Ultrastructure and function of stalks of the diatom *Didymosphenia geminata*

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Abstract One of the most striking features of the diatom *Didymosphenia geminata*, which has increased markedly in abundance in a number of countries in recent years, is the very large branched stalks. In order to help understanding their role, an ultrastructural study was carried out on two populations, one from a stream in northern England and the other from a river on Vancouver Island, Canada. In both cases, the main part of the stalk had a central reticulate core surrounded by an outer region with dense fibres. A longitudinal structure in the uppermost part of the stalk just under the collar surrounding the base of the cell may perhaps correspond to a tube. The structure of the septa formed where branches divide is also described. Phosphomonoesterase activity known to be present in the stalks was shown to occur in the inner peripheral layers of the stalks and especially in the collar area.

The results show that stalks have a complex structure suggesting their importance for their phosphatase activity to overcome low inorganic phosphate concentrations. Their large surface may function in herbivory avoidance, a better exposure of cells to turbulent conditions to increase nutrient uptake, adsorption of limiting elements and gas exchange.

Introduction

Didymosphenia geminata (Lyngbye) M. Schmidt has received much interest in recent years because of the increases in abundance known to have occurred in various countries (Blanco & Ector, 2009; Whitton et al., 2009) and the ecological problems they have caused in New Zealand in particular (Kilroy, 2008). The success of the species may be attributed in part to its long and persistent stalks which contribute to the formation of extensive mats reaching 3 cm or more in depth (Whitton et al., 2009) and forming up to 90% of the biomass (Lee et al., 2008).

Although a number of diatom genera form stalks, modern taxonomists have shown little interest in this feature and hence there are few ultrastructural studies. Daniel et al. (1987) and Wang et al. (1997, 2000) used electron microscopy to study the short stalks of

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Achnanthes longipes C. Agardh and reported a simple structure composed of a central ribbon and radially disposed fibres. A study by Moffat (1994) on ultra-structure of *D. geminata* dealt only with the cell. A light microscopy study by Ellwood & Whitton (2007) showed that stalks have high phosphomonoesterase activity, more intense in apical parts.

Extracellular polymeric substances (EPS) produced by diatoms have been studied by various methods including confocal microscopy and chemical analysis (Staats et al., 1999; Bahulikar & Kroth, 2007). Carbohydrates were the main component, but small amounts of proteins, uronic acids (Staats et al., 1999) and glycoproteins (Chiovitti et al., 2003) were also detected. Gretz et al. (2006) and Gretz (2008) reported that *D. geminata* stalks are composed of sulphated xylogalactanes (mainly formed by galactose and xylose), which are hydrophilic, and showed a structure in concentric layers with different chemical composi-tion; the outermost was the most resistant to degradation.

The aim of this study was to investigate the ultrastructure of *D. geminata* stalks using transmission electron microscopy and to establish the location of the phosphomonoesterase activity reported by Ellwood & Whitton (2007).

Materials and methods

Sampling

The colonies of *D. geminata* were collected from Stony Gill, Yorkshire, UK and R. Puntledge, Vancou-ver Island, Canada. Environmental data for Stony Gill are given by Ellwood & Whitton (2007) and Ellwood et al. (2008) and information about R. Puntledge by Sherbot & Bothwell (1993). Both the samples were collected in springtime.

The samples were transported in cold and dark conditions to the laboratory and then sent by courier service to Murcia cooled in liquid carbon dioxide. They were subsequently stored at −20°C, preserved with 2.5% glutaraldehyde in cacodylate buffer for electron microscopy (see below) or 3% formalin for later morphological and floristic studies and also inclusion in MUB Herbarium of Murcia University (ALG-3370, 3371).

Light microscopy

A microscope OLYMPUS BX 51 equipped with interdifferential contrast optics and a digital camera were used for light microscopic analysis.

Scanning electron microscopy (SEM)

For scanning electron microscopy the colonies were dehydrated in increasing concentrations of alcohol, with a final step of acetone, before critical point drying. Finally, the samples were covered with gold–palladium. The observations were made with a JEOL-6100 scanning microscope equipped with a digital camera.

Transmission electron microscopy (TEM)

For TEM preparations, colonies were fixed in 0.1 M cacodylate buffer with 2.5% glutaraldehyde at 4°C for 2 h and then rinsed in 0.1 M cacodylate buffer with 8% sucrose for one night at the same temperature. The samples were postfixed in 8% osmic acid for two and a half hours at 4°C, rinsed overnight and dehydrated in increasing concentrations alcohol (30, 50, 70, 90 and 100%, 10 min in each) at room temperature. The samples were then treated with propylene oxide (two rinses of 20 min each). They were traited at the same temperature with uranyl acetate for 2 h before rins-ing in distilled water for 5 min. The material was embedded in 1:2 epon plus propylene oxide, followed by 1:1 and finally 2:1 proportions of the same (1 h each step). Finally, the samples were left in pure epon overnight. The epon blocks were cut into ultrathin sections with a REICHERT-JUNG Ultracut, and the sections were contrasted with uranyl acetate and lead citrate. The observations were made with a PHILIPS-TECNAI-12 microscope equipped with a digital camera.

Histochemistry: alkaline phosphatase detection

The frozen colonies were sectioned at a thickness of 10 μm using a Reichert-Jung cryostat. The sections were incubated at pH 8.8 for alkaline phosphomonoes-terase activity (Gomori, 1952). Controls for the histo-chemical reaction included samples without substrate. The sections were mounted in glycerine and examined under an AXIOLAB microscope (ZEISS). All photo-graphs were obtained with a digital camera CoolSNAP

(RS Photometrics) using a Pentium III computer with a CoolSNAP (RS Photometrics) software.

Results

The stalks are branched several times, with some individual branches much longer than others. Using light microscopy the surface of the stalks appear smooth or slightly striated and are always expanded to form a collar near the base of the cell (Fig. 1). No significant differences were observed between the two samples.

The collar expansion eventually separates into two sections that indicate the start of a new pair of stalks (Fig. 2).

Fig. 1 Stalks of *D. geminata* with light microscope. **a** The presence of line/tube is obvious and the apical expansion of the collar. **b** The slight striation of the stalk and the apical collar is evident. *Scales* 20 μm
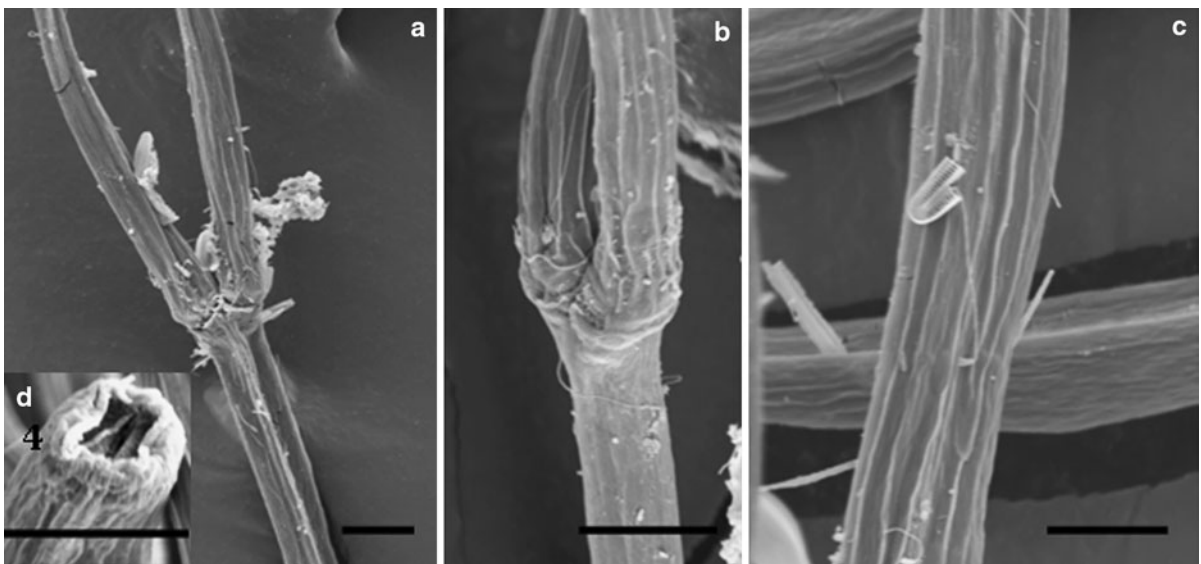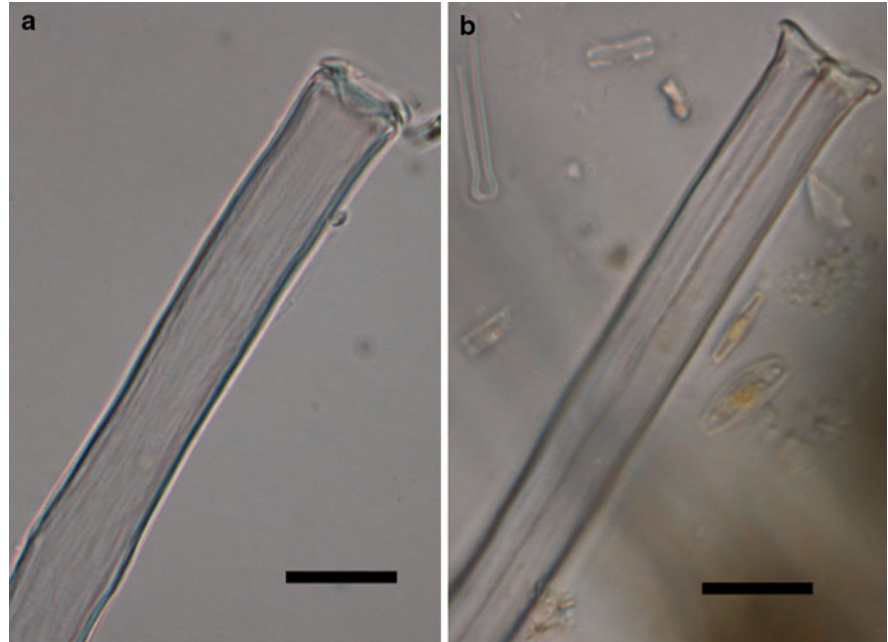

Fig. 2 Stalks of *D. geminata* with SEM. **a** General aspect of striated and branched stalks. **b** Striated branches on almost smooth stalk. **c** Detail of striation. **d** Detail of the upper parts of the stalk where the initial formation of a branch is observed. *Scales* 20 μm

Fig. 3 Ultrastructure of *D. geminata* stalks with TEM. **a** Transverse section of a stalk with a reticular structure and the accumulation of material in the outer part. **b** Detail of the outer part of the stalks. **c** Junction of the stalk with the frustule. **d** Intermediate portion of the stalk. *Scales* 1 μm
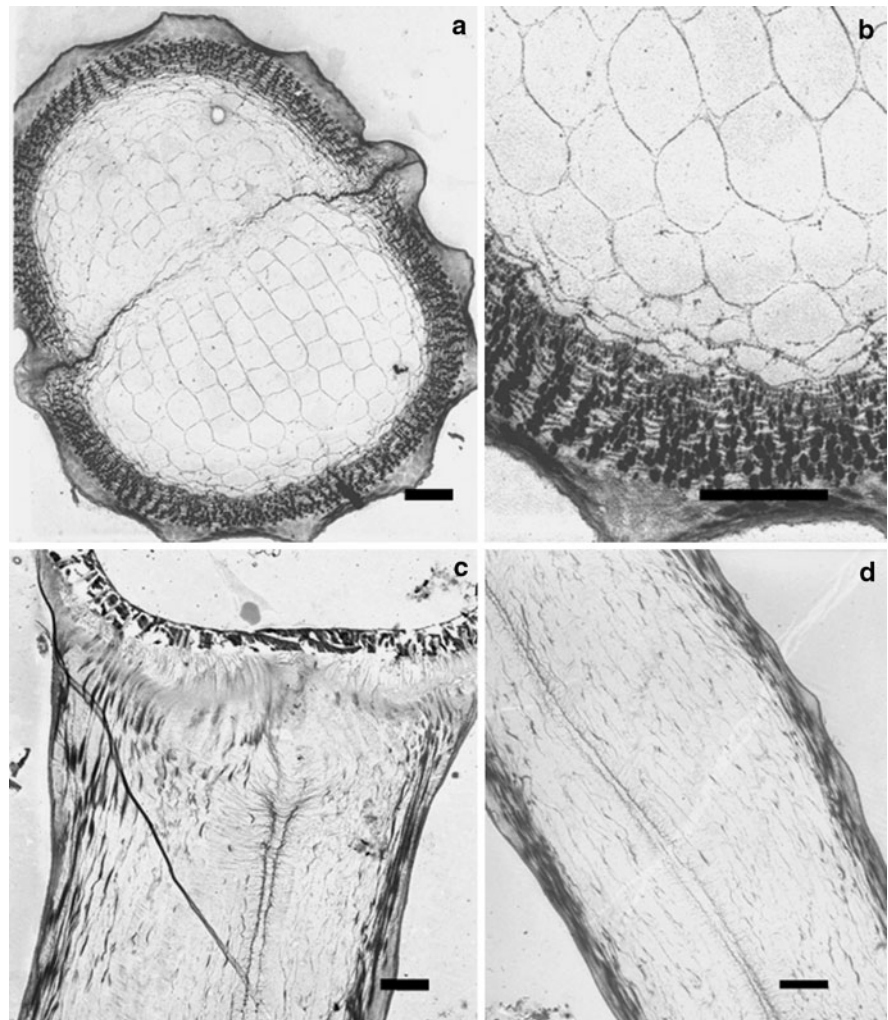
Internally, the stalks have a clear reticular structure (Fig. 3), which is evident in both the longitudinal and the transverse views. The fibres constituting the reticulum are much more densely disposed in the outer layers and form a distinct longitudinal layer of 1.5–2 μm. In transverse section, the progressive stages in centripetal formation of the septum that will divide the stalks into two branches were observed (Fig. 3).

The fibrillar material is expelled through the pores of the apical pore field of cells. The fibres are complex and seem to be composed of granular units. In some longitudinal sections a tubular-like structure is also evident (Figs. 3, 4).

Stalks showed strong phosphomonoesterase activity in the inner periphery layer, especially at the collar region (Fig. 5).

Discussion

The structure of *D. geminata* stalks is the most complex reported till now for diatom stalks. There are few ultrastructural studies of long stalked diatoms. Huntsman & Sloneker (1971), quoting Drum (1964), mentioned the hollow mucilaginous stalks of *Gomphonema olivaceum* (Lyngbye) Kützing without showing data or images; however, the stalks of *G. olivaceum* var. *calcareum* (Cleve) Van Heurck have since been shown to have a basic structure (authors, unpublished data). A collar-like structure was also reported at the apical part of *Achnanthes* stalks (Wang et al., 2000). The present observations confirm the suggestion of Kociolek & Stoermer (1988) that stalk material is secreted via the apical pore field of the cell.

Fig. 4 a Protrusion of fibrous mucilage from frustule apical pore field. **b** Apical part of a tube in the base of a cell. **c, d** Detailed views of the septum formation where granular fibres can be observed. *Scales* 1 μm
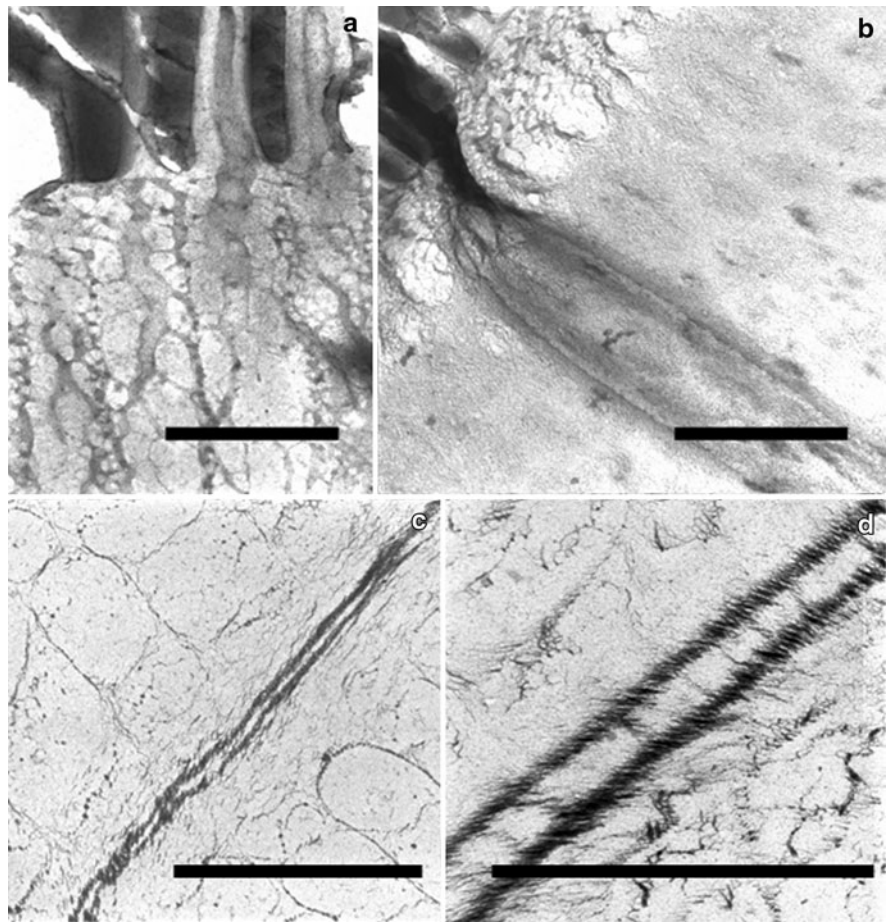
In the stalks of *D. geminata*, the fibres are organized in a complex structure but inner and outer concentric regions can also be clearly differentiated. A clear differentiation between peripheral and central areas has also been observed in *A. longipes* with a central ribbon parallel to the long axis of the stalks and radiating fibres oriented perpendicularly to the ribbon (Wang et al., 1997, 2000).

The presence of a central tube in the upper part of the stalks was already reported in *D. geminata* by Ellwood & Whitton (2007) with light microscopy. The function of this structure and whether the raphe is involved in its production as seems to be the case for the central ribbon in *A. longipes* (Wang et al., 2000) should be addressed.

The chemical composition of stalks is similar to *Cymbella cistula* (Ehrenberg) Kirchner (Wang et al., 2000; Gretz, 2008), *G. olivaceum* and carrageenan (Huntsman & Sloneker, 1971) and could be involved

in the adsorption of substances from ambient water including iron that would enhance the uptake of nitrate, silicate, Ca, Mg, S and phosphate (Novarino, 1993; Takeda, 1998). Accumulation of ions would require a gradient of the particular ion and a rapid metabolic uptake inside the cell to establish the gradient. This is very likely to occur for phosphate (Ellwood & Whitton, 2007). The passive adsorption of other ions has only been reported for amorphous EPS not for stalks but this possibility would represent an advantage in nutrient-limited habitats, especially taking into account the huge surface stalks present in *D. geminata* colonies, where there is no production of amorphous mucilage (Whitton et al., 2009).

Nutrient limitation usually induces or increases the extrusion of EPS in benthic diatoms (Granum et al., 2002). The excretion seems to be a physiological response to environmental factors in early spring, when the amount of fixed carbon exceeds the capacity
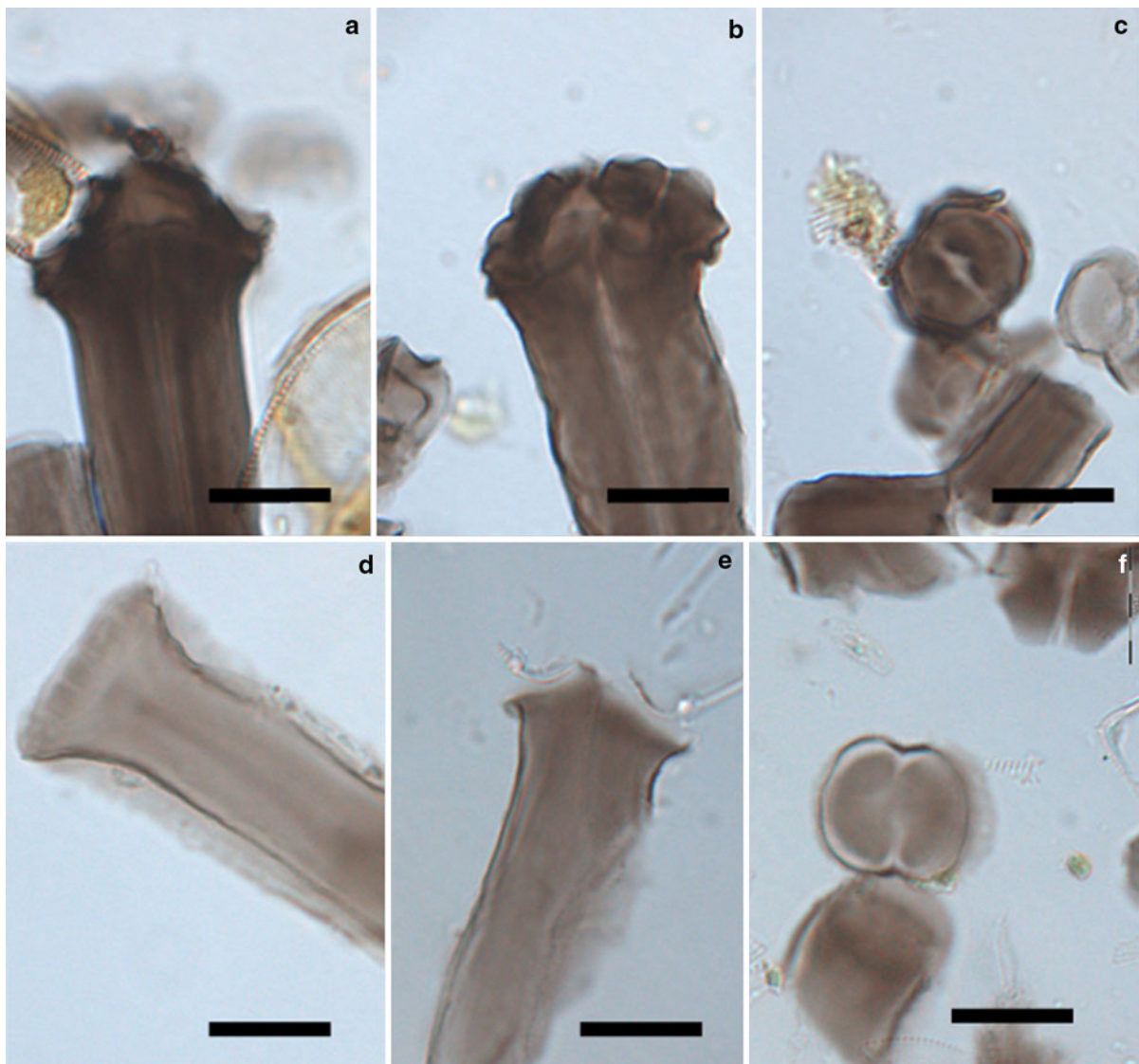
Fig. 5 Histochemical reaction (Gomori staining) to reveal alkaline phosphomonoesterase activity: **a**, **b** The *blackish colour* indicates the more intense presence of phosphomonoesterase in the apical part of stalks. **c**. Phosphomonoesterase activity in the inner part of the stalks, stronger around the inner core. **d**–**f** Controls *greyish* in colour. *Scales* 20 μm

of carbon storage within the cells. The composition of EPS may vary depending of the nutrient status of cells and environment (Underwood et al., 2004). In *A. brevipes*, the amount of released polysaccharides is higher under phosphate depletion (Guerrini et al., 2000). The stalks of some bacteria and multicellular hairs of cyanobacteria and eukaryotic algae increase in length in response to P limitation (Schmidt & Stanier, 1966; Whitton et al., 2005).

The stalks may represent a means of elevating cells above the substrate to avoid competition in dense biofilm (Lewis et al., 2002) and to have at the same time a better exposure to current speeds enhancing gas exchange and nutrient uptake (Whitford, 1960). Frequently, the stalked colonies of *D. geminata* are located near the water surface and may eventually be emerged without suffering any damage probably because they are protected by the huge amount of polysaccharides they produce.

The results presented here confirm the strong phosphomonoesterase activity of stalks reported by Ellwood & Whitton (2007), and their localization

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especially in collar area and in peripheral inner layers. The same authors also detected high phosphodiesterase activity in colonies. The presence and activity of several groups of phosphatases may permit the transformation of a wide range of organic phosphorus compounds and growth in low levels of inorganic dissolved phosphorus and could explain the success of a number of species (Whitton et al., 2005; Ellwood et al., 2008). If the hydrolysis of organic phosphorus occurs inside the stalks, as seems to be the case in *D. geminata*, inorganic phosphate could reach the cells simply by diffusion followed by a phosphate gradient associated with energy-requiring phosphate binding inside the cells (Ellwood & Whitton, 2007).

The results show that stalks have a complex structure suggesting their importance not only for their phosphatase activity to overcome low inorganic phosphate concentrations. Their large surface may function in herbivory avoidance, or a better exposure of cells to more turbulent conditions to increase nutrient uptake, adsorption of limiting elements and gas exchange. The observation that long stalks are clearly related with low in phosphorus conditions needs further investigations as their presence may represent a good monitoring characteristic for those conditions.

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