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Microcosm Experiment: The Relationship Between Salinity and Light on the Production of Extracellular Material by Synechococcus sp.

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Abstract

Synechococcus sp., the hypersaline Cyanobacterium form, produces extracellular products under high salinity and high intensity of light. The excretion of large amounts of extracellular material caused an additional increase in brine viscosity. An experiment was undertaken using twelve aquaria filled with brine. Measurements were made of salinity , viscosity and the amount of organic materials at the high and low light intensity . Relative viscosity and the amount of organic materials were higher in conditions of high salinity and high light intensity, because under these conditions *Synechococcus* produced more extracellular products. Therefore these can decrease the quality and quantity of salt produced in solar salt field which is economically important to understand the effect of these extracellular materials in these environments.

Keywords: *Synechococcus, salinity, Extracellular products, viscosity.*

Introduction

Synechococcus is a non-heterocystous unicellular Cyanobacterium that can fix free nitrogen under anoxic conditions or in low oxygen concentrations (Stal, 1991). It has a very variable size (2-10 μm) and shape (ellipsoidal, ovoid or cylindrical) and produces extracellular mucus in which its cells are found embedded (Borowitzka, 1981). *Synechococcus* colonies usually take the form of a slimy or gelatinous mat in the shallow littoral zone of salt lakes (Bauld, 1981), but may also appear as single cells suspended in the water column. Golubic

(1980) noted that it is the commonest genus in mucilaginous coatings in the benthos of many salt ponds.

Like many other bacteria and algae, *Synechococcus* produces extracellular products (ECPs). For taxa other than *Synechococcus*, a wide variety of ECPs are now known (e.g. O' Colla, 1962; Fogg, 1962, 1966 and 1971; Hellebust, 1974; Arad *et al*., 1985; De Philippis *et al*., 1993). ECPs are also known to play important roles in algal growth and physiology as well as more generally in food chains and ecosystems (Hellebust, 1974). Their rate of production depends on both internal physiological factors and external environmental ones (Hellebust, 1974). Some of this extracellular polysaccharide is secreted and liberated to the medium and can increase viscosity of the medium (Guillard and Hellebust, 1971 and Vincenzini et al. 1990a and b). The ECP produced by *Synechococcus* is in the form of a mucilage. Some forty years ago, Seshadri and Buch (1958) noted that the mucilageous ECP of *Synechococcus* decreased the quality and quantity of salt in solar salt ponds by adhering to salt crystals, coloring harvested salt, imparting a foul smell to it, and by increasing brine viscosity.

Despite the obvious economic significance of these effects, rigorous evaluation of them is lacking. To address this matter, experimental investigations were undertaken using material from the Dry Creek solar salt field located north of Adelaide, Australia.

In the first experiment, twelve aquaria were set up outside of the laboratories. This experiment was designed to determine the relationship between salinity and light on the amount of ECP produced by *Synechococcus*. An additional experiment examined the chemical composition of ECP from *Synechococcus*.

Methods

Experimental design

Twelve aquaria, each 40 x 30 x 50 cm (length, width, depth), were located outside of the Zoology building, University of Adelaide. Water from two ponds at Dry Creek solar salt field with average saltiness of 190.5 g/L and 285 g/L, respectively, was filtered through a net (mesh size ~ 50 μm) to remove large organisms and debris. Ten liters of

water, salinity 190.5 g/L, were added to six aquaria; they were designated low salinity (aquaria LS). Ten liters of water, of salinity 285 g/L, was added to other six aquaria; these aquaria were designated high salinity (aquaria HS). *Synechococcus* was collected from pond with average salinity of 190.5 g/L, drained of excess water and transferred to the laboratory. Approximately 700 g of this *Synechococcus* material was transferred to each aquarium that simulated conditions in solar salt fields. After 24 hrs, six aquaria, three LS and three HS, were covered by fine-meshed shade cloth to decrease light intensity (70% reduction); these aquaria were designated as low-salinity-covered (LSC), and high-salinity-covered (HSC) aquaria. The other aquaria were designated as low-salinity-uncovered (LSU), and high-salinity-uncovered (HSU) aquaria. HSU, HSC, LSU and LSC aquaria were randomly arranged and the position of each aquarium was changed every two days.

To avoid nutrient depletion, phosphorus (as K_2HPO_4) and nitrogen (as $NaNO_3$) were added daily to the aquaria. The amounts chosen (0.019 mg/L phosphate and 0.016 mg/L nitrate) approximated to the natural concentrations in the study area. To compensate for evaporation, distilled water was added daily. Additionally, aquaria walls were scrubbed daily to prevent the build-up of salts above water-level. The volume of evaporated water was also calculated daily.

Solar irradiance was measured with Lunasx3 (Gossen) daily at 2:00 pm. Maximum and minimum air temperature were recorded daily, as was water temperature in aquaria at 2:00 pm.

Sample collection

Water samples (30 ml) were collected weekly from each aquarium after mixing the aquarium slowly with glass rod to homogenize it.

Synechococcus material (approximately 5 gm) was collected from each aquarium at the end of the first, and last week of the experiment to measure chlorophyll *a* and estimate the amount of ECP. The *Synechococcus* was collected on paper filter (Whatman #1), drained by gentle suction for 2-3 minutes, and then placed on absorbent paper in a petri-dish to remove further brine. The filter paper was stored in a plastic bag and frozen until further analysis.

Laboratory measurements

The pH, conductivity and viscosity of water samples were measured. Conductivity measurements were converted to salinity using the regression equation of Williams (1986). Viscosity was measured with a capillary viscometer (PSL, Model C-3889, Type BS/U). Relative viscosity was obtained by comparing the viscosity of samples and the viscosity of seawater evaporated to the same salinity (for more detail see Ghassemzadeh, 1997).

Because it is difficult to determine the amount of ECP per cell of *Synechococcus*, estimates were made from *Synechococcus* cells embedded in mucilage by measuring chlorophyll *a* and ash-free dry weight (AFDW). AFDW gave an estimate of both organic material (cells + mucilage); chlorophyll *a* and also gave an estimate of cell biomass. Thus, AFDW yielded an estimate of mucilage. Deriving estimates, a 5g of frozen *Synechococcus* material was dried at 105 °C for 24 hours to give dry weight (APHA, 1992), then ashed at 550 °C for 4 hours in a muffle furnance (Aloi, 1990). AFDW was calculated as the difference between dry weight and the weight of the ash after ashing.

To drive estimates of cell biomass, chlorophyll *a* was first extracted from 1g stored *Synechococcus* material using the methods of Krishman (1991) who noted that the most effective extraction of chlorophyll *a* from *Synechococcus* is with 90% methanol. Pigments were extracted in 90% methanol by placing each sample with 5 ml of solvent at 4°C in the dark for 12-18 hours, and then transferring it to a 70°C water bath where it was boiled in methanol for 2 minutes. Short periods of boiling aid pigment extraction without converting significant amounts of chlorophyll *a* into phaeophytin (Tett *et al.*, 1975). The solvent reacted with the mucilage and white flakes appeared but stirrers improved extraction. The solvent and extract were then put into a centrifuge tube. After the first extraction, 5 ml methanol was added to the sample, boiled for 2 minutes and added to the first extraction in a centrifuge tube. It was then centrifuged at 2000 RPM. Immediate centrifugation provided rapid cooling of the extracts and aided prevention of pigment breakdown. Optical densities were measured against a methanol blank at 750 (before acidification) 665, and 750

(after acidification) nm using a Varian UV/visible spectrophotometer. After the initial reading, extracts were acidified with two drops of 8% (2N) HCl and optical densities measured at the same wavelengths. Chlorophyll *a* was estimated using the equation of Talling and Driver (1963) as post-acidification readings may increase with time, two background readings were used (Tett *et al.*, 1975).

To confirm the identity of organisms in the aquaria and estimate their densities, samples were collected for microscopic examination. Visual observation of colour changes in the aquaria were noted.

Solid state high resolution ^{13}C Nuclear Magnetic Resonance (NMR) with Cross Polarization and Magic Angle Spinning (CP/MAS) techniques were used to determine the chemical composition of ECP produced by *Synechococcus*. A portion of *Synechococcus* standing crop that had been stored frozen was freeze dried by Dynavac Freeze Drier, Model FD.5 for determination of ECP chemical composition.

Results

Physico-chemical parameters

Table 1 summarized data on air and water temperatures during the experiment. Solar irradiance at 14.00 hr varied from 1500 to 2500 $\mu Em^{-2}S^{-1}$ on cloudy and sunny days, respectively. Table 2 and 3 summarized data on pH, specific gravity, salinity and viscosity of water from ponds 1 (salinity 190.5 g/L) and 2 (salinity 285 g/L) and also in the aquaria.

Table 1- Daily measurements of mean air temperature and water temperature (±SD) in experimental aquaria, Values as ?C.

	T_a		T_w			
Date	**max.**	**min.**	**HSU**	**HSC**	**LSU**	**LSC**
Week 1	34.8 (7.19)	18.8 (4.35)	34.5 (6.11)	31.2 (6.56)	31.7 (6.40)	30.6 (6.76)
Week 2	37.93.(27)	14.0 (2.64)	38.8 (1.59)	34.0 (2.94)	36.7 (3.35)	33.8 (2.86)
Week 3	35.2 (5.05)	16.(5.22)	38.7 (3.13)	32.9 (3.67)	36.3 (3.39)	32.3 (4.00)
Week 4	34.6 (2.92)	16.7 (2.19)	36.7 (4.40)	31.2 (3.72)	34.1 (4.81)	30.4 (4.35)
Week 5	37.1 (5.72)	19.8 (4.14	36.5 (3.92)	32.6 (3.83)	35.1 (3.44)	33.6 (4.39)
Week 6	35.6 (1.81)	16.6 (1.81)	35.4 (2.96)	31.3 (1.64)	33.5 (1.96)	31.2 (1.92)

Ta, air temperature; Tw, water temperature; HSU, high salinity, uncovered; HSC, high salinity covered; LSU, low salinity, uncovered; LSC, low salinity, covered.

pH was 8.53 and 8.87 in ponds 1 (low salinity) and 2 (high salinity), respectively. The values for specific gravity were 1.14705 and 1.2132, corresponding to salinities of 190.5 g/L and 285.0 g/L. The viscosity of water from pond 2 was higher than water in pond 1. At the end of first week viscosity values in experimental aquaria were 1.51340 and 1.5790 for water from ponds 1 and 2, respectively. In brief, for high salinity aquaria, pH, specific gravity and salinity means ranged from 8.4 to 8.9, from 1.1894 to 1.1924 and from 251.0-255.5 g/L, respectively. For low salinity aquaria, the means were 8.4 to 8.8 for pH, 1.1445 to 1.1464 for specific gravity and 185-191 g/L for salinity (Table 3).

Values for salinity and relative viscosity in the experimental aquaria are presented in Figure 1. This figure shows that the salinity in each aquarium was almost constant throughout the experimental period. The relative viscosity was higher in HS samples in weeks 2, 3 and 4. Viscosity increased in HSU and HSC samples up to week 4, ranging from 1.79036 to 1.94342 and 1.78616 to 1.86914 centistokes, respectively. In LSU and LSC samples, viscosity ranged from 1.52368 to 1.58153 and 1.51764 to 1.55263 centistokes, respectively. The highest viscosity occurred in HSU samples in week four. The lowest viscosity was recorded in week one in an LSC aquarium.

The effects of low-high salinity and cover-uncover status on the relative viscosity over a 6 weeks period was examined from the two-factor analyses of variance (Table 4). It shows the relative viscosity differed between samples from high and low salinity treatments ($p<0.01$) through time (salt factor). There was also a significant difference ($P<0.01$) between the relative viscosity in samples from covered and uncovered treatments. The interaction factor of the ANOVA is also significant indicating interaction between salinity and cover on relative viscosity. In weeks 1, 2 and 3 the data indicate great relative viscosity for high-uncovered treatments than other treatments, but the differences are small shown by the flat nature of the plane (Figure 2). During and after week 4, the plane is more steeply tilted and relative viscosity decreased in high salinity-uncovered treatments.

Table 2 - Physical and chemical parameters of water from 2 ponds used to fill aquarai.

Sample	pH	Specific gravity	Salinity (g/L)	Viscosity (centistokes)
Pond 1	8.53 (0.00)	1.14705 (0.01)	190.5 (0.21)	1.51340 (0.00)
Pond 2	8.87 (0.011)	1.2132 (0.00)	285.0 (0.11)	1.5790 (0.02)

Each value is the mean of three measurements (±SD).

Table 3 - Means of weekly measurements from three aquaria.

Date	Aquarium	pH	Specific gravity	Salinity (g/L)	Viscosity (centistokes)
W					
E	HSU	8.6 (0.03	1.1913 (0.00)	253.7 (0.64)	1.8023 (0.01)
E	HSC	8.7 (0.01)	1.1910 (0.00)	253.3 (0.3)	1.7990(0.00
K	LSU	8.6 (0.01)	1.1463 (0.00)	189.5 (1.10)	1.5237 (0.00)
1	LSC	8.6 (0.01)	1.1460 (0.00)	189.1 (0.26)	1.5196 (0.00)
W					
E	HSU	8.6 (0.03)	1.1914 (0.00)	253.9 (0.81)	1.8862 (0.00)
E	HSC	8.6 (0.06)	1.1894 (0.00)	251.0 (1.6)	1.8250 (0.03)
K	LSU	8.7 (0.01)	1.1460 (0.7)	189.1 (0.11)	1.5303 (0.00)
2	LSC	8.7 (0.02)	1.1464 (0.00)	189.6 (1.52)	1.5500 (0.00)
W					
E	HSU	8.6 (0.04)	1.8961 (0.00)	251.4 (1.82)	1.8986 (0.05)
E	HSC	8.8 (0.03)	1.1897 (0.00)	250.5 (1.37)	1.8158 (0.00)
K	LSU	8.8 (0.03)	1.1461 (0.00)	189.2 (1.05)	1.5623 (0.02)
3	LSC	8.8 (0.03)	1.1451 (0.00)	186.9 (1.90)	1.5424 (0.00)
W					
E	HSU	8.7 (0.08)	1.1918 (0.00)	254.5 (0.25)	1.9293 (0.02)
E	HSC	8.8 (0.12)	1.1906 (0.00)	252.8 (1.2)	1.8432 (0.01)
K	LSU	8.6 (0.04)	1.1458 (0.00)	188.1 (0.86)	1.5602 (0.01)
4	LSC	8.6 (0.04)	1.1453 (0.00)	188.1 (0.96)	1.5197 (0.00)
W					
E	HSU	8.7 (0.20)	1.1912 (0.00)	254.1 (2.08)	1.8642 (0.01)
E	HSC	8.9 (0.03)	1.1924 (0.00)	255.4 (0.17)	1.8514 (0.001)
K	LSU	8.8 (0.01)	1.1464 (0.00)	189.6 (0.60)	1.5586 (0.01)
5	LSC	8.7 (0.03)	1.1456 (0.00)	188.5 (0.30)	1.5323 (0.01)
W					
E	HSU	8.6 (0.16)	1.1916 (0.00)	254.2 (0.85)	1.8516 (0.03)
E	HSC	8.8 (0.05)	1.1991 (0.00)	253.4 (2.30)	1.8006 (0.00)
K	LSU	8.7 (0.02)	1.1456 (0.00)	188.5 (0.88)	1.5686 (0.00)
6	LSC	8.4 (0.06)	1.1459 (0.00)	188.8 (0.30)	1.5277 (0.01)

Each value is the mean of three samples measurement (±SD).

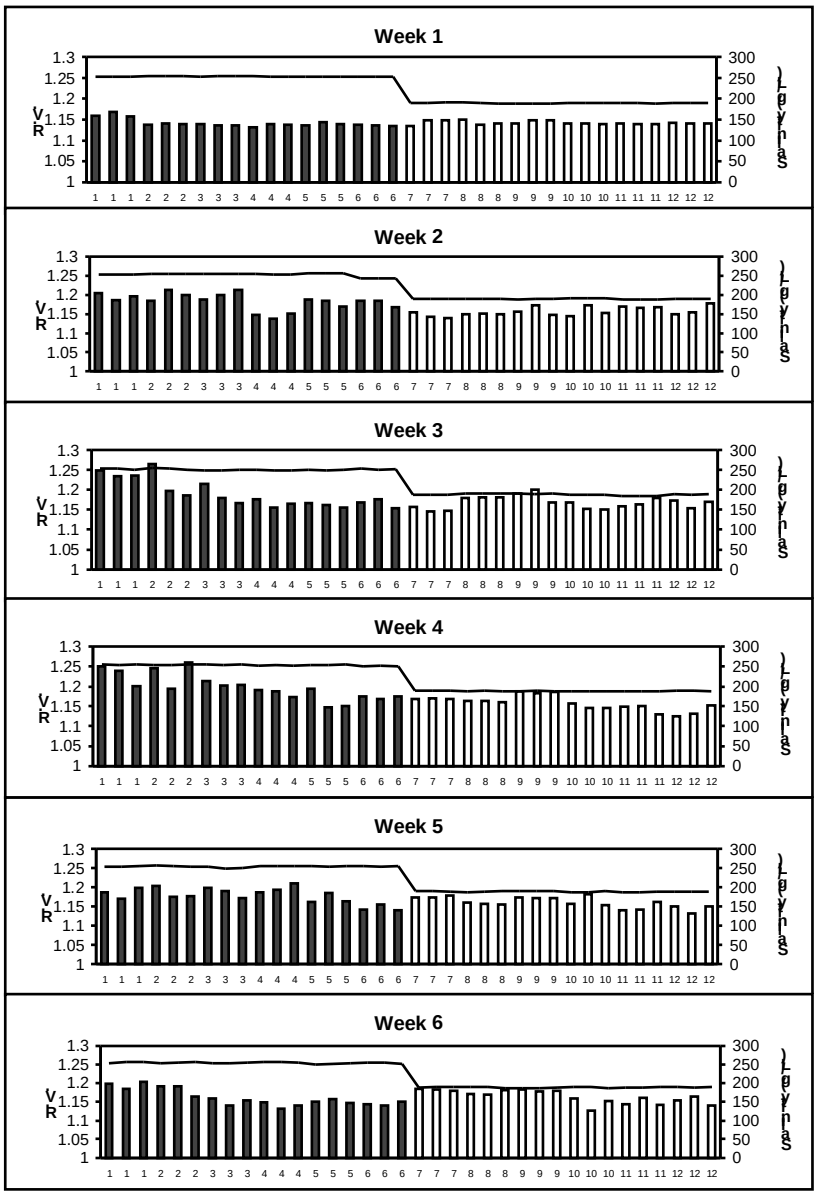


Figure 1 - Salinity (g/L, solid line) and relative viscosity (RV, bars) of brine during the experimental period. 1-3, high salinity-uncovered; 4-6, high salinity-covered; 7-10, low salinity-uncovered; 10-12, low salinity-covered.Triplicate samples from each aquarium.

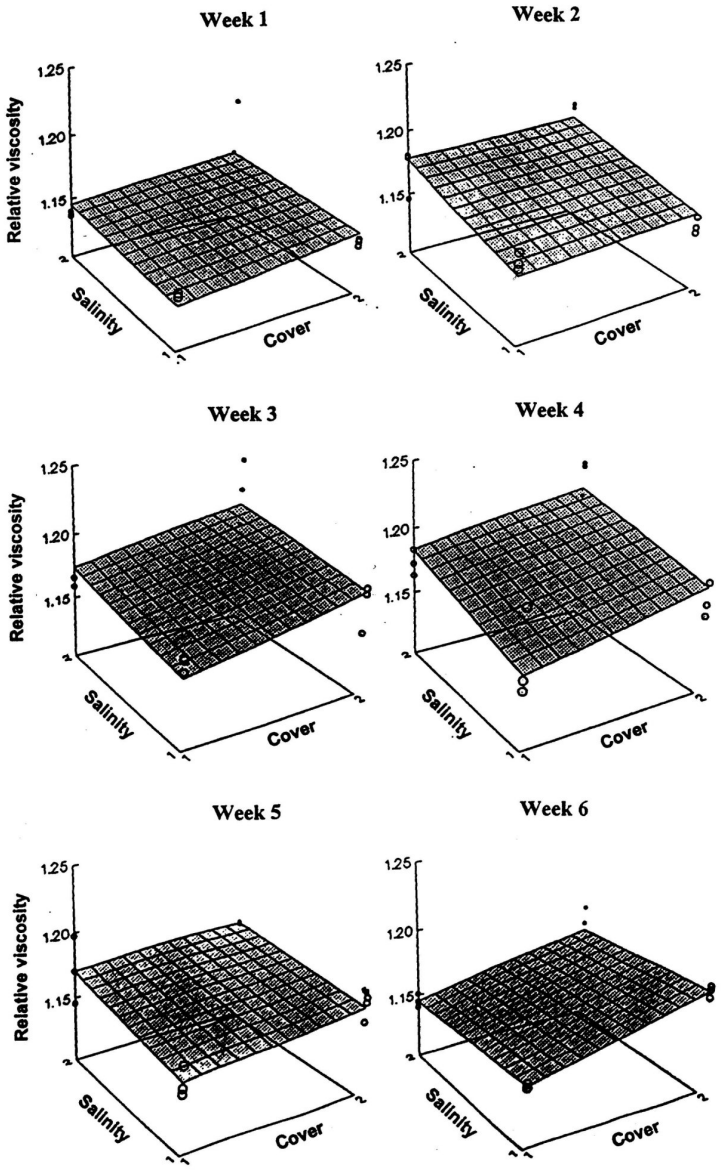


Figure 2 - Diagrammatic representation of the relationship between salinity and cover condition. Each point is average of three measurements for viscosity from one treatment. Numbers 1 and 2 in X

and Z axes are low and high salinity and covered and uncovered status respectively.

Table 4 - ANOVA Multivariate Repeated Measures Analysis on relative viscosity under salinity (high and low salinity) and cover and uncovered treatments over 6 weeks period.

Source	SS	DF	MS	F	P
Salt	0.0056	1	0.0056	14.7853	0.0049
Cover	0.0085	1	0.0085	22.3396	0.0015
Salt*cover	0.0028	1	0.0028	7.4115	0.0262
Error	0.0030	8	0.0004		

Microscopic observations

In all samples, microscopic examination revealed the presence of a unicellular green-colored Cyanobacterium, 1-10 (length) and 1-2 μm (width) respectively. Microscopic observations indicated morphological differences between organisms in uncovered and covered aquaria, but both were referable to the genus *Synechococcus*.

Microscopic observations provided estimates of the number of cells per 1 ml of *Synechococcus* standing crop in the sample. The densities of *Synechococcus* in LSU and LSC samples from weeks 1, 2, 3, 4, 5, and 6 were approximately $1.2x10^3$, $1.5x10^3$, $1.8x10^3$, $1.4x10^3$, $2.1x10^3$, and $2.4x10^3$ individuals per ml, respectively. For HSC and HSU aquaria, estimation of densities was difficult due to the high amount of ECP present and the high viscosity of the medium.

Microscopic examination of the samples indicated the presence of diatoms and *Dunaliella viridis* and *D. salina* as well as *Synechococcus* cells**.** Microscopic observation showed the high density of cocci and rods shaped bacteria in HSU aquaria after week 4. These cocci and rods were probably colonies of Halobacteriaceae and imparted a red color to the water. This was accompanied by increased in the viscosity of the HSU treatment after week 4. *Dunaliella salina* and *Stephanoptera* sp. were abundant in high salinity aquaria in week 4, 5 and 6.

Microscopic observations also revealed that unicellular Cyanobacteria were more abundant in high salinity than low salinity aquaria up to week 4. Filamentous and unicellular Cyanobacteria and diatoms were present in low salinity aquaria, and that diatoms were more abundant in those aquaria.

Synechococcus cells in LSC aquaria aggregated in short cylinders of large diameter (5-7 x 7-9 µm), while those dominant in HSU aquaria were narrow cylinders of varying length (2-4 x 3-8 µm). Approximately 10 or 25% (in high and low salinity, respectively) of the *Synechococcus* comprised paired cells, showing cell division. In undisturbed samples collected from HSU, some cells were in the form of chains and embedded in the ECP produced by the cells.

Laboratory examinations

Chlorophyll *a* mean ranged from 0.15mg/100g to 0.36mg/100g of wet sample in HSU aquaria and dry weight mean ranged from 24.06 % to 36.13 % of wet sample in HSC (Table 5). Chlorophyll a mean ranged from 0.16 to 0.33 mg/100g of wet sample in LSU aquaria and dry weight mean ranged from 23.4 % to 29.86 % of wet sample in HSC. There was no significant difference between the percentages of water in the samples; it ranged from only 70.13% to 76.11% in HSU and LSU Percentages of organic material were higher in samples from high salinity than low salinity aquaria at the end of week one. Organic matter was the lowest in samples from HSU in the last week of the experiment

Table 5 - Means of chlorophyll *a*, percentages of dry weight, water and organic material from standing crop of *Synechococcus* (AFDW) at week 1 and week 6 of the experiment.

Date	Aquarium	Chl *a* mg/100g wet weight	Dry weight %.	H_2O %	AFDW %
WEEK 1	HSU	0.36 (0.05)	24.06 (0.30)	75.62 (0.43)	28.50 (2.40)
	HSC	0.25 (0.01)	24.30 (0.01)	75.53 (0.63)	24.16 (1.09)
	LSU	0.32 (0.10)	23.40 (0.34)	76.11 (0.50)	20.30 (1.68)
	LSC	0.26 (0.01)	24.10 (0.53)	75.53 (0.30)	20.90 (0.98)

W					
E	HSU	0.15 (0.02)	36.13 (5.07)	73.84 (1.10)	18.53 (5.35)
E	HSC	0.21 (0.03)	35.83 (4.21)	74.13 (1.20)	22.74 (5.10)
K	LSU	0.16 (0.01)	24.96 (1.37)	75.00 (1.33)	25.18 (8.24)
6	LSC	0.33 (0.15)	29.86 (3.51)	70.13 (3.50)	21.11 (0.18)

The comparison between chlorophyll *a* concentration and the percentages of organic material from *Synechococcus* standing crop samples collected directly from field, and in the samples from the first and the last samplings are presented in Figures 3 and 4. Data for the first and the last samplings in these figures are the mean of triplicate treatments and three readings for each sample. The amount of chlorophyll *a* in samples at the end of the experiment was higher in covered than uncovered aquaria, especially in LSC. Water in aquaria under low light intensity (HSC, LSC) developed a green color, whereas aquaria under high light intensity (HSU, LSU) became yellowish or red.

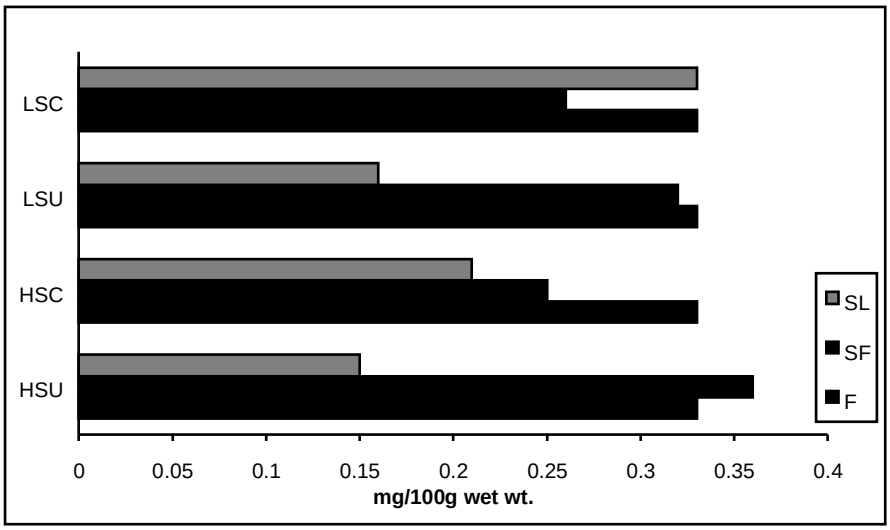


Figure 3 - Comparison between the amount of chlorophyll *a* from *Synechococcus* standing crop: samples collected directly from field (F), the first (SF), and the last (SL) samplings from each aquaria. (Values are the mean of three replicates).

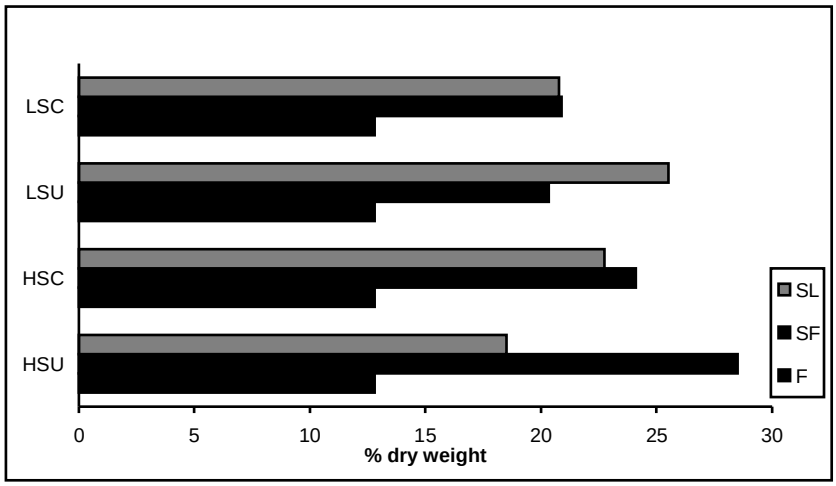


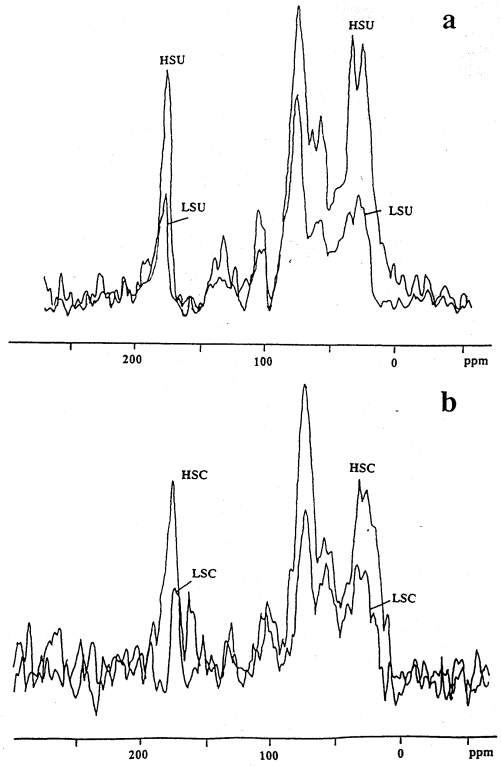
Figure 4 - Comparison between the percentages of organic material from *Synechococcus* standing crop: samples collected directly from field (F), the first (SF), and the last (SL) samplings from each aquaria. (Values are the mean of three triplicates).

Identification of the chemical structure of ECP by ^{13}C NMR spectroscopy

Figure 5 shows natural-abundance ^{13}C NMR spectra of ECP in *Synechococcus* from the four experimental treatments. The spectra were run under conditions which ensured that the peak heights accurately reflected the concentrations of various solutes in the extracts and peaks in these spectra show higher concentrations in high salinity than low salinity. The relative heights (or intensities) of their resonance reflect their relative concentrations in the samples. Therefore each sample displayed two or more well-resolved ^{13}C resonances which provided a useful check on relative comparisons between two samples and also provided a simple identification of the major organic composition of ECP.

All samples showed major signals at 175 ppm (carboxyl/ carboxyl), 129 ppm (aromatic protein residues/ lipid unsaturation), 103 ppm (anomeric carbon of sugars), 73 ppm (CHOH of carbohydrates other than anomeric), 65 ppm (carbohydrate CH_2OH), 56 ppm (protein alpha carbon), 31 ppm (lipid polymethylene), and at 24 ppm (protein methy groups). ^{13}C NMR studies indicated the presence of glycoprotein and glycolipids in the samples. Higher amounts of organic matter were found in samples from high salinity with covered and uncovered (HSC and HSU) than in low salinity covered and uncovered (LSC and LSU)

Figure 5



treatments (Figure 5). Some differences between uncovered and covered treatments, with peaks for HSU slightly greater than in HSC and for LSU slightly greater than LSC, can be seen by comparing Fig 5a and b.

Figure 5 - ^{13}C NMR spectra of relative amounts of ECP composition produced by Synechococcus standing crop. (a) samples from HSU (high salinity and uncovered) and LSU (low salinity and uncovered) aquaria; (b) samples from HSC (high salinity and covered) and LSC (low salinity and covered) aquaria.

Discussion

Most studies on coccoid Cyanobacteria inhabiting hypersaline waters are based on the examination of field material (e.g. studies reviewed by Bauld, 1981) or on laboratory studies of cultures in artificial media (e.g. Yopp *et al.*, 1978a and b). The present study was an attempt to simulate a natural field situation in easily controlled outdoor microcosms. Brine from ponds 1 and 2 supplied the low and high salinity water for experimental aquaria, respectively. The results for salinity show that this was almost constant for aquaria filled with low and those filled with high salinity waters. Thus, there were no physiological shocks resulting from rapid salinity fluctuation in any aquarium. Also, pH was always > 7, which is important in the survival of *Synechococcus* (Madigan, 1988).

Synechococcus responded to the environmental stress of high salinity, light and temperature by the production of ECP. Increased production of ECP under stress is also reported by other studies on this taxon and other Cyanobacteria under laboratories conditions not in natural environment as stated before (Painter, 1983; Panoff *et al.*, 1988; Philips *et al.*, 1989; Vincenzini *et al.*, 1990a and b; De Philippis *et al.*, 1991 and 1993, Witton *et al*. 2000). *Synechococcus* is a nitrogen-fixing bacterium, with photosynthesis occurring in the day when sunlight is available, and nitrogen-fixation occurring at night when oxygen levels within cells are lower (Stal, 1991). Thus, *Synechococcus* has advantages over other Cyanobacteria and algae in highly saline water with low oxygen concentration and low nitrate concentration (Mague *et al.*, 1980; Mitsui *et al.*, 1986; Grobbelaar *et al.*, 1986; Arad, *et. al*., 1988; Schneegurt *et al.*, 1994; Liu *et al.*, 1996; Michard *et al*., 1996; Ghassemzadeh, 1997; Seckbach, 1999).

The production of ECP is an osmotic response of *Synechococcus* in highly saline water; the ECP protect the cells from the stressful environment (Painter, 1983; Philips *et al.*, 1989; Vincenzini *et al.*,

1990a and b; De Philippis *et al.*, 1993). These molecules would have moderated the adjustment of the unicellular cyanobacterial cell to the hypersaline water: *Synechococcus* surrounds itself with this material that can act as a barrier and may be important in (1) limiting desiccation of the cells under high salinity, (2) balancing ions, and (3) aiding the uptake of nutrients in highly saline water. The results from ^{13}C NMR spectroscopy indicate that the extracellular product was organic material, probably, glycoprotein and glycolipid. Some of these ECP were liberated to the medium. The results demonstrate increasing relative viscosity due to the production of ECP by *Synechococcus* in hypersaline water as well as high light intensity. Economically, increasing brine viscosity is important in determining salt quality and quantity in solar saltfields (Ghassemzadeh et al., 1996a and b). They accord with published results for *Synechococcus* that show ECP increases at higher temperature, and in turn increases turbidity and viscosity (Yopp *et al.*, 1978a and b; Dor and Hornoff, 1985).

Conclusion

The results from ^{13}C NMR spectroscopy showed that the composition of the ECP produced by S*ynechococcus* under experimental conditions probably comprised glycolipid and glycoprotein. The amount of ECP produced by S*ynechococcus* affected brine viscosity which is important in the quality and quantity of the salt in solar saltfields. Relative viscosity and the amount of organic materials was higher in conditions of high salinity and high light intensity, because under these conditions S*ynechococcus* produced more ECP.

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