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NOTE

MORPHOLOGICAL FLEXIBILITY OF *COCCONEIS PLACENTULA* (BACILLARIOPHYCEAE) NANOSTRUCTURE TO CHANGING SALINITY LEVELS[1]

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Diatoms possess a silica frustule decorated with unique patterns of nanosize features. Here, we show for the first time from in situ samples that the size of the nanopores present at the surface of the diatom *Cocconeis placentula* Ehrenb. varies with fluctuating salinity levels. The observed reduction in nanopore size with decreasing salinity agrees with previous laboratory experiments. We also uniquely combined our observations with theoretical considerations to demonstrate that the decrease in the diffusive layer thickness is compensated for by the changes in pore size, which maintain a steady diffusive flux toward the diatom's cell at different salinities. This process allows diatoms to absorb similar amount of nutrients whatever the salinity and as such to increase their ecological competitiveness in fluctuating environments. These results further suggest that the overall ecological success of diatoms, and their ability to react to environmental changes, may be controlled by the flexibility of the morphological characteristics of their frustules.

Key index words: **diatoms; diffuse layer; passive diffusion; salinity**

Abbreviations: **FESEM, field-emission scanning electron microscopy; PSU, practical salinity units; SDV, silica deposition vesicle**

Diatoms (Bacillariophyceae) are among the most abundant phototrophs on earth (Falkowski et al. 1998) and account for 40% of the total primary production in the ocean (Nelson et al. 1995, Smetacek 1999, Tréguer and Pondaven 2000). They are seen as the main players in biogeochemical cycles (Buesseler et al. 1998) and as keystone organisms significantly affecting zooplankton grazing and recruitment (Irigoien et al. 2002, Ianora et al. 2004, Jones and Flynn 2005). In addition, they are increasingly recognized as useful biological indicators of water quality (Gell et al. 2002).

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Each of the estimated 10^5 diatom species has a specific frustule shape (or cell wall) similar to a petri dish with overlapping upper and lower halves, respectively referred to as epitheca and hypotheca (Hildebrand 2008). Capping the thecae are the valves, which are decorated with a unique pattern of nanosize features (i.e., pores, channels, ridges, spikes, spines; Round et al. 1990). The two valves are called the epivalve, which originates from the parent cell, and the hypovalve, which is formed after cell division. The nanosize features present at the valve surface, such as the pores, are species-specific and provide precise features for the identification of diatoms (Gell et al. 2005). In particular, the architecture of the valves of some diatom species (e.g., *Coscinodiscus* sp. and *Thalassiosira eccentrica*) shows fundamental morphological differences including the number of layers, size, shape, density, and geometric arrangement of pores (Losic et al. 2006). These pores allow the exchange between the internal cell and the external environment and are thus important features for diatom growth. Recent experimental work has shown that the architecture and distribution of the pores appear to be influenced by environmental factors, such as pH (Vrieling et al. 1999) and salinity (Gordon and Brodland 1990, Vrieling et al. 2000, 2007). However, to our knowledge, no study has addressed the issue of the impact of salinity on pore architecture, based on naturally occurring diatoms. The only reference to salinity in an investigation of diatom pores is the identification of salt crystals at the surface of *Biddulphia sinensis* taken from the North Sea (Vrieling et al. 2000).

In this framework, our study aimed (i) to assess the potential variability in the size of pores present at the surface of diatoms in contrasted salinity conditions and (ii) to infer their potential contribution to the diffusive fluxes through the frustule. This has specifically been addressed along the natural salinity gradient (from estuarine, $S = 25$ PSU, to hypersaline, $S > 150$ PSU; Schapira et al. 2009) occurring in a coastal lagoon extending over a distance of 100 km along the coastline of South Australia (Fig. 1), the Coorong wetlands.

Along the 100 km distance of the Coorong wetlands, six sites were monitored and showed a salinity gradient ranging from 27 PSU in the north lagoon (S1; Fig. 1) to 154 PSU in the south lagoon (S6; Fig. 1). At each site, diatoms were collected using a plankton net (60 μm mesh size). Hydrological parameters were determined in situ using a YSI-85 oxygen, salinity, and temperature meter (TPS WP Series, TPS Brisbane, Qld, Australia). Water samples were collected and frozen prior to assessing nutrient concentrations (i.e., nitrate, nitrite, ammonium, and phosphate) in the laboratory using an Aquaspex photometer (Aquaspex Water Testing Products, Blackwood, South Australia, Australia).

The only diatom species that was present at several sampling sites (S3 to S5) was *Cocconeis placentula*. However, the abundance of *C. placentula* at S5 was too small to be able to undertake further analysis on the population of that site. We then specifically focused on two sites, S3 and S4, with a salinity of 67 and 100 PSU, respectively. The organic material present on the surface of *C. placentula* was removed using sulfuric acid (50% concentration) following Losic et al. (2006). The architecture and distribution of the pores at the surface of the *C. placentula* frustules were determined using field-emission scanning electron microscopy (FESEM). FESEM images were acquired using a Philips XL30 field-emission scanning electron microscope (Philips Electronics, Andover, MA, USA) operated at 2–10 kV (Fig. 2). We then measured the nanopores of 20 diatoms from each of the sites (sites S3 and S4; Fig. 1). These 20 diatoms corresponded to the measurement of a total of ~400 pores per site. For each individual diatom, the size (i.e., length and width) of the frustules and pores was measured from the FESEM images using the MOTIC Images plus 2.0 software (MOTIC, http://www.motic.com).

Then, the total number of pores per valve and the related percentage of open surface area were determined. This was to characterize the surface of exchange between the cell and the environment. Comparisons between the two sampling sites were

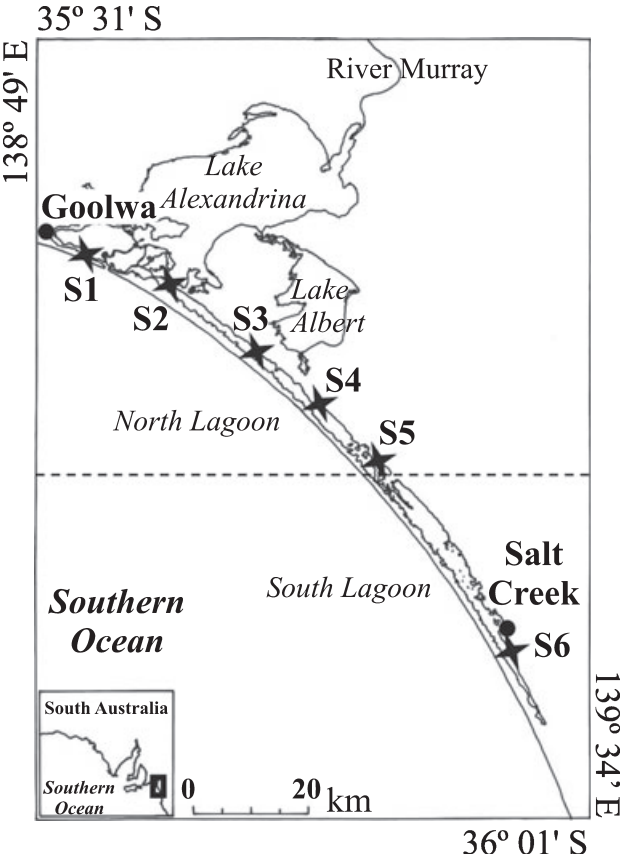

FIG. 1. Wetlands of the Coorong National Park in South Australia. The different regions (north and south lagoons) as well as the sampling sites (sites S1 to S6; 27–154 PSU) are indicated.

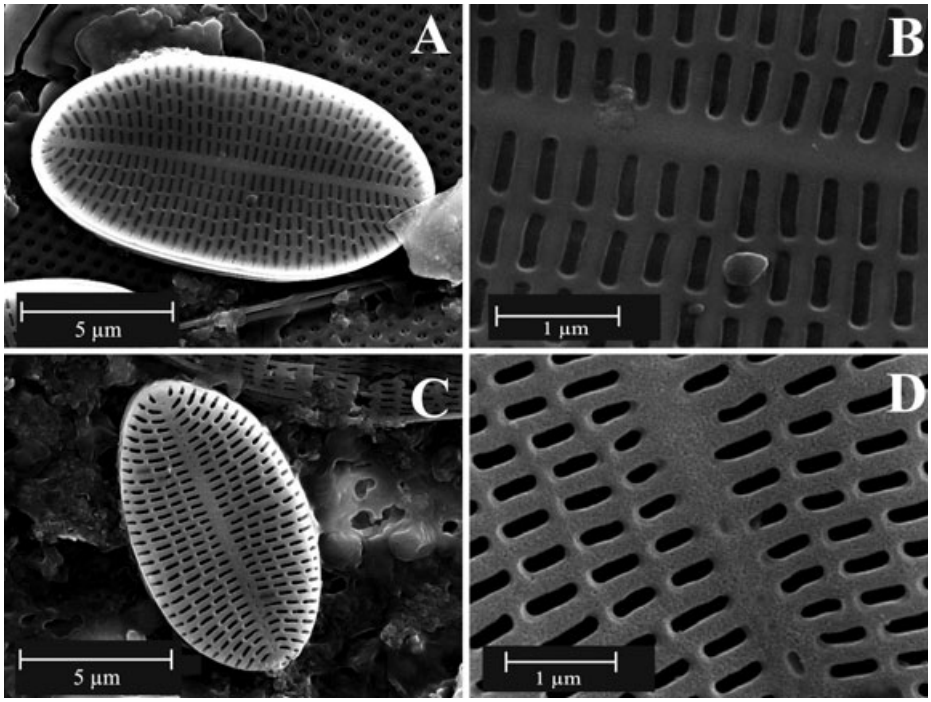

FIG. 2. Field-emission scanning micrographs of examples of the frustule morphology of *Cocconeis placentula* from two sites (sites S3 and S4) in the Coorong wetlands. (A) and (B) correspond to 67 PSU, and (C) and (D) correspond to 100 PSU.

conducted using the Wilcoxon–Mann–Whitney *U*-test (Zar 1999). In addition, the correlation between the size of the pores and the size of the diatoms was tested through the Spearman coefficient of rank correlation. Finally, the variability in the measurements of the length and width of the pores was assessed using the coefficient of variation (CV).

Results. The concentration of ammonium and phosphate, respectively, ranged between 20 and 103.33 μmol · L^{-1}, and 2.11 and 6.32 μmol · L^{-1}. The nutrients significantly increased between the two sites (Wilcoxon–Mann–Whitney, $P < 0.01$), with the highest concentrations consistently observed at 100 PSU. However, the concentration of nitrate was constant at both sites with 1.61 μmol · L^{-1}, and nitrite concentrations ranged between 1.09 and 1.30 μmol · L^{-1}. No differences were observed between the two sites for nitrate and nitrite ($P > 0.05$). The same observations were made for dissolved oxygen, which ranged between 3.86 and 4.29 mg · L^{-1}.

The length (L) and width (W) of the pores of *C. placentula* were measured at $L = 517 \pm 8$ nm ($\bar{x} \pm$ SD) and $W = 148 \pm 2$ nm for 100 PSU, and $L = 490 \pm 6$ nm and $W = 108 \pm 2$ nm for 67 PSU and showed significant differences between the two salinities at each site (Wilcoxon–Mann–Whitney, $P < 0.01$, Fig. 3). These findings indicate an increase in the size of the pores with salinity. Furthermore, the length/width pore ratio (*RPore*) at 100 PSU (*RPore* = 3.67 ± 0.08; $\bar{x} \pm$ SD) was significantly smaller than at 67 PSU (*RPore* = 4.78 ± 0.10, $P < 0.01$). Finally, the length/width valve ratio (*RValve*) did not vary between sites ($P > 0.05$). The variability (*CV*) in pore measurements was similar for both sites (*CVlength* = 17%–22% and *CVwidth* = 20%–21%).

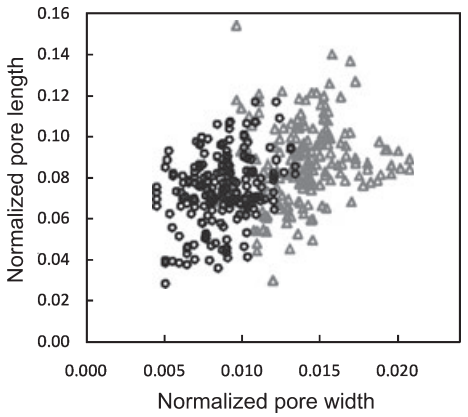

FIG. 3. Length and width of the pores located on the external side of *Cocconeis placentula* frustules for sites 3 (circles) and 4 (triangles), sampled along the Coorong wetlands. The length and width of individual pores have been normalized by the size (length and width) of the corresponding diatom frustule.

The valve and pore shapes were then approximated to ellipses, and the open surface area was calculated according to

$$S = N \times \pi \times \left(\frac{L}{2}\right) \times \left(\frac{W}{2}\right) \quad (1)$$

where N is the number of pores per valve, L is the average length of the pores, and W is the average

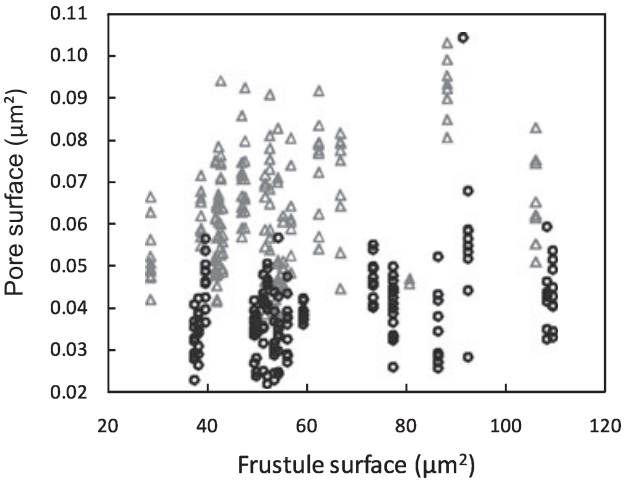

FIG. 4. Surface of the pores located on the external side of *Cocconeis placentula* frustules for sites 3 (circles) and 4 (triangles), sampled along the Coorong wetlands. The *x*-axis represents the surface of the valve from which the pore measurements are taken.

width of the pores. The percentage of ''open area'' of *C. placentula* was significantly different between the two salinities (Wilcoxon–Mann–Whitney, $P < 0.05$) and varied from 16.6% at 67 PSU to 28.6% at 100 PSU. In addition, the surface of the pores was consistently independent of the valve size at both sites (Fig. 4), as shown by the lack of significant correlation between the two parameters (Spearman coefficient of rank correlation, $P > 0.05$). This observation suggests that the processes driving pore size and frustule size may be distinct.

The variation in pore size with salinity might first be related to the silicification process. In particular, an increase in silicification has been reported for various diatom species at low salinity (Paasche 1980, Tuchman et al. 1984, Romero 1994). Freshwater species then tend to have a higher silica content and biovolume compared to marine and brackish species (Conley et al. 1989). This difference could reflect higher silicon availability or transport coupling effects due to reduced salinity (Hildebrand 2008). In particular, because salinity can promote diatom growth (i.e., cell division rate; Guillard and Ryther 1962, Olsen and Paasche 1986), the difference in silica content between marine and freshwater diatoms might be a consequence of the hastened cell cycle in marine diatoms, which simply allows less time for silicon transport and deposition (Flynn and Martin-Jezequel 2000, Martin-Jezequel et al. 2000). In addition, an increase in silicification under fluctuating pH (Vrieling et al. 1999) and salinities (Gordon and Brodland 1990, Vrieling et al. 2007) could also be related to changes in the physicochemical condition of the silica deposition vesicle (SDV) where the diatom silica formation processes occur (Hildebrand 2008). These changes in silicification imply the formation of a thicker frustule at lower salinity, which may lead to the reduction in nanopore size. This trend is congruent with our results and with previous experiments, which showed that the pore size of cultured *Navicula salinarum*, *Thalassiosira weissflogii,* and *Thalassiosira punctigera* become smaller with decreasing salinity (Vrieling et al. 2000, 2007). However, salinity-induced osmotic stress leads to an increase in silicification in diatoms (Olsen and Paasche 1986, Romero 1994). This change is especially relevant at the extreme salinities encountered in our study, which trigger osmoregulation, and hence are likely to affect both growth and silicification processes (Vrieling et al. 2007) and thus the thickness and nanostructure of the diatom frustules.

Changes in pore size are also related to the diffusive properties of the environment of the diatoms. Factors such as the surface chemistry of the pore wall and the size of the transported ions become increasingly important (Jirage et al. 1999). More specifically, as ions such as Na^+ and Cl^-, which make up the bulk solution of seawater, bind to the pore walls, they form an electrical double layer that impacts the amount of electrolytes diffusing through the pore. The quantity of electrolytes diffusing through the pore can be estimated by the diffuse layer, that is, the pore size reduced by the electrical double layer, estimated from the Debye screening length (Schmuhl et al. 2004). The Debye screening length (δ) is expressed as follows:

$$\delta = \frac{1}{zF}\sqrt{\frac{\varepsilon_0 \varepsilon RT}{2C_0}} \qquad (2)$$

where z is the ion valence; F ($F = 9.65 \times 10^4$ C $\cdot$ mol^{-1}), the Faraday constant; ε_0 ($\varepsilon_0 = 8.85 \times 10^{-12}$ F $\cdot$ m^{-1}), the dielectric constant; ε ($\varepsilon = 80$), the relative permittivity of the solution; R ($R = 8.31$ J $\cdot$ mol^{-1} $\cdot$ K^{-1}), the universal gas constant; T (K), the absolute temperature; and C_0 (mol $\cdot$ m^{-3}), the equilibrium water salinity (Titov et al. 2004). For a 1 M NaCl solution, the Debye screening length is 0.3 nm (Israelachvili 1985). The salinities measured in this study (67 and 100 PSU) can be approximated to 1.15 and 1.71 M NaCl, respectively. This leads to a Debye screening length within the pores of 0.345 nm at 67 PSU and 0.513 nm at 100 PSU. A similar change was observed on the frustule, with an increase in the surface area of the pores from 43.7% to 44.9% with increasing salinity. This finding suggests that the increase in pore size compensates for the decrease in diffuse layer; hence, diatoms maintain an equal diffusion capacity at any salinity. Through this process, diatoms always absorb similar amounts of nutrients whatever the salinity. However, diatom frustules are also covered by an organic casing (Vrieling et al. 2007) that is considered as a protection for the frustules in relation to, for example, osmotic changes (Gélabert et al. 2004), which might affect the above-mentioned relative changes in diffusive

layer thickness with salinity. This issue would require further investigations and is beyond the scope of the present work.

This work suggests salinity as a main driver to the morphology of diatoms frustules, but other environmental factors, such as temperature, light, pH, nutrients, and metal ions, may also impact the physiological state of the cells and therefore regulate uptake and deposition rates of silica (Tuchman et al. 1984, Brzezinski 1985, Blank et al. 1986, Olsen and Paasche 1986, Romero 1994, Vrieling et al. 1999, 2000). Further work is nevertheless needed to generalize the present work and unambiguously identify the parameters and/or processes driving the morphological flexibility of diatoms. This is particularly relevant to provide further understanding of the quasi-ubiquitous ecological success of diatoms and their ecological competitiveness.

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