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Calculating carbon biomass of *Phaeocystis* sp. from microscopic observations

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Abstract. Conversion factors for calculating carbon biomass of *Phaeocystis* sp. colonies and free-living cells were determined from microscopic observations and chemical analysis conducted on cultured and natural *Phaeocystis* sp. populations originating from the Southern Bight of the North Sea in 1986 and 1987. They allow calculation, in terms of carbon biomass, of the different forms of *Phaeocystis* sp. that succeed each other when the population is growing, on the basis of microscopic observations. The latter include enumerations of free-living cells (flagellated and non-motile) and colonies, as well as colonial biovolume measurement. Specific application to natural populations from Dutch coastal waters during spring 1986 shows that more than 90% of *Phaeocystis* sp. carbon biomass is under colonial form, most of it exceeding the grazing characteristics of current zooplankton at this period of the year. Detailed analysis of seasonal changes shows in addition that the size of the colonies greatly increases during the course of *Phaeocystis* sp. flowering, reaching sizes as high as 1 mm diameter at the top of the bloom when nutrients are depleted. Physiologically this corresponds to an enhanced synthesis of mucilaginous substances, with the decrease of available nutrients leading to an increasing contribution of the matrix to the total colonial carbon during the course of the bloom. Carbon content of *Phaeocystis* sp. colonies therefore greatly varies with their size, ranging from 0.3 to 1430 ngC colony^{-1}.

Introduction

Phaeocystis sp. is a very widespread phytoplanktonic alga that massively blooms mainly in temperate (Gieskes and Kraay 1975, Cadée and Hegeman 1986, Lancelot et al. 1987) and polar waters (Kashkin 1963, El-Sayed et al. 1983, Chang 1984, Eilertsen and Taasen 1984, Palmisano et al. 1986, Davidson and Marchant 1987). This prymnesiophycean is characterized by a complex polymorphic life cycle and occurs under at least two different morphological stages: unicellular and colonial. The former is characterized by free-living cells of 3 to 8 μm either flagellated or non-motile. The latter is composed of cells devoid of flagella, embedded in a mucilaginous matrix mainly composed of polysaccharides (Chang 1984, Lancelot unpublished data). These colonies originate either from one motile single cell that loses motility, secretes its mucilaginous substances (Kornmann 1955, Lancelot and Mathot 1985) and divides inside the colony or, later in the course of the life cycle, from true colonial division (Kornmann 1955, Verity et al. 1988). Colony size varies therefore by more than two orders of magnitude during the course of their development, ranging from 10 μm to 3 mm under natural conditions. Their cellular content varies accordingly, from 2 to about 10 000 cells, and their shape greatly changes with age (Bätje and Michaelis 1986). The two morphological forms cohabit in natural environments but feed different planktonic organisms: single cells are ingested by protozoa like tintinnids and other ciliates (Admiraal and Venekamp 1986), whereas colonies in the 10 to 300 μm size range can sometimes be grazed by zooplankton, depending on the species and its development stage (Weisse 1983, Daro 1985, Verity and Smayda 1989). Ungrazed, large colonies deposit onto the bottom (Wassman 1984, Jenness and Duineveld 1985), cover beaches as layers of seafoam (Bätje and Michaelis 1986, Lancelot et al. 1987), or are degraded in the water column by planktonic bacteria (Billen and Fontigny 1987).

Regarding the trophic rôle of *Phaeocystis* sp., and its importance as a dominant species in eutroph environments, either natural like Antarctic waters or anthropogenically influenced like the well known eutrophicated North European continental coastal zones, the accurate determination of single cell and colony numbers together with their respective carbon content is of prime importance for understanding of the dynamics of *Phaeocystis* sp. blooms.

Unfortunately, there is still presently no unifying criteria for accurately assessing *Phaeocystis* sp. abundance. This is explained by the lack of adequate proce-

dures for the sampling and preservation of intact colonies and for the mechanical separation of free-living cells and colonies. Current literature reports *Phaeocystis* sp. as single cell numbers (Eilertsen et al. 1981, Cadée and Hegeman 1986, Veldhuis et al. 1986, Weisse et al. 1986), as chlorophyll *a* (Gieskes and Kraay 1975, Cadée and Hegeman 1986, Lancelot and Mathot 1987) or as colony numbers (Jones and Haq 1963, Bätje and Michaelis 1986). The first two methods, because they do not distinguish between unicellular and colonial forms, greatly overestimate *Phaeocystis* sp. single cell abundance when colonial forms are predominant, as is usual in natural environments (Lancelot et al. 1987). Indeed chlorophyll *a* concentrations refer to both colonial and free-living *Phaeocystis* sp. cells as isolated by classical filtration procedures, whilst cell counts from classical Lugol's iodine or formalin preserved samples enumerate colonial cells as well because these preserving agents dissolve the mucilaginous matrix (Chang 1984, Admiraal and Venekamp 1986). Colony enumeration, on the other hand, is of minor ecological interest when not combined with colonial size and cell content evaluation.

An important step was, however, recently achieved by Davidson (1985) and Davidson and Marchant (1987), who established an experimental procedure for the estimate of free-living and colonial cell numbers. These authors not only used a suitable preserving agent for colonial forms but established a log/log regression between the diameter of *Phaeocystis* sp. colonies growing in the Southern Ocean and their number of cells. This relationship allows the assessment of the cell content of *Phaeocystis* sp. colonies from the knowledge of their size. From this, carbon content of colonies on the one hand, and of free-living cells on the other hand, should now be estimated provided the above relationship is general for *Phaeocystis* sp. and the conversion factors for the transformation of individual cells and mucilaginous matrix are determined.

In the framework of a joint EEC Research Project on the dynamics of *Phaeocystis* sp. blooms in the North European continental coastal zones of the North Sea, we have determined conversion factors that allow one to easily calculate *Phaeocystis* sp. single cell and colony biomass on the basis of single cell and colony counts and colonial volume measurements. These factors were statistically determined by combining numerous microscopic observations including cellular and colonial volume measurements and chemical and biochemical analysis. They were established from both pure cultures and field communities dominated at more than 95% by *Phaeocystis* sp. Ecological interest of this methodology is given by application to pure culture and field populations of *Phaeocystis* sp. at different stages of their development.

Materials and methods

Phaeocystis sp. samples

Phaeocystis sp. samples originated from both field populations and unialgal cultures. Field populations were sampled in Dutch and Belgian coastal waters respectively during spring 1986 and 1987 and were directly fixed for microscopic analysis. Subsamples were treated for chlorophyll *a* and dissolved organic carbon (DOC) analysis according to methods described below.

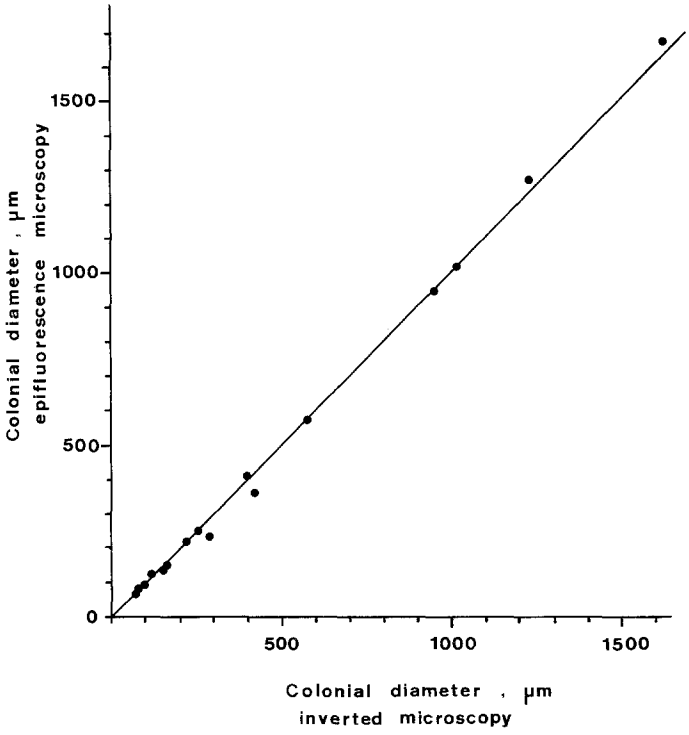

Fig. 1. *Phaeocystis* sp. Comparison between colony diameters measured by inverted and epifluorescence microscopy ($r^2 = 0.99$; $n = 16$; $y = 1.03\ x$)

Cultures of *Phaeocystis* sp. colonies were first inoculated with a suspension of free-living cells. The latter had previously been obtained by gentle filtration through a 10 µm sterile net of unialgal *Phaeocystis* sp. culture originating from the Channel (strain from Plymouth Marine Laboratory, UK). Culture medium was prepared with sterile, filtered seawater enriched as in Veldhuis and Admiraal (1987) except for nitrate, ammonium and phosphate whose concentrations were respectively 50, 25 and 5 µ*M*. Cultures were grown at 11 °C in an illuminated growth cabinet (Luminincube II, Analys) under a 12 h light:12 h dark cycle at 120 µE m^{-2} s^{-1}. Subsampling for microscopic and chemical (DOC and chl *a*) analysis was performed at short intervals during 2 wk in order to follow appearance of colonies and change in colony and single cell numbers.

Microscopic analysis

Cell and colony enumerations and biovolume measurements were carried out either by inverted (Leitz Fluovert) or epifluorescence microscopy (Leitz Laborlux D). The two methods are highly comparable as shown in Fig. 1 which compares the diameters of a large range of colony sizes measured by inverted and epifluorescence microscopy. Experimental procedures were the following: (1) Samples for inverted microscopy were preserved in glass bottles with a glutardialdehyde-lugol (35‰, v/v) solution (1 ml for 100 ml seawater) as prepared according to Thomas (personal communication) and stored in the dark at 4 °C. Fixed samples (10 ml) were sedimented for 12 h in Utermöhl plankton chambers. (2) Samples for epifluorescence microscopy (2 to 10 ml seawater) were stained with acridine orange solution as recommended by Hobbie et al. (1977). Colonies were sampled with open-end pipettes (Sterilin), collected on black-stained 0.2 µm filters using a gentle vacuum in order to avoid colony disruption. After filtration, filters were placed on microscope slides and stored in the dark at 4 °C until analysis.

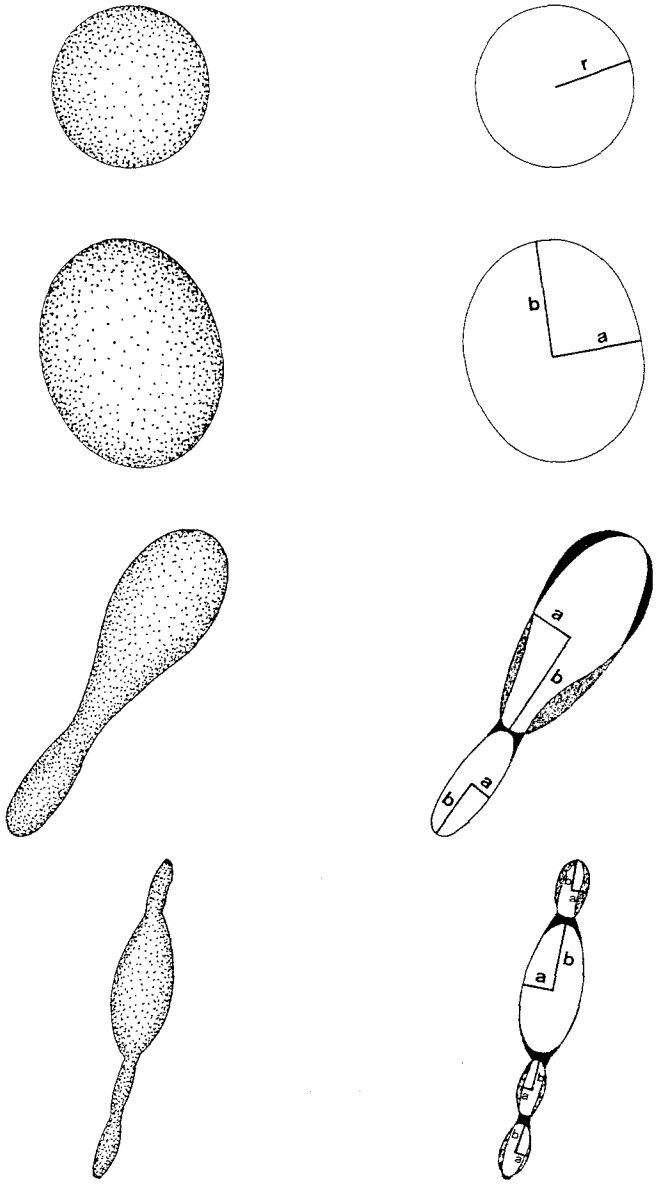

Fig. 2. *Phaeocystis* sp. Typical shapes of colonies observed in culture and field populations and their transformation in simplified geometrical forms. Volume over-estimations (stippled areas) and under-estimations (filled areas) are equivalent. Dimensions to be measured are also indicated. Ellipsoids are considered as rotating around their major axis; their volumes are then calculated by $V=4/3\,\pi\,a^2\,b$

In each case, all colonies ($n=10$ to 150) were enumerated and their size measured with a precision of ± 0.5 μm. Single cells were counted on several (10 to 30) randomly chosen fields (10 to 50 cells field^{-1}). Colony cell content determination was performed only by epifluorescence microscopy. Indeed, this method allows easy enumeration of stained cells because of their two-dimensional location. Cell and colony sizes were estimated visually by comparison to a calibrated grid. Cell volume was calculated by considering *Phaeocystis* sp. cells as ellipsoids, whilst colonial volume was calculated by considering colonies as spheres, ellipsoids or an arrangement of both. The hypothesis was formulated that ellipsoids are elongated, i.e. rotating around their major axis. Typical shapes of *Phaeocystis* sp. colonies that can be observed in culture and field populations and their transformation in a *collage* of geometrical forms are illustrated in Fig. 2.

Chemical analysis

Samples for chlorophyll *a* and DOC analysis were collected by filtration of small volumes on pre-ashed fiberglass filters (Whatman GF/C) using a high vacuum pressure in order to achieve complete colony disruption. Chlorophyll *a* was measured either by spectrophotometry (Lorenzen 1967) or by fluorescence (Yentsch and Menzel 1963) and dissolved organic carbon was determined by a persulfate, UV assisted wet oxidation, followed by infrared determination of CO_2 using Dohrmann 180 equipment.

Conversion factors

Relationship between colonial cell number and biovolume

The relationship between the colonial volume and cell number per colony was established on the basis of microscopic observations performed during the development of a pure *Phaeocystis* sp. culture, initially inoculated with single cells. Fig. 3 shows the size distribution of colonies at different stages of culture growth together with the change in colony number. Colony size, expressed in equivalent spherical diameter unit, increased with the age of the culture from 10 to 2000 μm. At the same time, a progressive change in dominant shapes from spheres to ellipsoids and irregular forms was observed. Detailed examination of temporal change in colony size and number clearly indicated that colonies are not only continuously initiated by free-living cells but also originate from the division of colonies themselves, in perfect agreement with Verity et al.'s (1988) data.

Fig. 4 shows a highly significant log/log relationship ($r^2=0.95$, $n=244$) between colonial volume and cell number per colony for the large range of colony sizes and shapes illustrated in Fig. 3. This relationship allowed us to estimate the cell content C of a colony from the knowledge of its volume V expressed in mm^3, according to the following equation:

$$\log C = 0.51 \log V + 3.67.$$

The slope of the regression line indicated that the relative importance of the mucilaginous matrix with regard to the cell number was increasing with the size of the colony.

The zero ordinate of the regression line, on the other hand, estimated at 8 μm the minimum size of a starting colony from a single free-living cell.

Validation of this empirical relationship and its applicability to *Phaeocystis* sp. colonies from several origins were examined in Fig. 5 where similar data relative to both natural populations and unialgal strains isolated from temperate and polar waters are compared with the regression line and its 99% confidence interval as calculated from data illustrated in Fig. 4. Examination of Fig. 5 suggests that the empirical log/log relationship is valuable for *Phaeocystis* sp. colonies from several origins. The higher deviation to the regression line for Southern Bight field data might be explained by the presence at the top of the *Phaeocystis* sp. bloom of large colonies characterized by highly diversified shapes whose geometrical form is sometimes difficult to define properly.

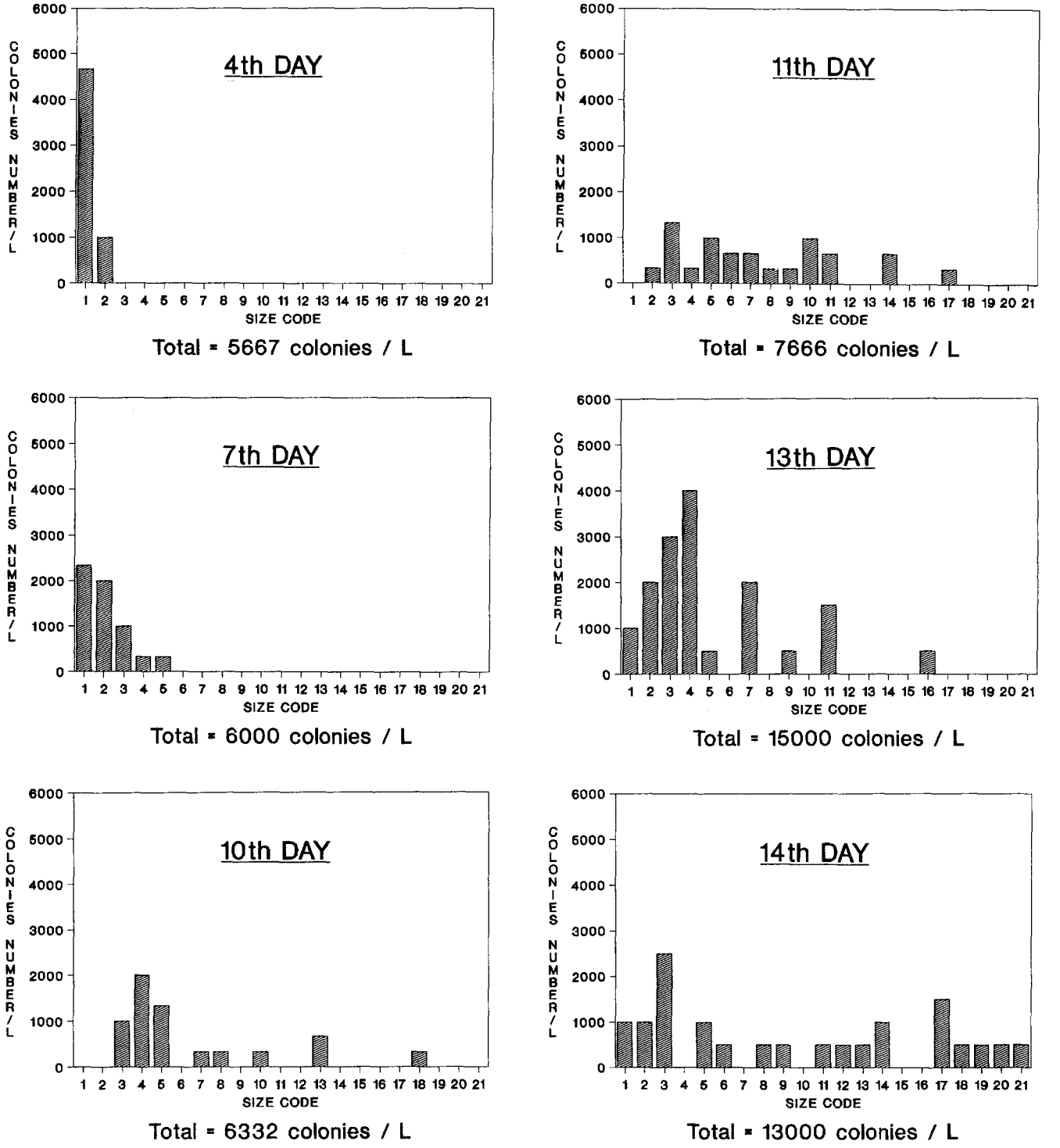

Fig. 3. *Phaeocystis* sp. Size distribution of colony number at six different stages of development of a pure culture. Sizes are expressed in terms of equivalent spherical diameter. Colonial diameter size code is as follows: (1) <50; (2) 50–100; (3) 100–200; (4) 200–300; (5) 300–400; (6) 400–500; (7) 500–600; (8) 600–700; (9) 700–800; (10) 800–900; (11) 900–1000; (12) 1000–1100; (13) 1100–1200; (14) 1200–1300; (15) 1300–1400; (16) 1400–1500; (17) 1500–1600; (18) 1600–1700; (19) 1700–1800; (20) 1800–1900; (21) 1900–2000 μm

Carbon content of free-living and colonial cells

Two procedures were used and compared to determine conversion factors for calculating carbon content of *Phaeocystis* sp. free-living and colonial cells from microscopic counts.

The most classical one is based on the determination of plasma volume from cell volume measurement and its transformation to carbon using the conversion factor 0.11 (pgC μm^{-3}) recommended by Edler (1979) for flagellates. Table 1 reports average value and standard deviation of *Phaeocystis* sp. cell carbon content calculat-

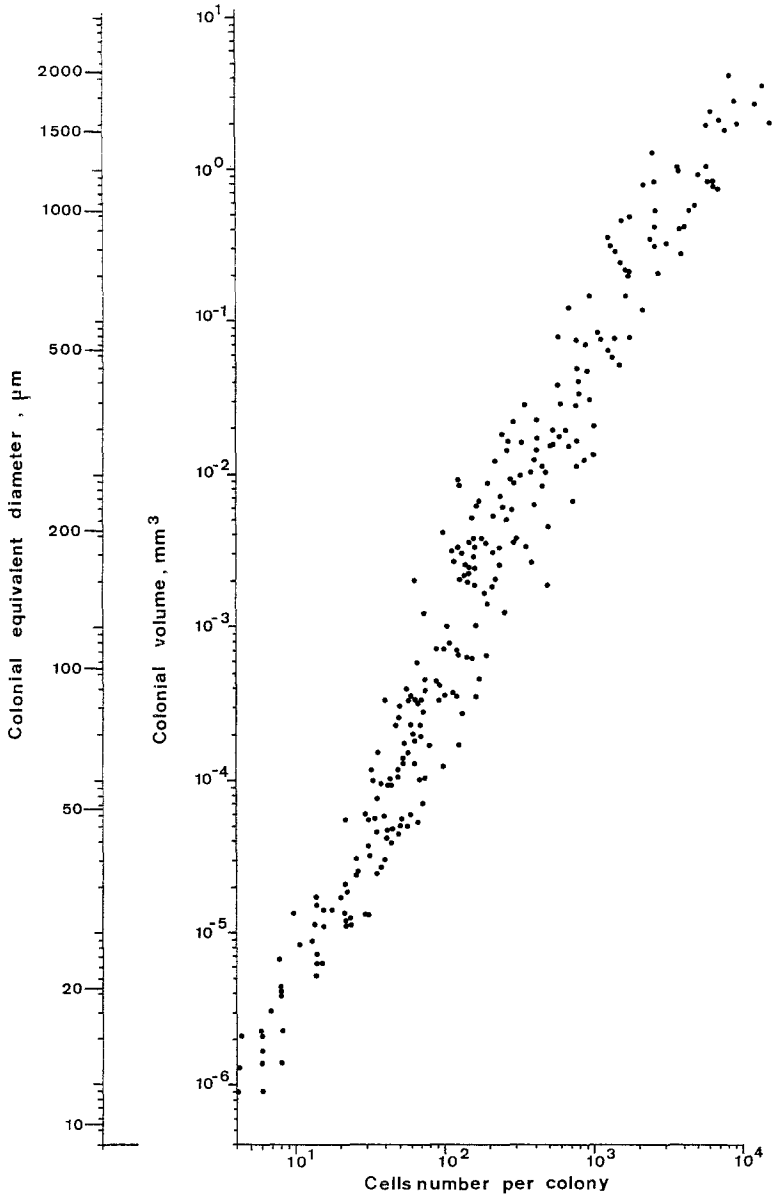

Fig. 4. *Phaeocystis* sp. Relationship between colonial volume and cell number per colony for the large range of colony sizes and shapes sampled during the batch culture development illustrated in Fig. 3. Colonial equivalent spherical diameter scale is indicated on left of volume scale

Table 1. *Phaeocystis* sp. Cell carbon content (pgC cell^{-1}) calculated from cell volume measurements

Cells	No. of observations	C content ±SD
Colonial cells	220	14.15±5.34
Free-living cells		
Flagellated	85	10.80±3.47
Non-motile	260	15.91±4.86

ed from cellular volumes relative to a large number of *Phaeocystis* sp. cells originating from pure culture and sampled in the Belgian coastal waters in spring 1988 and 1989, at different stages of the spring bloom development (Rousseau unpublished data). Examination of Table 1 shows that carbon associated with non-motile free-living cells is very close to that associated with colonial cells indicating their colonial origin. On the other hand, mean carbon content of flagellated single cells is lower, as expected from their smaller size.

The second procedure estimates a factor for the conversion of cell counts to carbon unit on the basis of the statistical regression analysis between biochemical carbon and cell number. Biochemical carbon is defined by the cell content in proteins, polysaccharides and lipids and is calculated from chlorophyll *a* concentrations using a 29 C:chl*a* (w:w) conversion factor as recommended in Lancelot-Van Beveren (1980) when *Phaeocystis* sp. dominates the community. This procedure does not discriminate, however, between colonial and free-living cells, as chlorophyll *a* concentrations refer to the whole population isolated by filtration. This statistical analysis was performed on *Phaeocystis* sp. populations sampled in the Belgian and Dutch coastal zones for different stages of the bloom development. The slope of the regression line estimates at 13.5±1.8 pg the *Phaeocystis* sp. cell carbon content ($r^2=0.81$, $n=29$, $p<0.01$). Good agreement was observed between the latter value and the carbon content of colonial and non-motile free-living cells calculated by means of the plasma volume method.

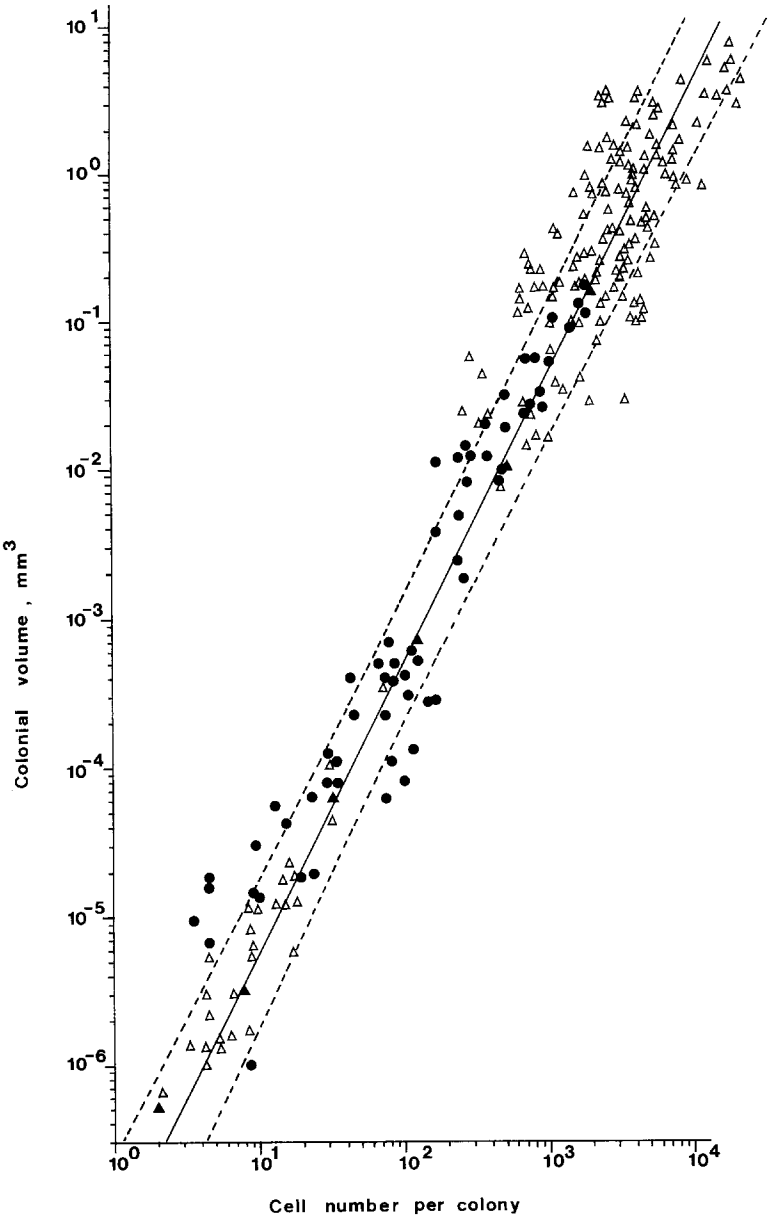

Fig. 5. *Phaeocystis* sp. Comparison between data relative to both natural populations (open symbols) and unialgal strains (filled symbols) isolated from temperate and polar waters, and the regression line and its 99% confidence interval calculated on the basis of data illustrated in Fig. 4. Data are from: (△) Belgian coastal waters (Rousseau unpublished data); (▲) German Bight (Kornmann 1955); (●) Prydz Bay (Davidson 1985)

Carbon content of mucilaginous matrix

Mucilaginous substances that compose the colonial matrix are currently separated from cells by vacuum filtration at high pressure through glassfiber (Whatman GF/C) filters (Lancelot and Mathot 1985). This mechanical procedure disrupts the colonies and solubilizes the colonial matrix into seawater. However, under these conditions, the dissolved mucilaginous compounds arising from living colonies cannot be distinguished from DOC of other origin without laborious chemical treatment including desalting, concentration of organic matter by dialysis and chemical precipitation of the mucilaginous substances.

This chemical procedure, unsuitable for routine analysis, was bypassed by utilizing statistical regression analysis between DOC concentration – a variable characterizing the total pool of dissolved organic substances – and the total colonial volume – a variable specific of the colonies. This statistical analysis was applied on several couples of data originating from both pure *Phaeocystis* sp. cultures and field communities dominated at 95% (in terms of cell number) by *Phaeocystis* sp. colonies. DOC data were, however, previously corrected for a background DOC value due either to culture medium initially enriched with vitamins or to refractory dissolved organic matter always present in natural seawater. The former has an average value of 2.8 ± 0.4 mgC l^{-1} whilst the latter reaches a mean background value of 1.1 ± 0.5 mgC l^{-1} in early spring in the coastal waters of the Southern Bight of the North Sea.

The relationship between DOC and total colonial volume of *Phaeocystis* sp. is illustrated in Fig. 6. A good correlation ($r^2 = 0.74$, $n = 24$, $p < 0.01$) is observed between total colonial volume of *Phaeocystis* sp. and its organic carbon content. The positive Y-intercept observed in Fig. 6 is due to the high variability of the background value. The slope of the regression line estimates

Table 2. *Phaeocystis* sp. Conversion factors for the determination of carbon biomass

Variable	Measurement	Conversion	Source
Colonial cell no. C	Colonial volume V	$\log C = 0.51 \log V + 3.67$	This paper Fig. 4
C biomass of			
Colonial cell	Cell no.	14.2 pgC cell^{-1}	Edler conversion factor (1979)
Motile isolated cell	Cell no.	10.8 pgC cell^{-1}	Edler conversion factor (1979)
Non-motile isolated cell	Cell no.	15.9 pgC cell^{-1}	Edler conversion factor (1979)
C content of mucilaginous matrix	Colonial volume	335 ngC mm^{-3}	This paper Fig. 6

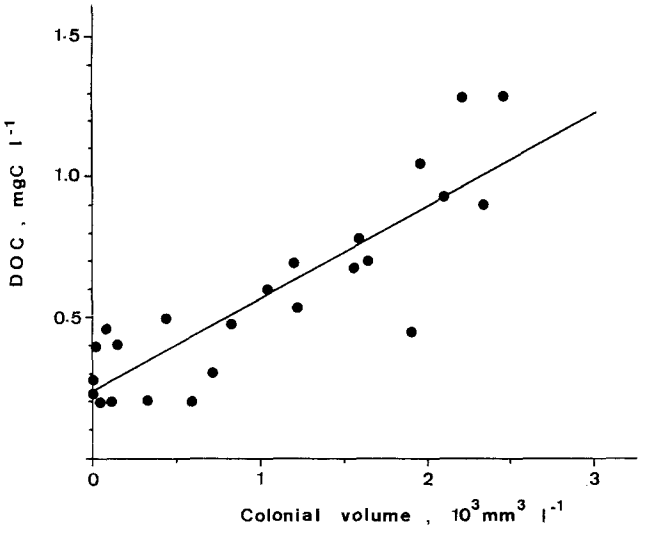

Fig. 6. *Phaeocystis* sp. Relationship between total colonial volume and corresponding dissolved organic carbon concentrations

at 335 ± 42 ngC (mm^3 colonial vol.)$^{-1}$ the factor for the conversion into carbon unit of mucilaginous biovolume.

Applications

The conversion factors recommended in the previous section are summarized in Table 2. They were used for calculating and comparing *Phaeocystis* biomass changes during its development in a pure culture and in the natural environment.

Fig. 7 shows the variations in carbon biomass of respectively free-living, colonial cells and colonies as calculated from microscopic counts and colonial volume measurements during the development of a batch culture of *Phaeocystis* sp. of which the frequency diagram of colonies is illustrated in Fig. 3. As indicated by Fig. 7, colonies develop and immediately dominate the population in terms of carbon biomass. Colonial biomass contributes indeed to 50 to 95% of total *Phaeocystis* sp. biomass during the course of culture development. Examination of Fig. 7 shows, on the other hand, that free-living cells and colonies have an identical carbon turnover rate. It indicates in addition that the specific growth rate of *Phaeocystis* sp. free-living and colonial cells, respectively 0.62 and 0.58 d^{-1}, are very close.

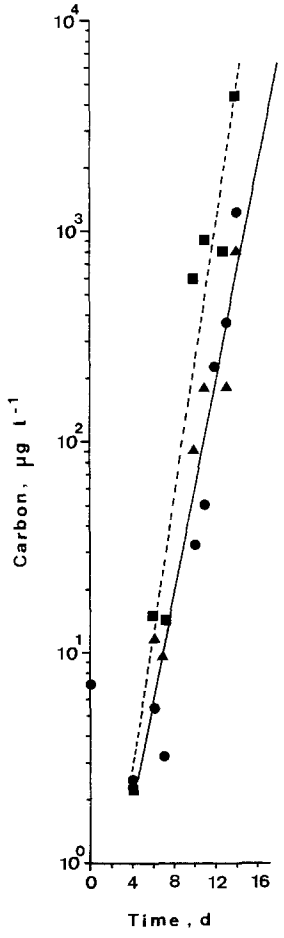

Fig. 7. *Phaeocystis* sp. Temporal change in calculated carbon biomass of free-living cells (●, continuous line), colonial cells (▲, continuous line) and colonies (■, dashed line) during the batch culture development illustrated in Fig. 3

A highly different feature is however observed when carbon data relative to *Phaeocystis* sp. colonies are discussed in terms of their size distribution. Fig. 8 shows the relative contribution per colony of different sizes of colonial cells and mucilaginous matrix to the total *Phaeocystis* sp. colonial carbon for four typical stages of the batch culture development. It clearly indicates that the specific carbon content of the colonies increases according to their size allowing a highly variable carbon/colony ratio ranging from 0.3 to 1436 (ngC colony^{-1}) for colonies whose diameters are respectively 10 μm and 2 mm. Although cellular carbon rises, the contribution of mucous carbon to total *Phaeocystis* sp. biomass is increasing much more rapidly when the size of the colonies is getting larger and becomes dominant once colonial diameter is higher than 400 μm. When colony diameter is higher than

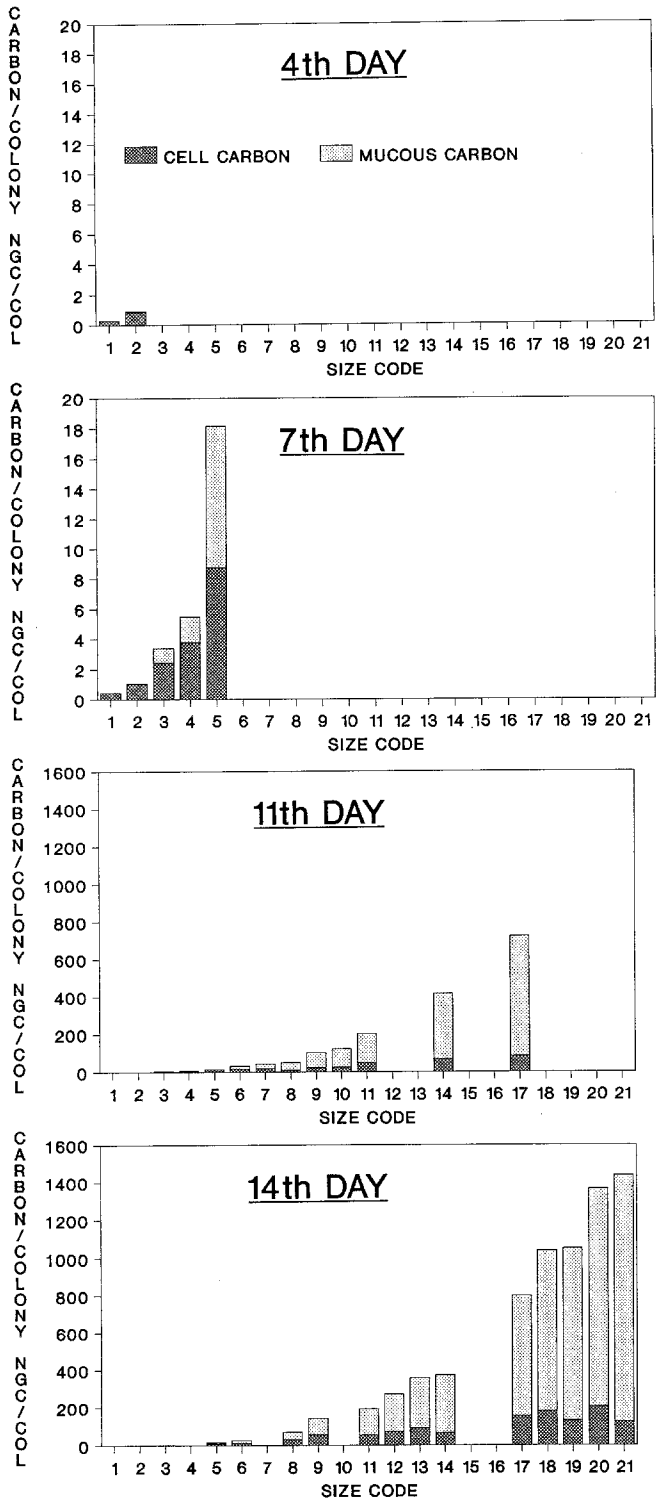

Fig. 8. *Phaeocystis* sp. Size distribution of colony carbon content with relative contribution of cellular and mucous carbon at four stages of batch culture development as illustrated in Fig. 3. For size code, see Fig. 3

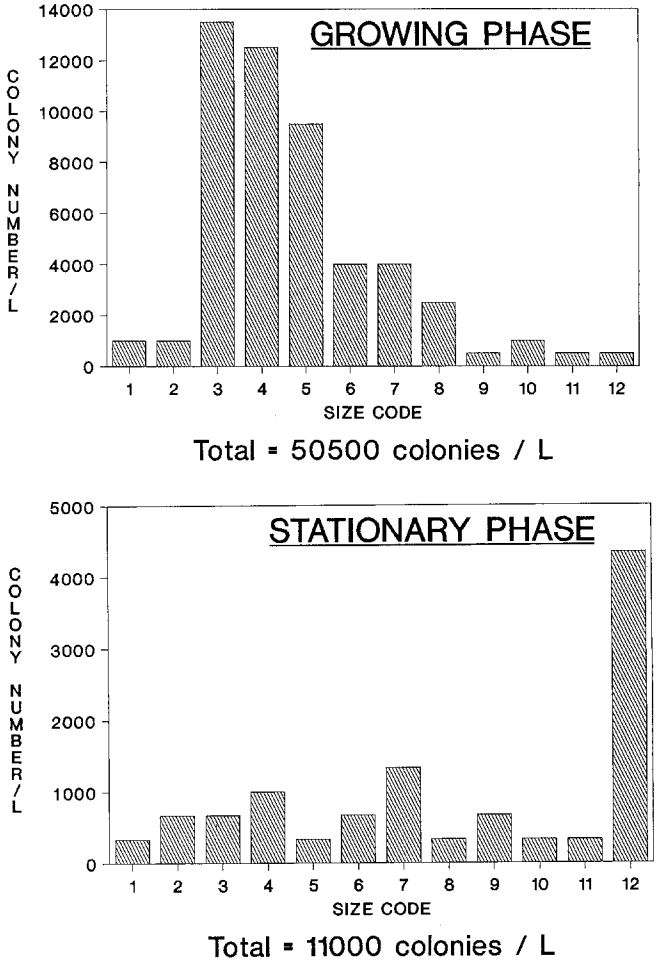

Fig. 9. *Phaeocystis* sp. Size distribution of colony number at two different stages of bloom development in Dutch coastal waters during spring 1986. Sizes are expressed in terms of equivalent spherical diameter. Colonial diameter size code is: (1) <50; (2) 50–100; (3) 100–200; (4) 200–300; (5) 300–400; (6) 400–500; (7) 500–600; (8) 600–700; (9) 700–800; (10) 800–900; (11) 900–1000; (12) >1000 μm

1 mm, mucous contributes up to 90% of *Phaeocystis* sp. colony biomass. This has important ecological implications for organisms of higher trophic level that feed on *Phaeocystis* sp. Cells and mucous are indeed of very different nutritional quality. The mucilaginous substances of the matrix being mainly composed of polysaccharides and devoid of nitrogen (Guillard and Hellebust 1971, Chang 1984, Braeckman personal communication), they are of less nutritional value.

Ecological implication of the size distribution of *Phaeocystis* sp. biomass in the natural environment was deduced from Figs. 9 and 10 which indicate, respectively, the size distribution of *Phaeocystis* sp. colony number and of carbon content at two different stages of its development in the Dutch coastal waters during spring 1986. *Grazable* and *ungrazable* colony size-classes were defined on the basis of copepods species and development stages present at the time of the *Phaeocystis* sp. bloom (Daro 1985) as well as their grazing characteristics determined under controlled conditions (Weisse 1983). According to this, a size limit of 300 μm of equivalent diameter was chosen.

Similarly to its development in batch culture, *Phaeocystis* sp. population is dominated by numerous small sized colonies during its growing phase, when nutrients are sufficient. At this time, the population is dominated by *grazable* forms of *Phaeocystis* sp. (Fig. 9, Table 3).

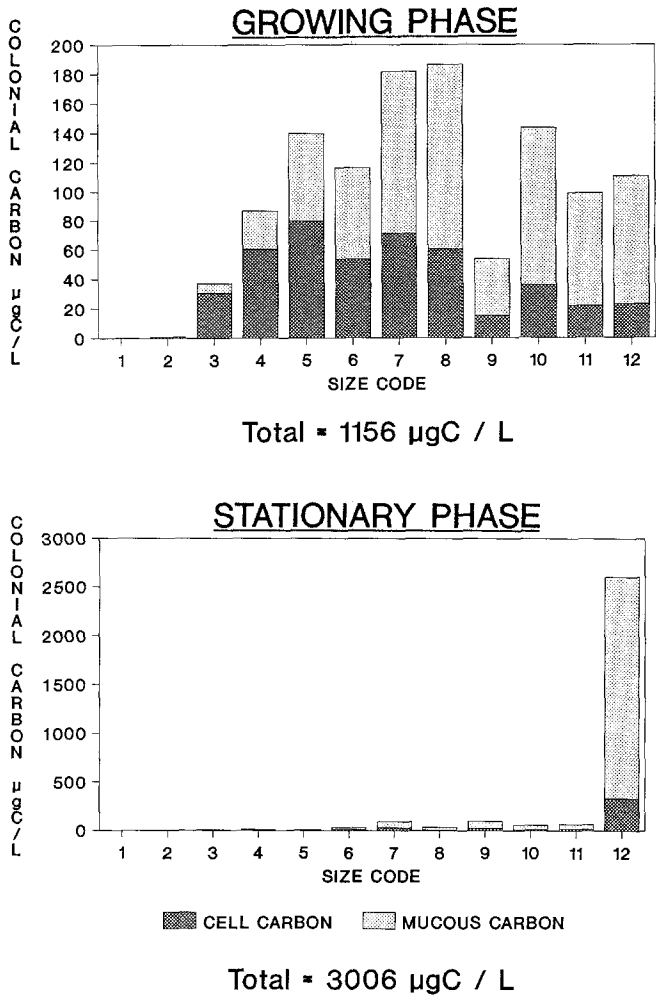

Fig. 10. *Phaeocystis* sp. Size distribution of colony carbon content with relative contribution of cellular and mucous carbon, at two different stages of bloom development in Dutch coastal waters during spring 1986. For size code, see Fig. 9

Table 3. *Phaeocystis* sp. Carbon content (μg C l^{-1}) of grazable and ungrazable forms at two periods of the spring bloom in 1986 in Dutch coastal waters. Numbers and percent mucous also given

Date	Free-living cells		Grazable (<300 μm)			Ungrazable (>300 μm)		
	No. $10^6 l^{-1}$	C	No. $10^3 l^{-1}$	C	Mucous %	No. $10^3 l^{-1}$	C	Mucous %
May 06	7.56	107	28	125	26	22.5	1031	65
May 13	1.82	26	2.7	23	38	8.4	2984	85

Reversely, at the stationary phase of its development, the population has decreased in terms of colony number but is dominated at 75% by big colonies whose size is greater than 1 mm.

A different feature can be observed when data of Fig. 9 are converted into carbon unit as illustrated by Fig. 10. Carbon content of small colonies is insignificant compared with the large size ones, these latter being composed of more than 50% of mucilaginous substances. At the top of the bloom when large colonies dominate the population, *Phaeocystis* sp. biomass is composed of 85% of mucilaginous substances. This corresponds physiologically to the enhanced synthesis of mucilaginous substances that compose the colonial matrix when available nutrients become scarce (Lancelot and Billen 1985). According to this, colony carbon content ranges between 0.4 and 813 ng in the Dutch coastal waters during the course of *Phaeocystis* sp. flowering. The ecological impact of the size distribution of *Phaeocystis* sp. biomass on heterotrophic growth in the Dutch coastal waters during spring 1986 is clearly shown by Table 3, which gives available food for specific grazers (ciliates and zooplankton) in terms of carbon and cell or colony numbers for the two stages of the bloom. Examination of Table 3 highlights the fact that most *Phaeocystis* sp. biomass is not *grazable* by zooplankton and thus escapes this trophic pathway. Ungrazed *Phaeocystis* sp. colonies can, however, be degraded by planktonic bacteria. The efficiency of this microbial trophic pathway is however highly questionable, as more than 50% of *Phaeocystis* sp. biomass is composed of mucilaginous polysaccharides, the biodegradability of which remains to be determined.

Conclusion

The conversion factors recommended in this paper for the calculation of *Phaeocystis* sp. carbon biomass, on the basis of microscopic observations, were shown to be useful both for determining the life cycle of *Phaeocystis* sp. during the course of its flowering, and for evaluating available food for ciliates and zooplankton that graze, respectively, on cells and colonies – provided that microscopic observations are detailed as follows: (1) counts of free-living cells and distinction between flagellated and non-motile cells; (2) counts of colonies and measurement of their respective biovolume for different colony size-ranges, determined on the basis of grazing characteristics of zooplankton present at the time of *Phaeocystis* sp. bloom. These colony size-classes vary, therefore, from one biotope to another.

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