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MOVEMENT MODALITIES AND RESPONSES TO ENVIRONMENTAL CHANGES OF THE MUDFLAT DIATOM *CYLINDROTHECA CLOSTERIUM* (BACILLARIOPHYCEAE)[1]

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Cylindrotheca closterium **(Ehrenberg) Reiman et Lewin is a raphid diatom widely distributed in mudflat assemblages. Video microscopy showed various movement modalities defined as smooth and corkscrew gliding, pirouette, pivot, rock and roll, rollover, and simultaneous pirouette and gliding. Z-axis projection analysis of images revealed a unique gliding motif with corkscrew motions, which may have important ecological implications for *C. closterium* movement in muds. The general response to salinity alteration was a decrease in gliding movements with a concomitant increase in other modalities listed above. Short-term responses to salinity change include dramatic alteration in modalities in hypo-saline conditions and cessation of motility in extreme hyper-saline environments. Modality changes were rapid and occurred within 5 s in response to hyper-saline conditions. Hypo- or hyper-saline conditions resulted in decreased gliding speed in standard media. Five- and 15-day acclimation to salinity changes resulted in a progressive reduction in gliding movement, increased non-gliding modalities and increased cell aggregation. Aggregation in hypo-saline conditions was accompanied by a large increase in the polymer extracted by hot bicarbonate- and ethylenediamine tetraaceticacid- fractions of extracellular polymeric substance (EPS), the polymers of which have been implicated in cell attachment/motility phenomena. The monosaccharide profiles of these fractions were altered in response to hypo-saline conditions. In general, monosaccharide profiles showed increased diversity upon cessation of motility and aggregation of cultures. The movement responses of *C. closterium* in response to environmental changes, accompanied by modifications in EPS, may form part of an adaptive strategy to survive in mudflats and could be useful as bioindicators of environmental changes.**

Key index words: **aggregation; corkscrew gliding; *Cylindrotheca closterium*; diatom; environmental effects; EPS; movement modality; nutrient; pirouette; pivot; salinity; video microscopy**

Abbreviations: **cEPS, media soluble polymer precipitated by 70% ethanol; EDTA, polymer extracted by EDTA; EPS, extracellular polymeric substance; f/2-20 or f/2-35, f/2 media at 20 or 35 psu salinity; 2F-20 or 2F-35, 2F media at 20 or 35 psu; HB, polymer extracted by hot bicarbonate; HW1 and HW2, polymers extracted by hot water for 30 and 90 min, respectively.**

The most widely studied movement modality in pennate diatoms is gliding, and models of the propulsion mechanism include secretion of extracellular polymeric substances (EPS) through the raphe (Gordon and Drum 1970, Edgar and Pickett-Heaps 1984). There have been few reports of non-gliding movements in diatoms. Pickett-Heaps et al. (1991) reported rolling movements exhibited by an araphid diatom *Ardissonea crystallina* (Agardh) Kutz. Shuffling and rocking movements were observed in the centric diatom, *Odontella sinensis* C. A. Agardh (Pickett-Heaps et al. 1986). In several studies (see Harper 1977), brackish-water species were described to exhibit pirouette movements on their apices under stressful conditions. Whether these movement attributes are characteristic of diatom species subjected to fluctuations in changing environments or if certain movement modalities are exhibited as a response to a discrete stimulus is not yet fully understood.

Diatoms have been shown to respond to external stimuli such as light, temperature, nutrient, and salinity with movement responses. Light plays an important role in rapid changes of cell direction, with a photodetection system appearing to be located at the tips of

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the cell (Cohn 1999, 2001, Cohn et al. 2004). In the field, the onset of vertical migration of assemblages within biofilms can be controlled by light (Consalvey et al. 2004), and low-temperature scanning electron microscopy (SEM) showed that different species have different thresholds that trigger migration. In UK mudflats, *Navicula* and *Nitzschia* spp. were the first to appear when photon flux density was low ($<0.585\,\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$), and *Scoleuplora tumida* A. Grunow dominates at higher light levels ($0.85\,\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$) (Paterson 1986, Paterson 1989). Using techniques described in Consalvey et al. (2004) coupled with high-resolution single-cell fluorescence imaging of PS II, species-specific migratory rhythms were demonstrated in the Colne estuary, UK. Shade-adapted species such as *Gyrosigma balticum* A. Hassal, *Pleurosigma vitrea* W. Smith and *Nitzschia* spp. dominated during early mornings but migrated away from the surface with increasing irradiance. At midday, *Pleurosigma angulatum* W. Smith was dominant in the surface layers of the biofilms, and there was an increase of taxa toward dusk (Underwood et al. 2005). While upward migration allows maximum light utilization for photosynthesis, movement to the deeper layers minimizes photoinhibition and limits the cells' exposure to the damaging effect of high irradiance. Downward migration of cells was observed by Underwood et al. (1999) in a diatom mat subjected to high levels of UV-B radiation.

Although a general pattern of diatom dominance in the topmost photic layer is generally observed, a large number of diatoms in different phases of cell division were found in the anoxic layer (2–3 cm deep) of sediments in Chernaya River Estuary (Kandalaksha Gulf, White Sea, Russia). This layer is rich in inorganic forms of nitrogen (NH_4^+ and NO_3^-), which may be suitable for growth and division. It was proposed that diatom migration is a physiological adaptation for cells to be able to undergo cell division under optimum conditions in spatially independent layers (Saburova and Polikarpov 2003). However, different species of diatoms vary in their trophic preferences. Underwood et al. (1998) showed in their field and mesocosm studies that at high ammonium concentration (380–450 µM NH_4^+) the population densities of *Gyrosigma fasciola* A. Hassal, *G. littorale* A. Hassal, *Pleurosigma angulatum*, *Navicula phyllepta* Kutzing, *Cylindrotheca signata* Reiman et Lewin, *Cylindrotheca closterium*, and *Nitzschia apiculata* (Gregory) Grunow decreased while populations of *G. limosum* Sterrenburg et Underwood, *Nitzschia sigma* (Kutzing), and *Scolioneis tumida* D. G. Mann significantly increased. In another mesocosm experiment, downward migration of *Euglena proxima* Ehrenberg was also shown to be influenced by subsurface nutrients (Kingston 2002). The mean depth of the population receiving inorganic nutrients was significantly greater than for those receiving only deep porewater. This indicates that vertical spatial distribution of species could be dependent upon nutrients available in the microlayers of the sediment and is effected by active migration. A direct chemotactic response was observed in *Amphora coffeaeformis* (Agardh) Kutz, where cells showed migration along a glucose gradient whereas cells moved randomly in the absence of glucose (Cooksey and Cooksey 1988).

Temperature is another factor that has been shown to influence diatom movement. *Craticula cuspidata* (Kutz.) Mann, *Stauroneis phoenecenteron* (Nitzsch) Ehrenb, *Neostrongylus linearis* (C. Agardh), and *Pinnularia viridis* (Nitzsch) Ehrenb. exhibited maximum speeds at 35° C, and speeds decreased above this limit with very little movement at 40° C (Cohn et al. 2003). Given this effect, rapid rises in temperature on intertidal flats over tidal exposure periods (Underwood 2002) could have an impact on the dispersal of diatoms.

Salinity has been shown to influence diatom species distribution within estuarine mudflats. In the Colne estuary, UK, *Navicula* spp. were abundant at oligo- and mesohaline sites while *Pleurosigma angulatum* and *Plagiotropis vitrea* were commonly found at the polyhaline sites (Underwood et al. 1998). In the tidal channels of Guerrero Negro, Baja California Sur, Mexico, Clavero et al. (2000) found that diatom diversity decreased with increasing salinity. Isolated strains from these hyper-saline environments showed that species of *Amphora*, *Nitzschia*, and *Entomoneis* grew well in salinities of 0.5–15% while *Pleurosigma strigosum* W. Smith was unable to grow in salinities <5%. Different species from the Colne estuary, UK, were also reported to grow at certain salinity levels in combination with nutrient concentration. *Navicula phyllepta* (10–20 psu) has a significantly lower growth rate at >400 µM ammonium concentration while *Navicula perminuta* (10–30 psu), *Navicula salinarum* Grunow (20–35 psu), and clones of *Cylindrotheca closterium* (10–25 and 25–35 psu) showed little ammonium toxicity even at >1 mM concentration (Underwood and Provot 2000). In studies of diatoms isolated from Wadden Sea, North Sea Shore, Dangast (Germany), maximum cell migration was observed at exposure to 35 ppm with a decrease at 5–60 ppm (Sauer et al. 2002).

It would appear likely that differential responses of individual diatom species to environmental changes, such as gradients in nutrients, salinity, and irradiance, occur in natural conditions. In a highly fluctuating estuarine environment, movement is an important mechanism for organisms to be able to find a suitable location in the habitat. Species vertical distribution has been attributed to differential patterns of vertical migration; however, the processes involved in establishing the pattern are not clearly understood. The problem lies in the lack of understanding the organism's direct movement responses to environmental cues.

The model mudflat species, *C. closterium*, has interesting features that relate to its migration in the sediments. The frustules are partially silicified and are strongly twisted spirally around the apical axis, which has led to speculation that the diatom follows a spiral path as it moves through the mud (Round et al. 1990).

The movement modalities that form the basis for responses to the prevailing conditions in the muds are not yet known. The purpose of this study was to examine the modalities of movement of *C. closterium* and determine how they were affected by varying salinity and nutrient concentrations and with varying acclimation to these environmental stressors.

MATERIALS AND METHODS

Culture. *C. closterium* was isolated from mudflat sediments of the Colne estuary, UK, and inoculated into f/2- enriched (Guillard 1975) autoclaved filtered seawater. Inocula were treated with an antibiotic mixture (Wustman et al. 1997) and maintained in axenic condition. High nutrient medium (2F) was prepared as described by Liang et al. (2000).

Cultures were maintained in 100 μmol photons $\cdot m^{-2} \cdot s^{-1}$ irradiance at 18° C with a 12:12 light:dark photoperiod for 5 and 15 days. Salinities of 5 and 20 psu were obtained by simple dilution of the normal f/2-35 solution with distilled water. Fifty, 70, and 140 psu were obtained by the addition of sodium chloride to the f/2-35 medium. Salinity was determined using an optical Abbe refractometer (AO Buffalo, NY, USA).

Direct observation of short-term movement patterns. Fifteen microliters of cell suspension from f/2-35 culture was washed with fresh f/2-35 and placed on a coverslip mounted on a flow-through stage chamber for 3 min observations on a Zeiss Axioskop (Zeiss, Thornwood, NY, USA) equipped with differential interference contrast. Sony DCX 930 3CCD camera output was fed to a Matrox RT.X100 capture card and the video was processed in Adobe Premiere Pro (Adobe Systems Inc., Mountain View, CA, USA). Five cells >75 μm in length were randomly chosen and tracked for movement modality changes at different salinity levels for 60 min. Cells were maintained under constant illumination from the condenser at 20° C. Media with the desired salinity and the f/2-35 were placed separately into sterile reservoirs fitted with a Y-type venocyclis set that allows simultaneous or selective flow of the media (Abbott Laboratories, North Chicago, IL, USA) equipped with a flow selector control. Selected media were passed through the cell by gravity flow at a constant rate of 0.3 mL s^{-1}.

Z-axis projection analysis and model generation: Sequential video frames were converted into stacks for *z*-axis projection with Image J (NIH Image, Scion Corp., Frederick, MD, USA) software using average intensity projection at the corresponding pixel location along the axis perpendicular to the image plane (the so-called "*z*-axis"). *z*-axis projection was used to measure the distance covered by the cell during gliding with intermittent corkscrewing and reversals in 60 s. Adobe Photoshop (Adobe Systems Inc.) was used to define the midpoint in each frame in preparation for *z*-axis projection analysis.

The video images acquired and electron micrographs of *C. closterium* (Round et al. 1990) were used in generating computer-assisted simulation models using Blender 3D (GNU Public License). The position of the cell relative to the *x*-, *y*-, and *z*-axes when exhibiting corkscrew gliding or pirouetting, which were difficult to discern in a 2D view, was clearly illustrated using this technology.

Long-term movement and aggregate measurements: Video analysis was as above with the exception that observations were conducted in slide culture chambers (Fisher Scientific,Versailles, KY, USA) using a water immersion objective. The percent cells exhibiting different movement modalities over a 5 min period in the 5- and 15 day cultures were computed. Speed of actively gliding cells from each treatment was determined by tracking 20 individual cells for 30 s intervals using 3D Studio Max (Autodesk Inc. Montreal, Canada) software. A dense portion of the cell was selected that would enable the program to follow and compute the distance traveled within the specified time.

Images of aggregates in culture chambers were obtained using a stereo microscope (Olympus SZ-CTV Lake Success, NY, USA) connected to a video camera (Sony DXC 151A CCD Sony Corp., New York, NY, USA) and processed in Sigma Scan Pro. The area of each aggregate was measured using Image J (NIH Image, Scion Corp., Frederick, NY, USA) software.

Carbohydrate analysis: Cells and associated mucilage were scraped from 20 flasks (500 mL) and media soluble components were isolated (cEPS) following the procedure of Underwood et al. (1995). The insoluble pellets were further extracted sequentially in two separate procedures as described by Wustman et al. (1997): (1) hot water (90 min at 95° C) to yield the hot water soluble (HW2) fraction followed by 0.5 M $NaHCO_3$ at 95° C for 1 h to yield the hot bicarbonate soluble (HB) fraction, and (2) hot water for 30 min at 95° C to yield (HW1) fraction followed by 0.2 M EDTA at 22° C for 30 min to yield the EDTA soluble fraction (EDTA). The HW1, HW2, and HB fractions were dialyzed overnight against distilled water and freeze dried. The EDTA soluble polymers were first dialyzed against 0.5 M imidazole (pH 7.0) overnight at 5° C, and then overnight against distilled water and freeze dried. The total carbohydrate content of each fraction was determined by phenol–sulfuric assay (Dubois et al. 1956) with glucose as standard. Monosaccharide analysis was carried out as described by Wustman et al. (1997) with trifluoroacetic acid (TFA) hydrolysis, reduction, and acetylation to alditol acetates. Alditol acetates were injected on an SP2330 column (30 m; 230° C, isothermal) and analyzed with Finnigan Magnum GC/MS operated in electron impact mode as described in Wustman et al. (1997). Identification and quantitation was accomplished by comparison with commercially available sugar standards (Wustman et al. 1997).

RESULTS

Movement modalities and short-term effects of salinity changes. *Z*-axis projection of video images obtained from observation of cells shows various modalities (Fig. 1). The distinguishing features of each movement modality of *C. closterium* are defined (Table 1) based on the position of the cell during movement relative to the *x*-, *y*-, and *z*-axes (Fig. 2, A and B). In addition to the common "smooth" gliding observed for other diatoms (Fig. 1, A and B), *C. closterium* exhibited a "corkscrew" gliding motion with rotation about the *x*-axis as evidenced by lateral displacement of cell tips (Figs. 1, C and D, and 2A). During both smooth and corkscrew gliding, reversals in direction were rapid (Fig. 1, B and D). Although speed increased during corkscrewing, no clear pattern in speed changes was observed in transition from corkscrew to smooth gliding or vice versa or as related to reversals (Fig. 3). Whether gliding or stationary, cells were capable of pirouetting with one tip attached and the other precessing around the *z*-axis (Figs. 1, E and F, and 2B). Pivot, rollover, and "rock and roll" (Fig. 1, G, H, and I) were not commonly observed in f/2-35. Tip flexion was easily observed when tips of gliding cells became lodged against debris (Fig. 1J).

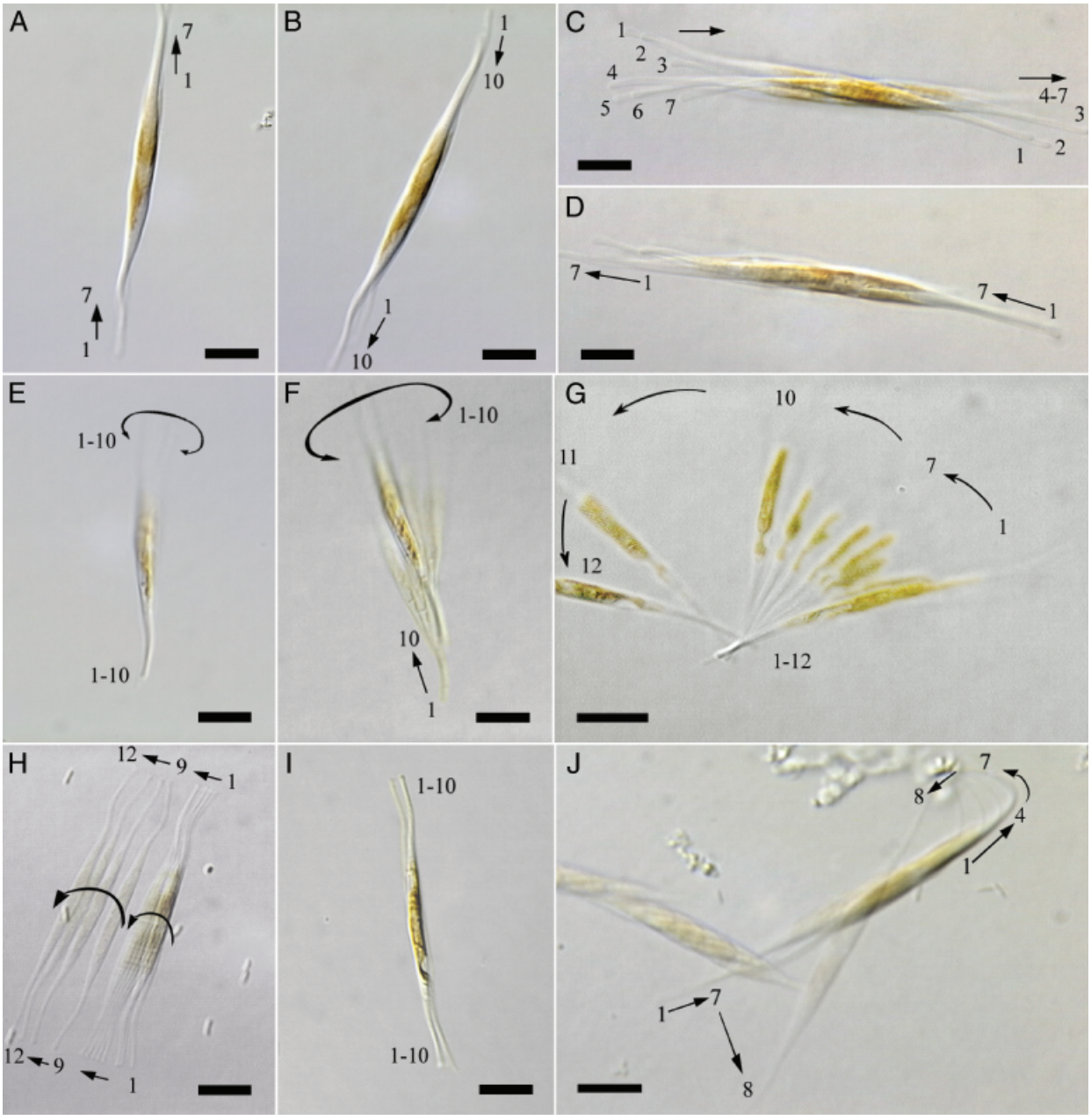

FIG. 1. *Z*-axis projection of video images showing various movement modalities exhibited by *Cylindrotheca closterium*: (A) smooth gliding forward, (B) smooth gliding reverse, (C) corkscrew gliding forward, (D) corkscrew gliding reverse, (E) pirouette, (F) pirouette while gliding, (G) pivot, (H) rollover, (I) rock and roll, and (J) tip flexion. Arrows indicate direction of movement of the cell tips and numbers indicate the number and sequence of frames. Scale bar, 10 μm.

Addition of f/2-35 to the flowthrough chamber (control) showed continuation of a gliding motif with sporadic pirouetting (Fig. 4). A decrease (5 and 20 psu) or increase (50, 70, and 140 psu) from this salinity level resulted in exhibition of various modalities with shorter periodicity of change from one modality to another. The change of modality pattern was noticeable immediately after addition of hyper-saline media (Fig. 4). Large changes in salinity from normal resulted in cessation of movement (e.g. f/2-5 and f/2-140) or detachment (e.g. f/2-70) by the end of the experiment. Under hyper-saline conditions (f/2-70 and f/2-140), long periods of "rocking and rolling" with limited pirouetting were observed (Fig. 4). Generally, at extreme hypo- (Fig. 4) and hyper-saline (Fig. 4) conditions, the characteristic response was "rocking and rolling," which persisted for longer periods. In salinity of 5 and 70 psu, cells re-established normal gliding after about 20 min of "rock and roll", while at 140 psu cells did not resume gliding (Fig. 4). Eventually, in all the foregoing salinity levels, cells exhibited rocking and rolling toward the end of the duration that resulted in non-mo-

TABLE 1. Characteristic movement modalities of the mudflat diatom *Cylindrotheca closterium*.

I. Gliding—movement along the line defined by the center of the long axis of the cell (designated as *x*-axis)
(A) Smooth—gliding without rotation about the *x*-axis
(B) Corkscrew—gliding with rotation about the *x*-axis
II. Non-gliding—movement without cell displacement along the *x*-axis
(A) Pirouette—one tip attached with movement of the other tip described by precession about the *z*-axis (non-gliding pirouette)
(B) Pivot—one tip attached and the other moving in the XY plane
(C) Rock and roll—partial roll about the *x*-axis
(D) Rollover—complete roll about the *x*-axis
III. Gliding pirouette—gliding with one tip adjacent to substratum and the movement of the other tip described as precession about the *z*-axis displaced along the X–Y-plane
IV. Detaching—losing contact with a substratum resulting in no directed movement

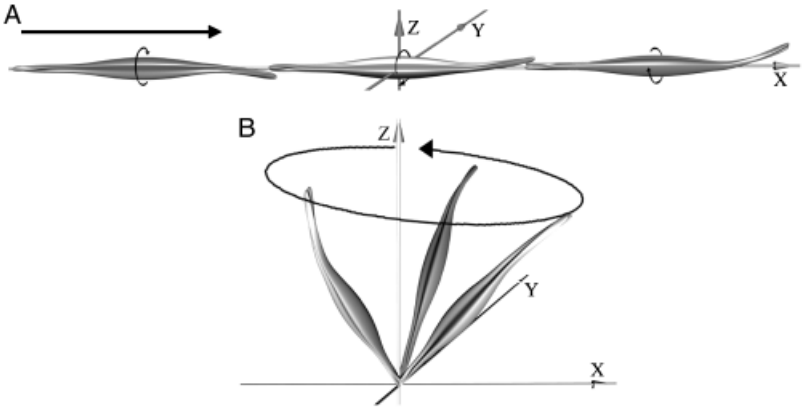

FIG. 2. Model of *Cylindrotheca closterium* movements showing changes in position relative to the *x*-, *-y*, and *z*-axes: (A) during corkscrew gliding. Thin arrows indicate direction of cell body rotation, while the thick arrow points to the overall direction of cell movement; (B) During pirouetting. Arrow indicates precession of one tip around the *z*-axis.

tility. In some instances, after a prolonged period of "rock and roll", the cells detached (Fig. 4). Although switching from one modality to another generally did not follow a specific order, rocking and rolling appears to be a prelude to non-motility or detachment. However, experiments designed to test reversibility revealed that cells that were exhibiting "rock and roll" after exposure to f/2-5 and f/2-70 regained normal gliding modality within 1 min of exposure to f/2-35. In a parallel experiment, cells exposed to 140 psu for longer than 1 min did not have the capacity to recover gliding motility under any condition. However, with exposure to 140 psu for <1min, cells regain gliding modality upon exposure to f/2-35.

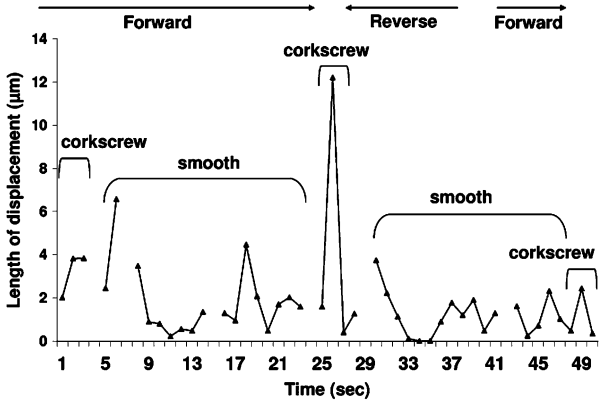

FIG. 3. Length of displacement of a typical *Cylindrotheca closterium* cell while smooth gliding with intermittent corkscrew gliding (each unique modality is indicated by brackets). Arrows indicate direction of movement.

The change in movement patterns was also reflected in the total percentage of time that cells spent exhibiting the different modalities within the exposure period (Fig. 5). In f/2-35, cells spent 90% of the time gliding (Fig. 5), which decreased in 20, 5, 50, 70, and 140 psu (Fig. 5) with a concomitant increase in other modalities, particularly pirouette, pivot, and rock and roll. In f/2-70, cells were detaching 13% of the time (Fig. 5), while at 140 psu, cells were either non-motile or exhibiting rock and roll 96% of the time (Fig. 5).

Movement. Long-term effects of nutrient and salinity changes. In f/2-35 cultures, cells exhibited predominantly gliding movements compared with those grown in other salinities ($P < 0.05$). When acclimated for 5 days at 5, 20, and 50 psu, gliding declined with an increase in rock and roll and more non-motile cells (Fig. 6). In 2F medium, cells exhibited different movement modalities in almost equal proportions (Fig. 6). A significant increase in pirouette modality ($P < 0.05$) from <10% after 5 days to >60% after 15 days of acclimation was observed particularly in f/2-enriched cultures (Fig. 6).

The gliding speed of cells in f/2-35 was significantly greater ($P < 0.05$) than those in 2F-35 after 5 days of acclimation. In f/2 media, decreased speed was observed in cells acclimated to altered salinity levels tested compared with f/2-35. In 2F-50 gliding speed was significantly increased (Fig. 7, $P < 0.05$), which was also indicated by frequency of cells gliding at higher speeds (Fig. 8). In f/2 media, the frequency of cells

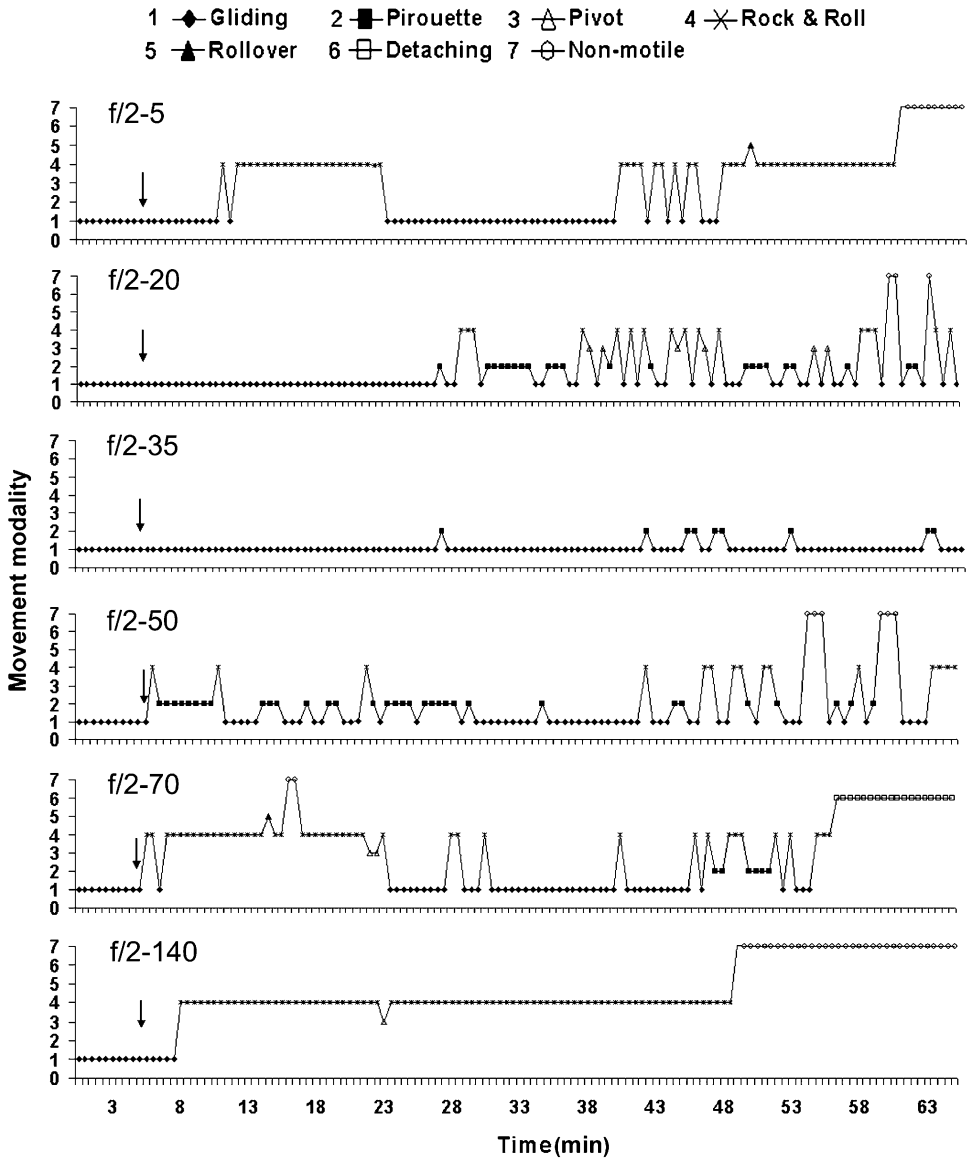

FIG. 4. Modalities of movement including gliding, pirouette, pivot, rock and roll, rollover, detaching, and nonmotility exhibited by *Cylindrotheca closterium* (initially acclimated in f/2-35) during short-term exposure to different salinities: f/2-5, f/2-20, f/2-35, f/2-50, f/2-70, and f/2-140 within 60 min of observation. Arrow indicates initiation of exposure to media of altered salinity. Each trace represents movements of a single cell, typical of patterns observed for at least five cells for each treatment.

gliding at slower speeds increased with altered salinities, but the effect was not as pronounced as in 2F media (Fig. 8).

Aggregation. Long-term effects of nutrient and salinity changes. Cells grown in standard media (f/2-35) for 5 days show a uniform distribution of aggregate sizes ($<0.2\,mm^2$), which increased to 10–50 mm^2 after 15 days (Fig. 9). Hypo-saline conditions increased aggregation as shown by a relative decrease in the proportion of aggregates below 0.2 mm^2 associated with the decrease in salinity from 35 to 20 psu, a broader distribution of aggregates of larger sizes, and an increase in the maximum aggregate size (Fig. 9). In f/2-20, for example, aggregate sizes after 5 days ranged from 5 to 10 mm^2, which was similar to those in 2F-35 (Fig. 9). Aggregates of 50–100 mm^2 were formed by cells grown in 2F-20 (Fig. 9). A general pattern of increased maximum aggregate size was observed after 15 days, but the proportions of aggregates $<0.2\,mm^2$ increased in some media (f/2-20, 2F-35) and decreased in other media (f/2-35, 2F-20).

Carbohydrate. Long-term effects of nutrient and salinity changes. Glucose, galactose, mannose, and ribose constituted the majority of the sugars detected, although the levels of these varied between fractions. Rhamnose, fucose, arabinose, and xylose were also detected. The f/2 cultures contained xylose, rhamnose, fucose, and ribose in addition to the major neutral sugars detected in all cultures. In 2F cultures, the increased diversity is accounted for by sugars such as arabinose and rhamnose. The HB, HW1, and HW2 fractions from 2F media contain higher levels of glucose than those of cells grown in f/2 (Fig. 10). The cEPS fractions of the 5-day-old cultures showed highly variable monosaccharides across media types. The HB fractions of the 5-day cultures grown in 2F-20 showed a substantial increase in galactose at the expense of mannose relative to 2F-35 media. After 15

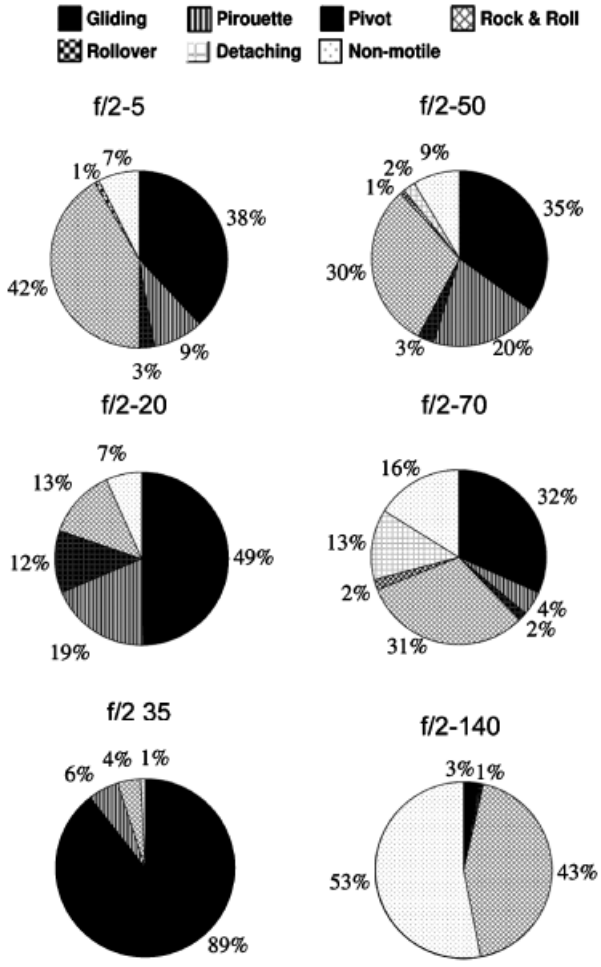

FIG. 5. Percentage of time *Cylindrotheca closterium* cells (initially acclimated in f/2-35) exhibit each movement modality during the 60 min sampling period in short-term exposure to different salinities: f/2-5, f/2-20, f/2-35, f/2-50, f/2-70, and f/2-140. Values represent the mean of observation of five cells.

days, monosaccharide diversity generally increased, with a decrease in the relative abundance of glucose, and most fractions also showed higher proportions of ribose. This is most evident in the HW1 fractions of all cultures except the f/2-35, in which ribose is mostly recovered in the HW2 and HB fractions. The HB fraction showed increased monosaccharide diversity in 20 psu salinity for both f/2 and 2F media after 15 days. In the EDTA fraction, exposure to hypo-saline conditions resulted in increased rhamnose and a decrease in galactose and mannose (Fig. 10). The fractions extracted from cultures grown in both salinities of 2F media and f/2-20 generally showed more diverse sugar composition after 15 days than after 5 days. The reverse appears to be the case for growth in the f/2-35 media.

The carbohydrate content (expressed in picogram per cell) was significantly ($P < 0.05$) higher in fractions from f/2- than in 2F-grown cultures (Fig. 11). Highest values were obtained from the HB and EDTA fractions from all treatments following 5-day acclimation. After 15 days, the carbohydrate content of the HB fraction increased, but that of the EDTA soluble fraction decreased significantly ($P < 0.05$). The carbohydrate content of HB fraction increased dramatically after 5 days exposure to f/2-20 and 15 days exposure to 2F-20 and the EDTA fraction showed similar trends. The cEPS content, on the other hand, was low for all salinities.

DISCUSSION

In diatom gliding, the raphe is considered an important morphological feature (Jarosch 1962, Drum and Hopkins 1966, Harper and Harper 1967, Gordon and Drum 1970, Edgar and Pickett-Heaps 1984) if not the true locomotory "organelle" (Hader and Hoiczyk 1992). In the majority of pennate species, the raphe is part of a relatively flat valve face, allowing close contact with the substratum as the organism glides. In *C. closterium*, the raphe follows the pronounced spiral twist of the frustule around the cell body. This morphology has led to speculation that *C. closterium* would exhibit rotation about the long axis of the cell as it glides along the substratum (Round et al. 1990). We have confirmed this unique form of gliding movement in *C. closterium* (corkscrew gliding, Fig. 2A) and have also observed that the more common gliding motif, gliding without rotation about the long axis of the cell (smooth gliding), also occurs. Given the frustule morphology of *C. closterium*, "smooth" gliding requires that the motive force be transferred across the frustule to the substratum through only a small section of the raphe in constant, close proximity to the substrate. This conforms to the observations of Houpt (1980) that only a small section of the raphe needs to be in contact with the substratum to enable movement in some naviculoids. Other modalities exhibited by *C. closterium*, such as pivot and pirouette while gliding, could easily be envisioned to be controlled through a small contact region of the frustule. The sudden reversals in gliding of *C. closterium* could be brought about by the occurrence of readily reversible mucilage attachment sites of membrane components near the raphe or by anti-parallel flow channels that operate alternately, as suggested by Edgar and Pickett-Heaps (1984).

The various movement modalities of *C. closterium* may represent adaptation to the mudflat habitat. Considering the cohesive nature of the mudflat sediment (Underwood and Kromkamp 1999), for example, corkscrew gliding may assist in passage through the fine layers, as observed for the spirochete *Leptospira* moving through a viscous medium (Goldstein et al. 1996). Pirouette and pivot movements, on the other hand, might be exhibited by the cells when seeking a chemical gradient to direct taxis under suboptimal conditions. This type of searching movement is exhibited in the twirling motion of the Apicomplexan parasite *Toxoplasma gondii* while invading a host cell (Frixione et al. 1996, Hakansson et al. 1999). Pirouette and pivot may also represent a response because of cell

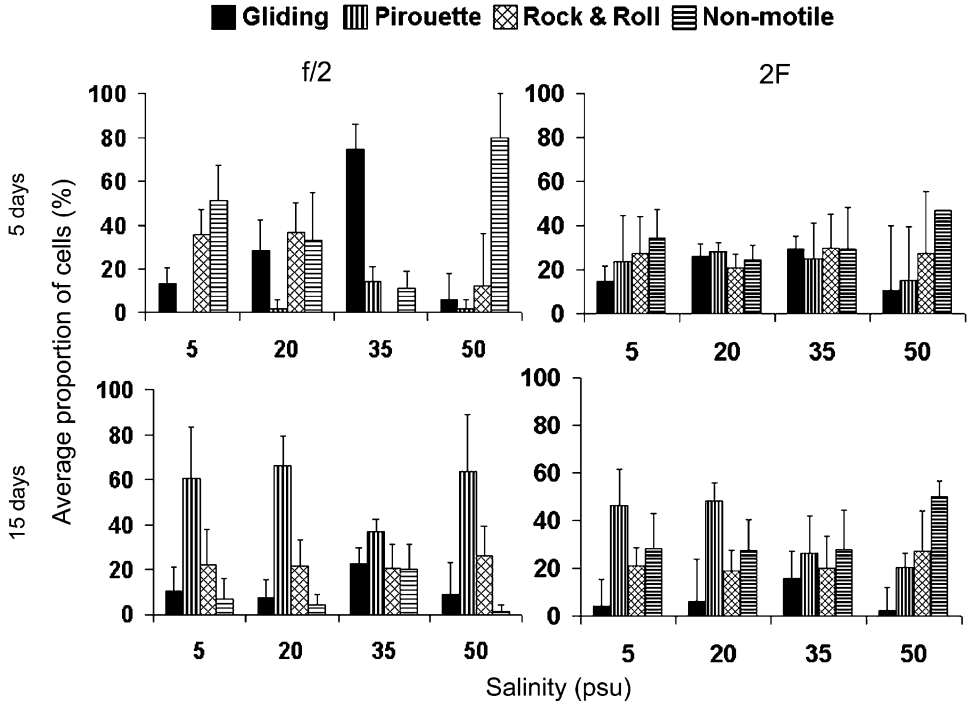

FIG. 6. Percentage of *Cylindrotheca closterium* cells exhibiting various modalities including gliding, pirouette, rock and roll, and non-motile following acclimation at different salinity levels (5, 20, 35, and 50 psu) in f/2 and 2F media for 5 and 15 days. Values are mean ± SE, $n = 15$.

tips having a sensory detection system (Cohn et al. 2004) that might not only be used for light but with other environmental cues as well. Pirouette movement has been observed in the field for estuarine species during stressful conditions (Harper 1977). The other movement modalities that are not frequently exhibited in normal conditions such as "rock and roll," rollover, and detachment appear indicative of the organism's response to salinity changes. Rocking, rolling, and shuffling movements have been observed in some centric and araphid diatom species. Movement in the centric, *O. sinensis* was an indirect result of mucilage secretion through the labiate process resulting in continuous small oscillations. In the araphid species *Ardissonea crystallina*, movement was associated with secretion of mucilage at one end of the cell that drives discontinuous jerky and rolling motions (Pickett-Heaps et al. 1986, 1991). Adhesion is a prerequisite to gliding (Wetherbee et al. 1998); hence, detachment would consequently result in passive transport of the cell. Whether this detachment is a passive process or a physiologically driven response requires further investigation.

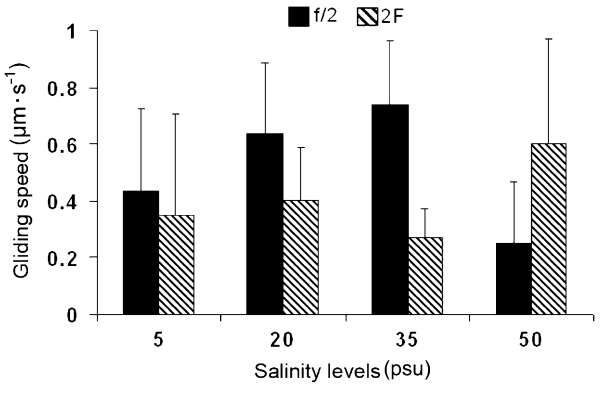

FIG. 7. Mean gliding speed of *Cylindrotheca closterium* cells following acclimation in f/2 and 2F media at different salinity levels (5, 20, 35, and 50 psu) for 5 days. Sampling period = 60 s. Values are mean ± SE, $n = 20$.

Cessation of gliding during salinity fluctuations could have a major effect on the migration of organisms and consequently their spatial distribution in the mudflat. This has been demonstrated in the findings of Sauer et al. (2002), where fewer cells were observed to migrate at the top layers of the sediment in 5 and 60 psu than in normal 35 psu seawater. Nevertheless, recovery from salinity stress was shown to be possible, depending on the salt concentration and length of exposure, with lesser probability of recovery in hyper-saline conditions. The immediate response to hyper-saline conditions in *C. closterium* suggests that the sensory mechanism used to detect this stimulus might be unique from that involved in sensing a hypo-saline environment. These phenomena were also observed in another pennate diatom *Phaeodactylum tricornutum* Bohl., which showed attenuated calcium responses to hypo- compared with hyper-osmotic concentrations (Falciatore et al. 2000). Our results also showed that gliding speeds decreased at salinity levels deviating from 35 psu for f/2-grown cells, observations in agreement with the recorded behavior of the freshwater diatom, *Craticula cuspidata*, in which speed changes were used as an indicator of toxicity levels in sediments (Cohn and McGuire 2000). Whether the change in movement modality or gliding speed is an adaptive strategy or a manifestation of some physiological deficiencies experienced by

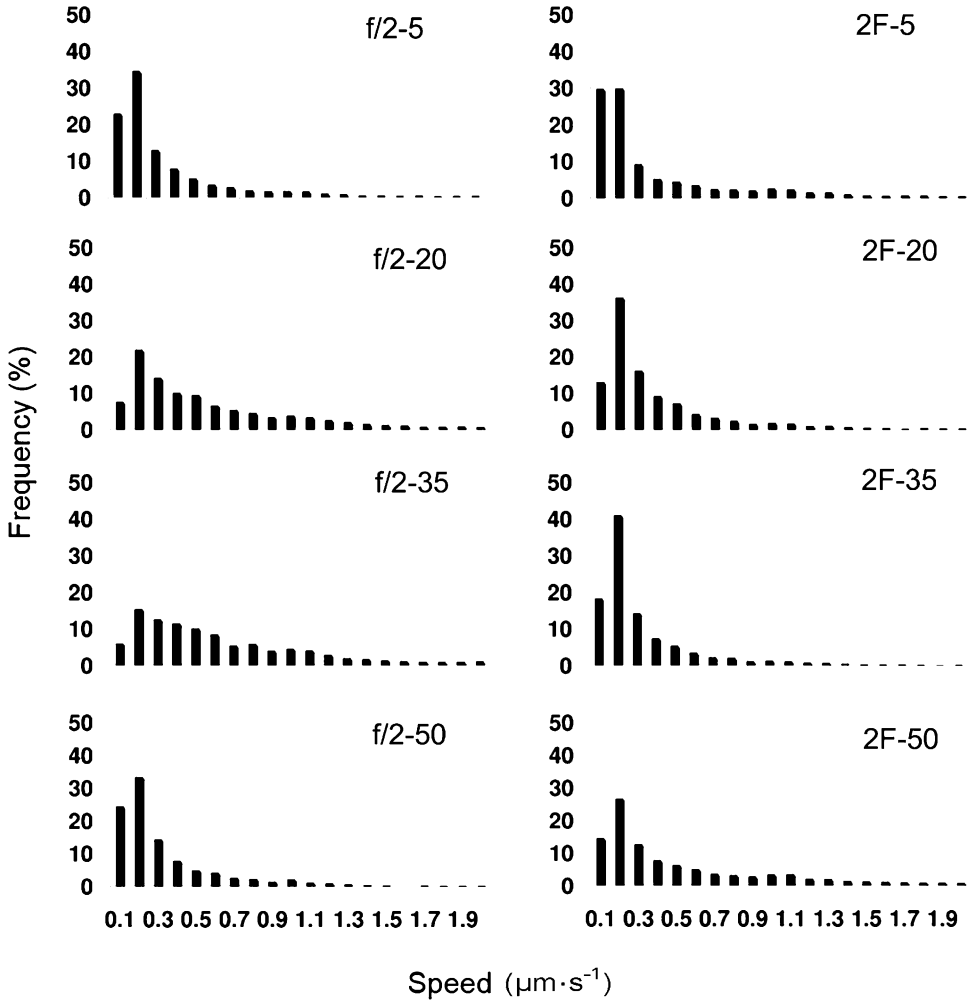

FIG. 8. Frequency distribution of speed in *Cylindrotheca closterium* cells while gliding (60 s sampling period). Cells were acclimated in f/2-5, f/2-20, f/2-35, and f/2-50 for 5 days before measurement. $n = 20$.

the cell during salinity stress can only be speculated. The detachment of cells in higher salinity, for example, could be a strategy for translocation to avoid longer exposure, which may disrupt their motility apparatus.

Aggregation of *C. closterium* increases with altered salinity and high nutrient levels in the surrounding medium. The causative factors in aggregate formation are still obscure (Herndl et al. 1992) although quorum sensing, which is the ability to communicate and coordinate behavior via signaling molecules, has been put forward as a possibility (Park et al. 2003). An interesting observation on surface sensing in diatoms was presented by Wigglesworth-Cooksey and Cooksey (2005). In a co-culture, *Pseudoalteromonas* sp. or its spent medium reduced adhesion and inhibited motility of *Amphora coffeaeformis* (Agardh) Kutz and *Navicula* strains. However, addition of galactose or mannan to the bacterial spent medium enhanced motility or cell aggregation, respectively, of the diatoms, suggesting that diatoms use their secreted polymers as surface probes.

The general assumption is that quorum formation derives from conditions favorable for growth and high cell density (Park et al. 2003). Therefore, increased aggregation in high nutrient concentration is anticipated. However, the enhanced aggregation observed in low-salinity conditions could be brought about by two factors: (a) decline in gliding, as shown in the results, that keeps cells in close proximity and (b) taxis, which is a behavioral response toward or away from an external stimulus, which is indicated by increased pirouetting during salinity stress. After a longer period of culture in f/2, aggregates formed, suggesting the possible influence of factors such as nutrient limitation. It has been suggested that stickiness of diatoms during aggregation is brought about by the EPS that they produce (Thornton et al. 2002), which can be increased under nutrient limitation (Smith and Underwood 1998, Underwood and Smith 1998, Staats et al. 1999, de Brouwer and Stal 2002, Underwood et al. 2004). Our results are in agreement with these findings, with lower carbohydrate content in nutrient-enriched cultures and carbohydrate content of all except the EDTA fraction increasing in nutrient-limited cultures.

The primary response in EPS production by *C. closterium* during salinity change was an increase in the amounts of HB and EDTA fractions. These increases were correlated with cell aggregation observed

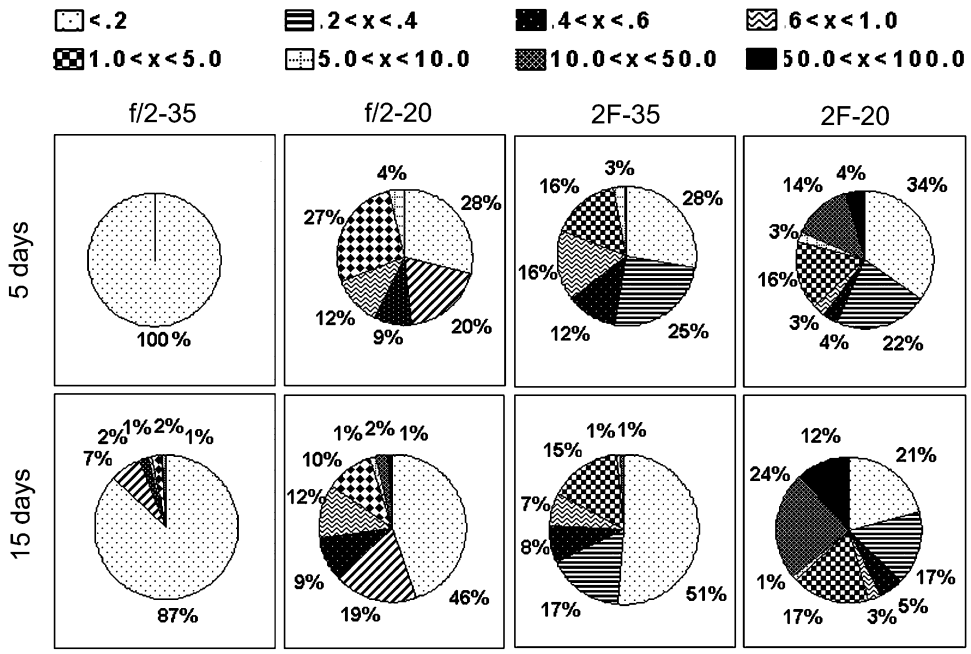

FIG. 9. Percentage surface area covered by different aggregate sizes <0.2, 0.4–0.6, 0.6–1.0, 1.0–5.0, 5.0–10.0, and 10.0–50.0 mm^2 formed by *Cylindrotheca closterium* following acclimation in f/2- 35, f/2-20, 2F-35, and 2F-20 for f5 and 15 days. $n = 3$.

in hypo-saline conditions. In studies of *Achnanthes longipes* Agardh, HB and EDTA fractions were shown to contain the polymers necessary for adhesion (Wustman et al. 1998), and recent studies (B. J. Bellinger, unpublished observations) indicated that detachment of mudflat diatoms from substrata was effected by HB and EDTA extraction. As monosaccharide profiles of HB and EDTA fractions isolated from *C. closterium* under salinity stress are unique from those extracted from f/2 cultured cells, it appears that not only do the amounts of these fractions change in response to salinity stress, but polymer chemistry is altered as well. Assignment of structure/function relationships of the HB and EDTA fractions produced in response to salinity changes must await more detailed chemical analysis, given the known complexity of EPS from estuarine environments (Staats et al. 1999, de Brouwer and Stal 2002, Underwood et al. 2004).

From these findings, it can therefore be inferred that changes in modalities of movement of the mudflat

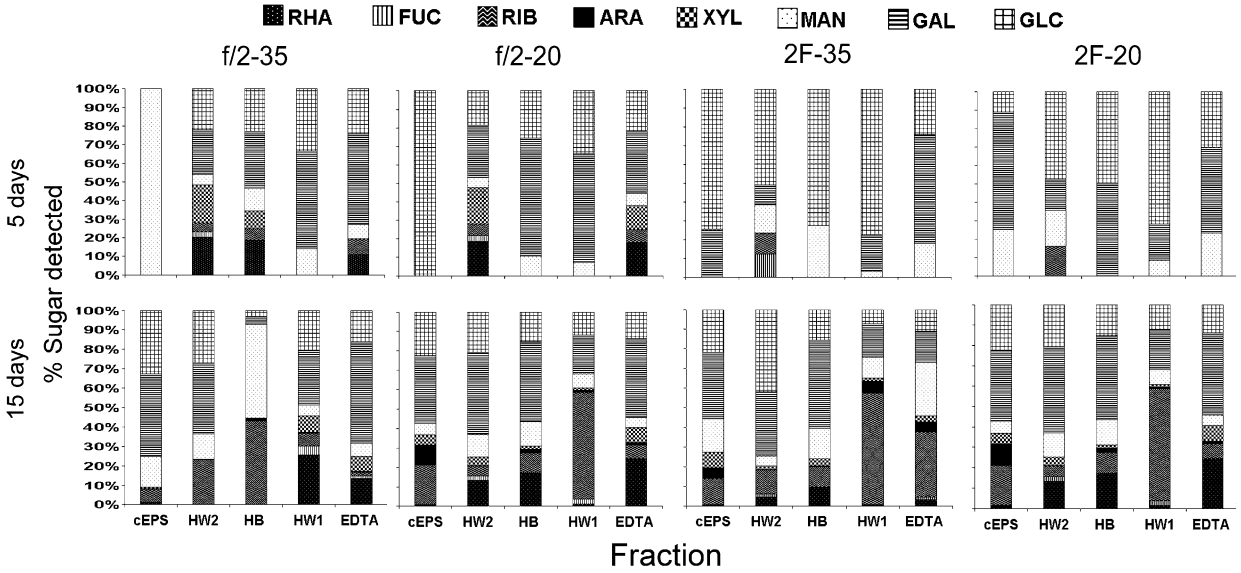

FIG. 10. Percent neutral sugar detected in the different carbohydrate fractions (media soluble polymer precipitated by 70% ethanol (cEPS), polymers extracted by hot water for 30 and 90 min, respectively (HW2, HW1), polymer extracted by hot bicarbonate (HB), and polymer extracted by ethylenediaminetetraaceticacid (EDTA) soluble) of *Cylindrotheca closterium* acclimated in f/2-35, f/2-20, 2F-35 and 2F-20 within 5 and 15 days. $n = 3$. Values expressed as percent of total neutral sugar detected.

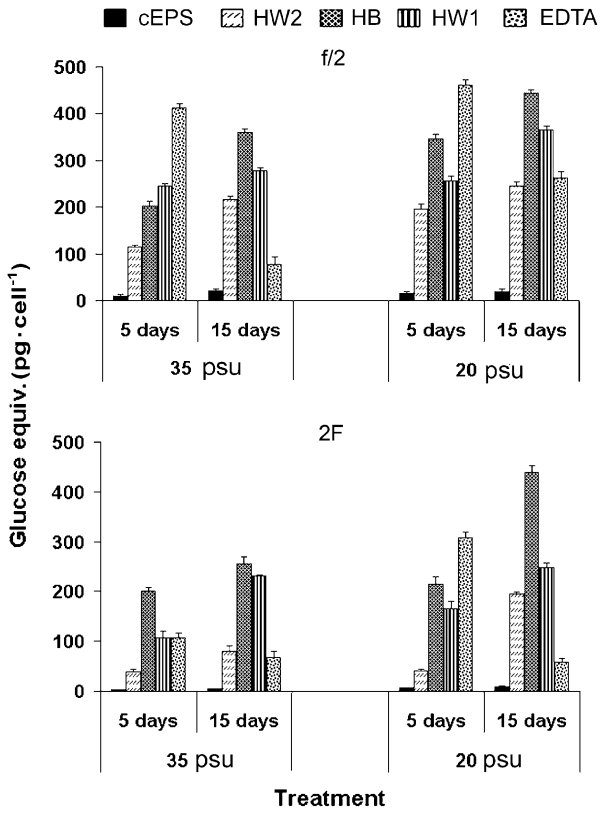

FIG. 11. Carbohydrate content of the different fractions: media soluble polymer precipitated by 70% ethanol (cEPS), polymers extracted by hot water for 30 and 90 min, respectively (HW1,HW2), polymer extracted by hot bicarbonate (HB), and polymer extracted by ethylenediaminetetraaceticacid (EDTA) soluble from *Cylindrotheca closterium* acclimated in f/2 and 2F media at 35 and 20 psu salinity for 5 and 15 days. Values are mean ± SE, $n = 3$.

diatom, *C. closterium*, are exhibited as responses to changes in salinity and nutrient concentration prevailing in the environment. Baseline information on how salinity and nutrient fluctuations directly affect movement of this species and speculation as to how these modalities may influence vertical migration in the mudflat are provided herein. These data should provide the basis for more accurate models predicting patterns of spatial distribution in the sediments during salinity fluctuations, as well as the formation of aggregates and the changes in the amount and type of EPS produced. Further investigations are required to establish the functionality of EPS and its relation to movement and aggregation in estuarine muds.

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