SHEATH PIGMENT FORMATION IN A BLUE-GREEN ALGAE LYNGBYA AESTUARII AS AN ADAPTATION TO HIGH LIGHT

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INTRODUCTION

The Great Sippewissett salt marsh has extensive areas of intertidal microbial mats. The top layer of the mats is composed primarily of blue-green algae Lyngbya aestuarii and Microcoleus chthonoplastes. These mats are subjected to long exposures of high light intensity which can potentially cause photodynamic damage to the microorganisms. L. aestuarii with heavily pigmented sheaths is often found in these exposed areas. The conditions for sheath pigment formation are not yet understood. Iron concentrations in the environment, periodic desiccation and light intensity are possible factors which may influence sheath pigment formation. From the results of the present study, however, exposure to high light appears to be the primary requirement for sheath pigment formation.

The yellow-brown sheath pigment has yet to be fully characterized. The staining of the Lyngybya sheaths is due to the pigment scytonemine (as cited in Birke, 1974) and is very effective in reducting incident light. There has been speculation that the sheath pigment evolved in the Precambrian, as a primitive form of protection from high light intensity, including ultraviolet radiation (Rambler et al., 1977). No recent or detailed chemical analysis of this pigment has been reported.

MATERIALS AND METHODS

Axenic cultures of Lyngbya aestuarii, grown in modified artificial Von Stosch medium (Table 1) were used for this project. To obtain a somewhat homogeneous inoculum, Lyngbya was put in a Waring blender for approximately 10 sec and 125 ml Erhlenmeyer flasks were inoculated with 50 ml artificial Von Stosch medium.

Table 1. Modified Artificial Von Stosch Medium with Instant Ocean.

Stock (20X conc.)
per liter distilled water

0.1 g EDTA
4 ml Nitsch's trace element mixture
20 ml $FeCl_3$ solution (0.29 g/l)
0.84 g $NaNO_3$
1.00 g NH_4Cl
0.08 g $NaH_2PO_4\ H_2O$
20 ml Na_2SeO_4 (0.01 mM)
5 ml $NiSO_4(NH_4)_2\ 6H_2O$ (0.01 mM)
33.0 g NaCl

Dilute 50 ml with 950 ml Instant Ocean Seawater Mix*, add 1 ml vitamin mix (D.N.), check and adjust pH 8.0-8.3, and autoclave

*Instant Ocean 33 q/liter distilled water

Three different light intensities were used for this study: (a) direct

each light situation were harvested simultaneously. To produce equal aliquots of material, each sample was blended with 10 ml of medium for 10 sec. and 5 ml of the mixture was washed with deionized water and filtered onto a 1.0 µm filter, desiccated in the dark for 24 hrs and the dry weights recorded. Another 5 ml was washed, filtered, and then extracted with absolute methanol 4-6 hrs at 4°C for cell pigment extraction, and analyzed on a Guilford 2600 spectrophotometer. Microscopic examination of the filaments showed that only the sheath pigments remaining, which were extracted with dimethyl sulfoxide (DMSO) for approximately 1 hr.

RESULTS

L. aestuarii grown in low light produces no noticeable pigmented sheaths. The DMSO extract of the sheath pigments does have some absorbance in the near ultraviolet range (Figure 1). Overall, the absorbance of the sheath pigment is much lower than the absorbance of the cell pigments for the low light culture. The methanol extract produces well defined peaks of the cell pigments (Figure 1). At 435 nm there is a combined chlorophyll and carotenoid peak, at 470 nm there is a carotenoid peak, and at 660 nm there is a distinct chlorophyll peak.

L. aestuarii grown in high light outside produces heavily pigmented sheaths. The absorbance of the pigment is highest in the near ultraviolet range (360-400 nm) as shown in Figure 2. There is also heavy absorbance through the violet, blue, and blue-green regions of the spectrum. A prominent secondary maximum occurs at 495 nm. The absorbance drops off considerably at wavelengths greater than 540 nm. The absorbance of the sheath pigments from the high light cultures generally is much higher than the cell pigments from the same amount of culture material (Figure 2).

The chlorophyll content of the cells from the various cultures was determined using the extinction coefficient, 13.33 µg/ml (R.W. Castenholz, personal communication) using the equation (Jensen 1978):

$$C_a = \frac{13.33\ \mu g/ml \times \text{absorbance} \times \text{volume of extract}}{\text{dry weight of sample}}$$

The chlorophyll content of the cells does appear to be regulated (Table 2), as chlorophyll content in the cells increases during growth with decreasing light intensities. The carotenoid to chlorophyll absorbance ratio decreases with decreasing outside light intensity. The slight relative increases in the ratio with the inside light may be possibly a result of the continuous exposure to light.

DISCUSSION

Exposure to high light intensity can potentially cause photodynamic destruction to microorganisms continually subjected to the sunlight. Cell pigment regulation is the only way many blue-green algae protect themselves from high light intensities. Blue-green algae typically show a decrease in chlorophyll content with an increase in light intensity, as protection from photo-oxidative damage. The decrease in carotenoid to chlorophyll ratio with decreasing light intensity also indicates cell pigment regultion during

growth. Cell pigment regulation is often more dramatic in other blue-greens, indicating that L. aestuarii may have an alternative means of protection from high light.

The sheath pigments which form in L. aestuarii exposed to high light intensities, absorb primarily in the near ultraviolet range, which is possibly the most damaging part of the spectrum normally reaching the earth's surface. This suggests that the sheath pigment may have a significant function in protection from high light intensities. It appears that Lyngbya aestuarii adapts to high light intensities inherent in its natural environment, by regulating chlorophyll and carotenoid contents in the cells, as well as by the formation of pigmented sheaths.

Clearly, more work needs to be done in order to form more concrete conclusions about sheath pigment formation in L. aestuarii. The nature of the sheath pigment still remains mysterious but at least some progress has been made.

Table 2. Cell pigment content of Lyngbya aestuarii.

	high outside light (620 watts/m^2)	low outside light (364 watts/m^2)	inside light (25 watts/m^2)
chlorophyll µg/mg	3.8	4.4	7.5
carotenoid/ chlorophyll absorbance ratio	3	2	2.4

Figure 1: Representative absorption curves for sheath and cell pigments of Lyngbya aestuarii grown in low outside light.

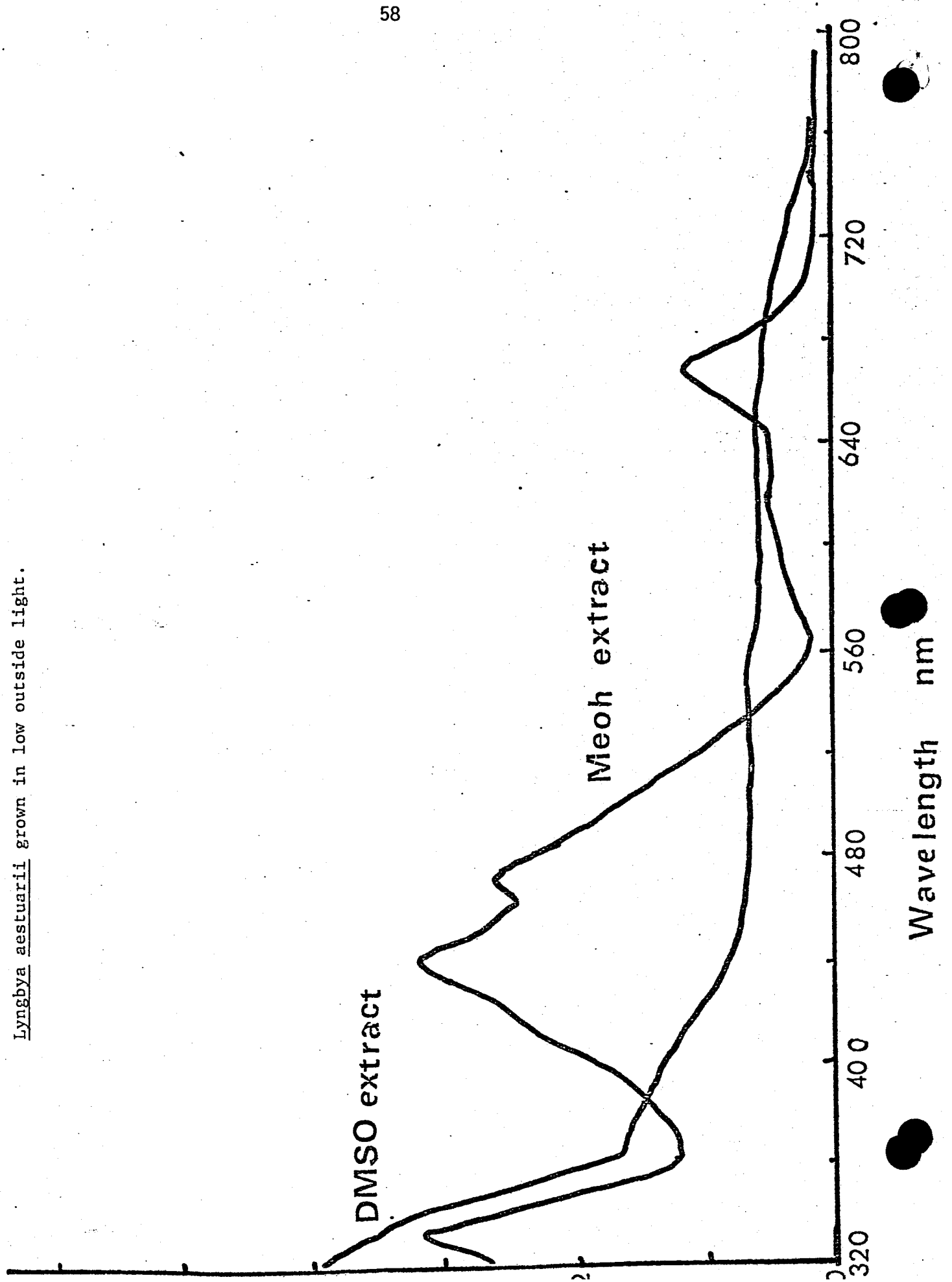
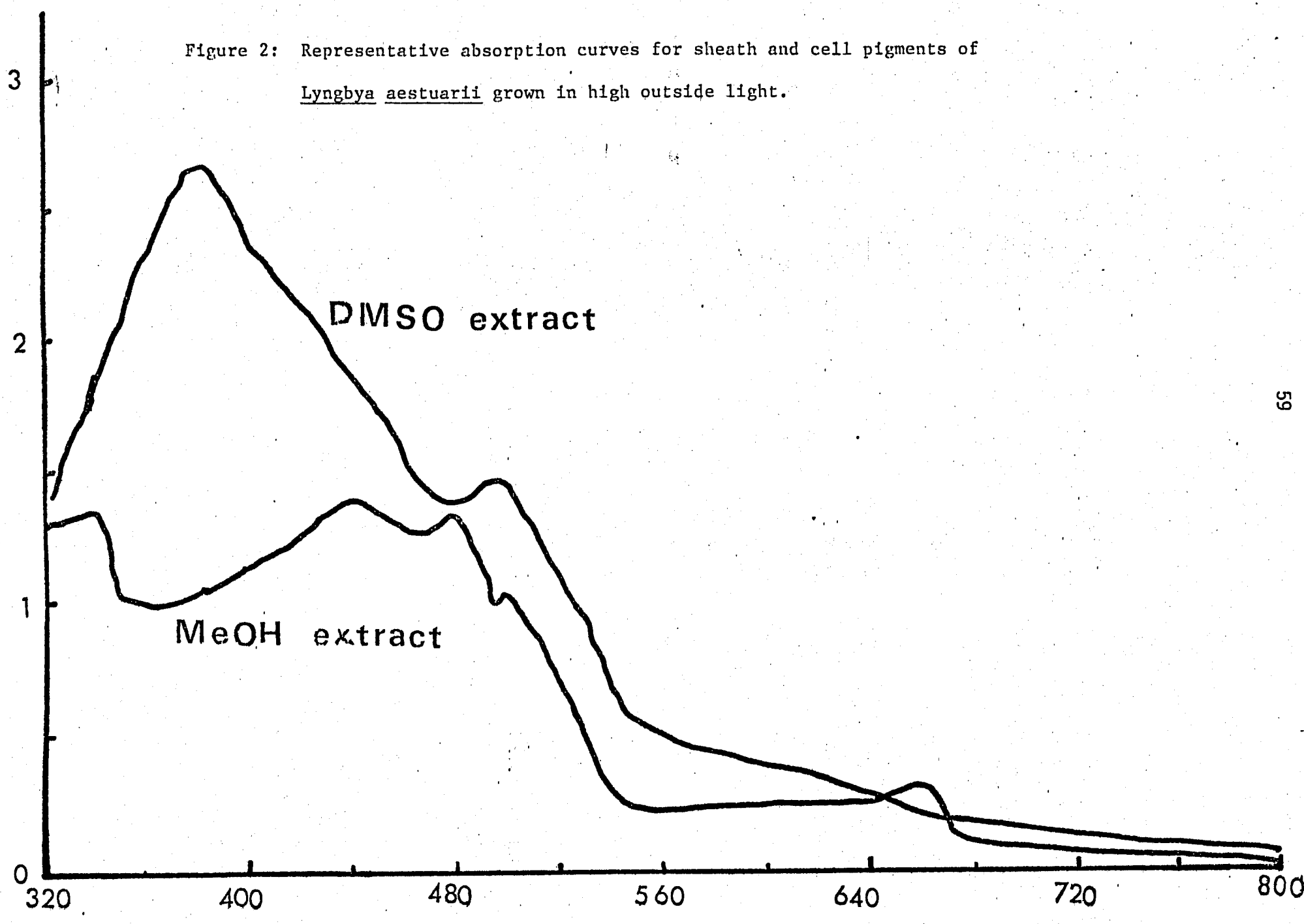


Figure 2: Representative absorption curves for sheath and cell pigments of Lyngbya aestuarii grown in high outside light.



DIEL VERTICAL MOVEMENTS OF BACTERIA IN INTERTIDAL STREAMS OF SIPPEWISSETT MARSH

Ken Noll

Environmental conditions within the sediment of intertidal streams of salt marshes change drastically in 24 hrs. Light intensity and quality; surface temperature; dissolved oxygen and sulfide concentrations in pore-waters; and depth of watercover all play a role in determining the survival of bacteria living in this environment.

The purpose of this report is to observe how bacteria in these areas respond to environmental changes in the course of a day and to attempt to determine what causes these responses. To simplify the system, the work described here was confined to monitoring the effects of light intensity and sulfide under laboratory and field conditions.

MATERIALS AND METHODS

The area studied was a stream approximately 20 cm wide (at low tide) which was covered by about 1 cm of water at low tide. The stream was composed entirely of loose sand with no interwoven mat material. For some studies, this area was recreated in the laboratory by filling aluminum pans (28 cm x 18cm x 4cm) with sulfide-rich sand from this area. Over the surface of this was deposited approximately 0.5 cm of the surface sand from this area. Seawater was allowed to flow across this continuously. One pan was set up outside to get maximal exposure to the sun. Other pans were set up in the laboratory under illumuniation from either a 150 watt or a 75 watt flood lamp from a distance of approximately one meter. These lamps were on for 14 hrs and off for 10 hrs per day.

Shading experiments were conducted in the marsh using filters made of mason jar lids with attached wire screens of varying thickness, a layer of dark plastic, or metal lids. After leaving these areas covered for 2 hrs, the surfaces of the shaded areas were sampled and examined microscopically.

Other experiments in the marsh involved covering areas with mason jar lids (38.5 cm^2) and applying 2.5 ml of 10^{-4} M DCMU to the surface. After 2 hrs under direct sunlight, the surface was collected and examined microscopically.

Cores of 0.5 mm diameter were collected from the laboratory pans and extruded onto a microscope slide for sectioning. Slices of 1 lmm were removed using a razor blade. These were disperses onto another slide and examined microscopically. Slices were refrigerated in vials if not examined immediately.

Slices of six replicate cores used for spectrophotometric studies were suspended in 0.5 ml of TSM buffer and attached cells removed by sonication in an ultrasonic bath while on ice for 5 min. The supernatant was decanted and the washing repeated a total of three times. To the pooled supernatants was added 2.1 of sucrose and the mixture centrifuged to pellet large debris. This supernatant was used to scan spectra from 650 nm to 1100 nm using a Zeiss M4QII/PMQII spectrophotometer.