenophyceae, Haptophyceae, Prasinophyceae).

The abundant presence of "unindentified flagellates or small organisms" is recognized in several studies in Brazilian coastal and oceanic waters from the tropics to warm temperate latitudes (Villac, 1990; Ribeiro, 1996; Bergesch & Odebrecht 1997; Odebrecht & Garcia, 1998; Gianesella *et al.*, 1999; Torgan *et al.*, 2000). Typical concentrations vary between 10^5–10^6 cells l^{-1}, being predominant in oceanic waters while this dominance is shared with diatoms and dinoflagellates in coastal and shelf areas. Electron microscope techniques were only applied in few studies like the oceanic region off the southeastern Brazilian coast (25-32° S; 38-50° W, between Santos and Santa Marta Grande Cape), and at the eastern Brazilian continental shelf (16-21,5° S; 36-40,5° W, Abrolhos) during the autumn season (Ribeiro, 1996). A total of 73 haptophyte, 2 silicoflagellate, and 9 other flagellate species were identified, with the predominance of coccolithophorids. In this study, cell concentration of haptophytes, prasinophytes, chrysophytes, euglenophytes, cryptophytes, dictyochophytes and craspedophytes attained 10^4 cell l^{-1} (Ribeiro, 1996). A maximum of 10^5 cells l^{-1} of coccolithophorids occurred in summer at the continental shelf between 23-24° S (Aidar *et al.*, 1993).

In the Lagoa dos Patos estuary (32⁰ S, 52⁰ W), nano- and picoplankton are relatively more important (annual mean »70 % of chlorophyll *a*) than microplankton during the year. The latter (chlorophytes and diatoms) is dominant in northern and central areas (Odebrecht *et al.*, 1987; Torgan *et al.*, 2000), while nanoflagellates are the most abundant organisms in estuarine shoals and channels (10^6–10^9 cells l^{-1}), highest densities occurring under special conditions. Generally, nanoplankton is less important in spring-summer in this estuary, when microplankton chlorophyll *a* increases (Bergesch, 1990). Primary production in the Patos Lagoon varies between 2–5 mg C m^{-3} h^{-1} in winter and 160-350 mg C m^{-3} h^{-1} in spring, most of the carbon being assimilated by nanoplankton (annual mean »70,3 % of total particulate primary production, Odebrecht & Abreu, 1997). Nanoflagellate photosynthetic capacity is higher under reduced light intensity (<50 µmol $m^{-2}s^{-1}$), while coastal diatoms and dinoflagellates seem to be adapted to higher light intensity in this environment (>400 µmol m^{-2} s^{-1}) (Abreu, 1992). Cryptophytes are the most diverse nanoplankton flagellates (18 species) and an important component of biomass, contributing with 28% of cellular countings in Lagoa dos Patos estuary and 19% in coastal waters. Other groups (prasinophytes, haptophytes, rafidophytes, chrysophytes and chlorophytes) are also important: only 3 species were identified up to present (*Heterosigma akashiwo, Fibrocapsa japonica* and *Prymnesium patelliferum*).

At the Lagoa dos Patos estuarine plume on the continental shelf, horizontal environmental gradients (salinity, suspended material, dissolved nutrients) are associated with distinct phytoplankton communities and bacterial biomass. A straight relationship among bacterial biomass and heterotrophic flagellates and ciliates was observed in turbid waters (Abreu *et al.*, 1995). Higher biomass (bacteria and phytoplankton) in turbid estuarine waters, probably result from the shallow nutrient-rich plume, enhancing the exposition of cells to light.

Table1: Flagellates (without dinoflagellates) observed in Brazilian coastal and marine waters.

Class	Genus or species	Reference
Raphidophyceae	*Chattonella cf. antiqua*	Odebrecht & Abreu, 1995
	Chattonella sp.	Sevrin-Reyssac et al., 1979; Tenenbaum et al. 1998; Prooença pers. comm.;
	Heterosigma akashiwo	Persich et al., 1998
	Fibrocapsa japonica	Odebrecht & Abreu, 1995; Persich et al., 1998
Dictyochophyceae	*Dictyocha fibula*	Ribeiro, 1996; Brandini, 1985, 1988; Brandini & Moraes, 1986;
	D. speculum var. octonarius	Ribeiro, 1996
	Distephanus sp.	Brandini, 1988
	Mesocena sp.	Brandini, 1988; Brandini & Moraes, 1986
Chrysophyceae	*Dinobryon faculiferum*	Ribeiro, 1996
	Meringoshphaera mediterranea	Ribeiro, 1996
	Tetraparma sp.	Ribeiro, 1996
	Triparma cf. retinervis	Ribeiro, 1996
Haptophyceae	*Anoplosolenia brasiliensis; Acanthoica cf. biscayensis;* *A. quattrospina; Algirosphaera oryza; A.robusta;* *Calcidiscus leptoporus; Calicasphaera diconstricta;* *Calyptrolithina multipora; C. wettsteinii;* *Calyptrolithophora papillifera; Calyptrosphaera oblonga;* *C. sphaeroidea; Corisphaera gracilis; C. sp.;* *Coronosphaera binodata; C. mediterranea;* *Cyrtosphaera leculiae; C. sp.; Daktylethra pirus;* *Discosphaera tubifera; Emiliania huxleyi var. corona;* *Florisphaera profunda; Flosculosphaera calceolariopsis;* *Gephyrocapsa ericsonii; G. oceanica; G. ornata;* *Gladiolithus flabellatus; Hayaster perplexus;* *Helicosphaera carteri; H. carteri var. hyalina;* *H. carteri var. wallichii; Helladosphaera cornifera;* *H. pienaarii; Homozygosphaera triarcha;* *Michaelsarsia elegans; Oolithotus antillarum;* *Periphyllophora mirabilis; Polycrater galapagensis;* *Poricalyptra aurisinae; P. magnaghii; P. guarderae;* *P. isselii; Poritectolithus maximus; P. cf. poritectum;* *P. tyronus; Reticulofenestra sessilis;* *Phaeocystis pouchetii; P. scrobiculata;* *Pontosphaera discopora; Rhabdosphaera clavigera;* *R. clavigera var. stylifera; Syracolithus catilliferus;* *S. confusus; S. quadriperforatus;* *Syracosphaera halldalii; S. lamina; S. molischii;* *S. nodosa; S. pirus; S. pulchra; S. sp.;* *Turrilithus latericioides; Umbellosphaera irregularis;* *U. tenuis; Umbilicosphaera hulburtiana;* *Zygosphaera bannockii; Z. hellenica;*	Ribeiro, 1996

	Calciosolenia murrayi	Brandini, 1988; Aidar et al., 1993; Ribeiro, 1996
	Emiliania huxleyi	Brandini, 1988; Brandini & Moraes, 1986; Aidar et al., 1993; Ribeiro, 1996
	Calciopappus caudatus	Aidar et al., 1993; Ribeiro, 1996
	Ophiaster cf. hydroideus	Aidar et al., 1993; Ribeiro, 1996
	Umbilicosphaera sibogae	Aidar et al., 1993; Ribeiro, 1996
	Pontosphaera siracusana	Brandini, 1988; Ribeiro, 1996
	Scyphosphaera apsteinii	Brandini, 1988; Ribeiro, 1996
	Chrysochromulina sp.	Bergesch & Odebrecht, 1998
	Prymnesium patelliferum	Bergesch & Odebrecht, 1998
Cryptophyceae	*Chroomonas vectensis*	Torgan, 1997
	Cryptomonas pusilla	Torgan, 1997
	C. tetrapyrenoidosa	Torgan, 1997
	C. parapyrenoidifera	Torgan, 1997
	Hemiselmis sp.	Torgan, 1997
	Rhodomonas minuta	Torgan, 1997
	R. minuta var. v. nanoplanctonica	Torgan, 1997
	R. cf. salina	Torgan, 1997
	R. sp.	Torgan, 1997
Euglenophyceae	*Euglena limnophila*	Torgan, 1997
	E. cf. agilis	Torgan, 1997
	E. gracilis	Torgan, 1997
	E. sp.	Brandini, 1985
Prasinophyceae	*Pedinomonas minutissima*	Torgan, 1997
	Pseudoscourfieldia marina	Ribeiro, 1996
	Pyramimonas grossi	Torgan, 1997
	P. sp.	Ribeiro, 1996
	Pterosperma cristatum	Ribeiro, 1996
	P. parallelum	Ribeiro, 1996
	P. undulatum	Ribeiro, 1996
	Scourfieldia cordiformis	Torgan, 1997
Chlorophyceae	*Carteria sp.*	Torgan, 1997
	Chlorogonium fusiforme	Torgan, 1997
	C. sp.	Torgan, 1997
	Coelastrum reticulatum	Torgan, 1997
	Dictiosphaerium ehrenbergianum	Torgan, 1997

Harmful flagellates

Potentially harmful flagellates in Brazilian waters have deserved little attention, although their presence was shown in Guanabara Bay, Rio de Janeiro in June/July 1978, when potentially toxic raphidophytes (probably *Chattonella*) formed a typical red tide (1,7 x 10^7 cells l^{-1}) (Sevrin- Reyssac *et al.* (1979) Raphidophytes of the genus *Chattonella* were also the main organisms (6,3 x 10^7 cells l^{-1}) in Sepetiba Bay (RJ) in January 1998, forming a 15 km wide phytoplankton patch turning the water greenish/brown for 15 days (Tenenbaum *et al.*, 1998). Fish mortality was not observed in none of these events but in 1998 skin problems were registered after direct contact of people with the water. In southern Brazil, potential harmful flagellates (the raphidophytes *Chattonella*, *Fibrocapsa* and *Heterosigma*) are observed in Santa Catarina state where aquaculture activities are progressing fast (Proença pers. comm.). The raphidophytes *Chattonella* cf. *antiqua* and *Fibrocapsa japonica* reached concentration of 10^5 cells l^{-1} in the estuary of Lagoa dos Patos in autumn (May 1995) (Odebrecht & Abreu, 1995). In this environment, other harmful flagellates species like *Heterosigma akashiwo* (Persich *et al.*, 1998) and haptophytes of the genera *Chrysochromulina* and *Prymnesium* (Bergesch & Odebrecht, 1998) are common, representing potential risks to human health, economic aspects in aquaculture and tourism activities, besides ecological stress. Large-scale massive mortality of intertidal shellfish in southern Brazil is a recurent phenomenon which is probably related to harmful phytoplankton (Rosa & Buselato, 1981; Tommasi, 1985; Odebrecht *et al.*, 1995). However, the causative species of these events must still be identified.

Thus, although scarce, the available information suggests that harmful flagellates are widely spread in coastal Brazilian waters and that more attention should be addressed to these organisms in order to better evaluate future environmental problems. Aquaculture activities, mainly of mollusks, is progressing fast in some areas and requires the implementation of governmental food quality control actions. Phytoplankton monitoring programs to detect harmful/toxic species, including flagellates, is an important tool for the management of marine resources (Hallegraeff *et al.*, 1995), which must be improved in our country.

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