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Photosynthesis and Calcification in the Articulated Coralline Red Algae *Amphiroa anceps* and *A. foliacea*

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Abstract

Net photosynthetic oxygen evolution in *Amphiroa anceps* (Lamarck) Decaisne is inhibited at high oxygen concentrations. Photosynthesis is highest between pH 6.5 and 7.5. At pH 9 to 10 there is still a significant photosynthetic rate, suggesting that this alga can use HCO_3^- as a substrate for photosynthesis. At pH 7.0 to 8.5, the photosynthetic rate saturates at a total inorganic carbon concentration (ΣC_i) greater than 3 m*M*. At pH 8.5 and 8.8, calcification rate continues to increase with increasing concentration of ΣC_i. Between pH 7 and 9, the calcification rate in the light in *A. foliacea* Lamouroux is proportional to the photosynthetic rate, whereas at higher pH where the photosynthetic rate is very low, the calcification rate is stimulated by the higher concentration of CO_3^{2-} ion. At all pH values examined, the calcification rate of living plants in the dark and of dead plants is directly proportional to the CO_3^{2-} ion concentration, suggesting little metabolic involvement in calcification processes in the dark, whereas calcification in live *A. foliacea* in the light is influenced both by the photosynthetic rate and the CO_3^{2-} ion concentration in the medium.

Introduction

The pioneering studies of Goreau (1963) and later workers have shown that light stimulates calcification in most calcareous algae (see Borowitzka 1977, for review). The mechanism for this light stimulation has been studied in detail by Borowitzka and Larkum in the green algae *Halimeda* spp. (1976 a, b, c; 1977).

The coralline red algae (Rhodophyta, Corallinaceae), however, differ from *Halimeda* spp. in that they deposit the calcite crystal isomorph of $CaCO_3$ rather than the aragonite isomorph. Furthermore, the calcite is deposited within the organic cell wall and the crystals show some organization with respect to the cell (Borowitzka *et al.*, 1974; Borowitzka and Vesk, 1978, 1979). The coralline algal $CaCO_3$ also has a different composition of the stable isotopes of oxygen and carbon from that of aragonite algal $CaCO_3$ (cf. Borowitzka, 1977). These differences between calcite and aragonite-depositing algae suggest differences in their calcification mechanisms.

Knowledge of the source of inorganic carbon for photosynthesis and calcification is important for understanding the calcification system of algae. Recently, Pentecost (1978) showed that calcification in the coralline alga *Corallina officinalis* is directly related to the photosynthetic rate, and Smith and Roth (1979) showed that the calcification rate is related to the inorganic carbon concentration in the medium. This paper is a study of the effects of the various species of inorganic carbon on photosynthesis and calcification in the coralline algae *Amphiroa anceps* and *A. foliacea*.

Materials and Methods

Amphiroa anceps (Lamarck) Decaisne was collected at 3 to 5 m depth at Dee Why, New South Wales or from a wave-washed area of an intertidal rock platform at Hole in the Wall, Newport, New South Wales, Australia. These algae were kept in outdoor aquaria at Dee Why for up to 3 wk. *Amphiroa foliacea* Lamouroux was collected at approximately 5 m depth at Nelly Bay, Magnetic Island, Queensland, Australia, and maintained for up to 4 months in outdoor flowing seawater aquaria at Cape Ferguson.

For each experiment, epiphyte-free branches of approximately 7 to 10 segments length, taken from one algal clump, were used. Dead plants were obtained by air-drying the thalli and then resoaking them in seawater.

For isotope measurements the branches were pretreated overnight in the experimental solution. Dark-treated branches were pretreated for 12 h in the dark.

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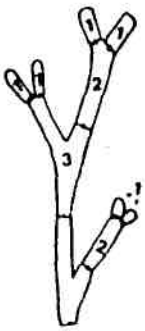

Fig. 1. *Amphiroa anceps*. Part of a branch, indicating system used for numbering segments. Segments 4 and 5 are next down from Segment 3 on the main axis

Table 1. *Amphiroa anceps*. Effect of oxygen concentration on photosynthetic oxygen evolution. Experiments were carried out at 18 °C, pH 8.2

Segment	O_2 concentration (μM)	Net photosynthetic rate (nmol g^{-1} min^{-1})
1	80.5	1925
	164.3	856
	198.8	471
2	82.1	1118
	174.1	447
	225.1	224
3	62.4	940
	142.9	592
	197.2	262
4 + 5	105.1	1000
	165.9	457
	239.9	100

Branches were then transferred to fresh medium and, after 30 min in a hermetically sealed labelling chamber, $NaH^{14}CO_3$ were added to give a final specific activity of 92.5 Bq mmol^{-1} ΣC_i (where $\Sigma C_i = [CO_2] + [HCO_3^-] + [CO_3^{2-}]$). The temperature was 28 °C and a light intensity of 600 μE m^{-2} s^{-1} was provided by overhead cool-white fluorescent lamps. At 90, 120, 150 and 180 min after addition of the isotope, 6 to 8 branches were removed and frozen at −90 °C. After freeze-drying, separation into individual segments and weighing, the segments were extracted and uptake of isotope into the inorganic ($CaCO_3$) and organic carbon fractions was determined according to the method of Borowitzka (1979). Only the second and third segments (intergenicula), (Fig. 1) of each branch were used except where noted to minimize variability due to age differences (Borowitzka, 1979). Photosynthetic and calcification rates were calculated by linear regression through the points at the 4 sampling times (i.e., a total of 45 to 65 points per treatment, Borowitzka, 1979). Experiments were carried out in Artificial Pacific Seawater (Borowitzka and Larkum, 1976a). During the course of an experiment, pH changes in the labelling chambers were less than 0.2 pH units.

Oxygen electrode experiments with *Amphiroa anceps* were carried out in aged seawater prepared from "Aquasonic" synthetic marine salts. For each set of measurements, 10 to 40 segments were used. Experiments were carried out in a Rank polarographic oxygen electrode at 18 °C (winter collections) or 22 °C (summer collections) at a light intensity of 700 μE m^{-2} s^{-1}, using a quartz-iodine lamp; this was sufficient to saturate photosynthesis.

The inorganic carbon concentration (ΣC_i) was adjusted with $NaHCO_3$ and pH with NaOH or HCl. O_2 concentration was adjusted by bubbling nitrogen gas

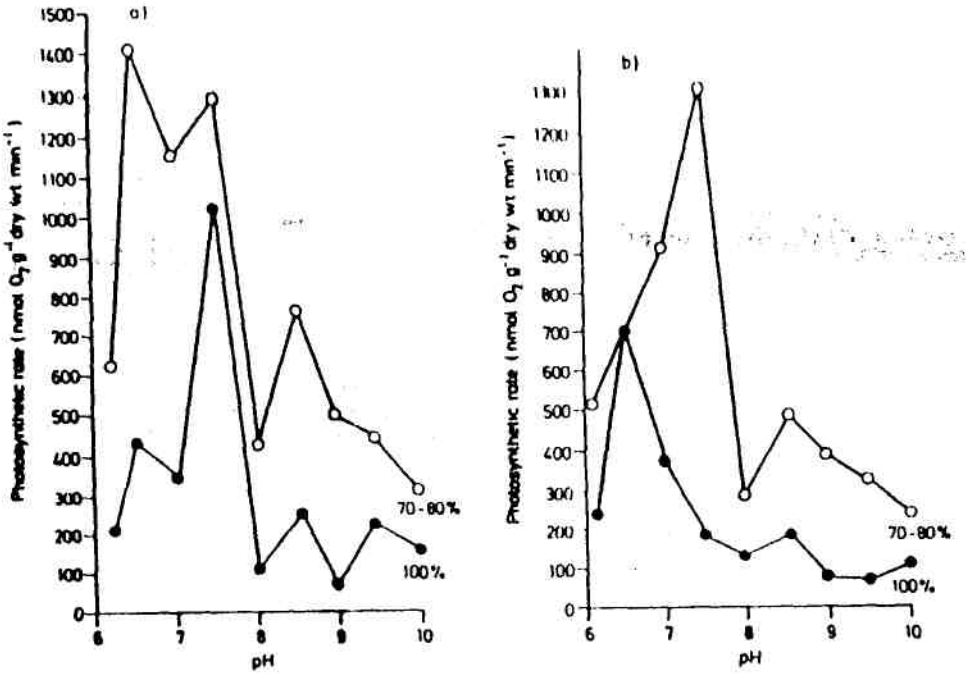

Fig. 2. *Amphiroa anceps*. Photosynthetic rate at different seawater pHs and oxygen tensions, and constant ΣC_i of 2 mM. (a) 1st (young) segments; (b) 2nd and 3rd (mature) segments (data combined)

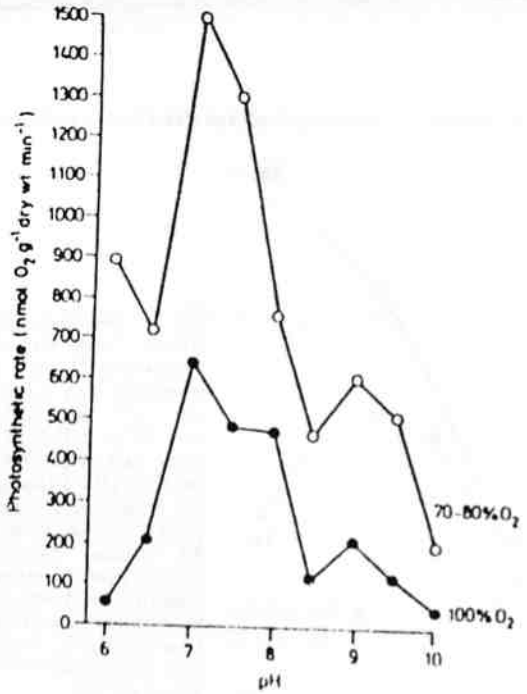

Fig. 3. *Amphiroa anceps*. Photosynthetic rate at different seawater pHs and oxygen tensions. Same seawater medium as used in Fig. 2 experiments with 2 m*M* ΣC_i added. 1st segments only

through the solutions. ΣC_i concentrations were determined by titration (Strickland and Parsons, 1972).

Results

Preliminary experiments with *Amphiroa anceps* showed a marked oxygen inhibition of photosynthetic oxygen evolution (Table 1). At pH 8.2, the photosynthetic rate at near-saturating oxygen concentration (225.1 μM O_2) was approximately half the rate at an oxygen concentration of approximately 72% saturation (174.1 μM O_2). It was therefore very important in all experiments to monitor the oxygen concentration and, unless otherwise noted, all experiments were carried out at 170 to 190 μM O_2 (i.e., at as constant a level of partial O_2 inhibition as could be maintained).

Fig. 2 shows the change in net oxygen evolution at constant ΣC_i, but different seawater pHs and O_2 tensions. The shape of the curve is independent of segment age; however, the first (apical) segments vary more than the second and third segments, probably due to greater variability in the stage of growth of the first segments. Photosynthetic rates are highest at pH 6.5 to 7.5 in all segments. Increasing the total inorganic carbon concentration (ΣC_i) in the medium by 2 m*M* has little effect on the shape of the pH-photosynthesis curve (Fig. 3), the maximum rate being at pH 7.0. Photosynthetic carbon fixation by *Amphiroa foliacea* seems to follow a similar pattern (Fig. 4a) between pH 7.0 and 10.0.

Calcification rates in *Amphiroa foliacea* in the light at pH 7.2 and 8.3 are almost the same, but at pH 9.0 the rate falls rapidly to a value below that of living plants in the dark or of dead plants (Fig. 4b). This fall in the calcification rate is similar to the fall in the photo-

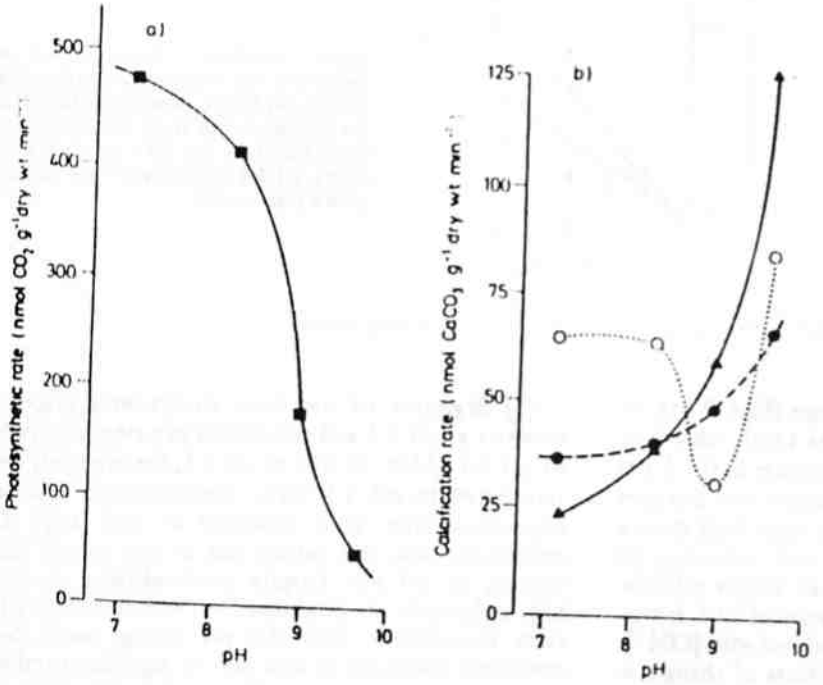

Fig. 4. *Amphiroa foliacea*. Mature segments only; 2.2 m*M* ΣC_i. (a) Photosynthetic rate at different seawater pHs; (b) calcification rate at different seawater pHs in the light (open circles), dark (filled circles) and in dead plants (triangles)

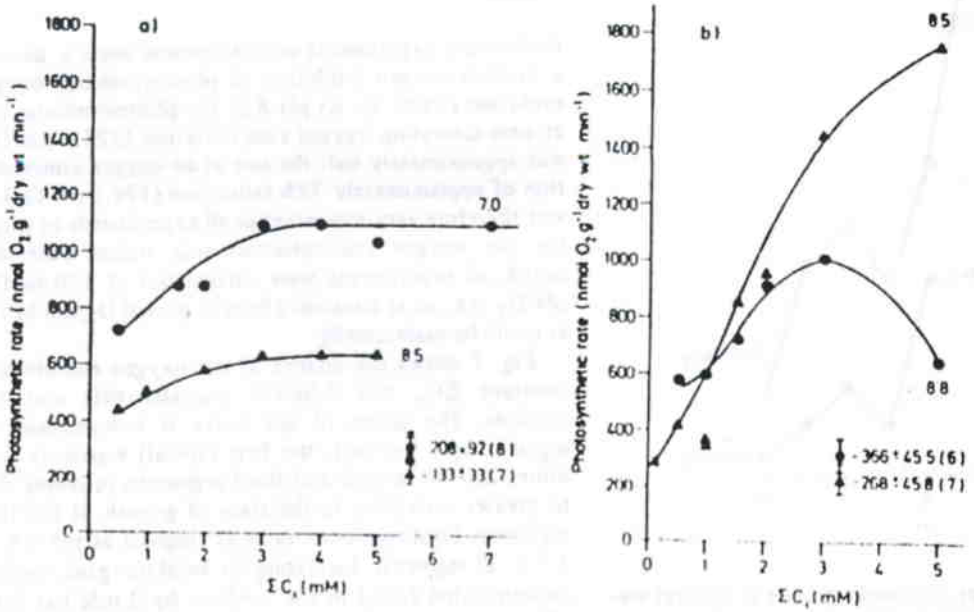

Fig. 5. *Amphiroa anceps*. Mature segments only. Photosynthetic rate with increasing ΣC_i. 1100 $\mu E\ cm^{-2}\ s^{-1}$ light intensity and 22 °C. (a) Photosynthetic rate at pH 7.0 (circles) and pH 8.5 (triangles); (b) photosynthetic rate at pH 8.5 (triangles), and pH 8.8 (circles). Respiration rates ± standard errors are indicated in lower right-hand corner of each graph. Note that $CaCO_3$ precipitation occurred above 3 mM ΣC_i at pH 8.8

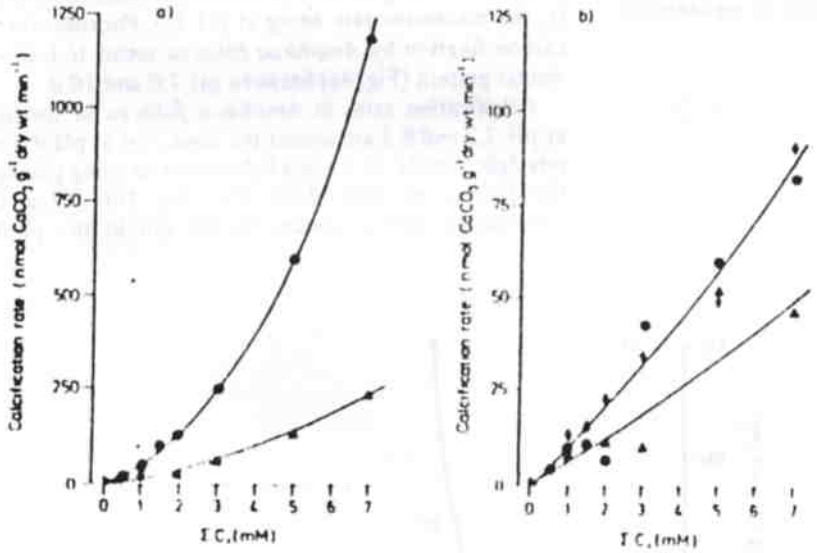

Fig. 6. *Amphiroa foliacea*. Mature segments only. Calcification rate with increasing ΣC_i. (a) Living plants in the light at pH 8.5 (triangles) and at pH 8.8 (circles); (b) living plants in the dark at pH 8.5 (triangles, pH 8.8 (circles) and dead plants at pH 8.8 (diamonds)

synthetic rate over the same pH range (Figs. 2–4a). At pH 9.7, the calcification rate reaches a high value (Fig. 4b), presumably due to the great increase in $[CO_3^{2-}]$ at this pH. In the dark, the calcification rate increases slightly with increasing pH, whereas dead thalli show a large increase in calcification rate with increasing pH (Fig. 4b). Regression analysis of dead thallus calcification rate with $[CO_3^{2-}]$ gives a correlation of 0.95, indicating that calcification is directly correlated with $[CO_3^{2-}]$.

In order to establish whether effects of changes in seawater pH on the photosynthetic and calcification system are largely due to changes in the relative proportions of the inorganic carbon species and not to pH acting on metabolism directly, two kinds of experiments were carried out:

(1) Segments of *Amphiroa anceps* were placed in seawater at pH 9.5 and transferred in a stepwise fashion to pH 6.1. After 30 min at pH 6.1, the segments were transferred to pH 9.5 again. Photosynthetic and dark respiration rates were measured at each step. The experiment was also carried out in the reverse order starting at pH 6.5. Despite pre-incubation at either high or low pH, the photosynthetic and dark respiration rates immediately after the pH change were those previously measured at that pH. No significant residual effect of pre-treatment at high or low pH values was detected.

(2) In the second series of experiments, photosynthetic and calcification rates at constant pH but varying levels of ΣC_i were studied. At each pH value the photo-

synthetic rate rose with increasing ΣC_i and eventually reached a plateau value. At pH 8.8 the photosynthetic rate fell when ΣC_i was greater than 3 m*M*, because of $CaCO_3$ precipitation in the medium. High values of ΣC_i could not be achieved at high pH due to precipitation of $CaCO_3$ at high pH and high ΣC_i. At pH 8.5 and 8.8 and ΣC_i higher than 3 m*M*, the actual value of ΣC_i must be considered as approximate, due to possible $CaCO_3$ precipitation. In all experiments there is considerable variation between plants, making direct comparison difficult (cf. Fig. 5); however, experiments conducted with branches from the same plant can be compared. Fig. 5a shows photosynthetic oxygen evolution at pH 7.0 and 8.5 with increasing ΣC_i. Fig. 5b shows a similar experiment at pH 8.5 and 8.8. In all experiments, photosynthesis appears to saturate at a ΣC_i value higher than 3 m*M*, except at pH 8.5 in one experiment (Fig. 5b) where photosynthesis had not yet saturated at 5 m*M*.

Similar experiments with *Amphiroa foliacea* showed that calcification rates at pH 8.5 and 8.8 continued to increase with increasing ΣC_i, with a higher rate at pH 8.8 (Fig. 6a). The non-linearity of these calcification graphs suggests that the increase in calcification rate is due to both the increased photosynthetic rate and the increase in $[CO_3^{2-}]$ with the increase in ΣC_i. This is further supported by the fact that the calcification rates in living plants in the dark and in dead plants increase almost linearly with increasing ΣC_i (Fig. 6b). Calcification rates in the dark at pH 8.8 were higher than those at pH 8.5. The gradients (k) of the regression lines of ΣC_i against calcification rate are in almost the same proportion ($k_{8.8}:k_{8.5} = 1.59$) as the concentrations of the CO_3^{2-} ion ($[CO_3^{2-}]_{8.8}:[CO_3^{2-}]_{8.5} = 1.66$). There is no significant difference between calcification rates of the living plants in the dark and the dead plants.

Discussion

The effect of oxygen concentration on photosynthetic oxygen evolution in *Amphiroa* spp. suggests the presence of photorespiration in these algae (Tolbert, 1974), although no O_2 burst was observed upon light/dark transition. Similar effects were demonstrated by Black *et al.* (1976) and Downton *et al.* (1976) for a wide range of tropical marine algae, though not for coralline algae. However, the studies of Lloyd *et al.* (1977) suggest that photorespiration does not occur in algae and that the O_2 consumption in the light may be due to the oxidation of some component in the photochemical electron transport chain rather than glycolate formation and oxidation, or CO_2 evolution. *A. anceps* appears to be especially sensitive to external oxygen tension. The cause of this oxygen inhibition of photosynthetic oxygen evolution in *A. anceps* as in other algae requires further study (see also Canvin, 1979). Irrespective of the cause of this inhibition, it is of great importance to monitor oxygen concentration in studies of photosynthesis and calcification carried out with this alga and probably all other calcareous algae.

The shape of the pH/photosynthesis curve of *Amphiroa* spp. is similar to that of other algae (e.g. Paasche, 1964; Borowitzka and Larkum, 1976b), with a maximum photosynthetic rate between pH 6.5 and 7.5. The shape of this curve is apparently not influenced by oxygen tension, and the apparent photosynthetic rate is clearly not wholly dependent on CO_2 concentration. The values for photosynthesis below pH 7 and above pH 8 are respectively lower and higher than would be expected if CO_2 only were being taken up (Fig. 2). The HCO_3^- of the external solution must, therefore, be a second source of inorganic carbon for photosynthesis. However the question of whether this HCO_3^- is taken up across the plasmalemma as HCO_3^- or as CO_2 remains unanswered, and cannot be answered in experiments where the external medium pH is altered. It is quite possible that localized pH changes in the cell wall or in the unstirred layer on the surface of the algal thalli, or even an extracellular carbonic anhydrase, could result in the extracellular conversion of HCO_3^- to CO_2 and OH^-, with subsequent uptake of the CO_2 (cf. Berry *et al.*, 1976; Miyachi and Shiraiwa, 1979). The possibility of such localized pH changes is of considerable importance in determining the mechanism of calcification, and requires study. The apparent inhibition of photosynthesis at low pH and high CO_2 concentration (e.g. Fig. 3) also requires further study. From studies to date, it is unclear whether this is an effect of CO_2 concentration or pH *per se* (Livingston and Franck, 1940; Steemann Nielsen, 1955; Swift and Taylor, 1967). In *A. anceps*, photosynthesis appears to saturate at about 3 m*M* ΣC_i irrespective of pH (Fig. 5) although there is some variation between plants. The fall in photosynthesis at 5 m*M* ΣC_i and pH 8.8 is probably due to reduced CO_2 and HCO_3^-, due to precipitation of $CaCO_3$ in the medium which could not be prevented.

Calcification in the light at medium carbon concentrations generally follows the photosynthetic rate, dropping between pH 8 and 9. Pentecost (1978) observed a direct relationship between photosynthetic rate and calcification in *Corallina officinalis* in a normal pH range. However, the rise in calcification observed at pH 10 can be attributed wholly to the increased concentration of CO_3^{2-} at this pH. This conclusion is supported by the correlation of $[CO_3^{2-}]$ with the calcification rate in plants at pH 8.5 and 8.8 (Fig. 6; see also Fig. 3 in Smith and Roth, 1979).

At very high pH (Fig. 4), some inhibition of calcification in living thalli in the dark compared to dead thalli is observed. This may be due to respiratory CO_2 evolution causing localized acidification (Borowitzka and Larkum, 1976b). Previous workers (Ikemori, 1970; Okazaki *et al.*, 1970; Pearse, 1972) using long incubation times (> 5 h) have obtained higher rates of ^{45}Ca deposition in living plants in the dark compared to dead plants. Their results are probably artifacts of the single time-point method used. Kinetic studies are more accurate (Böhm, 1978; Borowitzka, 1979), and show that the calcification rate (accretion rate *sensu* Goreau, 1963) of living thalli

in the dark is the same as for dead thalli in *Amphiroa foliacea* at normal seawater pH (Borowitzka, 1979).

Although experiments to date do not allow the formulation of a meaningful testable model for the mechanism of calcification in the Corallinaceae, some general properties of the calcification system can be described and evaluated in light of existing theories on algal calcification. Borowitzka (1977) lists a number of the theories which have been proposed to explain algal calcification.

Comparison with the aragonite-depositing green algae *Halimeda* spp. is of some value. Borowitzka and Larkum (1976b) proposed that the photosynthetic stimulation of $CaCO_3$ deposition in *Halimeda* spp. is due to localized changes in pH and $[CO_3^{2-}]$ due to photosynthetic uptake of CO_2 from the semi-isolated intercellular space. Coralline algae do not have as an extensive intercellular space (Borowitzka and Vesk, 1978, 1979) as does *Halimeda* spp. (Borowitzka and Larkum, 1977). The cell walls of coralline algal cells touch, and the $CaCO_3$ is deposited within the organic material of these cell walls. The nature and function of this organic material in coralline calcification are unclear, but it is probably of great importance to the mechanism of calcification. The organic material may act as a nucleating site for $CaCO_3$, either by acting as a template for epitaxial nucleation or by attracting Ca^{2+} ions electrostatically (Degens, 1976). Ca^{2+}-binding polysaccharides have recently been demonstrated in some calcareous red algae (Misonou *et al.*, 1980). One apparent effect of this wall material is that coralline algae deposit the calcite crystal isomorph of $CaCO_3$, whereas *Halimeda* spp. deposits the aragonite isomorph (Borowitzka *et al.*, 1974; Borowitzka, 1977). *Halimeda* spp. do not have an organic matrix within which the $CaCO_3$ is deposited, although some organic material has been observed to coat the aragonite crystals (Nakahara and Bevelander, 1978). Many organic compounds have been shown to influence the type of $CaCO_3$ isomorph precipitated in experimental solutions (Kitano and Hood, 1965).

Calcification rates in coralline algae are greatly influenced by the external carbonate ion concentration and by the photosynthetic rate (Goreau, 1963; Ikemori, 1970; Okazaki *et al.*, 1970; Pentecost, 1978; and present study). The role of metabolism may be to create changes in cell wall pH or $[CO_3^{2-}]$, favoring $CaCO_3$ precipitation [see Raven and Smith (1974) and Borowitzka (1977) for possible mechanisms]. Metabolic involvement may also be via continued synthesis of organic compounds favouring $CaCO_3$ precipitation or by actively transporting Ca^{2+} ions to the site of calcification (LaVelle, 1979). Okazaki (1977) has found a Ca^{2+}-dependent ATPase in some Corallinaceae but not in other calcareous and non-calcareous algae. On evidence to date, it appears that the mechanism of coralline algal calcification is more complex than that occurring in *Halimeda* spp., with both metabolic and physical factors directly contributing. It also seems likely that the relative importance of metabolism compared to physical factors varies with the stage of development of the geniculum. However, no meaningful model for calcification in the coralline algae can as yet be formulated.

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