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The Effect of Organic Material and Inorganic Ions on the Photosynthetic Rate of the Red Alga *Bostrychia binderi* from a Florida Estuary

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Abstract

Various constituents of spring water (calcium, bicarbonate, nitrate, phosphate, total organic material) influence the response of photosynthetic rate of *Bostrychia binderi* Harvey to changes in salinity. The rate of photosynthesis increased with a decrease in salinity. The rate of photosynthesis in low salinities was greater in seawater diluted with spring water than in seawater diluted with distilled water. Elevation of photosynthetic rates in the lower salinities (0 and 5 ppt) was partially due to increased levels of bicarbonate and various nutrients present in natural spring water. The higher calcium levels in spring water resulted in higher photosynthetic rates in plants held for 3 to 7 d in the lower salinities (0 to 5 ppt). Increased levels of calcium in salinities of 5 ppt or higher increased the photosynthetic rate only during the first 7 d of exposure, since acclimation occurred equally in individuals held for 2 to 8 wk in seawater diluted with distilled or spring water. This study suggests that the diverse algal floras, characteristic of estuaries on the west coast of Florida are in part the result of natural spring water mixing with seawater, sustaining the algae over short periods of low salinities.

Introduction

The algal flora of estuaries is usually lower in number of species than adjacent marine and freshwater environments (den Hartog, 1967), yet in some estuaries found on the west coast of Florida the algal flora is diverse and abundant (Dawes, 1974). Spring fed rivers may play an important role in the development and support of the algal flora (Phillips, 1960, Dawes *et al*., 1978, Hoffman and Dawes, 1980), particularly with regard to the higher levels of calcium (Yarish *et al*., 1980). A number of marine algae, such as the red alga *Bostrychia binderi* Harvey occupy both open coast, high salinity areas as well salt marshes and mangrove swamps on spring fed rivers. The present study examined the effects of various ions contributed by freshwater springs on the photosynthetic rate of *B. binderi*, which can be a dominant species of the intertidal zone in Floridian estuaries. This species is tolerant to a wide range of salinities (Almodovar and Biebl, 1962; Dawes *et al*., 1978). In this study, comparisons of photosynthetic rates were made on plants held in seawater diluted to various salinities with natural spring water, artificial spring water and distilled water. The purpose was to determine which components of spring water may be particularly important to the alga's survival in estuarine waters.

Materials and Methods

The Weeki Wachee Estuary is near Bayport, Florida (Long. 82°39′W, Lat. 28°35′N). The Weeki Wachee River has an average discharge of 102 MGD (million gallons per day) (Ferguson *et al*., 1947). A salinity range of 5–16 ppt was encountered at the site of plant collections. Three types of dilutents (distilled water, natural spring water and artificial spring water) were used in the preparation of designated salinities (0, 5, 10, 20, 30 ppt) from synthetic seawater (Instant Ocean) of 35 ppt. All salinity measurements were made with a refractometer that had been calibrated using Knudsen titration (Strickland and Parsons, 1968). Standard analyses were carried out to determine the concentration of HCO_3^- and CA^{++} (Carpenter *et al*., 1975) in seawater diluted with natural spring water and distilled water.

Artificial spring water was prepared by mixing $CaCl_2$ (calcium ion), $NaHCO_3$ (bicarbonate ion), $NaNO_3$ (nitrate ion), and Na_3PO_4 (phosphate ion) with distilled water in concentrations equal to those found in natural water. One or more ions were deleted in the artificial spring water in order to ascertain the effect of a particular ion on the photosynthetic rate of *Bostrychia binderi* (Table 1). The pH of all media was adjusted to

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Table 1. Ionic composition (mg l^{-1}) of artificial spring water

Ion	A	B	C	D
Bicarbonate	170.0	170.0	–	170.0
Calcium	50.0	–	50.0	50.0
Nitrate	0.4	0.4	0.4	–
Phosphate	0.1	0.1	0.1	–

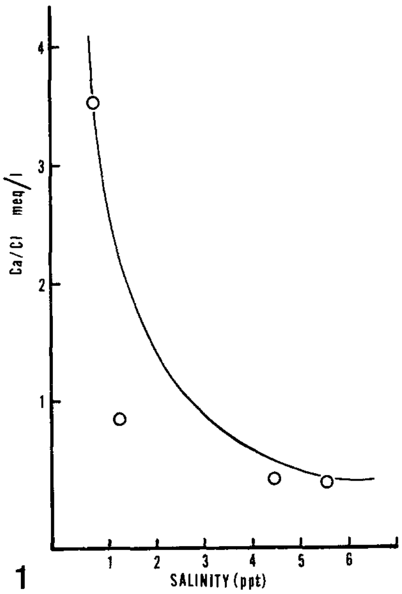

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Fig. 1. The Ca^{++}/Cl^{-} ratio in seawater diluted with spring water in the laboratory (solid line); and in water samples from the Weeki Wachee river estuary (single points)

8.0 by titration with HCl or NaOH on a daily basis. All salinities were maintained at the same osmolarity by the addition of NaCl using a Precision Osmette Osmometer. The ionic composition of each salinity was determined by an equation modified from Carpenter *et al.*, (1975). Organic compounds were removed from natural spring water by exposure to 1% activated charcoal for 2 d (Guillard and Ryther, 1962).

Bostrychia binderi in a cooler was transported directly from the field to the laboratory, where samples were cleaned of epiphytes, washed in filtered seawater, and placed in Erlenmeyer flasks containing 100 ml of the treatment media. The media was changed every second day. After a specified period (1 to 56 d) at the standard temperatures (20° or 28°C) and light (500 ft-c, Gro-Lux lamps), photosynthetic rates were monitored on a Gilson Respirometer (Model GRP-20, Dawes *et al.*, 1978) and are expressed as $\mu l\ O_2$ g dry $wt^{-1}\ h^{-1}$. Light intensity was maintained at 1000 ft-c (8710 $\mu W\ cm^{-2}$ 360-1100 nm^{-1}; 340 $\mu E\ m^{-2}\ s^{-1}$). A minimum of 5-10 replicates were used in every experiment.

Means between treatments of similar salinities were compared using Student's *t*-tests, whereas means of differing salinities within the same treatment were compared using Tukey's multiple range test. Analysis of variances (ANOVA) were carried out using a SASS computer program.

Results

The CA^{++}/Cl^{-} ratio water samples from Weeki Wachee estuary agree with the predicted ratio calculated from the simple mixture formula (Fig. 1) of Carpenter *et al.*, (1975). The ionic composition of water from the major spring on the Weeki Wachee river has apparently remained constant over the past 40 yr and is similar to other spring fed rivers of Florida (Table 2). The Weeki Wachee river water has three times the amount of calcium and bicarbonate as the average for rivers of the world (Table 2; Livingston, 1963). Potassium was much lower than the world average for rivers or when compared to other Florida springs, and 100 times lower than reported for the Mullieu River estuary (Yarish *et al.*, 1980). Bicarbonate and calcium levels resulting from Weeki Wachee spring water dilution of seawater differ more at the lower salinities when compared to seawater diluted with distilled water (Table 3).

Table 2. Levels of inorganic ion (mg l^{-1}) of various springs

Ion	Weeki Wachee springs			Other Florida springs		Average for rivers of world[2]
	1933[1]	1946[1]	1976	Silver[1] springs	Homosassa[1] springs	
Calcium	49.0	48.0	48.0	68.0	50.0	15.0
Magnesium	7.8	5.8	6.1	9.6	45.0	4.1
Sodium	3.7	4.0	4.1	4.0	308.0	6.3
Potassium		0.7	0.7	1.1	9.6	2.3
Bicarbonate	178.0	168.0	172.0	201.0	136.0	58.0
Sulfate	7.5	6.4	6.5	34.0	87.0	11.2
Chloride	4.7	4.8	5.0	7.8	5.7	7.8
Nitrate			0.4			
Phosphate			0.1			

[1] Ferguson *et al.* (1947)
[2] Livingston (1963)

Table 3. Concentration of Ca^{++} and HCO_3^- in seawater diluted with distilled or Weeki Wachee spring water

Salinity (ppt)	Distilled	Spring	% Difference
HCO_3^- (mg l^{-1})			
30	85	111	23
20	57	131	56
10	28	151	81
5	14	162	91
0	0	172	100
Ca^{++} (mg l^{-1})			
30	340	347	2
20	228	249	8
10	112	147	23
5	56	97	42
0	0	48	100

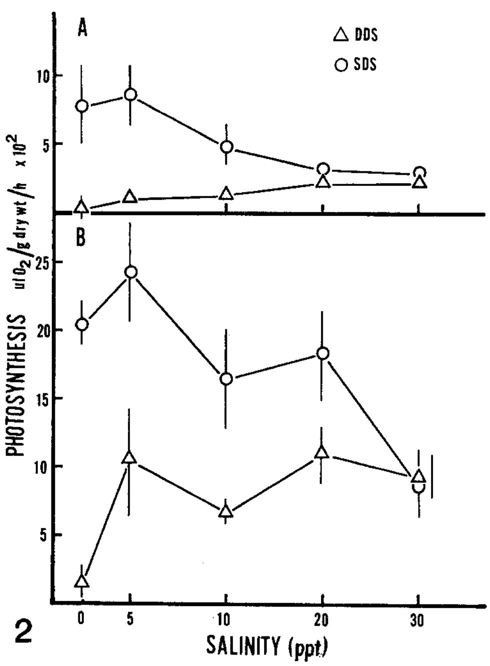

Fig. 2. *Bostrychia binderi.* Photosynthetic rates of *B. binderi* held for 3 d in seawater diluted with distilled water (DDS) and seawater diluted with spring water (SDS) measured at 20 °C (2A) and 28 °C (2B). The bars represent one standard deviation

The photosynthetic rate of *Bostrychia binderi* held for 3 d at low salinities (0, 5, 10 ppt) in spring diluted seawater (SDS) was significantly higher ($P < 0.05$; by two to three times) when compared to individuals held at 20°C (Fig. 2A) in distilled diluted seawater (DDS). The difference in the rate between SDS and DDS was even more pronounced at 28 °C (Fig. 2B). Individuals

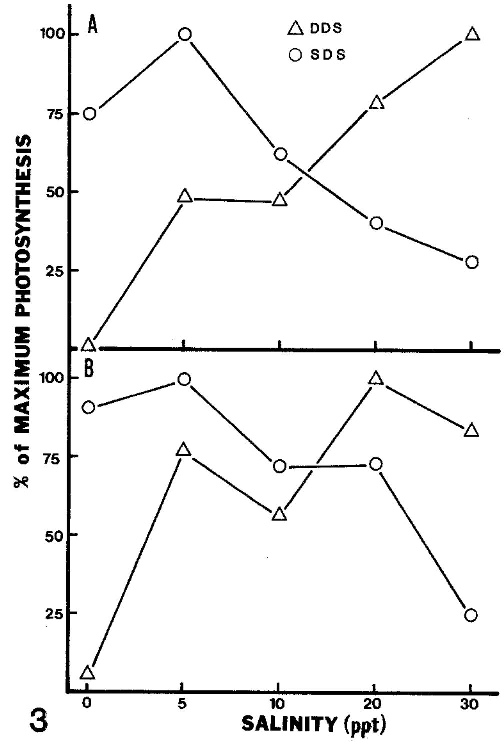

Fig. 3. *Bostrychia binderi.* The percent of the maximum photosynthesis for *B. binderi* held for 3 d in spring or distilled diluted seawater; (A) 20 °C (B) 28 °C

held in high salinities (20, 30 ppt) did not have significantly different photosynthetic rates. The ionic content was at a minimum in the higher salinities (Fig. 2A, B; percent difference, Table 3). Individuals maintained photosynthesis even after 3 d in 0 ppt in spring water but not in seawater diluted with distilled water (Fig. 2A, B). Using Tukey's multiple range test, the difference in photosynthetic rate was significant ($P < 0.05$) at both 20° and 28°C. Specimens held in seawater diluted with spring or distilled water had different photosynthetic maxima (Fig. 3A, B). The higher photosynthetic rates (expressed as percent of the maximum rate occurred at 5 ppt for individuals held in seawater diluted with spring water and either 20 or 30 ppt for individuals held in seawater diluted with distilled water. Individuals held at 20°C (Fig. 3A) had a response similar to those held at 28°C (Fig. 3B).

The addition of bicarbonate to seawater diluted with spring water resulted in an elevation of photosynthetic responses, especially at the lower salinities (Fig. 4). There was no significant difference ($P < 0.05$) in the photosynthetic response at 0 and 5 ppt within each treatments (with/without HCO_3^-, Fig. 4). There is a correlation between the increase in bicarbonate at the lower salinites and the increase in photosynthetic rates when seawater diluted with spring water is used (r = 0.99 at 20°C; r = 0.95 at 28°C).

After 1 wk in artificial spring water containing calcium, *Bostrychia binderi* maintained a photosynthetic

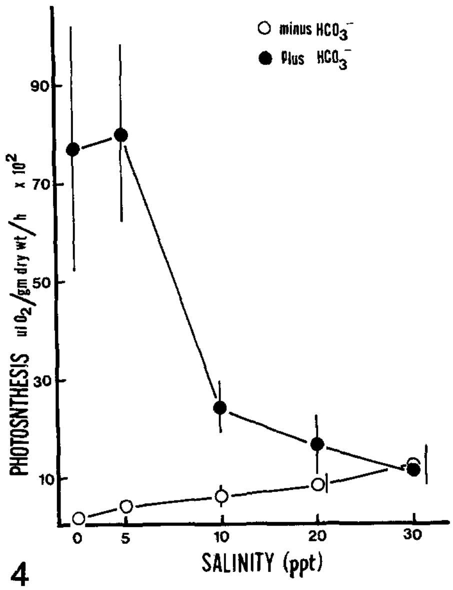

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Fig. 4. *Bostrychia binderi.* The effect of bicarbonate on the photosynthetic rate of *B. binderi* held for 3 d in different salinities at 20 °C. Artificial spring water with and without bicarbonate was used. The bars represent one standard deviation

Table 4. Effect of calcium in artificial spring water on the photosynthetic rate (mg 0_2 g dry wt^{-1} h^{-1}) of *Bostrychia binderi*

Duration	Artificial spring water	
	$-Ca^{++}$	$+Ca^{++}$
One day		
0 ppt	953 +/– 362	885 +/– 311
5 ppt	1108 +/– 353	1102 +/– 150
10 ppt	1184 +/– 95	805 +/– 180
Seven days		
0 ppt	897 +/– 306	1641 +/– 300
5 ppt	1238 +/– 172	1356 +/– 300
10 ppt	1240 +/– 38	1250 +/– 51

rate 32% higher than that of individuals held in artificial spring water without calcium (Table 4). The addition of calcium did not appear essential if *B. binderi* was exposed for a day or less to the lower salinities (Table 4). At higher salinities (5, 10 ppt; Table 4) the effect of calcium was not evident. The addition of natural spring water, which had a high level of calcium but a low level of potassium (Table 2, 3), also enabled *B. binderi* to maintain a significantly higher photosynthetic rate ($P < 0.05$) at the lower salinities even after 7 d. When calcium was deleted from the artificial spring water, the photosynthetic rate of plants held at 0 ppt was significantly lower ($P < 0.05$) than the rate at other salinities. The combination of bicarbonate plus calcium used as an artificial spring water never produced the same response as did natural spring water.

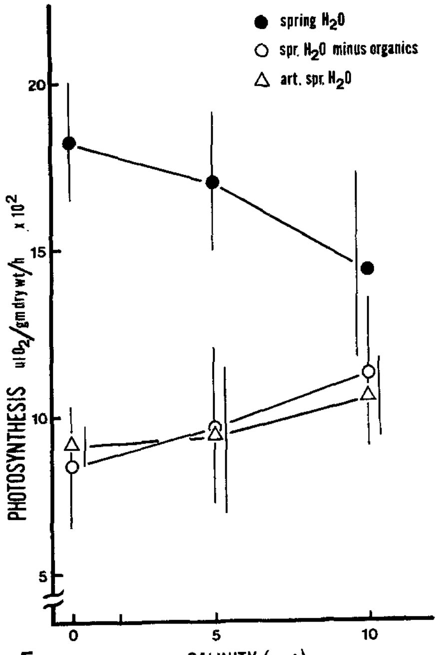

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Fig. 5. *Bostrychia binderi.* The photosynthetic rate of *B. binderi* collected in the summer and held for 3 d at 28 °C in seawater diluted with spring water with dissolved organic material removed, and diluted with artificial spring water that contained NO_3^- adn $PO_4^=$. The bars represent one standard deviation

An exposure during the summer to artificial spring water containing nitrate and phosphate ions did not result in a significant increase in photosynthetic rates similar to responses found in natural spring water (Fig. 5). At both 0 and 5 ppt there were significant differences ($P < 0.05$) between individuals held in natural spring water and individuals held in other media (spring water with nutrients removed, artificial spring water with nitrate and phosphate ions, Formula A, Table 1). At 10 ppt only a slight difference was noted (Fig. 6).

When photosynthetic rates of individuals collected directly from the field were monitored in filtered, natural seawater, the rate was approximately 20 μl 0_2 g dry wt^{-1} h^{-1}. *Bostrychia binderi* held for long periods (1, 2, 8 wk) showed marked differences in responses at the varied salinities (Fig. 6 A, B); lower rates were typical in the beginning (Days 1, 3) except for individuals held in salinities similar to the natural levels (*ca* 10 ppt). After 2 wk in 0 ppt individuals showed a decrease in photosynthetic rates whether cultured in pure spring or distilled water. At higher salinities a general increase in photosynthetic rate occurred over time, and the photosynthetic rate was significantly higher ($P < 0.05$) after 8 wk than that reported after one day. Photosynthetic rates for both sea water diluted with distilled water (Fig. 6A) and the sea water diluted with spring water (Fig. 6B) treatments showed no significant differences ($P < 0.05$) from 5 to 30 ppt after 8 wk.

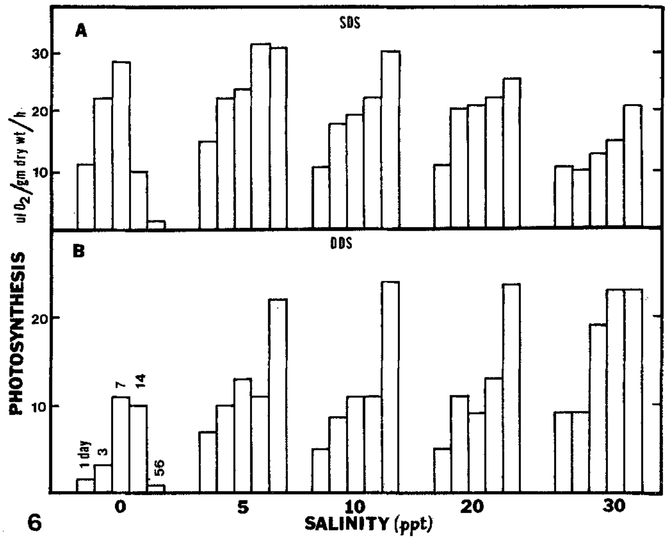

Fig. 6. *Bostrychia binderi.* The photosynthetic rate of *B. binderi* held at 28°C and different salinities of spring-diluted seawater (SDS), distilled water diluted seawater (DDS)

Discussion

Salinity is a major environmental variable limiting the distribution of marine algae in an estuary. In turn, many factors can modify the effects of salinity. Norton and South (1969) and Nygren (1975) pointed out that brackish water and seawater diluted with distilled water differ in composition and this can result in misinterpretations of salinity tolerances. Yarish *et al.*, (1980) found that both calcium and potassium play an important role in the adjustment for *Bostrychia radicans* and *Caloglossa leprieurii* to lower salinities in esturaries. Results from the present study substantiate these previous studies.

The photosynthetic rate of *Bostrychia binderi* dropped 70 to 100% but only 12 to 24% in spring water. Although *B. binderi* will die eventually if held either in distilled or spring water, the addition of spring water increased the length of time it tolerates the near freshwater conditions which commonly occur in Florida estuaries due to sudden rains or increased river flow. *B. binderi* does not show a reduction in tolerance to lower salinities at the higher temperature of 28°C, in contrast to reports of previous studies (Kjeldson and Phinney, 1972; Mathieson and Dawes, 1974).

Significant differences were noted between the photosynthetic rates of *Bostrychia binderi* held in seawater diluted with spring water and seawater diluted with distilled water at salinities below 10 ppt. Zavodnik (1975) also reported enhanced photosynthetic responses for algae exposed to seawater diluted with spring water. Our data support the importance of spring water dilution in increasing the primary productivity of low salinity estuarine areas during short periods of time (e.g. tidal changes, heavy rains). In part, this can be explained by the high concentration of calcium in estuary water diluted with spring water. The Ca^{++}/Cl^{-} curve above 6 ppt shows the decreasing influence of spring water on the ionic composition of the seawater at the higher salinities, and we suggest the effect of calcium is only important in the lower salinities. Long term effects of increased levels of calcium do not seem important as *B. binderi* held at various salinities had similar photosynthetic rates after 8 wk. These findings suggest that acclimation occurs after about 2 wk. Our longterm study (8 wk) differs from the short term study (4 d) of Yarish *et al.* (1980) of tetraspore germination.

Supplemental calcium maintained the photosynthetic rate at 0 and 5 ppt for periods longer than one day. Yarish (1976) and Yarish *et al.* (1980) found that at least 100 mg l^{-1} of calcium was required for *Bostrychia radicans* to reach its maximum growth rate. Dilution with spring water maintained calcium at 100 mg l^{-1} even in a salinity of 5 ppt. This level of calcium is maintained only down to a salinity of 10 ppt if distilled water is used. Significant differences in photosynthetic rates appeared between the dilutents used below 10 ppt; they were not significantly different in salinities of 10 ppt regardless of the dilutent photosynthetic rates.

There appears to be no direct evidence for the role of calcium in maintaining the photosynthetic rate. It is probable that increased calcium concentrations at the low salinities exert some effect both on maintaining the membrane integrity and cell wall polysaccharides. Membrane maintenance would be important for survival at low salinities over a long period.

Potassium modified the effects of lower salinities on algal growth (Yarish *et al.*, 1980). In the present study, the potassium content was low in the natural spring water and its effect was not tested.

Enhanced photosynthetic activity may be attributed to three other constituents found in high concentrations in the Weeki Wachee spring water: bicarbonate, nutrients (NO_3^-, $PO_4^=$) and dissolved organic material. Ogata and Matsui (1968) and Hammer (1968) indicated the importance of a carbon supply in supporting a sustained photosynthetic rate in marine algae. The higher levels of bicarbonate in natural spring water increased the photosynthetic efficiency of *Bostrychia binderi*. However, the higher bicarbonate levels did not increase tolerances to lower salinities when photosynthetic rates are compared within a salinity series (Fig. 4). This is in contrast to that reported by Ohno (1976) for *Fucus* sp. and *Enteromorpha* sp.

Dissolved organic material, nutrients and trace metals contributed by natural spring water elevate the rate of photosynthesis; nutrients had the greatest effect during the winter, while the effect of organic material was only noted during the summer months. The higher rates obtained during the winter through the addition of nutrients can probably be attributed to nutrient starvation. *Bostrychia binderi* had long periods of desiccation during January, probably resulting in diminished nutrient pools within the thallus and causing subsequently lower photosynthetic rates. Higher nutrient levels did not increase the tolerance of *B. binderi* to low salinities, in contrast to Prange's (1978) studies, or affect the photosynthetic maximum. However, out study did not examine trace metals and only nitrate and phosphate ions were used.

In contrast to the findings of Ogata and Schramm (1971), *Bostrychia binderi* acclimated to salinity. After 2 to 8 wk, the photosynthetic rate of *B. binderi* held in seawater diluted with distilled water increased and almost equalled the rate of individuals held in seawater diluted with spring water. The mechanism for this acclimation may involve two features. (1) The creation of a more efficient bicarbonate uptake. After 8 wk there was little difference in photosynthetic rates between plants held in carbon-rich SDS and carbon-poor DDS. (2) The creation of more favorable internal osmotic condition for photosynthesis. This is suggested in the similarity of photosynthetic rates of both DDS and SDS plants over a wide salinity range (5-30 ppt). Increased efficiency in bicarbonate uptake could be caused by alterations in carbonic anhydrase levels or the plasmalemma (Reed and Graham, 1977). Internal osmotic conditions could be adjusted by changes in ion, free amino acid or sugar levels.

When individuals were transferred from natural seawater with normal field salinities of 5-16 ppt to similar salinities in seawater diluted with spring or distilled water, a substantial rise in photosynthetic rates occurred after 3 d (except at 10 ppt) and continued through the 8 wk for all but those held at 0 ppt. In part, we believe the increased photosynthetic rates are due to an increase in available inorganic and organic ions, especially in the seawater diluted with spring water. Also the rise in photosynthesis may be caused by the stable, optimum culture conditions, lack of grazing and damage from prolonged desiccation characteristic of field individuals, and the lack of bacteria and sediment material on the individuals in culture. At the termination of the 8 wk experiment the individuals were clean, deeply pigmented and showed growth.

Bostrychia binderi is commonly found in seawater having open ocean salinities as well as in spring-fed low salinity estuaries. A number of estuarine algae can maintain higher photosynthetic rates at lower salinity over short periods of time by the addition of spring water which increases especially available calcium. The addition of bicarbonate, nutrients and organically enriched freshwater is partially responsible for the high productivity of estuarine algae (Dawes *et al.*, 1978; Hoffman and Dawes, 1980). However, the effect of spring water is temporary and only important at the low salinities.

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