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# The life cycle of *Phaeocystis* (Prymnesiophyceae): evidence and hypotheses

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**Abstract**

The present paper reviews the literature related to the life cycle of the prymnesiophyte *Phaeocystis* and its controlling factors and proposes novel hypotheses based on unpublished observations in culture and in the field. We chiefly refer to *P. globosa* Scherffel as most of the observations concern this species. *P. globosa* exhibits a complex alternation between several types of free-living cells (non-motile, flagellates, microzoospores and possibly macrozoospores) and colonies for which neither forms nor pathways have been completely identified and described. The different types of *Phaeocystis* cells were reappraised on the basis of existing microscopic descriptions complemented by unpublished flow cytometric investigations. This analysis revealed the existence of at least three different types of free-living cells identified on the basis of a combination of size, motility and ploidy characteristics: non-motile cells, flagellates and microzoospores. Their respective function within *Phaeocystis* life cycle, and in particular their involvement in colony formation is not completely understood. Observational evidence shows that *Phaeocystis* colonies are initiated at the early stage of their bloom each by one free-living cell. The mechanisms controlling this cellular transformation are still uncertain due to the lack of information on the overwintering *Phaeocystis* forms and on the cell type responsible for colony induction. The existence of haploid microzoospores released from senescent colonies gives however some support to sexuality involvement at some stages of colony formation. Once colonies are formed, at least two mechanisms were identified as responsible of the spreading of colony form: colony multiplication by colonial division or budding and induction of new colony from colonial cells released in the external medium after colony disruption. The latter mechanism was clearly identified, involving at least two successive cell differentiations in the following sequence: motility development, subsequent flagella loss and settlement to a surface, mucus secretion and colony formation, colonial cell division and colony growth. Aggregate formation, cell motility development and subsequent emigration from the colonies, release of non-motile cells after colony lysis on the other hand, were identified as characteristic for termination of *Phaeocystis* colony development. These pathways were shown to occur similarly in natural environments. In the early stages of the bloom however, many recently-formed colonies were found on the setae of *Chaetoceros* spp, suggesting this diatom could play a key-rôle in *Phaeocystis* bloom inception. Analysis of the possible environmental factors regulating the transition between the different phases of the life cycle, suggested that nutrient status and requirement of a substrate for attachment of free-living cells would be essential for initiation of the colonial form. Physical constraints obviously would be important in


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determining colony shape and fragmentation although autogenic factors cannot be excluded. Some evidence exists that nutrients regulate colony division, while temperature and nutrient stress would stimulate cell emigration from the colonies.

## 1. Introduction

*Phaeocystis* is one of the few marine phytoplankters exhibiting an heteromorphic life history. While two different cell types — vegetative cells and flagellates (zoids) — were already identified in the early beginning of this century as *P. globosa* Scherffel (Scherffel, 1900) and *P. pouchetii* (Hariot) Lagerheim (Ostenfeld, 1904), the first description of the general feature of the *Phaeocystis* life cycle is due to the detailed microscopic work of Kornmann (1955). This morphological study conducted on a cultured *P. globosa* strain isolated from Dutch coastal waters in the North Sea, evidenced the high complexity of the cycle, characterized by the alternance between different free-living cells and mucilaginous colonies of non-motile coccoid cells (the palmelloid stage). Colonies were shown to widely vary in shape and size, reaching several mm at the stationary stage of their growth. Apart from colonial cells, three different types of *Phaeocystis* flagellate free-living cells with likely different functions in the cycle were identified by Kornmann (1955): the swarmers, the microzoospores and the macrozoospores, varying between 3 and 9 $\mu$m in diameter. The occurrence of these various morphological cell types with additional reference to non-flagellate free-living cells, was later reported by Kayser (1970) and Parke et al. (1971).

Both morphological forms — free-living cells and colonies — have been reported to occur in the natural environment. Among the different species, the flagellate stage has been commonly recorded in absence of any colonies in oligotrophic waters of the Atlantic (Parke et al., 1971; Estep et al., 1984), Pacific (Moestrup, 1979; Booth et al., 1982; Hallegraeff, 1983; Hoepffner and Haas, 1990) and Mediterranean Sea (Delgado and Fortuño, 1991). Reversely, colony forms are predominant in nutrient enriched waters and are responsible for massive developments (El-Sayed et al., 1983; Eilertsen and Taasen, 1984; Rey and Loeng, 1985; Bätje and Michaelis, 1986; Weisse et al., 1986; Davidson and Marchant, 1987; Lancelot et al., 1987; Gunkel, 1988; Al-Hasan et al., 1990). The predominance of one morphological form on the other has been shown to have a strong influence on the trophodynamic structure of *Phaeocystis*-dominated ecosystems, due to the large size difference existing between both forms (Lancelot et al., 1987; Davidson and Marchant, 1992). *Phaeocystis* free-living cells, due to their small size, have been shown to be actively grazed by protozoa (Admiraal and Venekamp, 1986; Weisse and Scheffel-Möser, 1990) emphasizing the importance of the microbial food-web. Colonies, on the other hand, while little grazed in shallow environments (Hansen and van Boekel, 1991; Weisse et al., 1994), were shown to constitute a source of food for some mesozooplankton and metazoa species in deep-waters environments (Weisse et al., 1994).

The full knowledge of *Phaeocystis* life cycle, including the detailed description of all morphological forms as well as the factors controlling the transition from one form to another is thus prerequisite for understanding the ecological structure and functioning of *Phaeocystis*-dominated ecosystems. Numerous morphological studies were conducted for this purpose under laboratory conditions, using unialgal *Phaeocystis* cultures (Kayser, 1970; Parke et al., 1971; Veldhuis and Admiraal, 1987; Rousseau et al., 1990; Cariou, 1991; Riegman et al., 1992), mesocosm (Verity et al., 1988a, b) and field (Bätje and Michaelis, 1986; Veldhuis et al., 1986; Cadée, 1991) conditions. Surprisingly, nothing really new could be deduced from these investigations since the morphological description of some stages of *Phaeocystis* life cycle by Kornmann (1955). Even in recent laboratory studies, a great deal of confusion subsists about the different life forms, since they can be quite difficult to distinguish using conventional observation techniques. Flagellates are mentioned in numerous papers on *Phaeocys-*

*tis* (e.g. Riegman et al., 1992): in most cases, it is not clear, however, whether authors observed flagellates (swarmers) sensu Kornmann (1955) or microzoospores. An additional difficulty stems for the considerable taxonomic confusion and uncertainties about identity of *Phaeocystis* species or strains (Sournia, 1988; Baumann et al., 1994). In field observations, data interpretation is sometimes difficult due to the possible presence of different *Phaeocystis* species and of selective grazers feeding preferentially on one morphological stage.

In this paper, existing data on *Phaeocystis* life cycle are reappraised in the light of unpublished microscopic and flow cytometric observations in culture and field conditions. On this basis, evidence and new hypotheses about the *Phaeocystis* life cycle and its controlling factors are presented.

Referring to the criteria developed by Jahnke and Baumann (1987) and Baumann et al. (1994) for identifying the different *Phaeocystis* species, nearly all investigations made on cultured material refer to the only *P. globosa* Scherffel species. Although it is the most widely used taxa in literature, very few informations concern indeed the life cycle of *P. pouchetii* (Hariot) Lagerheim (Ostenfeld, 1904; Gunkel, 1988) and no reference to the life cycle of *P. scrobiculata* Moestrup, has been made in literature. Even, the colonial stage of this latter species, has, at the present time, never been observed. It is therefore questionable whether the sequence of events and regulating factors are the same for the different identified species. Here, we will always refer to *P. globosa* Scherffel, unless mentioned otherwise. Moreover, in order to avoid extending the confusion that already exists in literature, we will always use Kornmann's (1955) nomenclature for referring to the different cell types, despite the warning made by Sournia (1988) for a blind use of words such as spore, zoid, swarmer,... This deliberate choice is justified by the fact that Kornmann's (1955) observations constitute still today the most complete and the only comparative study of the different *Phaeocystis* cell types.

## 2. Observations in culture

### 2.1. The different Phaeocystis cell types

Beside colonial cells, four different *Phaeocystis* free-living cells have been described, based on their size, motility and DNA content:

*Free-living cells derived from the transformation of colonial cells released into the external medium*

At least two morphotypes of free-living cells originating from the transformation of colonial cells have been identified on basis of their size and motility:

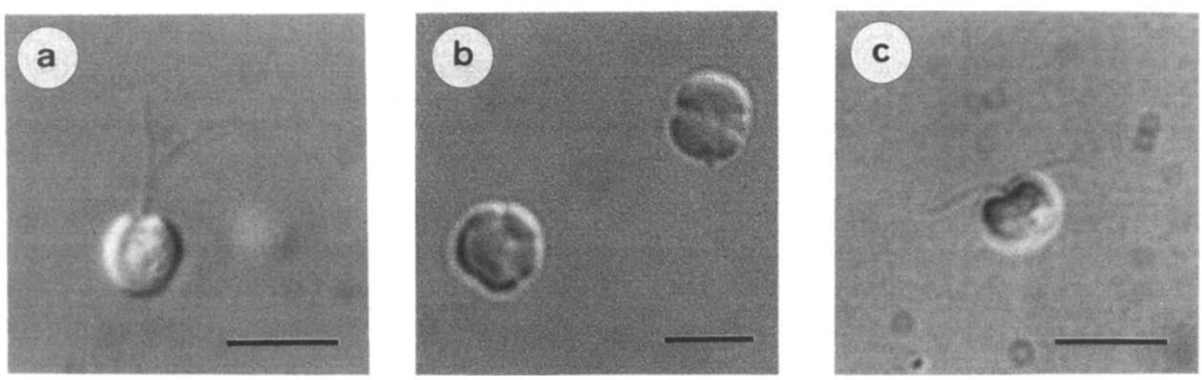


Fig. 1. Free-living cell types of *P. globosa*. Cells were fixed with glutaraldehyde 1% and viewed under Nomarski interference contrast. Scale bar = 5 $\mu$m. (a) Flagellate (swarmer) that appeared a few hours after the release of non-motile colonial cells due to colony disruption (strain ROSKO-A); (b) Non-motile cells (Strain NIOZ); (c) Microzoospore that appeared in a 2 month-old culture (strain PCC540) (photogr. by R. Casotti).

*Flagellates or swarmers*

These cells (Fig. 1a) were identified as flagellates produced after colony disruption, when initially non-motile colonial cells released from the colonial matrix in the culture medium, develop flagella within a few hours (Kornmann, 1955; Cariou, 1991). The life span of these flagellates is however very short (Kornmann, 1955: "Das kurzzeitige Schwärmerstadium...") and it is not clear whether these cells are capable of cellular division. These motile cells possess two flagella, one haptonema and their size is quite similar to that of original colonial cells, i.e. 4.5–8 $\mu$m (Kornmann, 1955).

*Non-motile free-living cells*

Visual observation gives evidence of the short life span of the swarmers: within 24 to 48 h, their majority (90%; Cariou, 1991) disperse in the culture flasks, lose their motility and settle usually on the walls and the bottom of the culture flasks (Kayser, 1970; Cariou, 1991). These non-motile free-living cells (Fig. 1b) are similar to colonial cells, in particular with respect to the cell size (Rousseau et al., 1990) and cannot be differentiated from colonial cells released into the medium immediately after colony disruption. The size similarity of swarmers and non-motile free-living cells is confirmed by data of light scattering (size index) measured by flow cytometry (Table 1). Moreover, comparison of flow cytometric signature (Table 1) indicates that both cell types are characterized by the same ploidy level. These cells have been shown capable of vegetative division (Kayser, 1970; D. Vaulot and R. Casotti, unpublished data) and have a strong ability to generate new colonies by secreting the polysaccharidic substances composing the colony matrix (Kayser, 1970). By successive divisions, the cell number increases in the colony while this latter is increasing in size (Kornmann, 1955). This sequential pathway constitutes the most common mechanism to induce the formation and growth of new colonies. However, there is presently not enough evidence that the flagellate cell stage is a necessary intermediate for initiating colony formation and that the above pathway constitutes the only mechanism to generate *Phaeocystis* colonies.

Table 1
Flow cytometric signatures of the different free-living cell types of *P. globosa*. Forward and right angle scatters (FALS and RALS respectively) are relative indexes of size, while the DNA level of G1 phase indicates the ploidy level of the cells. Mean ± standard error of each parameter are expressed relative to 2.07 $\mu$m fluorescent beads (Pandex). $n$ = number of samples analysed. Method: Analytical protocol slightly modified from Boucher et al. (1991) as follows: Preservation of cells in liquid nitrogen after fixation with glutaraldehyde 1% or paraformaldehyde 0.5%. Staining with 25 $\mu$g/ml of Chromomycin A3 (Sigma) and flow cytometric analysis at 457 nm and 100 mW [EPICS V (Coulter Electronics)]. (R. Casotti, unpubl. data).

| | Non-motile cells | Flagellates (swarmers) | Microzoospores |
|---|---|---|---|
| FALS | 8.51 ± 0.21 | 12.84 ± 0.54 | 4.51 ± 0.79 |
| RALS | 0.47 ± 0.01 | 00.44 ± 0.00 | 0.24 ± 0.05 |
| DNA level of $G_1$ phase | 1.14 ± 0.02 | 01.04 ± 0.09 | 0.58 ± 0.03 |
| $n$ | 128 | 2 | 6 |
| Strains | DCZ02<br>NIOZ<br>PCC540<br>ROSKO-A<br>ROSKO-E | PCC540 | PCC540<br>ROSKO-A<br>ROSKO-E |

Strains: DCZ02 and NIOZ (provided by M. Veldhuis and W. van Boeckel, Texel, The Netherlands); PCC540 (provided by the Plymouth Culture Collection, Plymouth, U.K.); ROSKO-A and ROSKO-E (isolated in Roscoff, France).

The property of these cells to adhere to solid surfaces explains their label "benthic stage" (Kayser, 1970; Parke et al., 1971; Sieburth, 1979; Chang, 1984; Sournia, 1988). There is nevertheless absolutely no evidence for a truly differentiated benthic stage, as observed in *Hymenomonas carterae* Braarud, another prymnesiophyte (von Stosch, 1967).

*Microzoospores*

Kornmann (1955) identified a second type of flagellate cells, called microzoospores because of their smaller size (3–5 $\mu$m). Microzoospores (Fig. 1c) have been identified in senescent cultures after colony disappearance (Kornmann, 1955) or in conjunction with non-motile cells and colonies (R. Casotti, unpubl. obs.). The process of microzoospore formation is presently unknown. Interestingly, flow cytometric signature indicates that

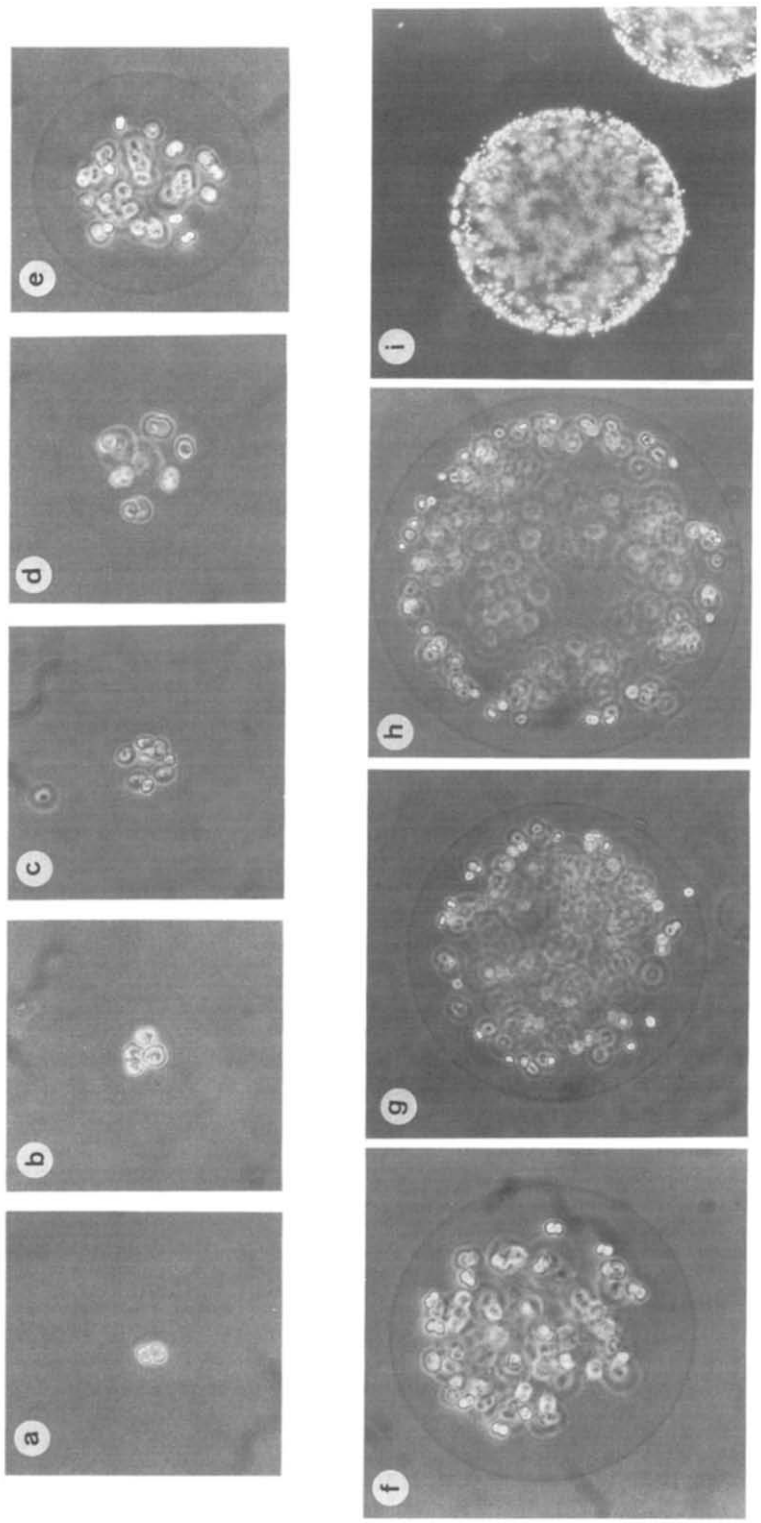


Fig. 2. Different development stages of *P. globosa* colonies (strain from Plymouth Culture Collection). Culture conditions: inoculum with colonial cells released from their matrix by mechanical disruption; culture medium of Veldhuis and Admiraal's (1987) with $NO_3^-$, $NH_4^+$ and $PO_4^{3-}$ concentrations: 50, 25 and 5 $\mu$M, respectively; temperature: 10°C; irradiance: 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ under 12 h light: 12 h dark cycle. Microphotographs were taken under inverted microscope (Leitz Fluovert) from living specimens sampled daily and representing the predominant stages of colony development in culture during a 10-day period. Colony diameters are respectively: a. 14 $\mu$m; b. 28 $\mu$m; c. 43 $\mu$m; d. 61 $\mu$m; e. 108 $\mu$m; f. 148 $\mu$m; g. 232 $\mu$m; h. 290 $\mu$m; i. 925 $\mu$m (photogr. by V. Rousseau).

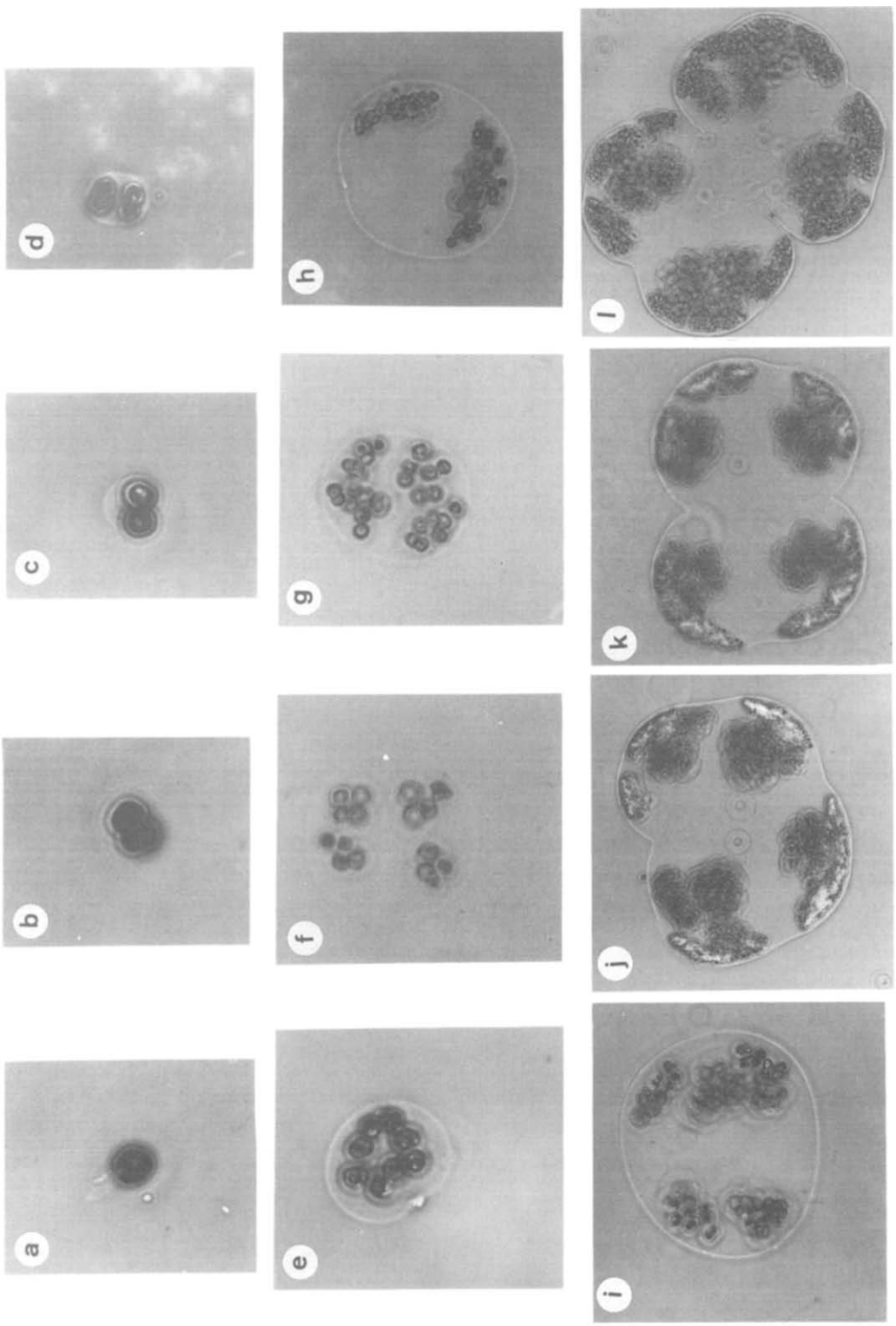


Fig. 3. Sequential development of *P. pouchetii* (strain isolated from the Greenland Sea) from free-living cells to cloud-like colonies. The characteristic grouping of cells within the mucilaginous matrix is clearly visible since the 16-cell stage (picture f). Culture conditions: inoculum with free-living cells originating from a culture in exponential phase; culture medium of Jahnke and Baumann (1987); temperature: (0°–2°C; continuous irradiance of 30–40 $\mu E \cdot m^{-2} \cdot s^{-1}$. Photographs were taken under inverted microscope from living specimens sampled daily and representing the dominant stages of colony development in culture during a 10-day period. Colony sizes are respectively: a. 8 $\mu$m; b. 12 $\mu$m; c. 15/18 $\mu$m; d. 16/24 $\mu$m; e. 40/48 $\mu$m; f. 68/68 $\mu$m; g. 60/75 $\mu$m; h. 125/140 $\mu$m; i. 150/180 $\mu$m; j. 370/470 $\mu$m; k. 400/560 $\mu$m; l. 700/890 $\mu$m (photogr. by J. Gunkel).

microzoospores distinguish themselves from above cell types by their significantly smaller size, and by their half DNA content (Table 1). Cells have been found in either $G_1$, S or $G_2$ phases of the cell cycle (R. Casotti, unpubl. data) confirming that they are capable of vegetative division (Kornmann, 1955). In 1971, Parke et al. published a very detailed ultrastructural study of cells referred as zoids, the most likely Kornmann's microzoospores, owing to their size range. Their work revealed two types of cells. Both types possess two equal heterodynamic flagella, a short stout haptonema with a distal swelling, an anterior depression, two types of organic body scales, chrysolaminarin vesicles and two chloroplasts. They differ by the presence, in only one type, of

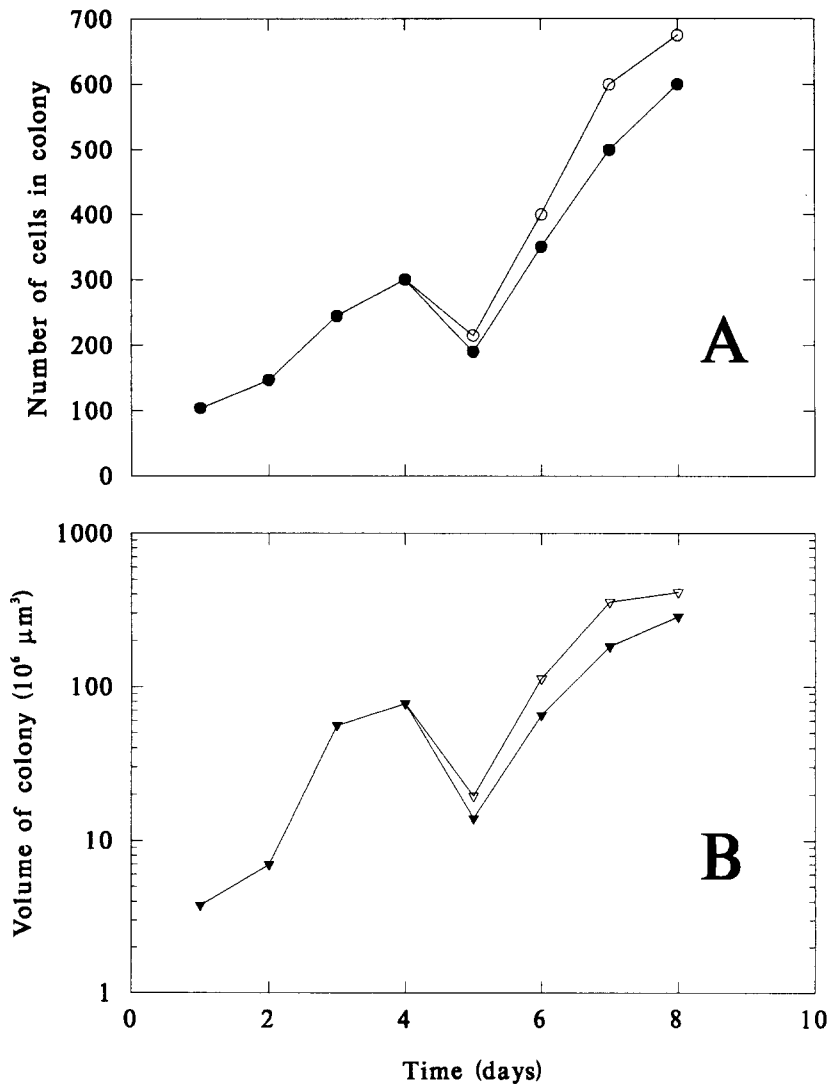


Fig. 4. Cleavage of a large colony into two daughters, each containing nearly the same number of cells. (A) Colony cell number and (B) Colony volume. Sum of volumes of daughter colonies is about 40% of the volume of the mother colony as an indirect evidence of colony matrix loss following colony cleavage. Culture conditions: inoculum with an isolated *P. globosa* colony (strain PCC540); culture medium of Veldhuis and Admiraal's (1987) with $PO_4^{3-}$ concentration of 2.5 µM; continuous illumination of 100 $\mu E \cdot m^{-2} \cdot s^{-1}$; temperature: 15°C. Daily monitoring of colony cell number and volume (V. Cariou, unpubl. data).

superficial vesicles that release a thread-like material forming a five rays star pattern. This feature has been used as an important taxonomic criterion to identify different species among *Phaeocystis* free-living cells (see in particular Moestrup, 1979).

*Macrozoospores*

In addition to the swarmers and the microzoospores, Kornmann (1955) observed in his cultures, a third type of flagellates: the macrozoospores. These cells were shown to appear inside colonies of 50 to 150 $\mu$m in diameter that did not further increase in size. Some of these cells regenerated new colonies, either inside the colonies themselves or, after emigration from the colonies into the external medium. It is not clear whether these cells were morphologically different from either swarmers or microzoospores. Their formation seems to be linked to inadequate growth conditions for colonial stage and would constitute an anomaly in colony development. Accordingly, macrozoospores have never been mentioned as such after Kornmann's description, although development of flagellate cells within colonies followed by emigration has been subsequently reported (Verity et al., 1988b).

*Colonial cells*

Colonial cells are non-motile cells which size ranges between 4.5 and 8 $\mu$m. They have two or four chloroplasts and contain a vesicle of chrysolaminarin (formerly leucosin) that can be stained with cresyl blue (Scherffel, 1900; Kornmann, 1955). Their ultrastructure has been studied by electron microscopy (Chang, 1984) although taxonomic identity of the described species is not clear (Baumann et al., 1994). This microscopic analysis showed that colonial cells are deprived of flagella and haptonema, possess a longitudinal groove, lack the organic scales covering microzoospores and are surrounded by a mucilage envelope composed of about 10 layers roughly 0.5 $\mu$m wide.

### 2.2. Colonial stage development

The sequential development of a colony from a free-living cell itself originating from colony disruption has been observed in culture for both *P. globosa* (Kornmann, 1955; Fig. 2) and *P. pouchetii* (Gunkel, 1988; Fig. 3). In its earliest stage, the colonial development is similar for both species with dividing cells remaining located in the centre of the colony. Differentiation in colony development occurs at the 16 cell-stage. At this stage, *P. pouchetii* colony transforms from a spherical to a cloud-like shape showing the well-known typical group arrangement of the cells within the mucilaginous matrix (Fig. 3). In a *P. globosa* colony, cells progressively migrate towards the edge of the colony and remain located on a spherical surface, 15–20 $\mu$m away from the border of the colonial matrix (Fig. 2). Usually, cells are regularly distributed on the periphery of the colony. However, polarized colonies with cells accumulated on one side have been occasionally observed in both undisturbed cultures (Kornmann, 1955; Cariou, 1991) and mesocosms (Verity et al., 1988b). As *P. globosa* colonies grow in size, some of them may progressively lose their spherical shape and become elongate, digitate or bladder-like (Kornmann, 1955; Rousseau et al., 1990). The division of a large colony into two smaller daughters of either similar sizes containing nearly the same numbers of cells (Fig. 4) or into several colonies of different sizes has been observed both in pure cultures (Kornmann, 1955; Rousseau et al., 1990; Cariou, 1991) and in mesocosms (Verity et al., 1988a). The regeneration of entire colonies from fragments has also been observed by Kornmann (1955).

Colony diameter varies from 10 $\mu$m up to 8 mm (Kornmann, 1955) or even 20 mm (Kayser, 1970) for *P. globosa*. The maximum size recorded for *P. pouchetii* does not exceed 2 mm (Baumann et al., 1994). A significant logarithmic relationship between cell number per colony and colony volume (Fig. 5) has been established for several *Phaeocystis* species from diverse origins: *P. globosa* (Kornmann, 1955; Rousseau et al., 1990), *P. pouchetii* (Gunkel, 1988) and *Phaeocystis* sp. from Antarctica (Davidson and Marchant, 1987). Fig. 5, which compares these data with the regression line established in culture for *P. globosa* by Rousseau et al. (1990), suggests that the calculated relationship is globally valid for the differ-

ent *Phaeocystis* species. The 1.96 exponent of the relationship indicates that the relative importance of the mucilaginous matrix dramatically increases with the size of the colony.

Within the colony, cell division occurs by usually synchronous binary fission (Kornmann, 1955). The resulting number of colonial cells is then expected to be a power of two. Some evidence of asynchronous colonial cells division has been

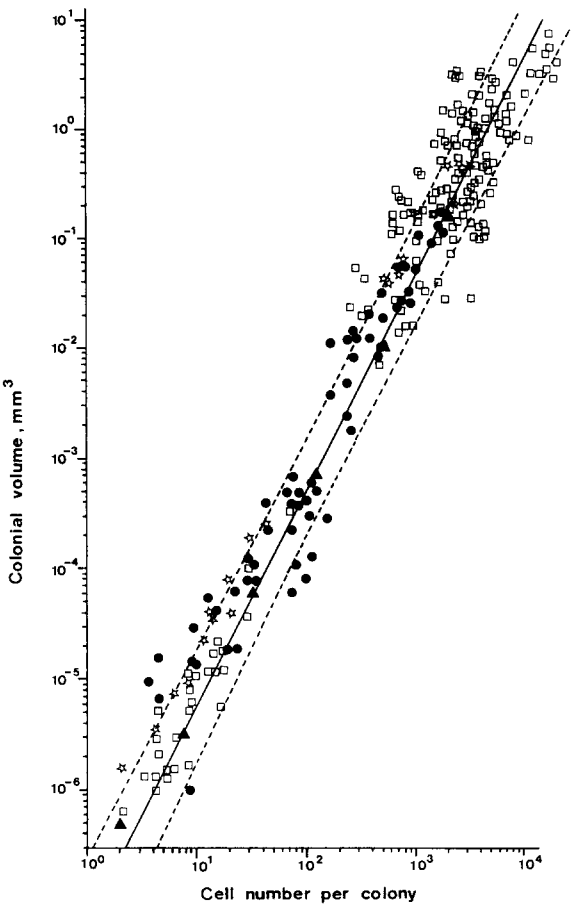


Fig. 5. Relationship between colony cell number and colony volume established for different *Phaeocystis* strains and compared with the regression line and its confidence interval at 99% calculated for a growing *P. globosa* culture (Rousseau et al., 1990); ▲ *P. globosa* (Kornmann, 1955), □ *P. globosa* (Rousseau et al., 1990), ● *Phaeocystis* sp. Antarctic strain (Davidson and Marchant, 1987); ☆ *P. pouchetii* (Gunkel, 1988). Equation of the regression line is: $\log C = 0.51 \log V + 3.67$ where $C$ is the colony cell number and $V$ is the colonial volume expressed in $mm^3$.

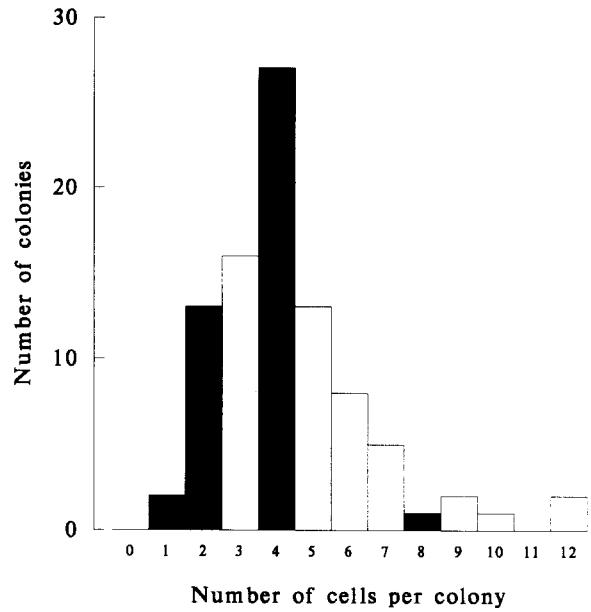


Fig. 6. Frequency histogram of cell number per colony in a *P. globosa* (strain PCC540) culture. The dark bars correspond to the numbers of cells per colony expected for synchronous cell division. Culture conditions: in K culture medium (Keller et al., 1987); temperature: 13°C; continuous illumination of 100 $\mu E \cdot m^{-2} \cdot s^{-1}$. *Phaeocystis* colony sampling in the exponential growth phase. Staining with Alcian blue and inverted microscopic examination (D. Vaulot, unpubl. data).

however reported for *Phaeocystis* examinated either under light microscopy (Kornmann, 1955; Fig. 6) or time lapse video microscopy (J.-L. Birrien, unpubl. data). From these observations, it is suggested that synchrony of the division inside colonies would be induced by the light regime.

Two phenomena are generally observed at the decline of a colony culture growth: (i) microaggregate formation through the progressive invasion of colony mucus by bacteria (Guillard and Hellebust, 1971; Davidson and Marchant, 1987) leading ultimately to the complete degradation of the colonies and (ii) "ghosts" colonies formation due to the emigration into the external medium of flagellates issued from the transformation of non-motile cells within healthy spherical colonies (Kornmann, 1955; Verity et al., 1988b; Cariou, 1991). It is not clear, however, whether these cells are diploid flagellates or/and haploid microzoospores.

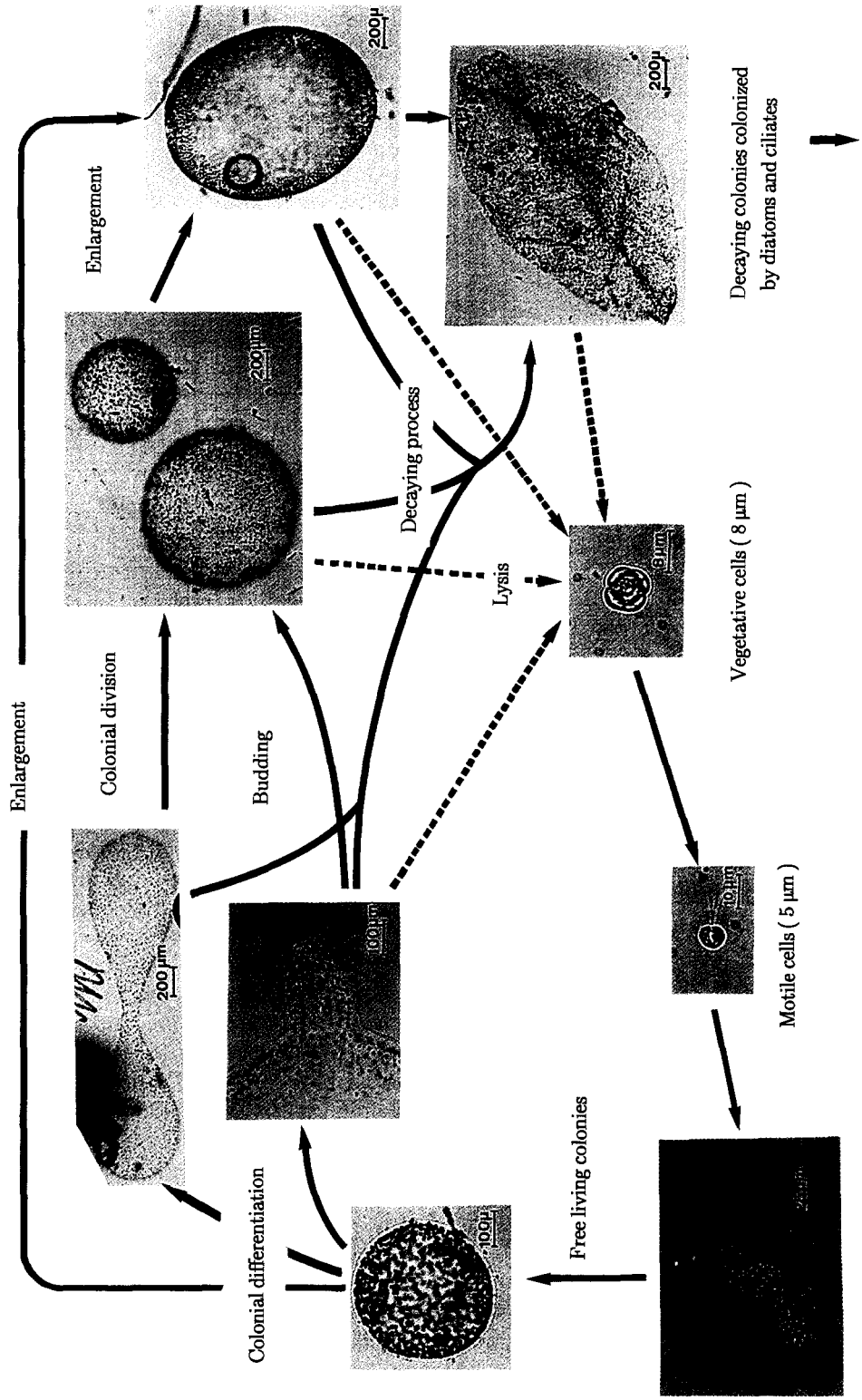


Fig. 7. Sequence of events occurring during a spring bloom of *P. globosa* in 1988 in the Belgian coastal waters of the North Sea as identified by a microscopic morphometric analysis of free-living cells and colonies. Surface seawater samples were collected 2–3 times/week at station N 51°26.05; E 002°49.08 with a bucket in order to avoid colony disruption and fixed with a lugol-glutaraldehyde solution. *Phaeocystis* colonies and free-living cells were enumerated under light microscope (Leitz Fluovert) using Utermöhl concentration method, at a magnitude of 40 or 100 and 1000 respectively. Morphological analysis was conducted as described in Rousseau et al. (1990) (V. Rousseau, unpubl. data).

## 3. Field observations

The sequence of events characterizing a *P. globosa* bloom development in natural environment has been identified through a detailed light microscopy analysis of morphological stages that succeeded each other during the spring bloom 1988 in the Belgian coastal waters (Fig. 7). This

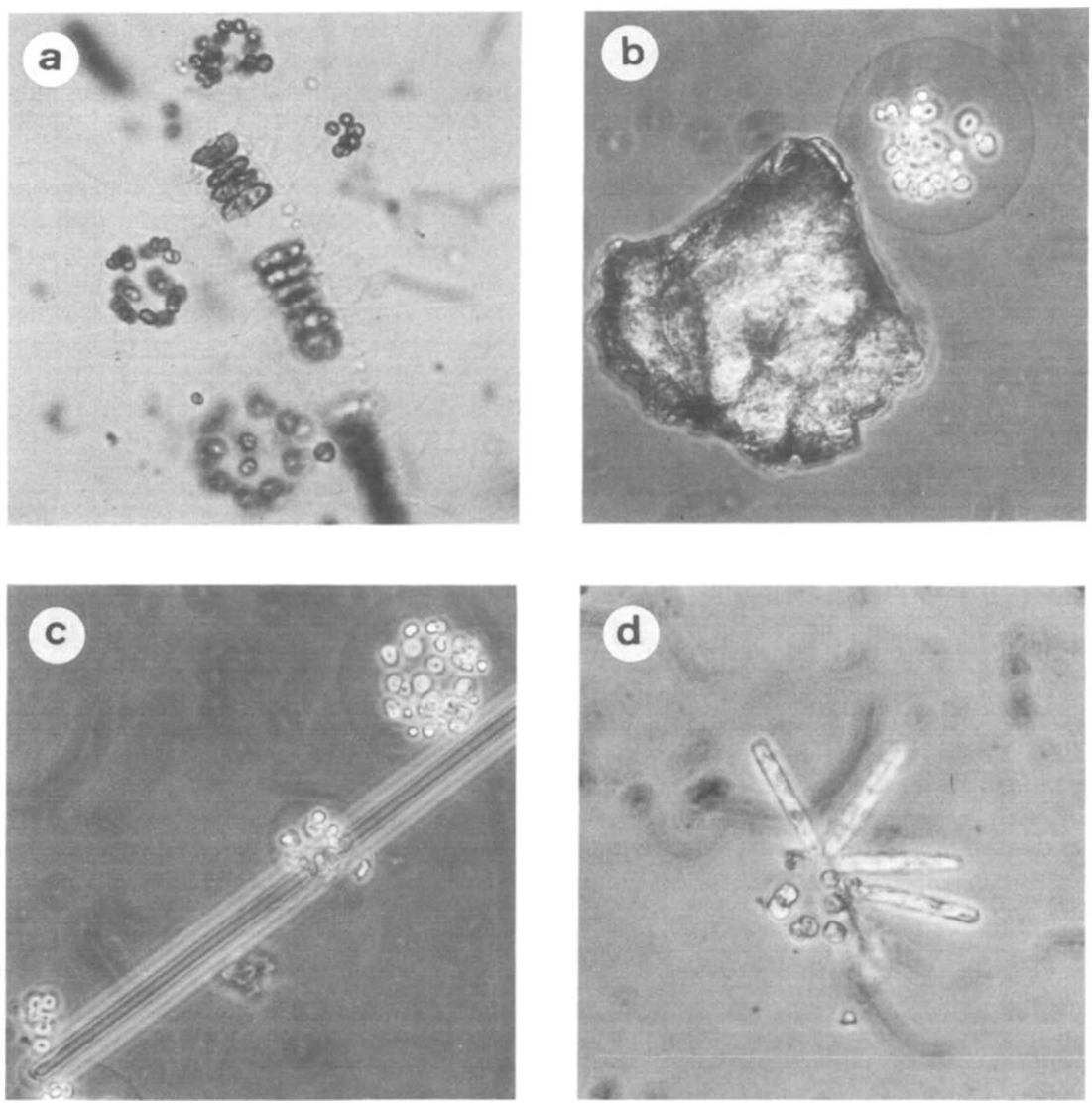


Fig. 8. Young spherical *P. globosa* colonies less than 50 $\mu$m in diameter attached to different solid substrates: (A) on *Chaetoceros* sp. setae as observed in the Belgian coastal waters during the early stage of the spring bloom 1988 (methods as in Fig. 7). (B) on living diatom *Asterionella* sp. (C) on a sand particle and (D) on a glasswool fiber. Culture conditions: inoculum with free-living cells obtained by mechanical disruption of colonies; culture medium of Veldhuis and Admiraal's (1987) with $NO_3^-$, $NH_4^+$ and $PO_4^{3-}$ concentrations: 50, 25 and 5 $\mu$M, respectively; temperature: 10°C; illumination of 80 $\mu E \cdot m^{-2} \cdot s^{-1}$ under a 12 h light–12 h dark cycle (V. Rousseau and T. Davies, unpubl. data).

study shows that the complex events evidenced in pure culture of *Phaeocystis* are also occurring under natural conditions.

### 3.1. Colony growth

The early stage of the bloom development is dominated by young healthy spherical colonies that succeed to a *Chaetoceros*-dominated diatom community. These colonies, less than 50 μm in diameter, are usually located on the setae of the diatom *Chaetoceros* spp. (Fig. 8a) whereas free-living colonies of this size are seldom observed. The formation of these small colonies seems to be strictly linked to the occurrence of *Chaetoceros* spp. This phenomenon, also observed in other *Phaeocystis*-dominated populations (Boalch, 1987), suggests that some *Chaetoceros* species would play a key-rôle in the development of *Phaeocystis* bloom by acting as a solid substrate. However, according to Cadée and Hegeman (1991), *Chaetoceros* cells would be too sparse to support all colonies, suggesting that, if required, other species or particles could act as substrate for colony development. As the bloom evolves, spherical colonies released from *Chaetoceros* setae undergo differing development. Part of them keeps spherical form and increase in size, covering a large range of diameter (50 μm–2 mm). Others change from spherical to elongate form and produce new daughter colonies by budding or division. This differentiation results, at the top of the bloom, in the complex coexistence of a high diversity of colony shapes and sizes, also observed in German coastal waters (Bätje and Michaelis, 1986).

### 3.2. Senescent stage and bloom termination

Decaying colonies are very scarce in the early stage of the bloom but appear in great numbers during the course of the bloom development. Senescent colonies are irregular in shape, less turgid and have a sticky mucus which appears less consistent compared to healthy colonies. Their large range of size (200 μm–2 mm) indicates that they originate from healthy colonies of different age. Senescent colonies are progressively invaded by various auto- and heterotrophic microorganisms and are covered by inorganic detritus, leading to the formation of aggregates of various size and composition at the end of the bloom. *Phaeocystis*-derived aggregates composed of mucus, *Phaeocystis* cells, diatoms, ciliates, dinoflagellates and heterotrophic nanoflagellates constitute micro-environments where a complete trophic food-web develops (Weisse et al., 1994). Their sudden disappearance from the water column may result from sedimentation, consumption, desintegration in the water column (Thingstad and Billen, 1994; Wassmann, 1994; Weisse et al., 1994) or advective transportation. Concomitantly with aggregate formation, at the end of the bloom, small flagellate cells similar to the microzoospores described in cultures, were observed to develop inside colonies and subsequently migrate outside. This has been observed for both *P. globosa* (Scherffel, 1900; Jones and Haq, 1963; Parke et al., 1971; Cadée and Hegeman, 1986; Veldhuis et al., 1986a) and for *P. pouchetii* (Gunkel, 1988).

### 3.3. The free-living cell stage

In the field, low densities of free-living flagellate cells were observed to precede the formation of the colonial form (Tande and Båmstedt, 1987; Davies et al., 1992) and persisted along the *Phaeocystis* colony development. The nature of the initial colony-forming cells could, however, not be identified due to the inadequacy of the light microscopy technique usually used in field studies for identifying the *Phaeocystis* cell types present in the water. In the same way, the nature of the over-wintering *Phaeocystis* form remains unidentified. Kornmann (1955) hypothesized that *Phaeocystis* survives as motile form throughout the year. Alternatively, Cadée (1991) regularly observed *Phaeocystis* colonies during winter in Dutch coastal waters of the North Sea. He suggested that these colonies could constitute the wintering form of *Phaeocystis* providing the inoculum for the next spring bloom through the release of motile cells. The presence, during the course of the bloom, of low density of free-living cells of the same size as colonial cells, whether motile or not, (Rousseau et al., 1990; Weisse and

Scheffel-Möser, 1990) suggests that part of *Phaeocystis* colonies are continuously disrupted along the course of the bloom development.

## 4. Factors regulating the different phases of *Phaeocystis* life cycle

### 4.1. Colony formation

Existing data on the factors controlling colony formation are very scarce. The nutient status is now believed to constitute a major factor driving colony formation from free-living cells. Phosphate concentration less than 1 $\mu$M has been suggested to induce massive formation of colonies from free-living cells in batch unialgal cultures of *Phaeocystis*. Actually, a careful reexamination of these data (figs. 1 and 2 in Veldhuis and Admiraal, 1987) indicates that colonies were yet present at a wide range of phosphate concentrations (0 to 70 $\mu$M). More recently, Cariou (1991) gave experimental evidence that a threshold phosphate concentration of 0.5–1 $\mu$M was a necessary condition to generate colonies from released colonial cells. Contrasting with these results, competitive experiments carried out by Riegman et al. (1992) under laboratory controlled conditions clearly showed that *P. globosa* colony forms were absent under phosphate or ammonium limitation but dominant under nitrate control. This indicates that massive blooms of *Phaeocystis* colonies may be expected in N-controlled environments with a high new production relative to regenerated production. Accordingly, *Phaeocystis* colonies are generally blooming in marine systems enriched in nutrients either naturally (El-Sayed, 1984; Smith et al., 1991) or through anthropogenic inputs (Lancelot et al., 1987; Al-Hasan et al., 1990). The rôle nutrients could play in colony initiation is still unclear. Several hypotheses have been suggested among which the induction of cellular differentiation (e.g. from free-living cell type to colonial type) and the selective enhancement pre-existing colonial-type cells (Riegman et al., 1992) are the most probable.

The requirement of a solid substrate has been suggested by several authors as triggering factor for colony formation in batch cultures and in natural environment. Both Kornmann (1955) and Kayser (1970) found that, in cultures, the flagellate cells liberated from disrupted colonies became attached before forming new colonies. From field observations, several authors (Boalch, 1987; V. Rousseau, this work) concluded that some diatoms and more particularly some *Chaetoceros* spp. may fulfill the rôle of substrate. However, recent experimental work under controlled laboratory conditions (Rousseau and Davies, unpubl. data), gives strong evidence that any microscopic particle, either biological (e.g. diatoms), organic or mineral (sand, glasswool) may act as substrate for colony development (Fig. 8b, c, d). Supporting this, young colonies attached to the diatom *Biddulphia* sp. were observed in the German coastal waters (T. Weisse, pers. commun.). Selective grazing of the small free-living colonies, preferential adhesion of diatoms to colonies due to specific attachment properties of surface polymers or release of attracting substances are hypotheses to be tested for explaining the localization of small colonies on diatoms and more specifically on *Chaetoceros* spp. setae in the natural environment.

### 4.2. Colony differentiation

The factors that regulate colony shapes (from spherical to elongate and budding colonies) are still unknown but the influence of physical forcing (water turbulence, particles) are strongly suspected (Kornmann, 1955). The process of colony division seems to be, at least partially, regulated by nutrient concentrations although autogenic factors cannot be excluded (Verity et al., 1988a). At the end of the bloom, nutrient limitation would induce physiological changes leading to the senescence of colonies and their invasion by various microorganisms.

### 4.3. Motility development and emigration of cells from the colonies

Motility development within the colony and subsequent release of cells from colonies have

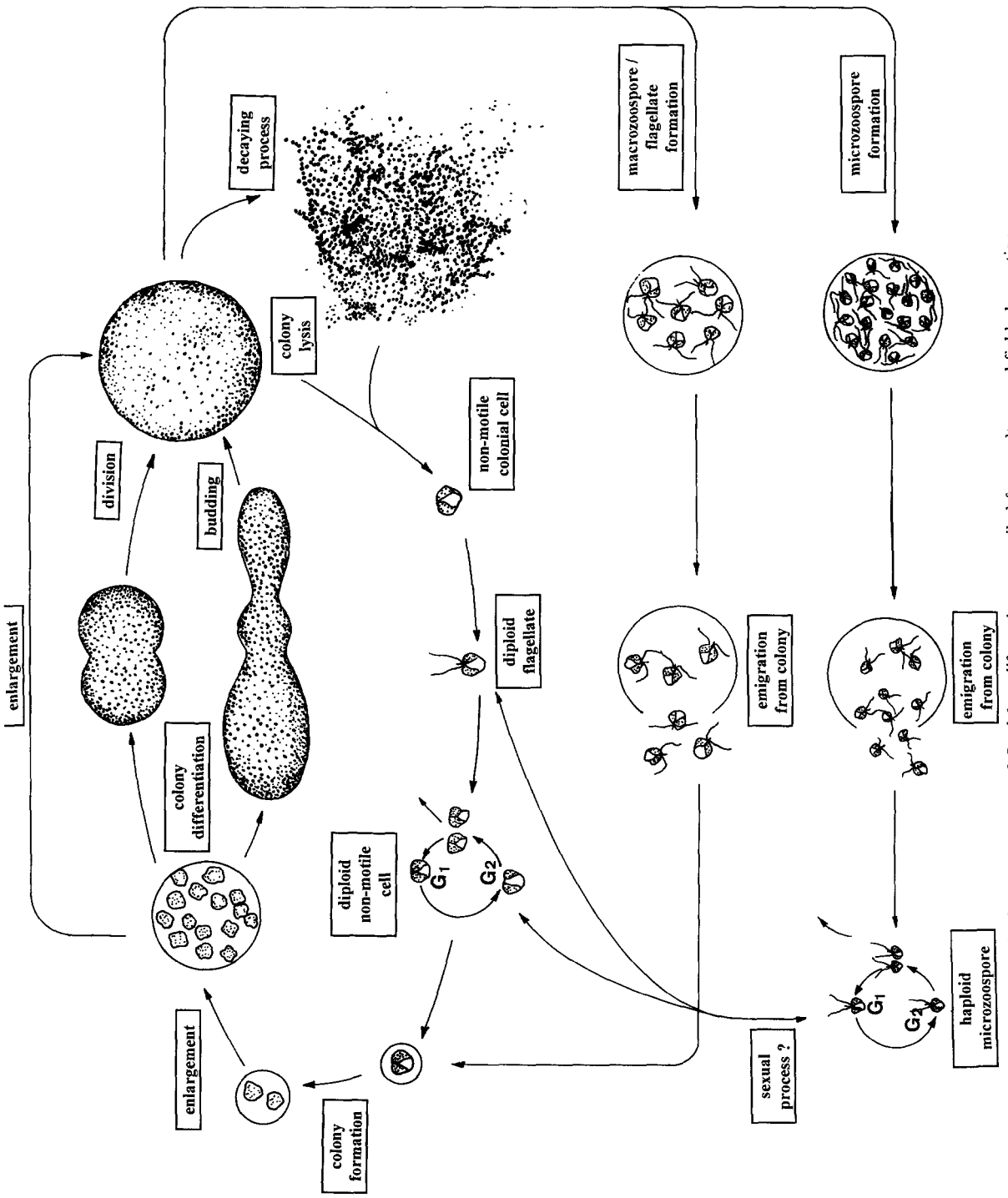


Fig. 9. Current status of *P. globosa* life cycle as compiled from culture and field observations.

been observed for colonies under stressed conditions. Nutrient limitation (Kornmann, 1955) possibly accompanied by significant temperature change (Verity et al., 1988b) have been showed to generate motility development within *Phaeocystis* colonies either under mesocosm or laboratory conditions.

## 5. Conclusions

The current knowledge of *P. globosa* life cycle synthetized from field and culture observations is illustrated by Fig. 9. At this stage, however, it is difficult to propose a coherent scheme for the place of the different cell types within *Phaeocystis* life cycle and to elucidate the pathways leading from one type to another. More has to be known about the mechanisms initiating *Phaeocystis* cellular differentiation and colony formation. As a first step in this direction, flow cytometric studies, by demonstrating ploidy differences between non-motile solitary cells and flagellates (diploid) and microzoospores (haploid), give strong support for the involvement of these latter in sexuality as already suggested by Kornmann (1955). Such alternation of haploid and diploid generations has also been observed in *Hymenomonas carterae* Braarud (von Stosch, 1967). Whether this give rise to an alternation of diploid and haploid colonies (the former originating from non-motile diploid cells and the latter from haploid microzoospores) or whether all colonies are diploid has not been demonstrated. However, no change in ploidy has been observed when diploid cells released from colonies give rise to new colonies (R. Casotti, unpubl. data), so corresponding to a vegetative multiplication of this alga favouring the further spreading of the colonial stage once initiated. This opens several questions that could be solved through further cytofluorometric investigations. Indeed, assuming all colonies are diploid, then colony formation from microzoospore-dominated cultures (Kornmann, 1955) implies either the sexual conjugation of haploid cells or alternatively the presence of a background of diploid cells in microzoospore cultures. Conversely, meiosis must intervene when microzoospores are formed in senescent cultures. None of these processes has been observed yet, suggesting that sexuality may involve a tiny percentage of the vegetative populations. In the meantime, the potential common occurrence of sexuality in *Phaeocystis*, resulting in high genetic plasticity, could be an explanation for its worldwide distribution.

Identification of the over-wintering form and of the first active form preceding colony formation, as well as the mechanisms involved in the transition between free-living cell stage and colony are essential for understanding the occurrence of *Phaeocystis* blooms. A better knowledge of processes of colony division and cell release from colony would allow to estimate the spreading of the colonial stage once initiated. Finally, the relative importance of motility development, of senescent colony and aggregate formation should be further investigated owing to their different ecological rôle in the bloom termination.

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