Morphological adaptations to small size in the marine diatom *Minidiscus comicus*

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Summary: *Minidiscus comicus* is a marine centric diatom that has cells with diameters as small as 1.9 μm, which brings it close to the lower limit of diatom cell size and also near to the lower limit of photosynthetic eukaryote cells. One of the questions that this raises is whether the cycle of size decline and size restoration used by most diatoms to time their life cycle can operate in such small cells. In samples collected from the western Mediterranean during 2009, *M. comicus* cells were found with diameters ranging from 1.9 to 6.0 μm. The larger cells were initial cells after size restoration, and these still had the valves of their parent cells attached, making it possible to determine the diameter of the threshold below which size restoration could be induced (3.1 μm). During size decline, *M. comicus* cell shape changed from discoid to spherical. This adaptation helped to reduce and even halt the rate of cell volume decrease, allowing cells to continue to use diameter decline as a clocking mechanism. The results show how adaptable the diatom cell wall can be, in spite of its rigid appearance.

Keywords: marine diatom; *Minidiscus comicus*; size change; size limit; size restoration.

Adaptaciones morfológicas al pequeño tamaño en la diatomea marina *Minidiscus comicus*

Resumen: *Minidiscus comicus* es una diatomea céntrica marina cuyas células pueden alcanzar diámetros mínimos de tan solo 1.9 μm, lo que las acerca al límite inferior de tamaño de las células de diatomeas y en general de las células eucariotas fotosintéticas. Una de las preguntas que surgen es si el ciclo de disminución y restauración de tamaño usado por la mayoría de las diatomeas para medir los tiempos de su ciclo de vida pueda realizarse en estas pequeñas células. En muestras del Mediterráneo occidental tomadas durante 2009, las células de *M. comicus* presentaron diámetros de 1.9 a 6.0 μm. Las células mayores eran células iniciales después de la restauración de tamaño y aún tenían pegadas las valvas de las células progenitoras, lo cual hace posible determinar el umbral de diámetro que puede inducir a la restauración (3.1 μm). Se observó que al disminuir el tamaño, la forma de la célula cambiaba de discoidea a esférica. Esta adaptación ayuda a reducir e incluso detiene la tasa de disminución del volumen de la célula, lo que permite a las células continuar usando la disminución del diámetro como un mecanismo cronometrador. Los resultados muestran cuán adaptable puede ser la pared celular de las diatomeas, a pesar de su rígida apariencia.

Palabras clave: diatomea marina; *Minidiscus comicus*; cambio de tamaño; tamaño límite; tamaño de restauración.

INTRODUCTION

Planktonic diatoms vary in diameter by over three orders of magnitude, from *Ethmodiscus* cells that can be over 2 mm in diameter (Round et al. 1990) down to species with cells that have diameters of 2 μm (e.g. Vaulot et al. 2008). Size is important in planktonic environments for a variety of reasons, including trade-offs in terms of nutrient uptake, light interception, growth rate, grazing, and suspension (e.g. Geider et al. 1986, Vaulot et al. 2008, Winder et al. 2009, Litchman et al. 2009, Barton et al. 2013, Reavie and Barbiero 2013, Clark et al. 2013, Narkov et al. 2014, Jewson et al. 2015). However, for most diatoms, an additional

factor is that cell diameters in a population usually decrease with time. This was first observed by Macdonald (1869) and Pfitzer (1869) and was thought to be an inevitable consequence of their having a rigid silica cell wall structure. However, we now know that this decline can be avoided (see Chepurnov et al. 2004), but that it is retained in most species as an elegant solution to timing the length of their life cycles (Lewis 1983, 1984, Jewson 1992a, b, D'Alelio et al. 2010, Mann 2011, Jewson and Granin 2015). What we do not know is whether the cycle of size decline and subsequent size restoration still operates in a similar way in species at the lower size limit, when cell volume approaches the theoretical minimum for eukaryote cells of just under one micron in diameter (e.g. Raven 1998).

Minidiscus comicus Takano, order Thalassiosirales, is one of the smallest marine diatom species. It is widespread along the Pacific and Atlantic coasts, as well as in the Adriatic and Mediterranean Seas (Takano 1981, Lange 1985, Hasle and Syvertsen 1997, Tomas 1997, Bérard-Therriault et al. 1999, Shevchenko and Orlova 2002, Kaczmarska et al. 2009, Percopo et al. 2011, Lee et al. 2012). *Miniduscus comicus* belongs to a small marine genus of seven species (Aké-Castillo et al. 2001, Kang et al. 2003, Quiroga and Chrétiennot-Dinet 2004, Kaczmarska et al. 2009). The first report of *M. comicus* was in large aggregated flocks in Tokyo Harbour (Takano 1981), but since then it has usually been found as solitary cells or in short chains of 2 to 3 cells (Kaczmarska et al. 2009). Kaczmarska et al. (2009) reported cells of *M. comicus* with diameters of 2.1 to 3.2 μm and Percopo et al. (2011) found cells of 2.7 to 4.3 μm, but Quiroga and Chrétiennot-Dinet (2004) found broader diameter ranges of 1.9 to 7.0 μm and Takano (1981) 2.0 to 7.0 μm. In this study, we used samples of *M. comicus* collected from the western Mediterranean (see Estrada et al. 2014), to investigate how a change from discoid to spherical cell shape in small cells can help offset loss of volume when diameter decreases as part of the life cycle.

MATERIALS AND METHODS

Detailed site and sampling information are reported in Estrada et al. (2014), so only a brief summary is given here. The samples were collected during three cruises of the FAMOSO project carried out in mid-March, late April-May and September 2009, on board the R.V. *Sarmiento de Gamboa* in the central zone of the NW Mediterranean. The region was delimited approximately by coordinates 41°30' to 42°N and 4° to 5°E. Water samples were obtained with 12-L Niskin bottles mounted on a rosette. The samples in this study were collected from 20 m depth on 21 March 2009, from 25 m on 22 March 2009, from 80 m on 11 May 2009, and from 80 m on 19 September 2009. For each sample, either 150 or 250 mL of water was filtered onto 0.8- or 2-μm polycarbonate Nucleopore filters. These were stored dry in Petri dishes, which were kept in the dark and under partial vacuum. The filters were attached to stubs using a colloidal silver gel and then sputter coated (Polaron SC-500) with a gold-palladium

layer less than 10 nm thick. The scanning electron microscope (SEM) was a Hitachi S-3500N operated at 5 Kv. Cells were orientated and photographed in both girdle and mantle views to enable measurements of cell shape. All estimates of cell volume were based on non-dividing cells. In the largest cells, with diameters above 5 μm, their shape approximated to a cylinder, with the girdle band region having a height of 0.8 μm. In cells with smaller diameters (below 4 μm), the valves of non-dividing cells abutted (see below), so no allowance was needed for the girdle region. Most small cells below 3 μm approximated to the shape of a sphere and their cell volume was then estimated using the standard formula. However, for cells with valves that were domed but not completely hemispherical, their volume was calculated using the formula for a 'spherical cap' of a sphere, $V=0.33h^2(3r-h)$, where h is the height of the domed valve and r is the radius of the sphere that would be formed if the curvature of the valve was extended to form a full sphere. This could be estimated from the SEM photographs, because the curvature of the doming was consistent in all but a few cells, enabling simple geometry to reconstruct the appropriate circle size for each individual valve in a cell.

Site details

On 21-22 March 2009, the water column was well mixed, with temperatures of 13.1°C to 13.2°C in the upper 200 m. On 11 May 2009, the water column consisted of a shallow mixed layer (12 m) and a gradient of increasing density down to about 100 m; water temperature was 16.0°C at the surface and 13.1°C at 80 m depth. In September 2009, the water column was strongly stratified, with a marked pycnocline at about 50 m depth and temperatures of 22.7°C at the surface and 13.3°C at 80 m depth.

The March sampling took place during a well-developed winter-spring phytoplankton bloom, which is a typical feature of the study region. On 21 and 22 March 2009, chlorophyll *a* (Chl *a*) concentrations in the upper 25 m ranged between 2.4 and 2.5 mg m⁻³. The larger (nano and micro size classes) phytoplankton was dominated by dinoflagellates and prymnesiophytes, including both healthy and senescent colonies of *Phaeocystis* sp. and coccolithophores (*Emiliana huxleyi, Calcidiscus leptoporus, Helicosphaera carteri*, among others), but later, in waters with higher salinities and Chl *a* concentrations, diatoms such as *Chaetoceros* and *Pseudo-nitzschia* were more prevalent, in addition to *Lauderia annulata* and *Dytilum brightwellii* (Estrada et al. 2014). By 11 May, Chl *a* had decreased to 0.6 mg m⁻³ at the surface and 0.15 mg m⁻³ at 80 m depth. Diatoms were scarce and dinoflagellates and *Phaeocystis* sp. dominated. On 19 September 2009 there was a marked Chl *a* maximum of 0.68 mg m⁻³ at 60 m depth, but a decrease to 0.24 mg m⁻³ at 80 m depth. The most abundant phytoplankton groups were dinoflagellates and diatoms (especially small *Chaetoceros* species). The picoplankton in March was dominated by *Synechococcus* and picoeukaryotes (Mouriño-Carballido et al. 2016). *Prochlorococcus* was not found in
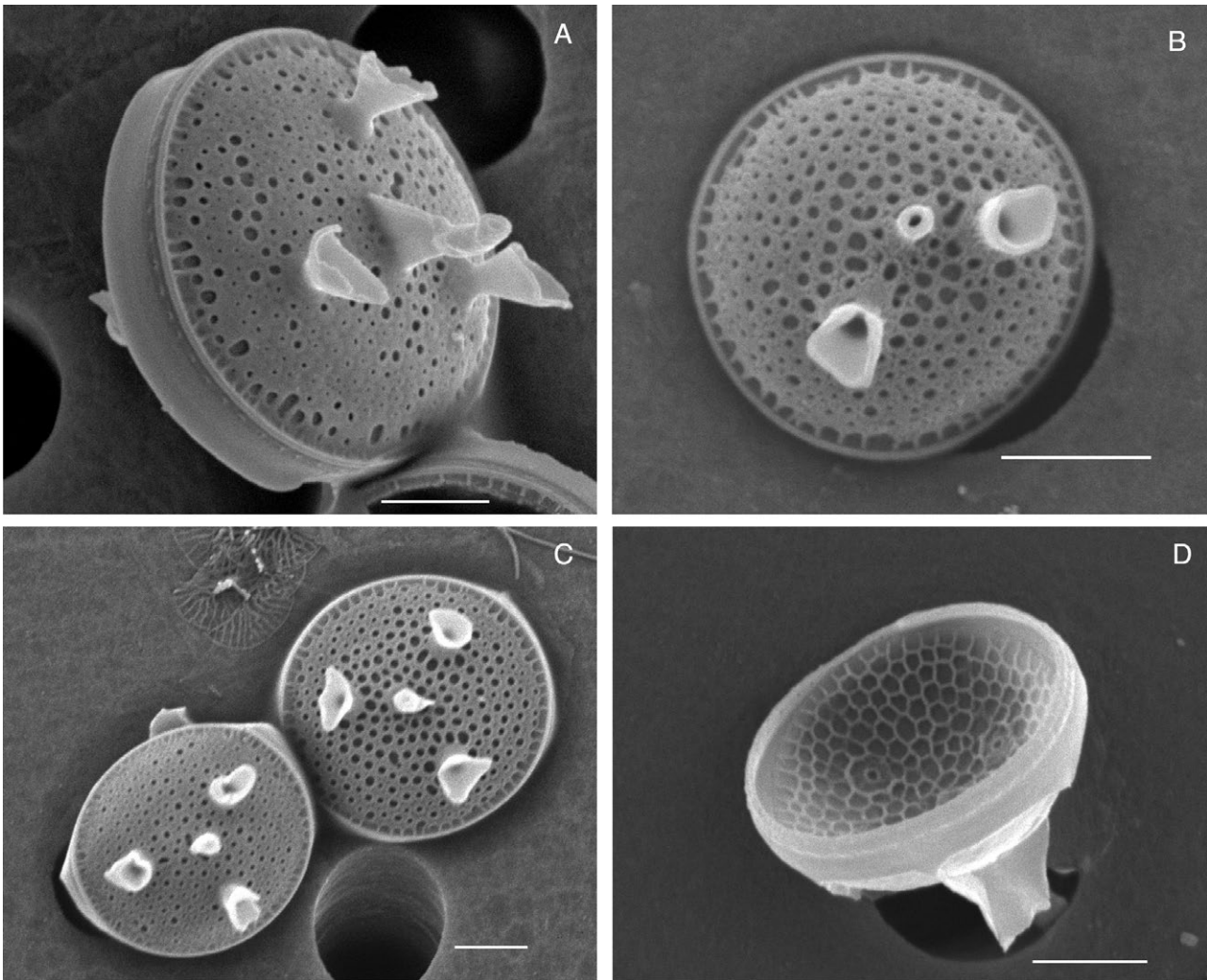
Fig. 1. – *Minidiscus comicus* showing the positioning of the central rimoportula and the arrangement of the surrounding fultoportulae. There are usually three fultoportulae per cell, but in the narrowest diameter valve, there are only two. A-C are external views of silicified cells. D is also an external view but of a partly silicified cell where the cell wall has become concave during preservation. It shows the hexagonal structure of the side walls of the pseudoloculi, which are underlain by a basal membrane. A-C, 21 March 2009; D, 11 May 2009. Scale bar 1 μm.

May, but was present in March and September, where it was more abundant than *Synechococcus* below 25 m. *Parmales* sp. of similar size to *Minidiscus* were also recorded, especially in March and September.

RESULTS

Morphology

Minidiscus comicus cell diameters varied in size from 1.9 to 6.0 μm (Figs 1-4). In the larger diameter cells of 5 μm or above, the mantle face was flat or slightly domed (Fig. 2B, D), but as cell diameters declined, doming of the valve face became more pronounced (Fig. 3), until the smallest cells (with diameters of 2-3 μm) became spherical (Fig. 4B, C) or even oblong (Fig. 4A, D).

In the cell wall of fully silicified valves, the external foramina of the loculate and/or pseudoloculate areolae became small (Fig. 1A). These valves retained their shape during preservation and observation on the SEM. However, on 11 May 2009, many valves (over 50%) just had the basal layer and/or the partially formed hexagonal sidewalls of the areolae. When dried out for SEM study, the valve faces of these cells often became concave, showing the flexibility of the cell wall at this stage (Fig. 1D).

Most valves had relatively large external processes, consisting of a central rimoportula surrounded by three fultoportulae (Figs 1A, C, 2D, 3A, C). However, some of the broadest cells had four fultoportulae (Fig. 2C) and the smallest cells only two (Figs 1B). The outer, fluted exterior tube-structure of the fultoportula was very well developed, rising to over 0.5 μm in height above the mantle. The final shape was apparent before the main silicification of the cell wall (Fig. 2A). To accommodate their formation and the doming of valves during cell division, daughter valves separated by up to 5 μm while still surrounded by copulae. The central rimoportula could reach up to 2.4 μm in height in wide-diameter cells (Figs 2B, D, 3B, D). The copulae that protect the dividing cells were characteristically very thin and collapsed around the valves during preservation and preparation of the samples (Fig. 3).
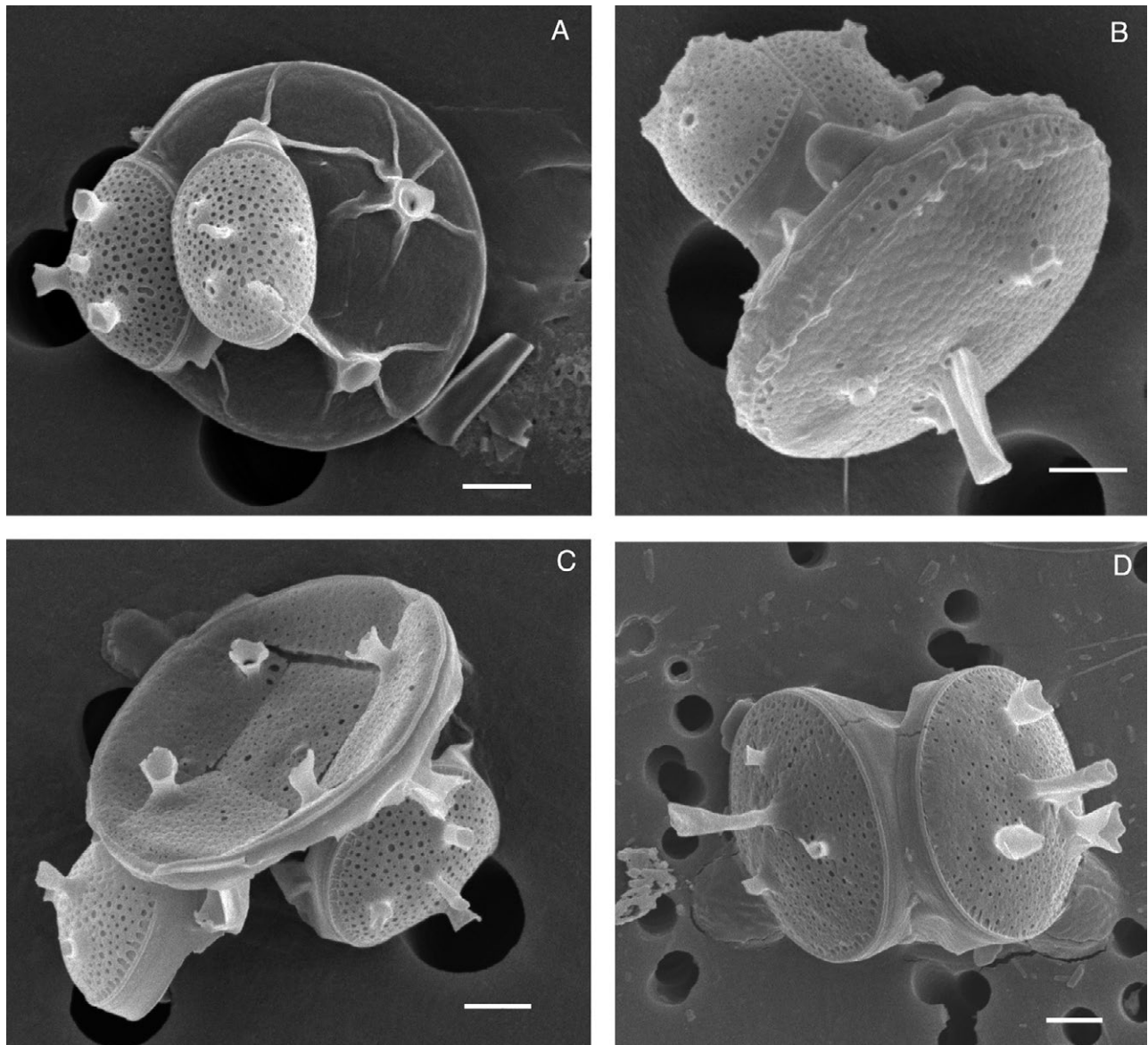
Fig. 2. – Size regenerated initial cells of *Minidiscus comicus* on 11 May 2009 with the valves of a parent cell still attached. A-C, 11 May 2009; D, 19 September 2009. Scale bar 1 µm.

Size restoration

On 11 May 2009, size restoration was taking place (Fig. 2). The valves of the parent cells stayed attached to the initial cells (Fig. 2), which made it possible to estimate in five cases the size of diameter change and the upper limit for inducing size restoration. The parent cells had diameters of 2.1, 3.0, 3.0, 3.1 and 3.1 µm, and the initial cells of 5.8, 5.7, 6.0, 5.7 and 5.8 µm, respectively. Therefore, the increase in diameter ranged from 1.8 to 2.8 times and the threshold diameter below which size restoration could be induced was 3.1 µm.

Diameter distribution

The size distributions of *M. comicus* cell diameters were measured on 21-22 March, 11 May, and 19 September 2009 (Fig. 5). On 21-22 March 2009, there was one main peak at 3.47 µm and a second smaller peak at 2.48 µm (Fig. 5A). These were above and below the size threshold for size restoration that was estimated above. However, there was no evidence of size regeneration until 11 May, at which time there was a relative decrease in the number of the small diameter cells at 2.48 µm and an increase in cells above 5 µm diameter (Fig. 5B). Also, the diameter of the majority of cells in the population, which were dividing asexually, decreased to around the threshold (3.1 µm) for inducing size restoration. By 19 September, most of the small cells below the threshold were gone, leaving a large peak of newly size-restored cells at 5 µm and the remainder of the population at 3 µm.

Shape change with size decline

The cell shape of *M. comicus* changed as diameter declined. In the largest-diameter cells the shape was discoid but it became spherical in the smallest cells
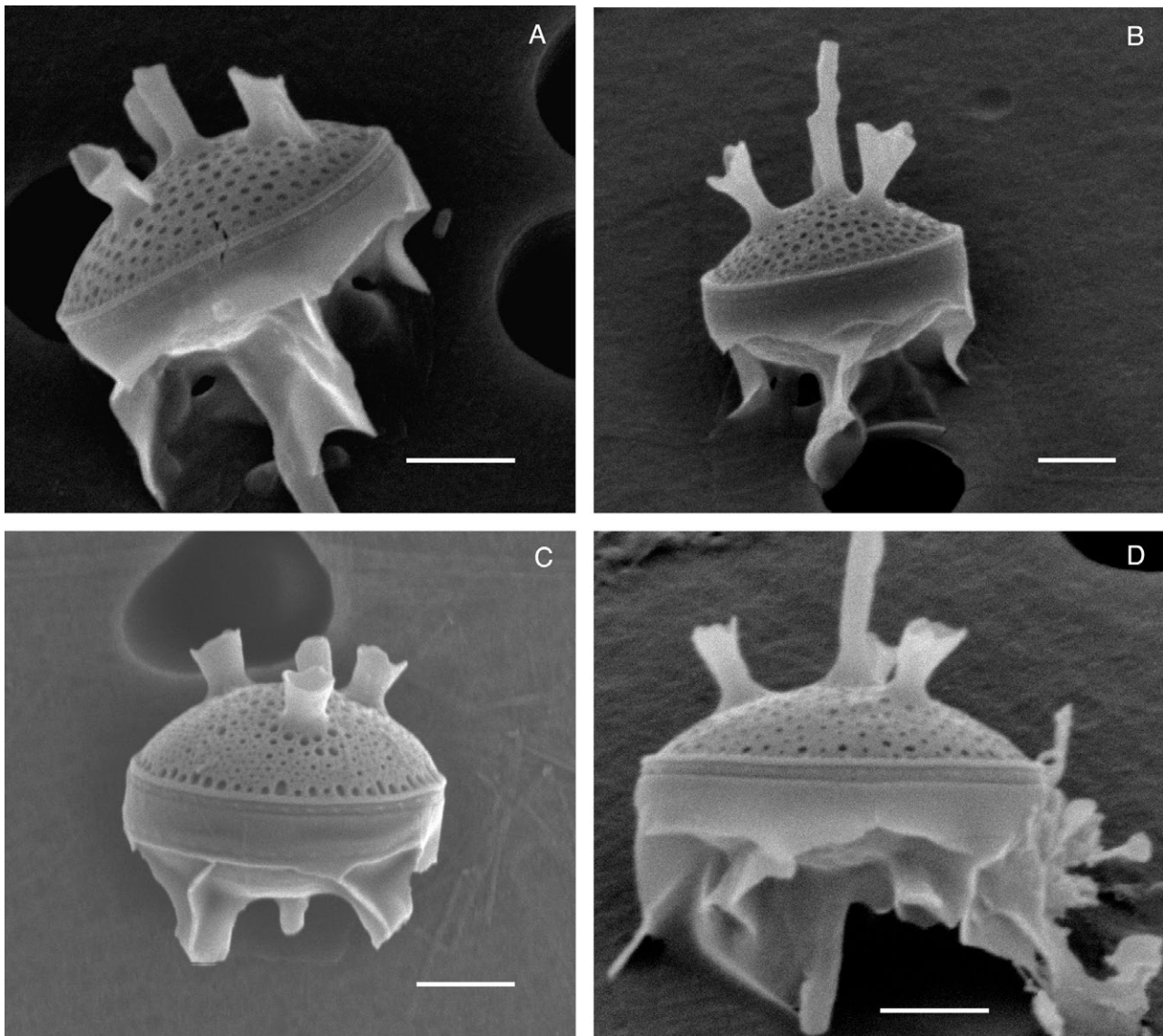
Fig. 3. – Girdle (side) views of *Minidiscus comicus* showing the varying degrees of doming of the valves. A, B, D, 11 May 2009; C, 21 March 2009. Scale bar 1 µm.

(see Figs 1-4). Such alterations to cell shape were only possible at the time of cell division, when new daughter valves were being formed. Cell volumes were calculated for the different diameters and their different degrees of valve doming (Fig. 6). Generally, cells with diameters above 4 µm were discoid with slightly curved mantle faces, but as the diameter decreased and approached the size restoration threshold (3.1 µm), then both valves became domed, making the cell shape spherical (Fig. 4). The change in shape of the valves was due partially to an increase in valve height from 0.7 to 1.3 µm, but also to an added curvature of the valve as the diameter decreased. In some of the very smallest cells with diameters below 2.5 µm, some valves reached a mantle height of 1.8 µm, which meant that the cell shape became oblong (Fig. 4D).

The overall effect of the change in shape is that a spherical cell at the size restoration threshold, with a diameter of 3.1 µm, had three times the cell volume (15.6 µm³) as a cell of the same diameter if it had re-mained discoid (5.7 µm³). The volume was also nearly the same (14.7 µm³) as a larger discoid cell of 5 µm diameter. Once cells took on the spherical shape, their volume then declined with diameter along the upper curve shown in Figure 5. They were still more than twice the cell volume of an equivalent flat-mantle cell at the minimum diameter size of 2 µm. However, because some cells of this size became oblong (Fig. 4), they increased their cell volume. For example, the volume of a spherical cell of 2.3 µm was 6.7 µm³, but an oblong cell of the same diameter was 9.4 µm³ (e.g. see arrowed point above the spherical curve in Fig. 6).

DISCUSSION

Size is a key factor in planktonic life and many studies have looked at its functional implications (e.g., Geider et al. 1986, Vaulot et al. 2008, Winder et al. 2009, Litchman et al. 2009, Barton et al. 2013, Reavie and Barbiero 2013, Clark et al. 2013, Jewson et al.
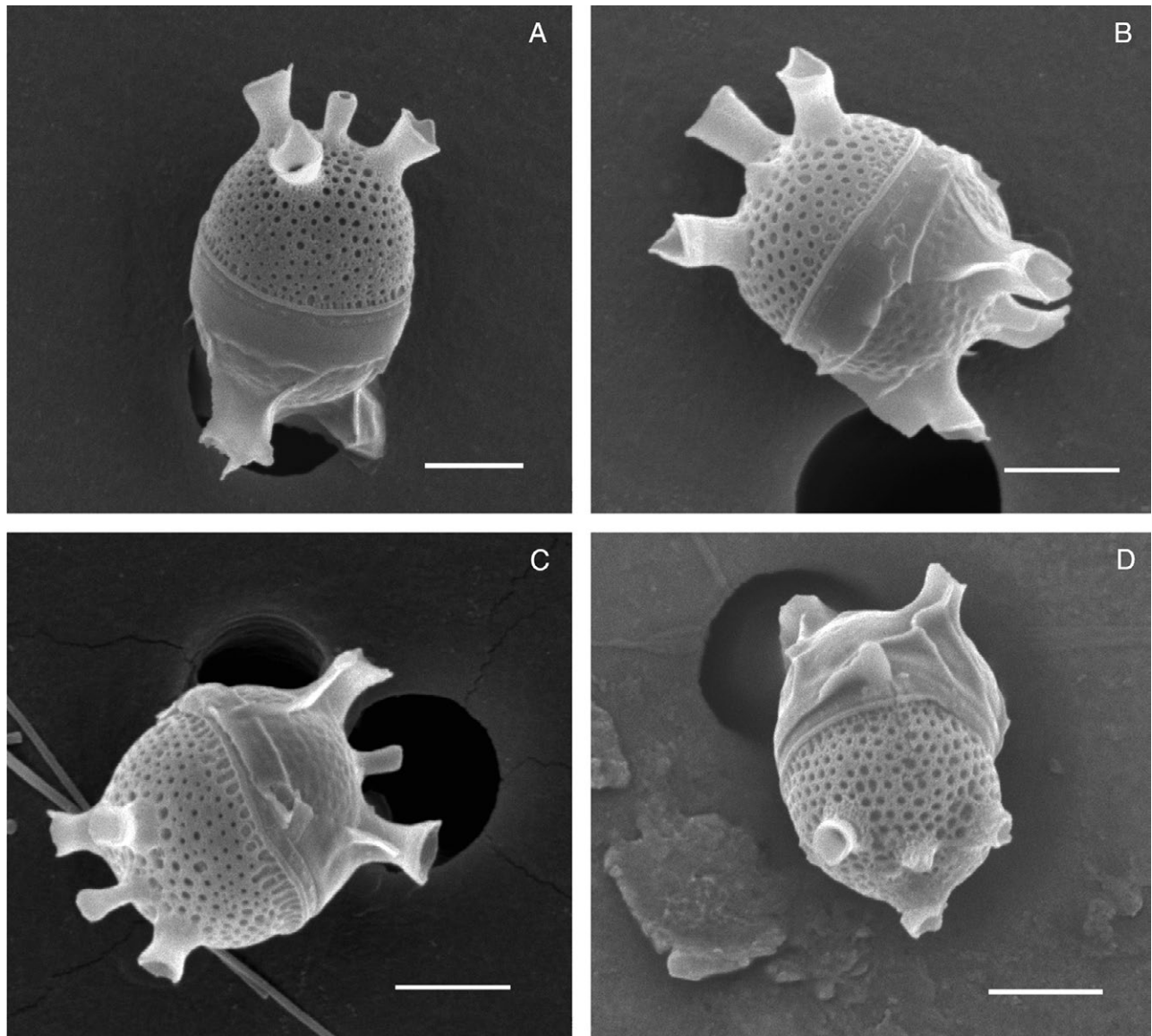
Fig. 4. – Small diameter cells of *Minidiscus comicus* showing the increased doming of the mantle to form spherical cells (A-C) and even oblong cells (D). A-C, 11 May 2009; D, 21 March 2009. Scale bar 1 μm.

2015). Among diatoms there is the added complication of having to cope with the cycle of size decline and size restoration used to time the length of their life cycle over periods longer than one year (Lewis 1983, 1984, Jewson 1992a,b, Jewson and Granin 2015). The interest in *M. comicus* was to see whether this cyclic clocking mechanism could still operate in very small cells, where the rigid cell wall structure might be expected to constrain the volume necessary for cell organelles, such as nuclei, mitochondria and chloroplasts, and for gamete production. The solution used by *M. comicus*, which avoids interfering with diameter decline, is to become spherical or even oblong to maintain cell volume as diameter declines. Essentially, it is a change in cell length, which is an adaptation used by some other centric diatoms, such as *Aulacoseira baicalensis,* to make room for storage products during dormancy (e.g. Jewson et al. 2010, Jewson and Granin 2015).

A longer sequence of sampling would be needed to accurately determine the length of the life cycle of *M.*

comicus, but from the rate of size decline observed during 2009, it is probable that some cells could reach the 3.1 μm threshold after size restoration in two years. For example, in September 2009 there were predominately two age classes (Fig. 5C). The size-restored cohort in September had a modal diameter of around 5 μm, while the second peak at 3 μm was probably mainly made up of cells that had regenerated their size in the previous year (2008). The diameter of these cells would then have declined further to be below the threshold for inducing size restoration by March 2010, and thus be ready to begin completing the cycle of size restoration by May, with cell diameter distributions similar to those found, respectively, for March 2009 (Fig. 5A) and May 2009 (Fig. 5B). This is just the 'average' situation, because the population would contain cells from other age classes, as the rate of size decline depends very much on conditions for growth and division experienced by individual cells, with some declining at slower rates and some at higher rates, which ensures an overlapping of age classes (see
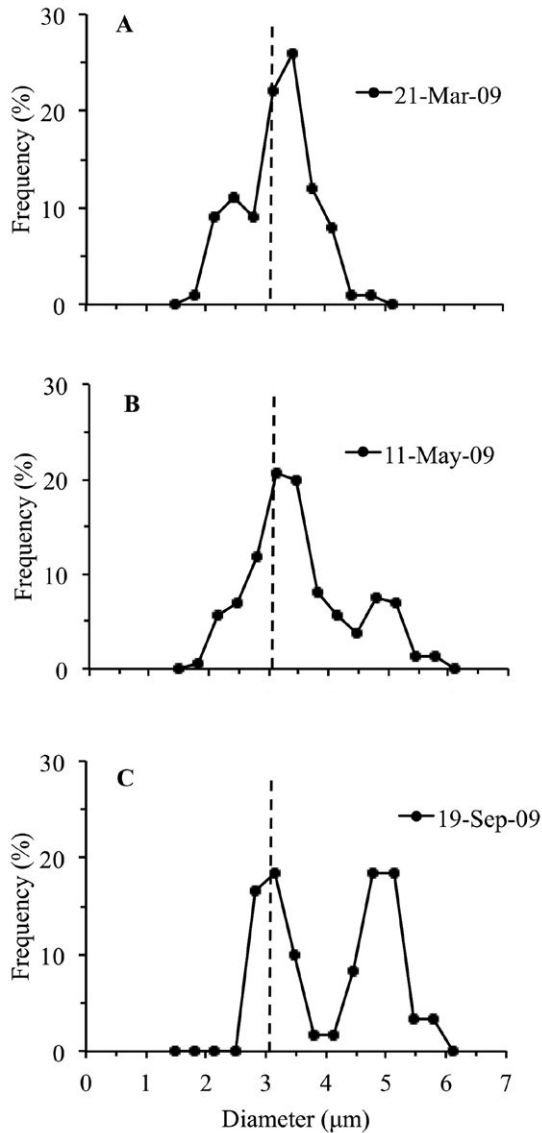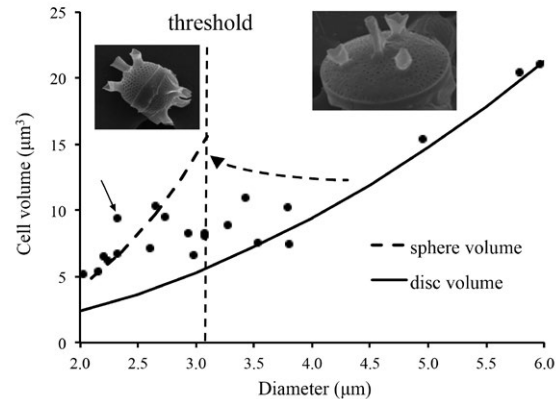
Fig. 6. – The relationship between diameter and cell volume in *Minidiscus comicus*. The lower curve shows the decline in cell volume for discoid cells and the upper curve shows the cell volume of spherical cells that are found when the diameter diminishes below the threshold at which size restoration can be induced (3.1 μm). The 'outlier' cell below 2.5 μm (arrowed) was a cell that had increased its volume by becoming oblong rather than spherical (see text).

Fig. 5. – Cell diameter size distribution of *Minidiscus comicus*, expressed as relative occurrence, in a) 21/22March (n=100), b) 11 May (n=160) 2009 and c) 19 September 2009 (n=60). The size class used was 0.33 μm. The vertical dashed line indicates the threshold below which size restoration can be induced.

Jewson 1992a). The likely length of the life cycle of *M. comicus* is therefore probably two or three years. The timing of size restoration in *M. comicus* is also interesting because it occurred in summer when larger diatoms were scarce and it was likely to have been a nutrient-poor period, especially for silica, which suggests that *M. comicus* can survive and reproduce at times when other, larger diatoms have ceased growth. Although it seems likely, we do not know yet whether size restoration in *M. comicus* is linked to sexual reproduction.

In addition to the large changes in cell shape, another striking feature of the *M. comicus* cell wall was the relatively large size of its exterior processes (Figs 1-4). There were two types. One was the exterior fluted tube-structures of the fultoportulae. Kaczmarska et al. 2009 showed that these structures produce chitin filaments. Similar chitin filaments are found in other small *Thalassiosira* (Martin-Cereceda and Cox 2011) and *Cy-*

clotella (Jewson et al. 2015), and they have been linked to reducing sinking rate (e.g. Walsby and Xypolyta 1977, Walsby and Reynolds 1980). However, in *M. comicus* it is unlikely that this is their sole purpose, because in such small cells, sinking is minimal. An alternative possibility is that the chitin filaments could interfere with grazing by flagellates and ciliates, because *M. comicus* lives in a nanno-habitat along with other small species, such as *Parmales*. Other possible functions might come from an observation by Kaczmarska et al. (2009) that cells of a close relative, *Minidiscus chilensis*, which were collected from surface sediments, had projecting chitin filaments. It is possible that the filaments, which can be over 30 μm long, could help in the resuspension of cells back into the water column by bridging across the boundary layer at the sediment surface. One property of the external tubes is that they restrict and support the angle at which the chitin filaments are deployed. However, the reason for this and their function is yet to be determined.

One consequence of the doming and production of the large external fultoportulae tubes during division is that they create a problem for keeping cells together. They require a relatively large distance of up to 5 μm, which is the same as or bigger than the cell diameter. In this study, the cingula protecting the lengthening cell during division offered little rigid support. Therefore, holding the two daughter valves together is probably the role of the other prominent structure, the central rimoportula. One reason for this adaptation may be that *M. comicus* grows after the main spring diatom bloom, when there is a lack of sufficient silica to build rigid cingula, so an alternative solution is required to keep daughter cells together during division. However, in nutrient sufficient conditions, such as in culture, *M. comicus* does appear to form cingula with a rigid structure (Percopo et al. 2011).

In conclusion, the main result from this study is that *M. comicus* demonstrates the versatility of diatoms and shows how their apparently rigid cell wall is not restrictive, with changes in shape possible over their life cycle. In the case of *M. comicus*, this is done by

changing from discoid to spherical shape to maintain cell volume during diameter decline, thereby making it possible to avoid interference with the life cycle clocking mechanism in a very small species.

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