Pressura et Lucida: High light intensity effects on *Eremosphaera viridis* turgor[1].

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OBJECTIVE

To determine if light intensity has an effect on the turgor of *Eremosphaera viridis*

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INTRODUCTION

Eremosphaera viridis, a unicellular alga, provides an excellent subject for measuring internal parameters of a cell; the large diameter (approximately 120 microns) of the cells provides an easy target for impalements. Their spherical geometry makes biophysical calculations easier. As an alga, *E. viridis* conducts photosynthesis in its chloroplasts. At high light intensities, the chloroplasts migrate towards the center of the cell and surround the nucleus (a process called systrophe) (Gasumova et al., 2009; Lew, 2010). Theories exist to explain this process; either it is an attempt by the cell to shield the nucleus from the light or that it reduces the amount of photosynthesis occurring in the cell by decreasing the cell's absorbance cross-section.

All cells, including *E. viridis,* are strongly dependent on regulating their internal water content through osmosis for survival. Osmosis is the phenomenon of water flow across a water permeable membrane from low solute concentration to high. This process can put a large strain on the walls of the cell with pressures in excess of 0.5–1 MPa (5–10 times atmospheric pressure) in the unicellular algae *E. viridis*. Quantitation of the cell wall's ability to cope with the pressures is based on Young's Modulus of Elasticity, ε, which measures the stiffness of the material (i.e., how well the material maintains its volume to pressure changes) and is described by:

$$\varepsilon = V\frac{dP}{dV} \qquad (1)$$

Where V is the total volume enclosed by the material and dP/dV is the differential change in pressure with respect to volume. For small changes in both pressure (ΔP) and volume (ΔV), ε can be approximated as:

$$\varepsilon = V\frac{\Delta P}{\Delta V} \qquad (2)$$

In theory, the osmotic pressure (Π) across a membrane is governed by:

$$\Pi = RT(C_i - C_o) \qquad (3)$$

Where R is the gas constant, T is the temperature, C_i is the internal concentration of the solute and C_o is the external solute concentration. Since solutes are constantly being transported across the cell's membrane, internal concentration of solutes are always varying in time, bringing with it changes in pressure. In particular, Lew, RR (2010) showed that during photosynthesis, there are significant ion fluxes in across the membrane of *E. viridis*. So, we investigated whether high light intensity treatments can cause concentration changes that will have a significant effect on the overall pressure in the cell.

MATERIALS AND METHODS

Pressure Probe Micropipette Fabrication. To obtain a wide aperture micropipette, borosilicate capillaries (1.00 mm OD, 0.58 mm ID, with internal filament) were double pulled. The first pull —at a high heat— stretched the capillary to create a small diameter 'pinch' in the glass. The second pull at lower heat (and no selenoid pull) created a large aperture tip (about 1–1.5 micron) suitable for pressure measurements. A plug of silicon oil (1.5 centistokes polydimethylsiloxane, Dow Corning, Midland, MN) was added to the back of the micropipette with a fine bore syringe needle. After the oil had migrated and filled the pipette tip (2–5 minutes), the rest of the pipette was filled with the silicon oil. Care was taken to ensure no air bubbles were left in the capillary. The pipette was then mounted in the pressure probe.

The pressure probe consisted of a subminiature pressure transducer (XT-140–300G, Kulite Semiconductor Products, Leonia, NJ) that was housed adjacent to the pressure-probe micropipette in a small brass holder connected to a micrometer-driven piston by thick-walled Teflon tubing (1.59 mm OD × 0.254 mm ID; Chromatographic Specialities, Brockville, Ontario, Canada). The piston, tubing, holder, and micropipette were filled with silicon oil.

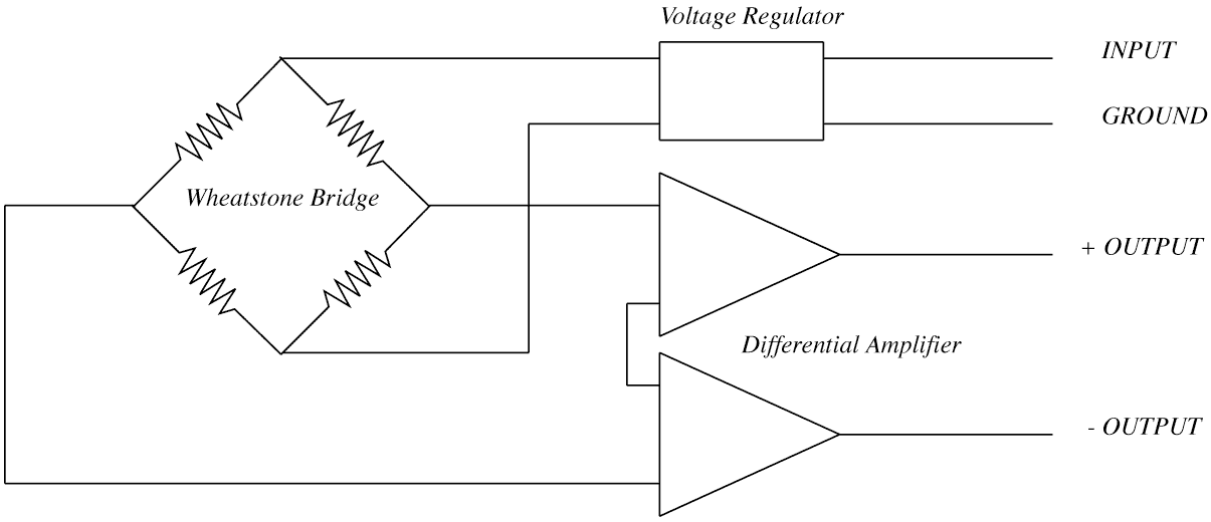

Figure 1: **The Wheatstone bridge.** A voltage regulator supplied a constant voltage of 10 VDC. One of the resistors in the Wheatstone bridge would change in response to changes in pressure. The potential from this resistor and its pair could then be fed into a differential amplifier that subtracted the voltage from the second —unaffected— resistor pair of the Wheatstone bridge, effectively cancelling the electrical noise from the output signal to allow measurements of the relatively small resistance changes.

The transducer was connected to a voltage regulator as shown in Figure 1 where the Wheatstone bridge represents the transducer. A pressure change caused a change in the resistance in one of the resistors. This potential is then sent through a differential amplifier to remove the background noise. The output signals were on the order of volts and fed to a digital storage oscilloscope (Tektronix TDS 2001C) with a digitization rate of 50 Hz. Pressure measurements could be read off of the LCD monitor of the oscilloscope.

Cell-Holding Micropipette Creation. It was necessary to hold the cell steady during impalements, for which a holding micropipette was used. The capillary used to fabricate the holding micropipette was double-pulled as described for the pressure probe micropipette. Under a ×10 objective, the location where the capillary was approximately 100 microns in diameter was found and —using a pair of fine point tweezers— the pipette tip was broken at this location. A micro-grinder was then used to polish the pipette tip and widen the bore to about 120 microns (Figure 2). Periodically, the pipette tip was viewed under a 10x objective to check the size and ensure the glass was polishing to a fine finish. The pipette was then mounted on the suction apparatus, which comprised a pipette holder held in place by a micromanipulator. A chamber containing a plunger that could compress or reduce the volume in the chamber to create or alleviate a vacuum in the pipette was attached to the pipette holder.

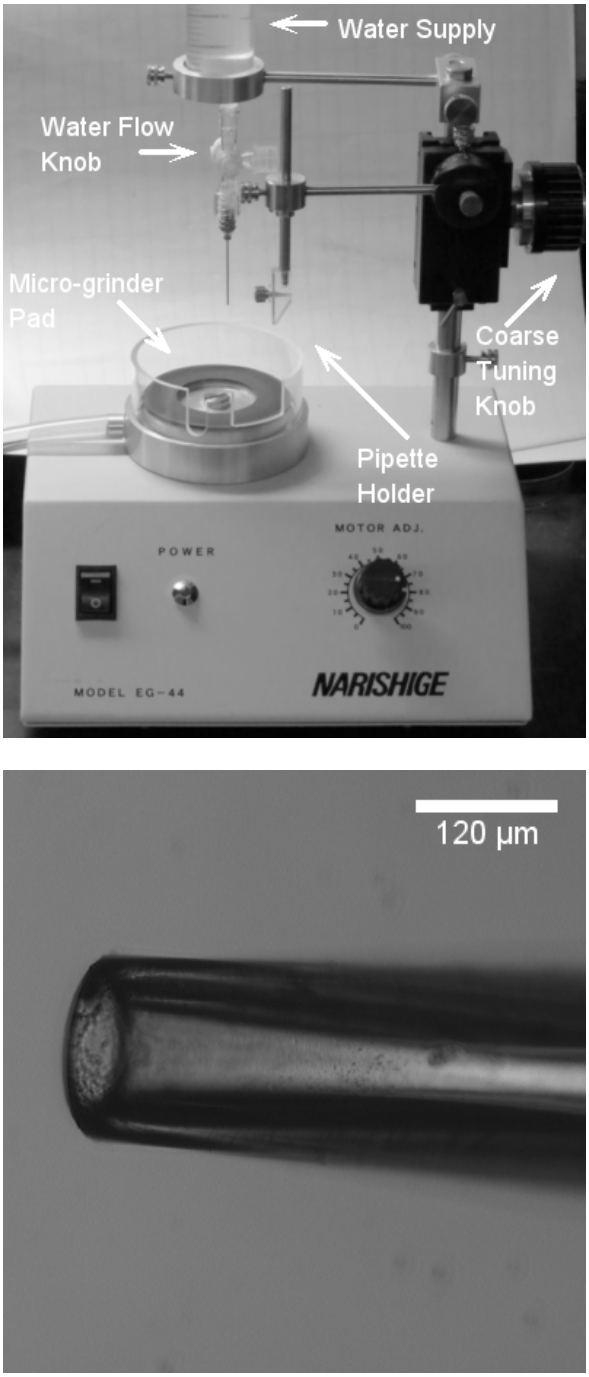

Figure 2: **Holding Micropipette Preparation.** (*upper panel*) The micropipette was first attached to a holder by using a screw that placed a plastic plate over top the pipette at the un-sharpened end. The water flow knob was set to allow double distilled water to flow from the water supply in a controlled manner onto the micro-grinder pad (for lubrication). Initially and periodically during micro-grinding, distilled water was added by hand to the pipette through a squeeze bottle in a generous amount. The pipette was slowly lowered at first using the coarse adjustment knob till it resided just above the pad. Using the fine adjustment knob, which was housed in the interior of the coarse adjustment knob, the pipette was slowly lowered onto the pad. The pad was rotating at a high speed. It was coated with diamond dust to polish the pipette. A weak scratching sound could be heard when the pipette and the pad met and slowly the pipette was lowered further to grind. Every once in a while, the micropipette was raised so that it could be examined under the microscope to determine its dimensions. The final product (*lower panel*) is a suction pipette with a smooth bore of 120 microns across.

Cell Preparation. In the lid of a culture dish, 3 mL of Bold's Medium was dispensed and 1 mL of *E. viridis* cell suspension (in Bold's medium) added with a pipette.

Impalements. The suction pipette was initially centered in the focus of the microscope using a micromanipulator. The stage was slowly raised so that the cells lying along the bottom of the culture dish could be observed. A cell without neighbours was chosen and was positioned close to the opening of the suction pipette. The plunger in the suction apparatus was retracted, pulling the cell to the tip of the micropipette. The stage was carefully lowered and the pressure probe pipette was brought near the cell wall. The cell's vertical axis was adjusted so that its most medial axis was in focus. The pressure probe pipette was then slowly inserted into the cell.

Light Measurements. Under a ×10 objective with Kohler illumination, the field diaphragm was adjusted to completely fill the field of view. The diameter of the field of view was measured with a calliper to be 2.03 cm (0.8 in). The light source used consisted of the microscope's tungsten-halogen lamp filtered with 3.5% w/v cupric sulfate to remove infrared light and provided a maximum transmission wavelength of 532 nm. A radiometer was placed on the stage and the total light intensity was measured before and after the experiment (the average of the two measurements was used as the irradiance level). From this, the total photon flux density could be determined. The high light intensity had an average photon flux of 7800 ± 300 μmol/m^2/sec and the low light intensity was 230 ± 20 μmol/m^2/sec. Cells were viewed under ×40 water immersion and an initial pressure measurement (at an intensity of 150 μmol m^{-2} sec^{-1}) was taken about 2 minutes prior to the high intensity light treatment. Pressure measurements were taken at approximately 3 minute intervals for 20-50 minutes.

Osmolyte Treatment. For approximately 15 minutes, the cell's internal pressure was monitored under a ×40 water immersion lens. With a pipette, 1 mL of 30 mM KCl in Bold's medium (which contains 2.26 meq/l of K^+ and 3.17 meq/l of Cl^-, Lew 2010) was added and roughly the same amount was removed via a suction apparatus (a Bernoulli pump connected through silicon tubing to a glass pipette). This was repeated twice. The pressure was then monitored for approximately 90 minutes at intervals of approximately 3 minutes.

Young's Modulus. Under ×10 magnification, the cell's initial radius and pressure was recorded. The pressure was slowly reduced so that the meniscus was drawn into (and visible) in the pressure probe micropipette (Figure 3). Care was taken to ensure that the meniscus position was due to the pressure and not a plug in the pipette's tip. Images of the meniscus location and pipette were taken by a Hamamatsu Orca C4742-95 camera mounted on the microscope and operated by OpenLab software. Using OpenLab spatial calibration tools, the meniscus' position, cell radius, and pipette diameter could be measured.

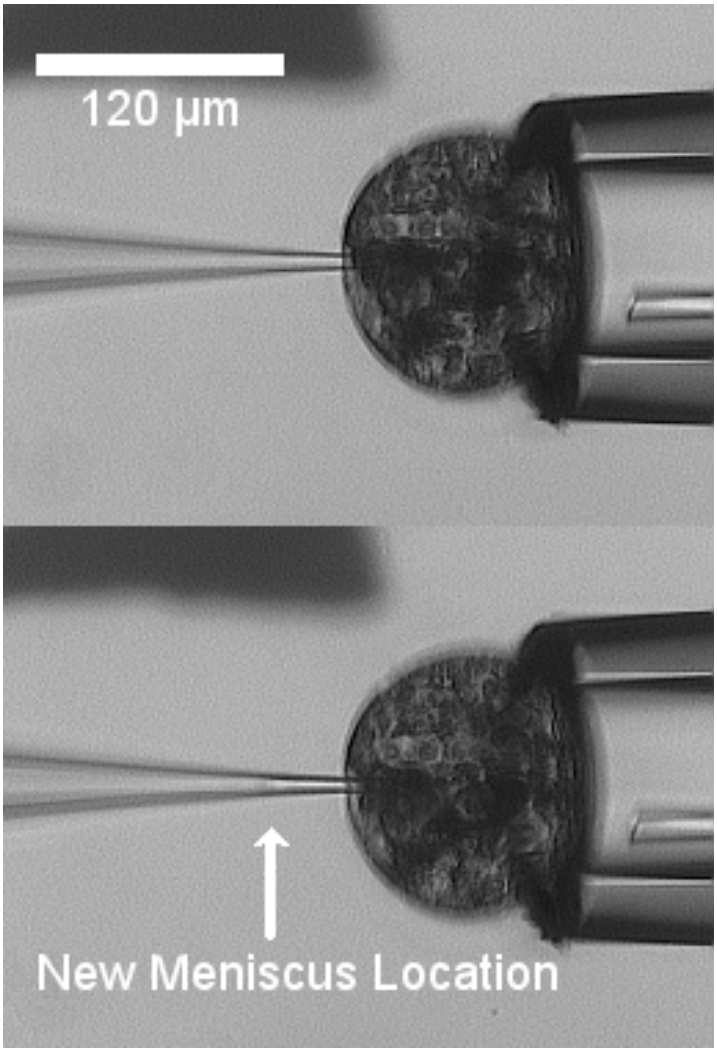

Figure 3: **Measuring Young's modulus of elasticity.** The left panel show an impaled cell in the suction pipette. The meniscus in the pressure probe pipette was brought up to the cell wall. The right panel shows the meniscus after the pressure in the pipette has been relaxed.

RESULTS

Esimating the Modulus of Elasticity. Our goal in measuring ε was to validate the measurements with the apparatus based on previous results from Frey et al. (1988). Initial pressure measurements, were taken by forcing the meniscus up to the cell wall. Overall, the initial turgor was 868 ± 102 kPa (n=27). The pressure was then lowered by 50 ± 20 kPa and the volume between the new position of the meniscus and the cell was taken to be the change in volume. Assuming the cell to be a sphere, its radius could be measured to obtain its total internal volume. For 18 trials, the elasticity constant had an average value of 33.5 ± 10.7 MPa while suspended in Bold's medium, a value in the range of Frey et al. (1988) who recorded between 20.3-35.5 MPa. Steudle, et al (1977) has noted that for the approximation in equation 2 to be valid the ratio of the change in pressure and elasticity constant must be significantly less than one; our ratio is 0.001, meaning that this technique for estimating ε should be reasonably accurate. In preliminary attempts to measure ε, the cells were pressure clamped at almost double their resting pressure and showed no discernible change in volume (figure 4), consistent with the high elasticity modulus determined with small volume changes.

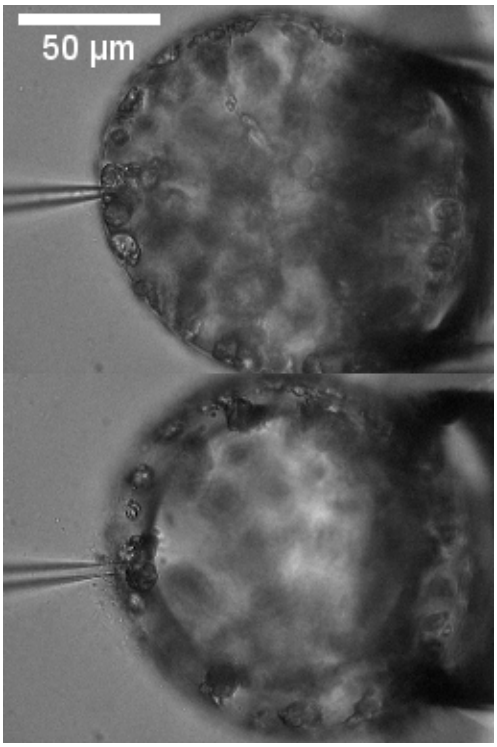

Figure 4: **Pressure Clamp.** A cell had an initial pressure of 0.76 MPa (top panel). Using the pipette, silicon oil was forced into the cell via the pressure probe to pressure clamp it to 1.20 MPa (bottom panel). There was no discernible change in the cell diameter, consistent with the high modulus reported by Frey et al. (1988) and our results with small volume changes.

Does *E. viridis* Turgor Regulate? To determine whether *E. viridis* regulates its turgor, the cell was treated with a high external osmoticum and turgor monitored over an extended period of time. The cell turgor was measured for 15 minutes before the osmolyte was added. Over the course of about an hour and a half after adding the osmolyte the pressure was monitored and showed no significant recovery to its original turgor (figure 5). On average, the turgor dropped to 84% of its initial value within the first 5 minutes of osmolyte addition and slowly decreased to

74% after 90 minutes. To ascertain whether the pressure changes had any discernible effect on the cell's volume, the internal pressure measurements were correlated with their volume normalized to their resting volume (Figure 6). The volume appears to be indifferent to any pressure changes and remain close to their original value.

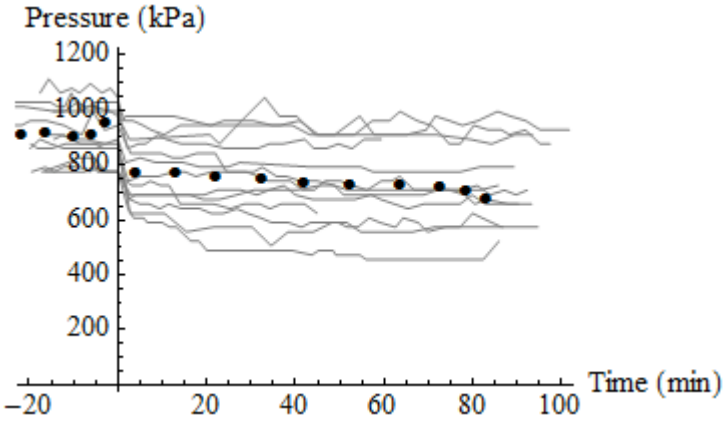

Figure 5: **Pressure relations in the presence of an osmolyte.** 30 mM of KCl in Bold's Medium was added at time zero. Average of the trials is shown as black dots.

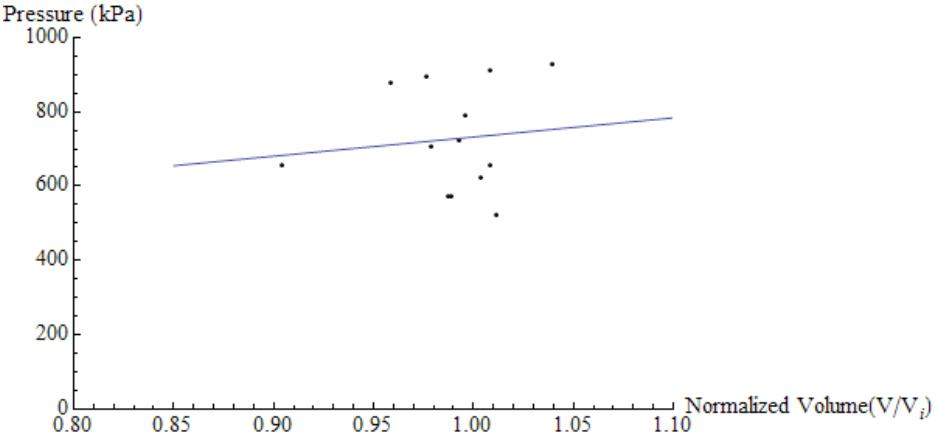

Figure 6: **Relation of the normalized volume to the internal turgor of the cell.** Values were for the final pressures and volumes for all 13 trials. The slope of the best fit provides an estimate for ε of 0.52 MPa (about 1.5% of our previous measure), but the data are scattered over a relatively small range of normalized volume, rendering the estimate suspect.

Light Irradiation Effects on Turgor. An initial measure of the cell's internal pressure was taken with a light flux of 150 μmol/m^2/s for both the controls and the high light intensity treatments. Figure 7 shows that there was very little deviation from the initial turgor for either the control or the high light intensity treatment. During high intensity irradiation, 6 cells showed complete systrophe in the time frame of the recording (Figure 8), 3 in partial phase with some translocation and 3 showed no signs of translocation. For the control light intensity, 2 showed complete systrophe, 3 showed migration and 4 showed no movement (note: 3 trials for the control had no visual data recorded).

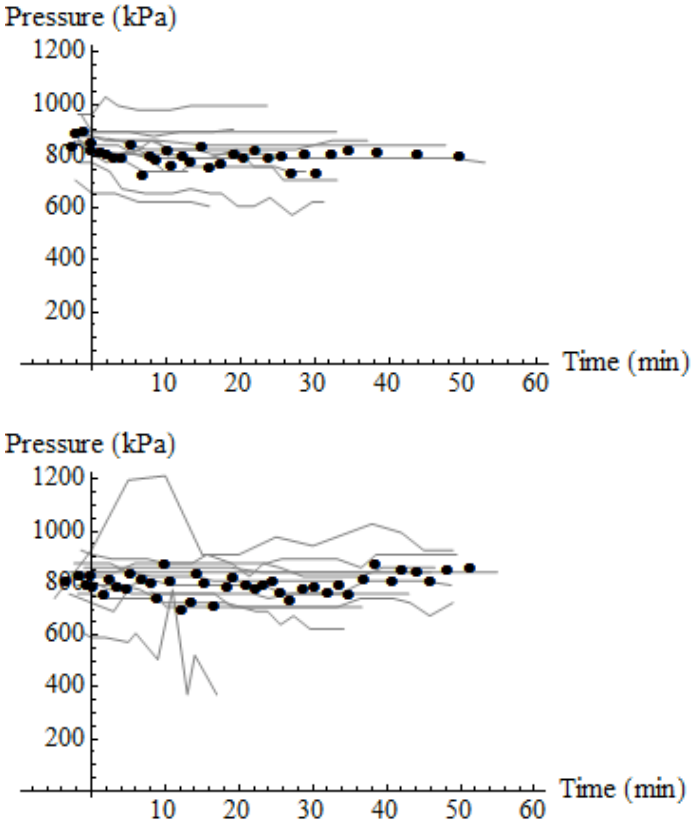

Figure 7: **High Light Treatment**. The upper panel shows the control trials (n=13) with a photon flux density of 200 μmol m^{-2} s^{-1}. The lower panel shows turgor during high light intensity treatment (n=12) with an average photon flux density of 7800 μmol m^{-2} s^{-1}. Binned averages are shown with filled circles. Time '0' is when the light treatment.

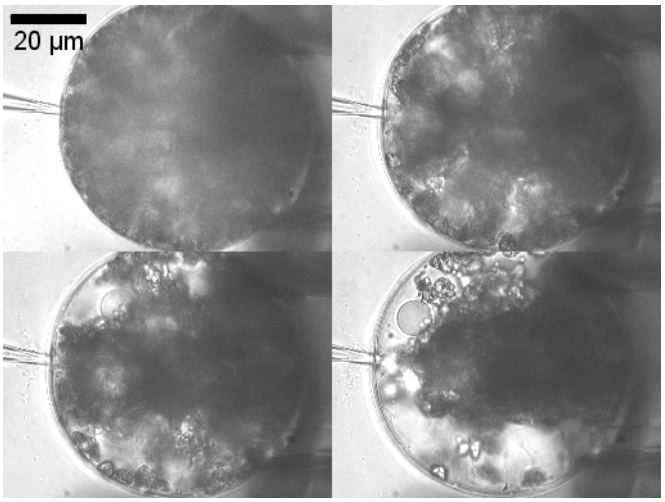

Figure 8: **Systrophe.** Top left panel shows a cell prior to light treatment showing no signs of chloroplast translocation. Top right and bottom left show visible signs the migration of its chloroplast. Bottom left shows near complete migration of chloroplast to the center of the cell (complete systrophe).

DISCUSSION

During the course of its life a cell needs to be able to adapt to changes in environmental conditions, of which one is osmotic stress. For a freshwater alga like *E. viridis*, osmotic challenges would occur when growing on soil or even in puddles that underwent drying cycles. Then, it's quite likely that the alga would face osmotic challenges in its natural environment. We mimicked a hyperosmotic condition through the addition of KCl to the extracellular bath and saw no recovery within 90 minutes. So, it appears that the alga does not turgor regulate. Frey et al. (1988) found that after osmolyte addition *E. viridis* required 24 hours before a complete or partial recover was observed. This slow response appears to be common as Okazaki et al (1984) found that *Lamprothamnium succinctum*, a brackish water alga, exhibited a similar slow recovery from a hyperosmotic treatment (1-2 days). Recovery from a hypoosmotic treatment was much shorter (≈ 2 hours), suggesting that the cell has a greater tolerance for low internal pressures than large. We did not explore hypoosmotic responses with *E. viridis*, but it would be something of interest for future testing.

We found that there was little volume change with pressure changes in the algae which results in the large modulus of elasticity (ε) value that we measured. ε was much larger than its internal pressure (34 MPa compared to 868 ± 102 kPa, n=27), indicating that the cell wall is very rigid and would require a very large pressure change before a significant volume change would occur. The rigidity of the wall is probably due to cellulose fiber sheets (M.D. Guiry 2013). These are 0.5 μm thick on average and 5-13 layers thick (Frey et al 1988). Other works show a similar range for ε in algae: Zimmermann et al. (1976) reported a value of 10–70 MPa in *Valonia utricularis*, a marine alga.

Previous work by Lew (2010) has shown that ion fluxes do occur in *E. viridis* during high light intensities. By van Hoft's law, a pressure change is expected and seems plausible. However appears not to be the case. The absence of change between the control and the treatment could be explained by the sensitivity of the pressure probe. With the system we used, we were able to measure pressure changes on the order of 10 kPa. Had the pressure change been smaller than 10 kPa, it would have been undetectable by our methods. Lew (2010) reported influxes in the range of 150 nmole m^{-2} s^{-1} (K^+), 100 nmole m^{-2} s^{-1} (Cl^-) and 20 nmole m^{-2} s^{-1} (Ca^{2+}), which —for a cell 110 μm diameter— would result in a concentration change of 53 mM over 60 minutes under similar high light intensities. This corresponds to a pressure change of 130 kPa. Clearly, a decrease in other internal osmotically active solutes must be occurring (probably due to efflux from the cell) to maintain the pressure at a steady level near the initial value.

The frequency of systrophe in the algae appears to be similar to previous work by Gasumova et al. (2009). They found that at a flux of 7935 μmol m^{-2} s^{-1}, 30-50% of the cells were found to be in state of partial systrophe on a time frame of 30-50 minutes and 50-70% were in a completely systrophed state, similar to our values of 25% and 50%, respectively. Likewise, at 223 μmol m^{-2} s^{-1} they found 50% were in partial stage of translocation and 0% were in complete

systrophe which matches our partial systrophe value of 33% but not the 22% for complete systrophe. The higher frequency of complete systrophe could then be attributed to outliers, since we viewed fewer cells than Gasumova et al. (2009) (who examined 50-60 cells per trial), and possibly to some effect of the impalement into the cell in our experiments.

References:

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