

Colonies of *Phaeocystis globosa* are protected by a thin but tough skin

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ABSTRACT: Colonies of the prymnesiophyte marine microalga *Phaeocystis globosa* were tested for mechanical properties, permeability and biochemical composition using the micropipette aspiration technique. We found that the *Phaeocystis* colony is enclosed by a thin, yet very strong, semi-permeable skin (pore size between 1 and 4.4 nm diameter) with plastic and to a limited extent also elastic properties. Qualitative staining of single colonies with selective fluorescent dyes indicated absence of lipophilic compounds and chitin but presence of amino groups in the colony skin. Individual cells in the colony appear to be weakly connected with one another and attached to a very dilute, peripheral gel. Suction applied to the colony resulted in volume loss due to expulsion of water and squeezing together of the cells within the skin into a tight pouch; the presence of any firm gelatinous matter within the colony was not discernible. On increasing suction pressure, the skin eventually ruptured and the cells were sucked out of the hole leaving the empty skin behind. We propose that the skin effectively protects the colony cells from grazing and infection by viruses and other pathogens. The unsuspected presence of a skin is probably the main reason why *Phaeocystis* colonies have reduced mortality relative to solitary cells and form large blooms in many regions of the world's ocean. Our findings indicate that the colonies should be viewed as 'bags of water' rather than 'balls of jelly'.

KEY WORDS: Phytoplankton colony · *Phaeocystis* · Grazer deterrent · Colony skin · Micropipette aspiration technique

INTRODUCTION

The marine microalga *Phaeocystis* is ubiquitous in coastal and shelf areas as well as in nutrient-rich regions of the open ocean and can dominate phytoplankton blooms for extended periods of time (Lancelot et al. 1998). Although its life cycle includes solitary cells and colonies, massive blooms consist almost exclusively of the smooth-surfaced, globose colonies (Lancelot & Rousseau 1994). Since growth rates of colony cells are reported to be similar to those of solitary cells (Guillard & Hellebust 1971, Grimm & Weisse 1985) it follows that accumulation of biomass can only be due to differences in the ratio of the growth/mortality rates between solitary and colonial life stages. Even though this has been long suspected, experimental evidence on the defense strategy of

Phaeocystis colonies has not been straightforward to obtain. Weisse et al. (1994) reviewed the literature on grazing of *Phaeocystis* colonies by zooplankton and concluded that the majority of reports indicated lower grazing rates on colonies as compared to other types of phytoplankton including solitary cells. Further, Jacobsen et al. (1996) recorded mass viral infection accompanied by mortality of a population of solitary *Phaeocystis* cells and hypothesized that colony cells were protected against viruses.

Although the structure of *Phaeocystis* colonies has been studied since the last century, there is no consensus on presence and location of chemical constituents. Lagerheim (1896) proposed that the colony structure and spherical shape is maintained by a thin, but dense skin, whereas Scherffel (1900) assumed that the whole colony was gelatinous. The latter view of the colonies as 'balls of jelly' as opposed to 'bags of water' is now widely believed. Recently, van Rijssel et al. (1997)

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showed that the colonies are hollow with a gelatinous periphery in which the cells are embedded.

In the present study we examined the mechanical and chemical properties of individual *Phaeocystis* colonies using the micropipette aspiration technique developed by Mitchison & Swann for sea urchin eggs (Mitchison & Swann 1954). The method and its applications—it is also used to examine blood cells (Rand & Burton 1964)—have been reviewed by Evans (1989) and Needham (1993). The technique enabled us to study the mechanical response and stability of the colony to large forces. Using 2 pipettes, we were able to rapidly transfer individual colonies between different solutions and to thus assess colony permeability as well as chemical constituents of its components by selective fluorescent stains. We used dyes that selectively stain lipids, amino groups, chitin and chitobiose.

The results clearly show that Lagerheim's view of *Phaeocystis* colony structure is essentially correct. We found that the colonies are enclosed in a very thin, but tough, skin with a dilute gelatinous lining to which the cells appear to be attached. The surprising strength of the skin strongly suggests that it is the major defense mechanism of the colony against grazing and infecting organisms.

MATERIAL AND METHODS

Cultures. *Phaeocystis globosa* was isolated from the North Sea and cultured at 10°C in enriched seawater (f/2) (Guillard & Ryther 1962). Three days prior to the experiment, the culture was transferred to natural North Sea winter water (taken in February 1997, osmolarity: 984 mOsm, salinity 33.1) to exclude possible effects of EDTA or high nutrient concentrations. Colonies were grown at 10°C in glass bottles under artificial light (approx. 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and a 16:8 light:dark cycle. Young colonies of appropriate size (50 to 100 μm) for the experiments were grown as described by Cariou et al. (1994). Colonies from the culture were rapidly transferred to the micropipette system or the staining buffers.

Microscopy and micropipette techniques. An inverted microscope (Axiovert 135TV, Carl Zeiss, Jena, Germany) equipped with an Achromat 20 \times /0.4 long distance lens (Carl Zeiss) and a long distance condenser was used. Bright field, phase contrast, differential interference contrast (DIC) and fluorescence microscopy were applied as contrast methods. Images were recorded at video rate with a standard CCD camera (C5403-1, Hamamatsu, Hamamatsu City, Japan) and stored on video tape. For fluorescence microscopy a Fluor 40 \times /1.3 oil lens and an extremely sensitive SIT camera (C2400-08, Hamamatsu) were used. Fluores-

cence was excited with a 100 W mercury arc lamp, excitation and emission wavelengths were separated with the appropriate filter sets (Zeiss filter set 10/No. 4879210-0000 for blue excitation, excitation: 450–490 nm, dichroic mirror: 510 nm, emission: 515–565 nm; Zeiss filter set 20/No. 487920-0000 for green excitation, excitation 546 nm, bandpass, dichroic mirror: 560 nm, emission: 575–640 nm).

Micropipettes were drawn from borosilicate capillaries (Hilgenberg, Malsfeld, Germany) with a micropipette puller (P-87, Sutter Instrument Company, San Rafael, CA). These raw pipettes had to be opened. This was done under a dissecting microscope by insertion of the pipette tip in a molten drop of soda lime glass, cooling down the glass drop and shearing off the pipette. Such pipettes were of cylindrical shape with an internal diameter between 6 and 15 μm and showed no visual irregularities. The pipettes were filled with the respective buffer, inserted into the micromanipulator (MMW-22, Narishige, Tokyo, Japan) and connected to the pressure reservoir. The experiments were performed on the microscope stage in a thermostated chamber that consisted of 2 coverslips held at a distance of 1 mm. The chamber was held at a temperature of 10°C by circulation of temperature controlled water. A schematic layout of the setup is shown in Fig 1.

We manipulated 9 *Phaeocystis globosa* colonies of different size to explore their structure and mechanical properties by subjecting them to suction with the micropipettes. The strength of the skin was also studied by stabbing a colony held in place by gentle suction of the larger pipette with a glass tube of 4 μm outer diameter from the opposite end.

Transfer for staining, exposure and permeability experiments. For staining or permeability measurements transfer of single colonies from the medium to the experimental solution and back is necessary. The principle of the transfer experiments (Evans 1989) is shown in Fig. 2. A split double chamber was used. Both chambers were separated by a narrow air gap. In order to transfer a colony 2 pipettes were employed. The so-called transfer pipette had a much larger inner dia-

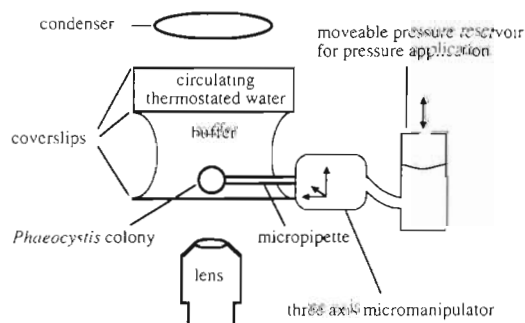


Fig. 1. Schematics of the micropipette setup, not to scale

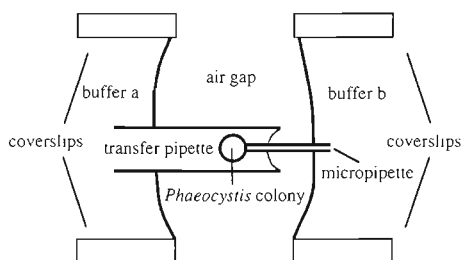


Fig. 2. Schematic view of a transfer experiment

meter than the usual pipettes and was filled with the experimental solution as well. After aspiration of a suitable colony with the measuring pipette, it was maneuvered into the transfer pipette. This assembly was then moved through the air gap by moving the microscope stage with the colony protected from air by the transfer pipette. Subsequently the colony was removed from the transfer pipette in the second chamber and moved several millimeters away from the air gap. The amount of medium co-transferred during the procedure was negligible for the experiments described here.

Permeability tests. Permeability was tested by transferring single colonies from natural seawater with an osmolarity of 982 mOsm into solutions of different compositions and osmolarities. These were deionized water, natural seawater and sucrose (1503 mOsm), natural seawater and dextran 6000 (1584 mOsm), and deionized water and dextran 6000 (183 mOsm). Colonies were exposed to these buffers for approximately 5 min and transferred back into natural seawater. Initial colony contractions, invoked by elevated extracolony osmotic pressures, were expected to be rapidly reversed if the colony skin was permeable for the compounds causing the pressure difference. Conversely, compounds too large to enter the colony skin were expected to cause persisting shrinkage.

Osmolarities of the solutions used were measured with a freezing point depression osmometer (Micro-osmometer, model 3MO, Advanced Instruments, Needham Heights, MA). The hydrodynamic radius of dextran was determined by quasi-elastic laser light scattering, also termed 'particle sizing' (Chu 1991). We used a commercial instrument described by Piekenbrock & Sackmann (1992). Dextran with a molecular weight of 6000 Da (Fluka) was dissolved in distilled water, filtered with a 200 nm pore filter to remove dust, and measured at scattering angles of 50° and 90°. The data were analyzed using the contin algorithm (Provencher 1982). At both scattering angles a narrow distribution of sizes centered at 2.2 nm was found.

Staining. In all cases the colonies were transferred into the staining buffer, left there for approximately

5 min, transferred back and moved away from the transfer pipette. The fluorescent regions within the colony were then immediately examined under the microscope.

Studied constituents: fluorescent probes and staining buffers:

(1) Plasma membrane: DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; green excitation) was dissolved at 1 mg ml⁻¹ in DMSO (dimethyl sulfoxide, Merck, Darmstadt, Germany) and 50 µl of this preparation were added to 1 ml seawater.

(2) Hydrophobic compartments: NBD hexanoic acid (6-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-amino) hexanoic acid; blue excitation) was dissolved at 1 mg ml⁻¹ in ethanol (Merck) and 50 µl of this preparation were added to 1 ml seawater.

(3) Amino groups: TAMRA-SE (5-(and-6)-carboxy-tetramethylrhodamine succinimidyl ester; green excitation) was dissolved at 1 mg ml⁻¹ in DMSO (Merck) and 50 µl of this preparation were added to 1 ml seawater.

(4) Chitin and chitobiose: FITC-labeled WGA (Fluorescein isothiocyanat-labeled wheat germ agglutinin; blue excitation) was dissolved at 50 µg ml⁻¹ in seawater.

All fluorescent probes (except WGA) were purchased at Molecular Probes (Eugene, OR) and used as received. WGA was obtained from Sigma, Deisenhofen, Germany.

Exposure experiments. Detergent treatment was also performed as a transfer experiment. Colonies were transferred from seawater into a 3% (w/w) solution of the detergent n-octylpolyoxipropylene (Sigma, Deisenhofen, Germany) in seawater, left there for approximately 5 min and transferred back. Colonies were also exposed to a saturated solution (280 g l⁻¹) of Na-EDTA (ethylenediaminetetraacetic acid, Sigma) titrated to seawater equivalent pH 8.2 with KOH.

RESULTS

Colony structure

The series of events recorded during a representative aspiration experiment is shown in Fig. 3. At first, the outer skin of the colony together with 1 cell entered the pipette, the remaining cells clumped into a pouch at the other end. The skin was tightly pressed against the cells and became invisible, its presence and location can be inferred from the outer margins of the cells inside and outside the pipette. The obvious loss in colony volume indicates rapid expulsion of water through the skin. Increasing suction dislodged cells individually from the pouch which tightened simultaneously. The cells were greatly elongated during pas-

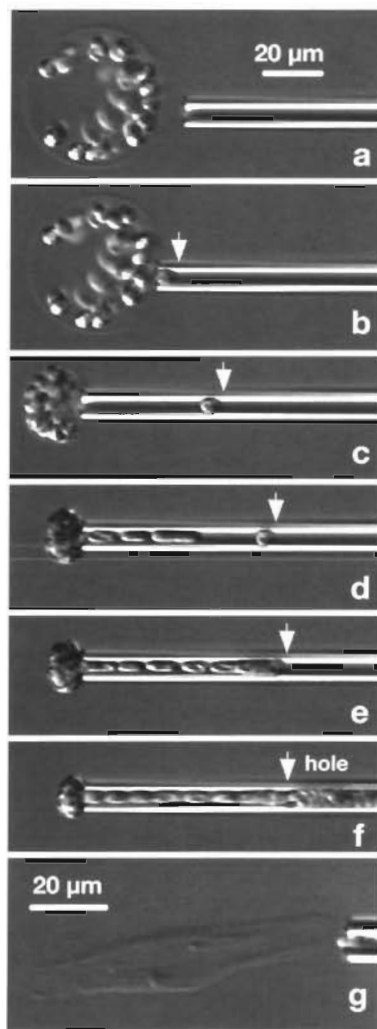


Fig. 3. Sequence of high pressure aspiration of a *Phaeocystis* colony (diameter ca 50 μm). (a) colony and pipette before aspiration, (b) commencement of aspiration, (c) a single cell entering the pipette, colony volume outside has significantly decreased, (d) additional cells, highly deformed by the extreme pressure gradient, entering the pipette; the initial cell remains in a stationary position, (e) buildup of the pressure head indicated by deceleration of the following cells, and extreme bunching of the cells outside which prevent their suction into the pipette, (f) rupture of the tip of the skin within the pipette with cells passing through the hole (marked by the arrow), (g) shows the tube-shaped empty colony skin (lateral view). Note the abundant longitudinal folds on the skin and the absence of visible space attributable to a firm gelatinous matrix within the colony immediately prior to its rupture

sage but squeezed together against the skin in the pipette. As cells are usually not or only barely compressible (Evans 1989) a very high tension developed in the skin. The entire discernible volume of the colony now comprised only the cells. Finally, the outermost extremity of the skin ruptured and all the cells were sucked out of the hole. The tip of the skin inside the

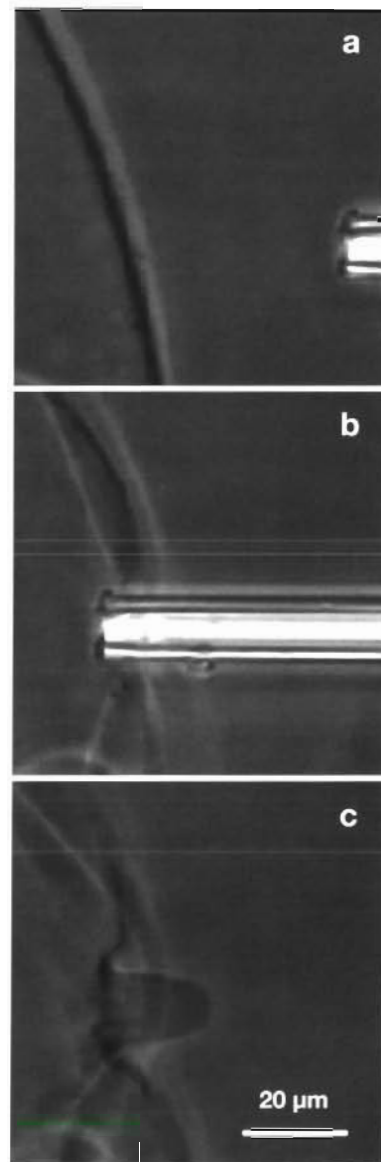


Fig. 4. A large *Phaeocystis* colony (ca 500 μm): (a) before aspiration, (b) during aspiration, and (c) after release

pipette remained more or less stationary till breakage, indicating that the skin is not distendable. On exhaling the contents of the pipette, the skin, followed by the individual cells, emerged as an empty, transparent, bag-like structure. Although its thickness was well below 1 μm , it appeared to be fairly stiff as it retained the shape it had on emerging from the pipette despite considerable movement in the agitated medium. There was no indication of the presence of any firm gelatinous matter either during compression of the colony or following exhalation of the contents of the colony.

Nine colonies of different sizes were tested with the microaspirator. Three smaller colonies (<50 μm) were

each sucked into the pipette without skin breakage, and emerged following exhalation in rod shape with serially oriented cells. Within 30 min, these compressed colonies approximately doubled their diameter. All 4 medium-sized colonies (50 to 100 μm) behaved exactly as described above (Fig. 3). The 2 larger colonies (150 and 500 μm) withstood the maximum suction of about several 10000 Pa (several Newton cm^{-2}) without breakage; only a nipple was drawn into the pipette (Fig. 4) which retained its shape after pressure release. Slight relaxation of the nipple shape commenced some 10 s later.

The strength of the skin was also studied by stabbing a colony held in place by gentle suction of the larger pipette with a tube of 4 μm outer diameter from the opposite end (Fig. 5). The skin withstood the pressure of being pushed through the colony. Retraction of the pipette left a deep crater-like pit that slowly filled out again after some seconds. The skin broke only after the pipette pushed through the skin inside the larger pipette. Skin breakage resulted in rapid disorganisation of the colony contents.

While the characteristics of the skin around intact *Phaeocystis* colonies are difficult to define optically because of its transparency and its thinness, its pres-

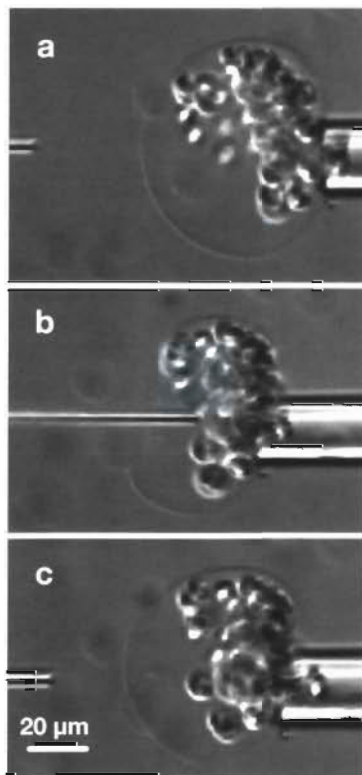


Fig. 5. Failed attempt to pierce the skin with a pipette: (a) before, (b) during, (c) and after the attack. Note that the pipette was rapidly stabbed into the *Phaeocystis* colony

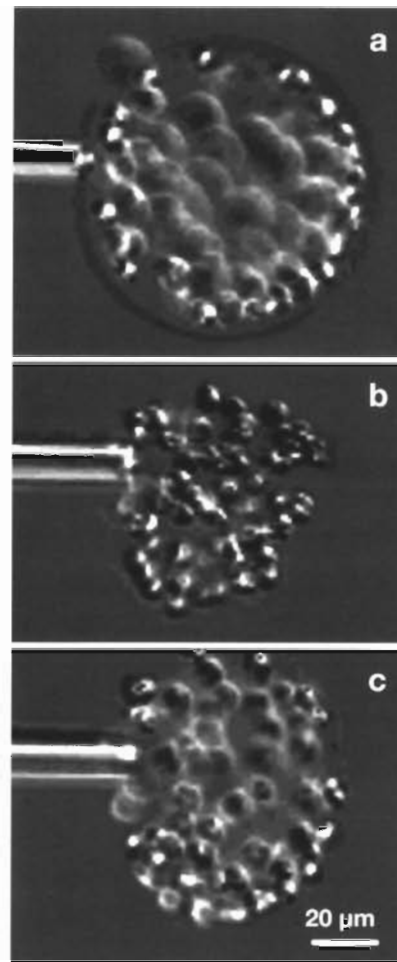


Fig. 6. Effect of transfer of a *Phaeocystis* colony into a hyperosmotic solution of dextran in seawater. The same colony in (a) seawater, (b) after transfer into a solution of dextran 6000 in seawater and (c) after transfer back into seawater

ence can be well discerned under DIC field. However, its thickness could not be resolved by light microscopy, and its presence as a determinate structure could only be examined following removal of its contents which proved its autonomy and indicated it to be a plastic deformable structure.

The colony skin was found to be permeable for inorganic ions and small organic molecules, but impermeable for large organic molecules. This was inferred from the following effects:

(1) Transfer of *Phaeocystis* colonies from seawater to deionized water had no visible effect on the colony skin, but a rapid decrease of intracolony osmolarity could be inferred from the rapid swelling and bursting of the colonial cells. This implies that inorganic ions pass freely through the colony skin. (2) Transfer of *Phaeocystis* colonies to a hyperosmotic solution (1503 mosm) containing seawater and sucrose (MW =

186, hydrodynamic radius 0.47 nm, Weast 1970) caused a brief contraction of the colonies which lasted for approximately 2 s. This indicates that the colony skin is permeable for sucrose, thus it must contain pores larger than 1 nm. (3) Transfer of *Phaeocystis* colonies to a hyperosmotic solution (1584 mOsm) containing seawater and dextran (MW = 6000, hydrodynamic radius 2.2 nm) caused rapid colony shrinkage to a considerably reduced size (Fig. 6). The compression was not reversed while the colony stayed in this solution (several minutes), which indicates that the colony skin was completely impermeable for dextran 6000. Hence the largest pores of the colony skin were smaller than 4.4 nm. Reinflation of the colony after retransferring it to the original seawater (Fig. 6c) confirmed this, and indicated the presence of large osmolytes in the colony. (4) Transfer of *Phaeocystis* colonies into a hypoosmotic solution (183 mOsm) containing deionized water and dextran (MW = 6000) led to a rapid shrinkage of the colony. This was followed by swelling and bursting of the cells and a simultaneous reinflation of the colony, indicating that the cells contained enough large soluble molecules to increase turgor and overcome plastic deformation. The skin was permeable for all other chemical compounds used in our study, because the cells were affected (lysed or dyed) by all of them.

A dilute gel layer of several micrometer thickness underlies the skin. Direct imaging of this layer was only possible for large (500 μm) colonies, where it appeared as a slightly darker zone in the colony periphery in phase contrast micrographs. Since the gel layer was not visibly stained with any of the fluorescent probes used in this study, and the colonies could be compressed until virtually no space was left between the cells and the colony skin (Fig. 3), the gel layer cannot be very substantial. Individual cells appear to be connected by a highly elastic structure, as pulling out 1 cell from a ruptured colony resulted in its being followed by other cells. The connection presumably consists of thread-like links. The adhesion of the colonial cells to the gel must be very weak because it could be broken by the viscous drag (estimated, according to Stokes law, to approx. 1 pN) of the cells outside the skin. Visible Brownian motion of single colonial cells relative to each other confirms that the cells must be held by a very flexible structure. The recovery of original shape after deformation implies that the interior of the colony is filled with seawater and some large-molecular osmolytes.

Biochemical composition

Immersion in the detergent n-octylpolyoxipropylene rapidly resulted in disintegration of the cells inside the

colony, but had no effect on colony shape (Fig. 7), suggesting that lipids are the dominant structure-stabilizing compounds of the cells, but not of the colony skin. However, the colonies strongly adhered to hydrophobic polystyrene surfaces of Petri dishes used for independent colony observation during the study, implying that the colony surface is hydrophobic. Yet, both lipid dyes did not label the colony surface. The amphiphilic fluorescent probe DiI is known to selectively label the plasma membrane of eucaryotic cells and is widely used for this type of experiment (Haugland 1996). The dye NBD-hexanoic acid is poorly soluble in water and therefore should be enriched in hydrophobic compartments. Both dyes stained exclusively the colonial cells. DiI labeled the plasma membrane only (Fig. 8a), NBD-hexanoic acid the internal membranes as well (data not shown). From this finding we conclude that the skin neither contains hydrophobic compartments (e.g. droplets) in more than trace amounts, nor an amphiphilic layer like a lipid membrane.

Succinimidyl ester, the reactive group of TAMRA-SE, attacks free amino groups (e.g. lysine residues in proteins) and forms stable chemical bonds with them. The optimum pH for this reaction is around 8.5, close to

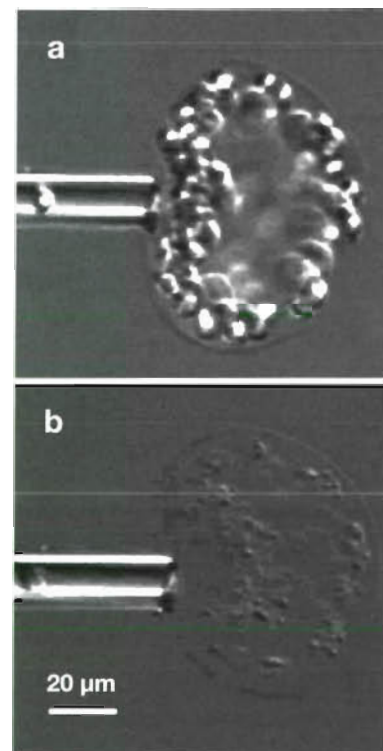


Fig. 7. Transfer of (a) an intact *Phaeocystis* colony into (b) the detergent n-octylpolyoxipropylene, resulting in rapid cell disintegration. The cell in the pipette was affected approximately 6 s later

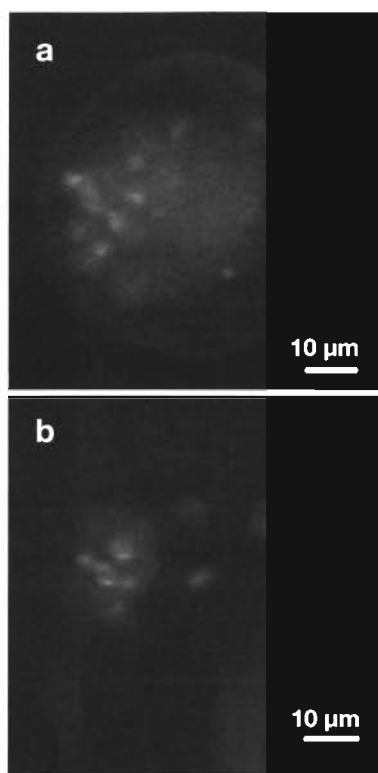


Fig. 8. Fluorescence micrographs of (a) a *Phaeocystis* colony that was stained with TAMRA-SE with a clearly visible colonial skin, and (b) a colony of the same size after staining with DiI, only the cells were labeled

the pH of seawater (Haugland 1996). The distinct labeling of both cells and skin by TAMRA-SE (Fig. 8b) shows that the *Phaeocystis* colony skin contains amino groups in high density. FITC-labelled lectin WGA which binds to chitin and chitobiose in seawater, (Montgomery et al. 1990), labelled only the colony cells, but not the skin. Thus, chitin is not present in the skin, and the amino groups most likely due to amino acids present as proteins in the colony skin.

Transfer of colonies into deionized water or highly concentrated EDTA solutions resulted in cell disintegration, but the skin and colony shape remained optically unchanged (data not shown). Thus, calcium bridges are not crucial for maintaining the integrity of the outer skin on a time scale of minutes.

DISCUSSION

The presence of a thin, tough skin encasing the *Phaeocystis* colony was not suspected when we commenced the experiments. Our findings now show that the skin must function as a highly effective defence against a wide range of planktonic grazers but also

bacterial and viral pathogens. In view of the fact that cell growth rates within colonies are similar to those of solitary cells, it would appear that skin formation is not unduly costly. Further, colony cells do not possess flagellae, scales or chitin fibrils typical for the solitary form (Baumann et al. 1994), which would free additional resources for skin formation. Although we studied only the warm-water species *Phaeocystis globosa*, it can be assumed that the properties we describe are common to the other colonial species *P. pouchetti* and *P. antarctica* as their colonies are superficially very similar and both species are reported to have evolved from *P. globosa* (Medlin et al. 1994). We argue below that protection by the skin is the key factor responsible for the extraordinary ecological success of this genus, enabling its colonial form to regularly build up extensive blooms in very diverse oceanic regimes (Verity & Smetacek 1996).

The colony skin as a grazer deterrent

Colonial organisation has been suggested to protect *Phaeocystis* cells against grazing (Weisse et al. 1994, and reference therein) although the nature of the protective mechanisms other than increase in size was not specified. Current knowledge on the mechanical properties of *Phaeocystis* colonies is controversial. It has been hypothesized that protection is of mechanical nature (e.g. Smaal & Twisk 1997). Whereas some studies suggest that *Phaeocystis* colonies are resistant to disruption (e.g. Schnack et al. 1985), others imply that the colonies are fragile (Cariou et al. 1994, Hansen et al. 1994). Attempts to pierce a large *Phaeocystis* colony with a needle failed and created a folded dent (Kornmann 1955), but *Phaeocystis* colonies are reported to have been successfully penetrated with microelectrodes (Lubbers et al. 1990). Our results indicate a high resistance of the colony to suction pressure and piercing even with very pointed objects.

It is now known that the major grazers of phytoplankton are heterotrophic protists which feed by very different mechanisms (Schnepf & Elbrächter 1992). The most common types ingest their prey and would be deterred by large prey size. However, those groups which feed by enveloping food items in a feeding veil (the pallium) or penetrate it by means of peduncles and similar extensible structures can overcome the size barrier and feed efficiently on much larger prey, in particular diatoms. However, we know of no reports of protists feeding on intact, healthy *Phaeocystis* colonies. In contrast, cells in disintegrating colonies and single cells are readily eaten by various protists (e.g. Admiraal & Venekamp 1986, Estep et al. 1990, Weiss & Scheffel-Moeser 1990, van Boekel et al. 1992). Solitary

cells are also susceptible to bacterial and viral attack (Thingstad & Billen 1994, Jacobsen et al. 1996) but cells in colonies appear to be more or less immune to pathogens. Hence, when colony cells migrate out of the colonies following peaking of the bloom, *Phaeocystis* biomass rapidly declines due to grazing pressure and cell mortality. Our findings strongly suggest that mechanical protection by the tough skin with its small pore size is the major reason for low mortality of colony cells rather than chemical protection by means of deterrents such as acrylic acid derived from the breakdown of DMSP (*sensu* Wolfe et al. 1996) or poor nutritional value of the cells (*sensu* Claustre et al. 1990 or Nicols et al. 1991).

Larger zooplankton such as euphausiids and large copepods are reported to feed readily on *Phaeocystis* colonies (Weisse et al. 1994) presumably because they possess the means (gastric mills and sharp mandibles respectively) to rupture the skin. Mechanical protection is also indicated by size dependent ingestion of *Phaeocystis* colonies by different developmental stages of *Calanus finmarchicus* (Hansen et al. 1990): while small copepodites (C1 to C3) ingested large (>100 μm) colonies less effectively than diatoms or smaller colonies, larger copepodites (C4 & C5) did not select against large *Phaeocystis* colonies. However, even these large forms are likely to be deterred by colonies because they will have to cope with the large amount of water within it. Such an increase in handling time will also help in reducing grazing and hence mortality rate. As the larger grazers are now known to consume only a small percentage of the phytoplankton, their feeding pressure will not suffice to prevent a bloom from building up.

Structure

The structure of *Phaeocystis* colonies has been discussed since the last century (see van Rijssel et al. 1997 and reference therein). Lagerheim (1896) proposed that the colonies are enclosed in a thin, but dense skin, lined from the inside with a loose gel in which the cells are located and with the colony lumen devoid of solid matter. In contrast, Scherffel (1900) assumed that the whole colony was gelatinous. As the presence of a skin is not evident under scrutiny with a microscope, it has been widely assumed that the shape and firmness of the colonies is due to a homogeneous matrix of gel-forming mucopolysaccharides (Lancelot et al. 1998). Presumably, the view of a firm gelatinous consistency was also supported by the fact that colonies can attain 10 mm diameter and still retain their shape. However, it has been recently shown that polysaccharides are concentrated in a relatively thin layer around the

periphery of the colonies (van Rijssel et al. 1997). As we were only able to separate the skin from colonies below 100 μm diameter, it is possible that larger colonies accumulate more polysaccharides over time; nevertheless the larger colonies we manipulated also possessed the skin which in itself, combined with internal turgour, would suffice to maintain the characteristic smooth, spherical appearance of the colonies and their resistance to attack by the wide variety of grazers in pelagic systems.

Our results support the colony structure proposed by Lagerheim (1896). It was not possible to determine the thickness of the skin with the methods we used. While van Rijssel et al. (1997) found a 8 μm thick layer where FITC-labeled Con-A bound to the skin, this study suggests that the mechanically tough and optically dense skin has a thickness well below 1 μm (Fig. 3a). However, this is not necessarily a contradiction, as the visibly stainable material might extend further into the colony than the very dense and mechanically tough outer layer. The existence of a dilute gel layer in the colony periphery as proposed here and mentioned by Lagerheim (1896), Kornmann (1955) and van Rijssel et al. (1997) would explain the low optical density, elasticity, and connective properties of this region observed by us.

Biochemical composition

Hydrophobicity of the colony skin has been observed by Janse (pers. comm.), and inferred from stronger adhesion of the colonies to polystyrene surfaces than to glass surfaces. As specific dyes indicated the absence of lipid in the skin, polysaccharides or proteins must be responsible for the adsorption of *Phaeocystis* colonies to hydrophobic surfaces. For instance, many proteins strongly absorb to polystyrene, which is used for immunoassays in polystyrene microtiterplates. In contrast to our expectations, Ca^{2+} , which has been shown to be important for colony formation (van Boeckel 1992), was not essential in maintaining their structural integrity.

The amino groups we found to be compounds of the colony skin could be derived from various sources, the most likely candidates being amino sugars and amino acids. The presence of chitin, which has been shown to form the fibrils of the *Phaeocystis* flagellates (Chretiennot-Dinet et al. 1997), can be excluded because the colony skin was not stained by FITC-labeled lectin WGA (Montgomery et al. 1990). Likewise, glucosamine, which is a structure-building component in cell walls of some green algae (e.g. Takeda 1991), has not been found in *Phaeocystis* colonies (Janse et al. 1996). Under the assumption that the colony skin of *Phaeocystis* has

a certain similarity to cell walls (see below), the detected amino groups could belong to proteins like the extensins, which have been found in the colonial freshwater alga *Volvox* (Ertl et al. 1989). An alternative explanation for our results could be that the mechanically tough structure is coated with a thin layer of different materials containing aminogroups (e.g. a carbohydrate layer coated with protein slime) or consists of glycoproteins.

Mechanical properties

In spite of its thinness, the outer skin is a very tough structure. Extreme forces were necessary to rupture the colony skin (Figs. 3 to 5). A similar observation was reported by Kornmann (1955). We observed predominantly plastic behavior of the skin, but reversible deformations and the formation of folds (Figs. 3 to 5) indicates a certain degree of stiffness (elasticity), therefore the reaction of the skin to mechanical deformation is elastoplastic. In contrast to the skin, the gel layer and the cell-cell connections appear to be mainly elastic.

While we could relate the colony structure to a protective function, the molecular structures of the colony architecture as well as development and growth of the colony skin are not known. It is of interest to note that some properties of the *Phaeocystis* colony, i.e. the mechanical properties (toughness and elasticity), pore sizes between 1 and 4 nm, sugar composition (Janse et al. 1996), the potential presence of a structural protein, and the potential to produce intracolony osmotic pressure with large organic molecules (Veldhuis & Admiraal 1985) resemble typical features of plant cell walls (see Kutschera 1996 for mechanical properties, Alberts et al. 1983 for sugar composition and pore size, Mc Queen-Mason 1995 for growth by osmotic pressure). Thus, the cell wall might serve as a model for further studies on the *Phaeocystis* colony skin.

Ecological and biogeochemical implications

In the following, we list some of the impacts of *Phaeocystis* blooms that differentiate them from other phytoplankton: (1) formation of large blooms also in the absence of silica (Smith et al. 1991), (2) low abundance of micro- and small mesozooplankton during blooms (van Boekel et al. 1992), (3) efficient sedimentation of *Phaeocystis*-derived organic matter in faecal material of large zooplankton (Hamm et al. unpubl. res.), but not in aggregates (Riebesell 1993), (4) rapid decline of the bloom once the colonies start breaking up (Davies et al. 1992), coinciding with (5), increase of the microbial commu-

nity and massive cell lysis (van Boekel 1992, Brussaard et al. 1995), potentially as a result of microbial (viral!) activity (Jacobsen et al. 1996) accompanied by buildup of dissolved organic matter in the water column (Eberlein 1985, Davidson & Marchant 1993), and (6) formation of surface slicks and foams at the water surface (Lancelot et al. 1998, Hamm & Rousseau unpubl. res.). We conclude that the presence of a colony skin — which protects otherwise susceptible colony cells from mortality and that is discarded following the bloom peak — might be largely responsible for these unique ecological and biogeochemical characteristics of *Phaeocystis* blooms. To our knowledge, this is the first report of an algal colony skin tough enough to provide effective refuge to the enclosed cells.

Acknowledgements. We thank Anke Dauelsberg for supplying the *Phaeocystis globosa* strain, Tobias Storz for measuring the hydrodynamic radius of dextran 6000, and Erich Sackmann for generous support. Sven Günther, Dieter Wolf-Gladrow and 4 anonymous reviewers provided valuable comments on the manuscript. This work was funded by the Deutsche Forschungsgemeinschaft via the group grant Sonderforschungsbereich 266 and by the Alfred Wegener Institute for Polar and Marine Research. This is publication No. 1620 of the Alfred-Wegener-Institute for Polar and Marine Research.

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Editorial responsibility: Otto Kinne (Editor),
Oldendorf/Luhe, Germany

Submitted: November 24, 1998; Accepted: April 12, 1999
Proofs received from author(s): September 24, 1999