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Salinity Effects on Photosynthesis, Carbon Allocation, and Nitrogen Assimilation in the Red Alga, *Gelidium coulteri*[1]

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ABSTRACT

The long-term effects of altered salinities on the physiology of the intertidal red alga *Gelidium coulteri* Harv. were assessed. Plants were transfered from 30 grams per liter salinity to media with salinities from 0 to 50 grams per liter. Growth rate, agar, photosynthesis, respiration, and various metabolites were quantified after 5 days and 5 weeks adaptation. After 5 days, growth rates were lower for plants at all altered salinities. Growth rates recovered from these values with 5 weeks adaptation, except for salinities of 10 grams per liter and below, where tissues bleached and died. Photosynthetic O_2 evolution was lower than control values at both higher and lower salinities after 5 days and did not change over time. Carbon fixation at the altered salinities was unchanged after 5 days, but decreased below 25 grams per liter and above 40 grams per liter after 5 weeks. Respiration increased at lower salinities. Phycobiliprotein and chlorophyll were lower for all altered salinities after 5 days. These decreases continued at lower salinities, then were stable after 5 weeks. Chlorophyll recovered over time at higher salinities. Decreases in protein at lower salinities were quantitatively attributable to phycobiliprotein loss. Total N levels and C:N ratios were nearly constant across all salinities tested. Carbon flow into glutamate and aspartate decreased with both decreasing and increasing salinities. Glycine, serine, and glycolate levels increased with both increasing and decreasing salinity, indicating a stimulation of photorespiration. The cell wall component agar increased with decreasing salinity, although biosynthesis was inhibited at both higher and lower salinities. The storage compound floridoside increased with increasing salinity. The evidence suggests stress responses to altered salinities that directly affected photosynthesis, respiration, and nitrogen assimilation and indirectly affected photosynthate flow. At low salinities, respiration and photorespiration exceeded photosynthesis with lethal results. At higher salinities, although photosynthesis was inhibited, respiration was low and carbon fixation adequate to offset increased photorespiration.

Intertidal seaweeds are subject to salinity stress when exposed at low tide or trapped in tide pools, where fresh water from rain or stream flows may lower salinity, or where evaporation may raise salinity. Also, salinity stress may occur for submerged plants under estuarine conditions, where tidal flows may yield large changes in salinity. The short- and long-term effects of salinity on the physiology of these plants have been examined primarily in terms of their physiological accommodation to this stress (1, 2, 6–9, 15, 17–20). In the red algae, altered salinity yields changes in turgor. In *Porphyra purpurea*, turgor increases with decreasing salinity, while volume decreases as salinity increases in a passively regulated system (18). On the other hand, in *Griffithsia monilis* and other species, turgor is maintained actively via ion pumping and perhaps synthesis of digeneaside (1, 8). However, the role of low molecular weight organic compounds such as digeneaside or floridoside as osmotica in accommodation of salinity stress is generally unclear. Reed *et al.* (19) showed increased floridoside levels with increased salinity and decreased levels with decreased salinity in *P. purpurea* but determined that these levels were insufficient to return cells to their original volume. These changes may require at least several hours at the altered salinity, as Kremer (9) was unable to detect changes in ^{14}C labeling of floridoside within the first hour. Kauss (6) suggested that changes in isofloridoside levels in response to salinity changes in *Ochromonas malhamensis* were a function of both synthesis and degradation of this compound.

Photosynthesis and respiration have also been shown to be affected by salinity changes. Coudret *et al.* (2) demonstrated decreased photosynthesis and metabolism and increased respiration with decreased salinity in *Aglaothamnion chadefaudii*. Yarish *et al.* (20) found decreased photosynthesis with decreased salinity in *Bostrychia radicans* and *Caloglossa leprieurii*, but not in *Polysiphonia subtilissima*. Kirst (7) showed decreased photosynthesis and respiration with increased salinity in *G. monilis*. Kremer (9) found decreased carbon fixation at both lower and higher salinities for six red algae. Reed *et al.* (17) demonstrated decreased photosynthesis at higher and low salinities for *P. purpurea* after both 1 and 7 d, with significant cell death occurring at the lowest salinities.

The body of reported work thus supports salinity effects on the net availability of photosynthate and its movement into specific compounds and suggests that this movement is directed at relieving osmotic stress. The work reported here examined accommodation to salinity changes after days to weeks exposure in the intertidal red alga, *Gelidium coulteri*, with respect to photosynthesis and respiration and the flow of photosynthate into various end products. Since photosynthetic carbon fixation and the partitioning of photosynthate into compounds such as floridoside, agar, amino acids, and protein had been previously shown to be affected by nitrogen levels (12), it was of interest to examine the effects of salinity on nitrogen metabolism. It appeared possible that salinity effects on photosynthate partitioning were indirect and mediated via changes in assimilated nitrogen.

MATERIALS AND METHODS

Plant Material and Culture Conditions. *Gelidium coulteri* Harv. in unialgal culture was graciously provided by John A. West (JAW 2604). This strain originated from northern Baja California. Plants were maintained in filtered, steamed seawater in 16-L carboys under constant aeration as previously described (13). Salinity was 30 g L^{-1} (0.51 M NaCl). Fluorescent light was

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provided at 250 μmol $m^{-2}s^{-1}$ PFD[3] and a 16-h photo period. Culture temperature was 26 ± 1°C. Cultures were replenished weekly with one-eighth strength PES (14), equivalent to 82 μM NO_3^- and 1.5 μM NH_4^+. PES also contained trace metals, vitamins, and glycerophosphate. Under experimental conditions of altered salinities, filtered seawater was diluted with distilled H_2O or mixed with NaCl prior to steam sterilization and the addition of PES.

Growth was measured every 3 to 4 days as changes in fresh weight. Plants were blotted dry and weighed immediately. Growth rate was calculated and reported as exponential growth rate, μ = 100% ln (final weight initial weight^{-1}) days^{-1}.

O_2 Polarimetry. Measurements of O_2 evolution and uptake were made using an O_2 electrode (Rank Brothers). Plants were placed in air-saturated seawater at experimental salinities in a Plexiglas chamber surrounded by a transparent, temperature-controlled flowing water bath at 26°C. Measurements were taken for 30-min periods in the light and the dark after a linear response was established. All readings were corrected for salinity.

[^{14}C]Bicarbonate Labeling. Adult plants were placed in vials containing 20 mL of sterile seawater at experimental salinities. Approximately 150 mg fresh weight of tissue, corresponding to 4 to 10 plant clusters, were used per vial. Cultures were preincubated for 30 min at 300 μmol m^{-2} s^{-1} PFD on a rotary shaker at 100 rpm. Sodium[^{14}C]bicarbonate (New England Nuclear) was added to each culture to an initial specific activity of 1.0 μCi μmol^{-1} of C. Initial bicarbonate concentration in the seawater was 2 mM.

An entire culture served as one sample. After a 2-h incubation period, plants were separated from the medium by vacuum, washed briefly with seawater, then placed in DMSO to kill the plants and extract soluble compounds. Aliquots were acidified and taken to dryness under N_2 gas. Residues were resuspended in 80% methanol, and amino acids were separated from other metabolites by ion exchange chromatography (10). Both fractions were chromatographed on paper in two dimensions as described by Pedersen *et al.* (16). The compounds were located and identified by autoradiography, cochromatography with known compounds, and use of selective stains. Authentic floridoside was graciously provided by James S. Craigie. The radioactive material was eluted from the paper with water and quantified by liquid scintillation.

Agar extraction was by a modification of Craigie and Leigh (3) described previously (13). Protein was measured on dried, ground plants by the method of Lowry *et al.* (11). Chl was quantified from DMSO/methanol extracts of fresh material by the method of Duncan and Harrison (4). Phycobiliprotein isolation and quantification were performed by a modification of the method of Glazer *et al.* (5) described previously (13). Total C and N were quantified using a Perkin Elmer model 240B elemental analyzer.

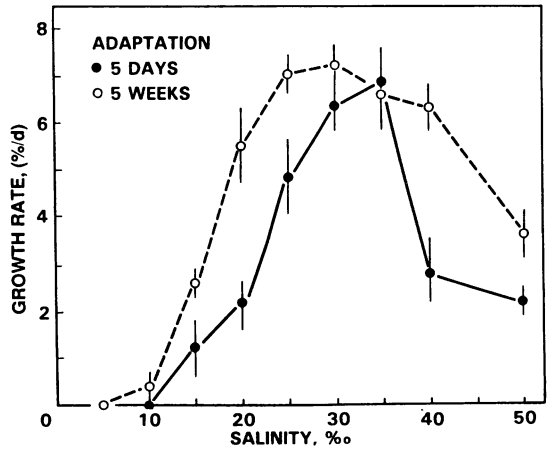

FIG. 1. Growth rate *vs.* salinity. Values are averages of six replicates ± SE.

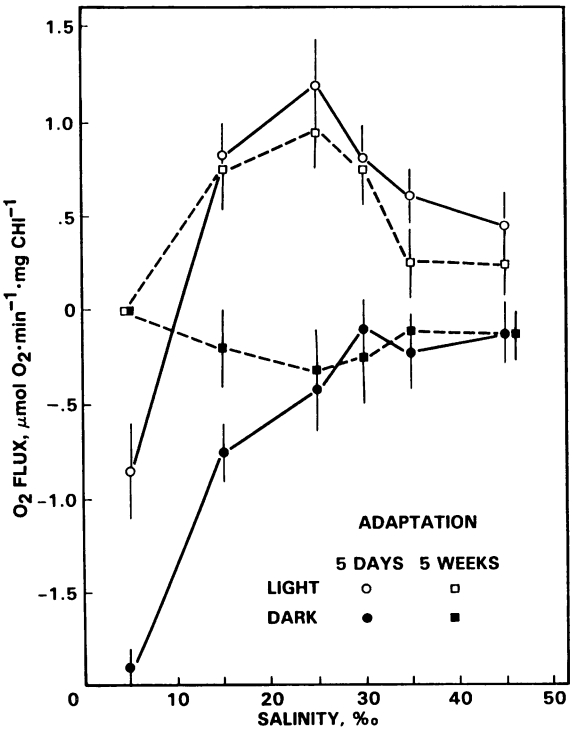

FIG. 2. O_2 uptake and evolution *vs.* salinity. Values are averages of three replicates ± SE.

RESULTS

Effects of Altered Salinities on Growth Rate. When plants initially cultured at 30 g L^{-1} (0.51 M NaCl) salinity were placed in salinities from 0 to 50 g L^{-1}, growth rates were lower at all changed salinities except 35 g L^{-1} (Fig. 1). This inhibition occurred within 3 to 5 d and was greater at more extreme salinity values. For salinities of 15 g L^{-1} and above, growth rates increased gradually over several weeks toward control values. After 5 weeks in culture, growth rates had stabilized for cultures that had not died outright. Salinities of 10 g L^{-1} or below were lethal. Since maximal inhibition was observed at about 5 d and maximal recovery after 5 weeks, subsequent experiments used tissues with these exposures.

Photosynthesis and Respiration. Measurements of O_2 evolution and uptake after 5 d and 5 weeks adaptation showed similar results (Fig. 2). Respiration (O_2 uptake) after 5 d adaptation increased with decreasing salinities below 30 g L^{-1} and was constant for salinities above. After 5 weeks, respiration was essentially unchanged for plants at 25 g L^{-1} or above. At 15 g L^{-1}, plant respiration decreased over time (data not shown) and was 70% lower at 5 weeks than after 5 d. After 5 weeks exposure, plants at 5 g L^{-1} had died. Photosynthesis (O_2 evolution) after 5 d adaptation was maximal at 30 g L^{-1} salinity and decreased with decreasing salinity to negative values at 5 g L^{-1}. Above 30 g L^{-1}, photosynthesis decreased with increasing salinity, but had positive values to 45 g L^{-1}. After 5 weeks, plants at salinities above 30 g L^{-1} showed additional inhibition of photosynthesis.

[3] Abbreviations: PES, Provasoli's enriched seawater; PFD, photon flux density.

Photosynthetic carbon fixation based on acid-stable [^{14}C]bicarbonate labeling was essentially constant on a Chl basis over the entire salinity range tested after 5 d (Fig. 3). Since Chl levels were substantially lower at lower salinities (see below), carbon fixation on a dry weight basis decreased with decreasing salinity. After 5 weeks, fixation rates were markedly lower at both lower and higher salinities. At 10 g L^{-1} and 50 g L^{-1}, fixations rates were, respectively, 15% and 60% of maximal values.

Salinity Effects on Pigment, Protein, and Agar Biosynthesis. Chl and phycobiliprotein levels decreased with decreasing salinity for plants at salinities below 30 g L^{-1}, with values approaching zero at salinities of 10 g L^{-1} and below (Fig. 4). These pigment losses occurred during the initial week in culture, then stabilized over time. Pigment levels decreased initially at higher salinities, then recovered to control values by 5 weeks.

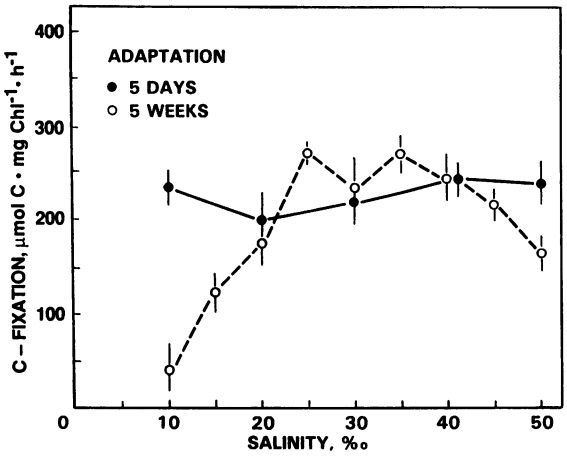

FIG. 3. ^{14}C fixation *vs.* salinity. Plants were labeled for 2 h in 2 mM [^{14}C]bicarbonate at indicated salinities in the light. Values are averages of three replicates ± SE.

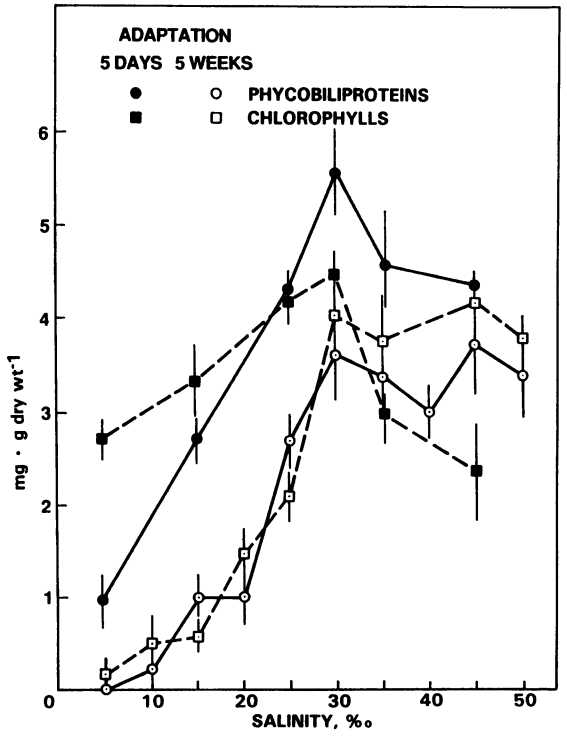

FIG. 4. Phycobiliproteins and Chl *vs.* salinity. Values are averages of three replicates ± SE.

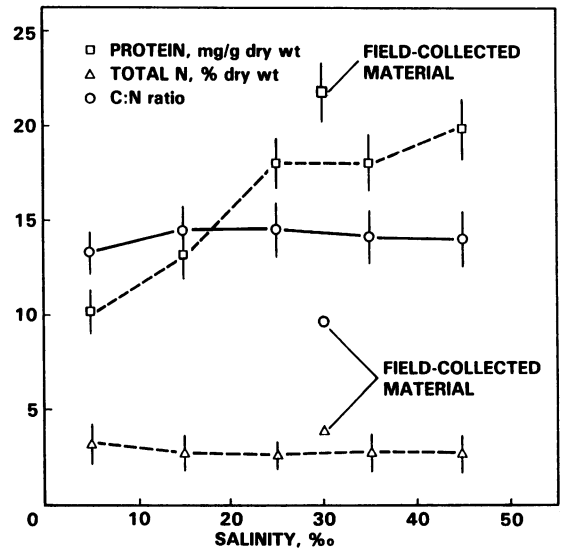

FIG. 5. Effects of salinity on plant N status. Values are averages of three replicates ± SE.

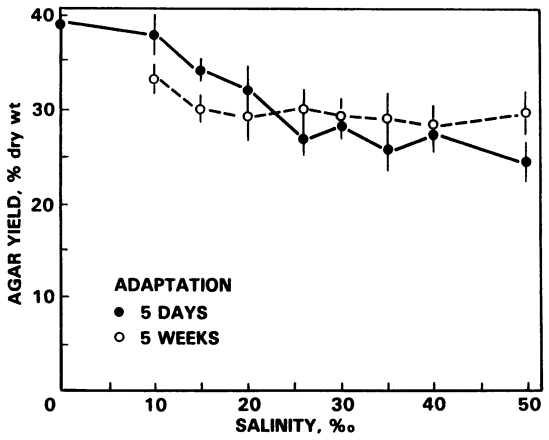

FIG. 6. Agar yield *vs.* salinity. Values are averages of four replicates ± SE.

Protein decreased with decreasing salinity over the range tested after 5 weeks exposure (Fig. 5). Most of this loss could be attributed to decreases in phycobiliprotein and Chl. Total N and the C:N ratio were constant over all salinities.

Agar yields on a percent dry weight basis increased with decreasing salinity below 30 g L^{-1} and remained constant at salinities above after 5 d adaptation (Fig. 6). This trend was dampened out after 5 weeks in culture, with agar values nearly constant from 15 to 50 g L^{-1}.

Partitioning of [^{14}C]Photosynthate. [^{14}C]-Labeled metabolites from 2-h incubations with [^{14}C]bicarbonate were separated and quantified. Label in floridoside (2-*O*-glycerol-α-D-galactopyranose), the primary low molecular weight storage compound, increased with increasing salinity after both 5 d and 5 weeks adaptation (Fig. 7). Labeling of agar decreased with increasing salinity after 5 d (Fig. 8). After 5 weeks, labeling at lowest and highest salinities had decreased markedly.

Total label in amino acids decreased with increasing salinity after 5 d, but had levels above control values at both higher and lower salinities after 5 weeks. Specific groups of amino acids varied independently. Labeling of glycine + serine (and glycolate, not shown) was higher after 5 d for salinities <30 g L^{-1} (Fig. 9). After 5 weeks, labeling was increased for both higher and lower salinities. Labeling of glutamate and aspartate decreased after 5

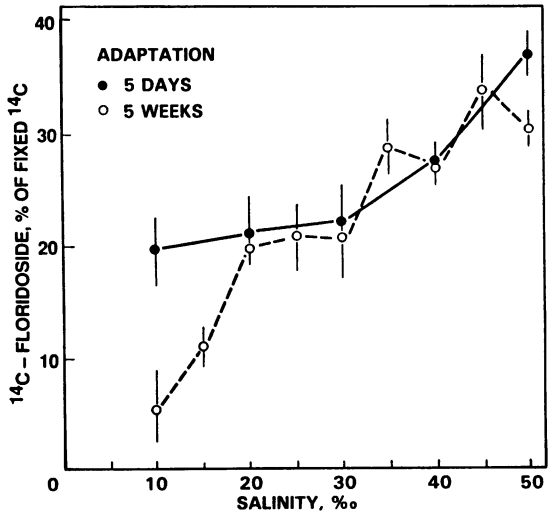

FIG. 7. ^{14}C labeling of floridoside *vs.* salinity. Plants were labeled for 2 h in 2 mM [^{14}C]bicarbonate at indicated salinities in the light. Values are averages of three replicates ± SE.

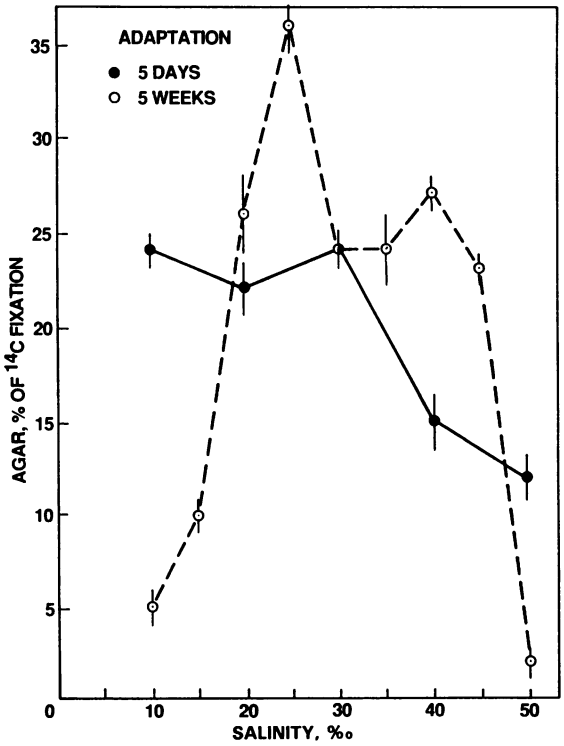

FIG. 8. ^{14}C labeling of agar *vs.* salinity. Method was as in Figure 7. Values are averages of three replicates ± SE.

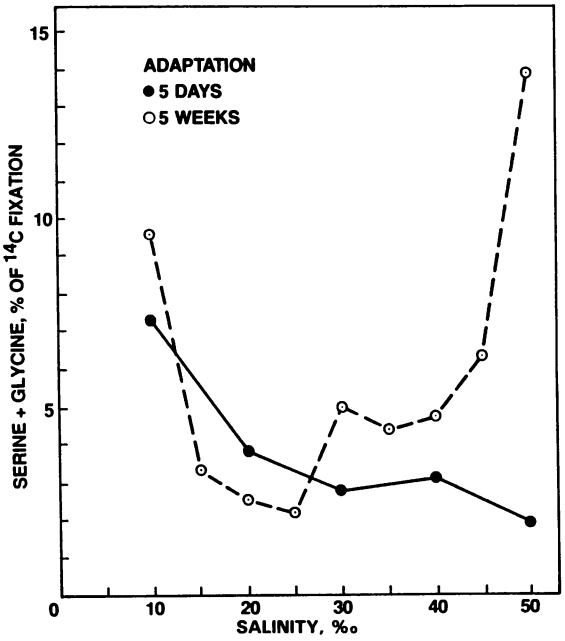

FIG. 9. ^{14}C labeling of glycine + serine *vs.* salinity. Method was as in Figure 7. Values are averages of two replicates.

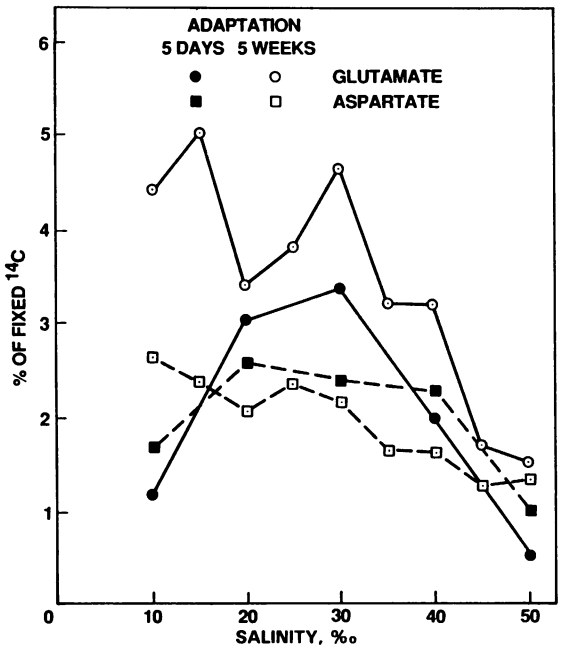

FIG. 10. ^{14}C labeling of glutamate and aspartate. Method was as in Figure 7. Values are averages of two replicates.

d for both higher and lower salinities (Fig. 10). Labeling rates at lower salinities had returned to control values after 5 weeks adaptation.

DISCUSSION

The data generated in these experiments suggest a complex response to changes in salinity for *G. coulteri*. They affect both the light and dark reactions of photosynthesis and the subsequent flow of photosynthate. The responses to a change to lower salinity are both qualitatively and quantitatively different from those after a change to higher salinity. At low salinity, photosynthetic O_2 evolution and carbon fixation are inhibited, while respiration is stimulated. Photorespiration appears to be stimulated as well, based on increases in glycolate, serine, and glycine. Fixed carbon does not appear to be moving into growth or storage compounds such as protein, agar, or floridoside, nor into plant pigments. While agar increased at lower salinities, biosynthesis decreased. Since agar carbon cannot be recovered by *G. coulteri*, this most likely represents the relative loss of other cellular constituents. The majority of [^{14}C]photosynthate (>80%) under these conditions was found in the low molecular weight solubles pool. However, only about 50% of this was recovered on paper chromatograms, which lose volitile compounds such as formic, hy-

droxypyruvic, and glyoxylic acids. Since these are elements of the C-2 cycle, it is possible that much photosynthate is lost to the plant via photorespiration. High photorespiration would be consistent with continued, although reduced, biosynthesis of glutamate and aspartate, which would be necessary for the transamination of glyoxylate to glycine directly, or indirectly through serine. Increases in the total amino acid pool are quantitatively similar to losses of protein under these conditions, suggesting a shift in N allocation and an inhibition of protein synthesis, but not an inhibition of inorganic N assimilation, as total N and C:N ratios were unchanged.

For salinities higher than normal, respiration was low and constant with salinity increase. Photosynthetic O_2 evolution and carbon fixation decreased, and photorespiration increased, with the result of less photosynthate available to the plant. However, plants grown at salinities up to 50 g L^{-1} maintained positive growth, indicating a net surplus of photosynthate. Metabolic patterns were consistent with this in that protein, pigments, and floridoside were maintained. The plants behaved as if N assimilation had been stimulated, although total N and the C:N ratio were unchanged. Previous work had shown that increases in available N result in increases in amino acid, protein, and floridoside biosynthesis and a decrease in agar biosynthesis (12), which were observed at higher salinities in the work reported here. While glutamate and aspartate levels fell with increasing salinity, this was more than offset by large increases in the serine + glycine pool.

From an ecological perspective, the ability to tolerate higher salinities is consistent with the intertidal habitat of *G. coulteri*. Dessication on exposure to the air can be frequent, if relatively short term, and amounts to a progressive increase in salinity. In this laboratory (BA Macler, unpublished data), exposure to H_2O-saturated air led to complete inhibition of ^{14}C fixation within 2 h. Air-exposed plants showed increased levels of ^{14}C label in floridoside, a decrease in agar labeling, and up to sixfold increases in total amino acids.

The data also indicate that *G. coulteri* can adapt to salinity changes within broad limits and that this adaptation may require up to several weeks to complete. This speaks to the results of other workers who examined algal responses to salinity stress after exposures of minutes to days. While changes in photosynthesis and respiration were noted in *G. coulteri* immediately upon exposure to changed salinities (BA Macler, unpublished data), maximal effects on protein, pigments, and agar were much longer in developing, and adaptive recovery was not completed for weeks. Therefore, discussions on the ecological significance of particular short-term observations must bear in mind longer term effects as well.

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