Movement Modalities As Adaptive Response To Salinity Changes Of The Mudflat Diatom *Cylindrotheca closterium (Bacillariophyceae)*

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I Introduction

The most widely studied movement modality in diatoms is gliding. Models of the propulsion mechanism include secretion of extracellular polymeric substances (EPS) through the raphe. Few reports of non-gliding movements include rolling, shuffling, rocking, and pirouette. Whether these movements are characteristic of diatom species subjected to fluctuations in changing environments or if certain modalities are exhibited as a response to a discrete stimulus is not yet fully understood.

The model mudflat species, *C. closterium* has features that relate to its migration in the sediments. The movement modalities that form 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.

II Materials & Methods

Cultures were maintained in 100 µmol $m^{-2}s^{-1}$ irradiance, 18 °C, 12:12 h light: dark photoperiod (5 & 15 days). Salinities of 5 & 20 practical salinity units (psu) were obtained by simple dilution of f/2-35 growth medium with distilled water. 50, 70 & 140 psu were obtained by NaCl addition to f/2-35. 2F media = 4x f/2-35. Salinity was measured with an optical refractometer.

Randomly chosen cells mounted on a flow-through stage chamber were tracked for movement modality changes at different salinity levels for 60 min at 20° C under constant illumination. Media with the desired salinity was produced using two sterile reservoirs fitted with a Y-type venocyclis set that allowed simultaneous or selective flow.

In slide culture chambers, the percent cells exhibiting different movement modalities over a 5 min period in the 5- and 15-day cultures was computed. Speed of actively gliding cells from each treatment was determined by tracking 20 cells for 30 sec intervals using 3D Studio Max software.

III Results

- Table 1 lists characteristic movements & Figs. 2A & B show the the position of the cell during movement relative to the x-, y- and z-axes.
- In addition to "smooth" gliding (Figs. 1A & B), *C. closterium* exhibited "corkscrew" gliding motion with rotation about the x-axis evidenced by lateral displacement of cell tips (Figs. 1C-D & 2A).
- Gliding or stationary, cells were capable of pirouetting with one tip attached & the other precessing around the z-axis (Figs. 1E-F & 2B).
- Speed increased during corkscrewing but no clear pattern in transition from corkscrew to smooth gliding or vice versa or as related to reversals was observed (Fig. 3)

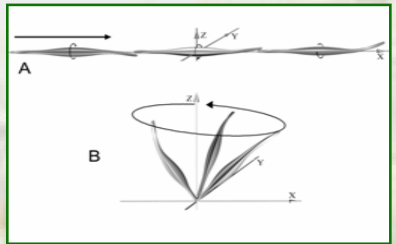


Fig. 2. Model of *C. closterium* movements showing changes in position relative to the x, y and z axes: (A) during corkscrew gliding and (B) pirouetting.

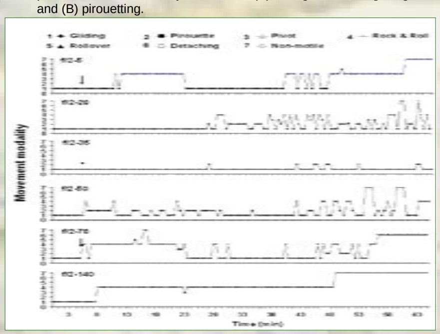


Fig. 4. Movement modalities including gliding, pirouette, pivot, rock and roll, rollover, detaching and non-motility exhibited by *C. closterium* during 60 min exposure to different salinities: f/2-35, f/2-20, f/2-5, f/2-50, f/2-70 and f/2-140. Arrow indicates initiation of exposure to media of altered salinity. n = 5.

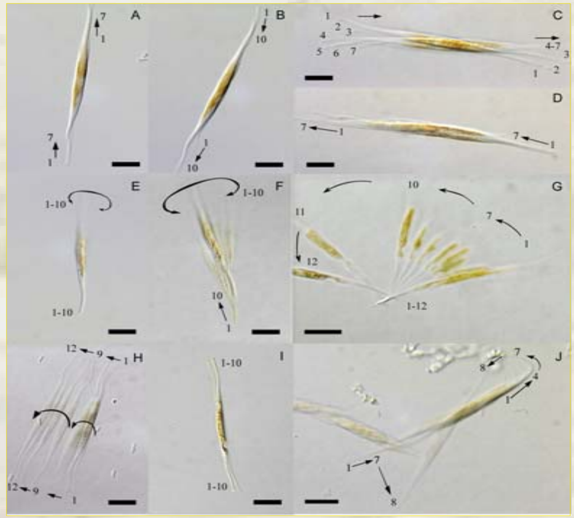


Fig. 1. Z-axis projection of video images showing various movement modalities exhibited by *C. closterium*: (A) smooth gliding forward, (B) smooth gliding reverse, (C) corkscrew gliding forward, (D) corkscrew gliding reverse; (E) pirouette; (F) pirouette while gliding; (G) pivot; (H) rock and roll; (I) rollover; 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.

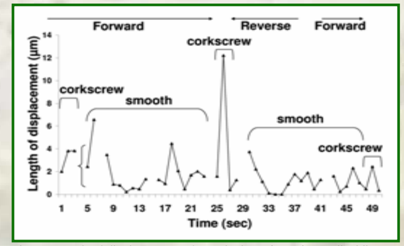


Fig. 3. Length of displacement of a typical *C. closterium* cell while smooth gliding with intermittent corkscrew gliding. Arrows indicate direction of movement.

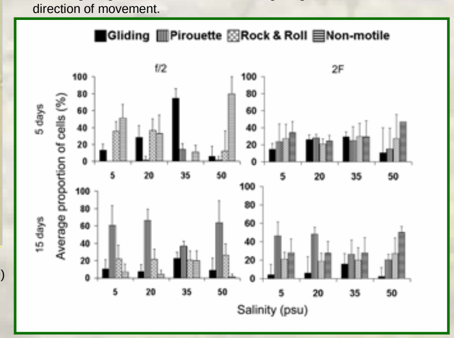


Fig. 5. Percentage of *C. closterium* cells exhibiting various modalities including gliding, pirouette, rock and roll, and non-motile following acclimation at different salinity levels (5, 20, 35 & 50 psu) in f/2 and 2F media for 5 and 15 days. Values are mean ± SE, n = 15.

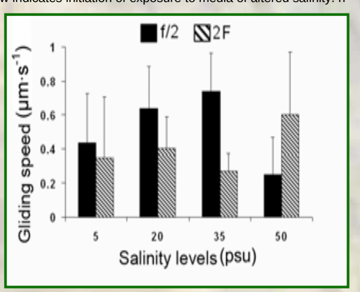


Fig. 6. Mean gliding speed of *C. closterium* cells following acclimation in f/2 and 2F media at different salinity levels (5, 20, 35 and 50 ppt) for five days. Sampling period = sixty seconds. Values are mean ± SE, n = 20.

Table 1. Characteristics of movement modalities of the mudflat diatom *Cylindrotheca closterium*

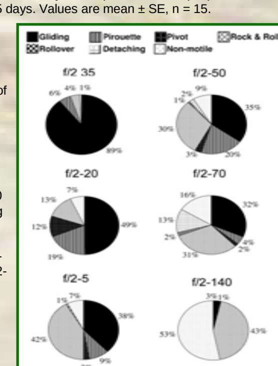
I. Gliding – Movement along the line defined by the center of the long axis of the cell (designated as X-axis)
- A) Smooth – gliding without rotation about the X-axis
- B) Corkscrew – gliding with rotation about the X-axis

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

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 X-Y plane
- C) Rock and roll – partial roll about the X-axis
- D) Rollover – complete roll about the X-axis

IV. Detaching – losing contact with a substratum resulting in no directed movement

Fig. 7. Percentage of time *C. closterium* cells exhibit each movement modality during the 60 min sampling period in different salinities: f/2-35, f/2-20, f/2-5, f/2-50, f/2-70 and f/2-140. n = 5.



- Pivot, rollover & "rock & roll" (Figs. 1G-I) were not commonly observed in f/2-35. Tip flexion was observed when tips of gliding cells became lodged against debris (Fig. 1J).
- In f/2-35 cells showed continuous gliding with sporadic pirouetting. A salinity decrease or increase resulted in exhibition of various modalities with shorter periodicity of change. In 5 & 70 psu, cells re-established normal gliding after 20 min of "rock & roll", while in 140 psu cells did not resume gliding (Fig. 4).
- Cells in f/2-35 exhibited more gliding than those in other salinities. After 5 days in 5, 20 & 50 psu, gliding decreased, rock & roll & non-motility increased. In 2F-35, gliding was reduced and cells exhibited other modalities in equal proportions. Pirouette modality increased from <10% after 5 days to >60% after 15 days in all salinities for f/2-enriched media (Fig. 5).
- Gliding speed of cells in f/2-35 was greater than those in 2F-35 after 5 days of acclimation. Speed decreased in cells acclimated to altered salinity levels. In 2F-50 gliding speed increased. (Fig. 6).
- In f/2-35, cells spent 90% of the time gliding which decreased in 20, 5, 50, 70 & 140 psu with a concomitant increase in other modalities. In f/2-70, cells were detaching 13% of the time, while at 140 psu, cells were either non-motile or exhibiting rock & roll 96% of the time (Fig 7).

IV Discussion

The various movement modalities of *C. closterium* represent adaptation to the mudflat habitat (Apoya-Horton et al. 2006). Considering the cohesive nature of the mudflat sediment (Bellinger et al. 2005), corkscrew gliding may assist in passage through the fine layers. Pirouette and pivot movements may represent a response to chemical gradients and directed taxis under suboptimal conditions. These responses may be due to cell tips having a sensory detection system (Cohn et al. 2004) that is not only used for light but with other environmental cues as well. Pirouette movement was observed in the field during stressful conditions (Harper 1977).

Rock & roll, rollover and detachment are indicative of the organism's response to salinity changes which we have also shown to affect polysaccharide synthesis (Abdullahi et al. 2006).

The immediate response to hyper-saline conditions in *C. closterium* suggests that the sensory mechanism used to detect this stimulus is unique from that involved in sensing a hyposaline environment.

Adhesion is a prerequisite to gliding (Wetherbee et al. 1998), hence detachment would result in passive transport of the cell. Whether the detachment process is passive or a physiologically driven response requires further investigation.

V Literature Cited

Abdullahi, A. S., Underwood, G. J C. & Gretz, M. R. 2006. Extracellular matrix assembly in diatoms (Bacillariophyceae). V. Environmental effects on polysaccharide synthesis in the model diatom, *Phaeodactylum tricornutum*. *J. Phycol.* 42(2): 363-378.

Apoya-Horton, M. D., Underwood, G. J. C. & Gretz, M. R. 2006. Movement modalities and responses to environmental changes of the mudflat diatom *Cylindrotheca closterium* (Bacillariophyceae). *J. Phycol.* 42(2): 379-390.

Bellinger, B. J., Abdullahi, A. S., Gretz, M. R. & Underwood, G. J. C. 2005. Biofilm polymers: relationship between carbohydrate biopolymers from estuarine mudflats and unialgal cultures of benthic diatoms. *Aquat. Microb. Ecol.* 38: 169-180.

Cohn, S. A., Bahena, M., Davis, J. T., Ragland, R. L., Rauschenberg, C. D. & Smith, B. J. 2004. Characterisation of the diatom photophobic response to high irradiance. *Diatom Res.* 19(2): 167-179.

Harper, M. A. 1977. Movements. *In* Werner, D. [Ed.] *The Biology of Diatoms*. Blackwell Scientific Publications, Oxford, pp. 224-249.

Wetherbee, R., Lind, J.L., Burke, J. & Quatrano, R. S. 1998. The first kiss: establishment and

Acknowledgements

We thank MTU for a Graduate Fellowship to MDAH and support from the National Science Foundation (Grant IBN-0110875 to MRG and GJCU).