Marine Biology 117, 129–132 (1993)

Marine Biology
International Journal on Life in Oceans and Coastal Waters

Calcification in the articulated coralline alga *Corallina pilulifera*, with special reference to the effect of elevated CO_2 concentration

K. Gao [1]*, Y. Aruga [2], K. Asada [3], T. Ishihara [4], T. Akano [4], M. Kiyohara [4]

[1] Kansai Environmental Engineering Center, Nakazaki-nishi 2-3-39, Kita-ku, Osaka 530, Japan
[2] Tokyo University of Fisheries, Konan-4, Minato-ku, Tokyo 108, Japan
[3] Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan
[4] Technical Research Center, The Kansai Electric Power Co. Inc., Nakoji, Amagasaki, Hyogo-ken 661, Japan

Received: 24 February 1993 / Accepted: 21 April 1993

Abstract. Calcification in *Corallina pilulifera* Postels et Ruprecht displayed diurnal variations in aerated (350 ppm CO_2) culture media, with faster rates during the light than during the dark period. Addition of CO_2 (air + 1250 ppm) inhibited calcification. This was attributable to the decreased pH resulting from CO_2 addition. Both photosynthesis and calcification were enhanced in seawater, with elevated dissolved inorganic carbon concentrations at a constant pH of 8.2.

Introduction

Atmospheric CO_2 from combustion of fossil fuels has been reported to have been taken up by oceanic waters over the past 20 yr (Quay et al. 1992). The dissolution of CO_2 in the ocean can result in a decreased pH of the seawater; e.g. an increase in atmospheric CO_2 from 330 to 660 ppm would chemically lower the pH of mixed surface seawater (15 °C, alkalinity 2.469 m*M*) from 8.2 to 7.9 (Stumm and Morgan 1981). The rate of a algal calcification is dependent on the pH of the environment, which affects the $CO_3{}^{2-}$ concentration of the seawater (Borowitzka 1981). Thus, an increase in atmospheric CO_2 would be expected to lower the pH of seawater and to affect the process of marine biological calcification. Information on the effects of elevated CO_2 concentrations on biological calcification would be useful in evaluating the ecological impacts of increasing CO_2 emissions from industrial activities.

The photosynthesis and calcification processes of calcareous macroalgae make these important components of the carbon and calcium cycles of the coastal ecosystem. Species of the green algae *Halimeda* have been well studied (Stark et al. 1969, Borowitzka and Larkum 1976a, b, c, 1977, Buesa 1977, Littler et al. 1983, Jensen et al. 1985, Payri 1988, Ballesteros 1991), as have some red coralline algae (Digby 1977, Smith and Roth 1979, Akioka et al. 1981, Borowitzka 1981, Okazaki et al. 1982, Baba et al. 1988). However, little is known about daily variations in the calcification of calcareous macroalgae. The present paper reports on the calcification and its diurnal variation in the articulated red coralline alga *Corallina pilulifera* Postels et Ruprecht.

* *Present address:* University of Hawaii, Hawaii Natural Energy Institute, 2540 Dole Street, Honolulu, Hawaii 96822, USA

Material and methods

Specimens of *Corallina pilulifera* Postels et Ruprecht were collected from April to June 1991 at a depth of 0.3 to 0.5 m below mean lower low water from Takahama, Wakasa Bay, facing the Sea of Japan to the north. After collection, the samples (2 to 5 cm long) were cleaned of obvious epiphytes.

Experiment 1

Cultures of *Corallina pilulifera* were aerated (500 ml min^{-1}) with air (350 ppm CO_2) or air + 1250 ppm CO_2 (1600 ppm CO_2) in two cylindrical vessels (15 cm high, 14 cm diam, with screw cap and air inlet and outlet). These vessels were placed in two incubators at 20 °C under a 12 h light:12 h dark cycle [light period from 08.00 to 20.00 hrs at 30 µmol photons $m^{-2}s^{-1}$; light intensity >40 µmol photons m^{-2} s^{-1} has been suggested to lead to tissue death in indoor cultures (Dr. M. Baba personal communication)]. The CO_2 supply and monitoring is described in detail in Gao et al. (1991). Variations in CO_2 concentration were <5%. For the culture medium, seawater was collected off the Miyazu shore of the Sea of Japan (2.1 µM inorganic N, 0.2 µM inorganic P), and was filtered (Whatman GF/C) and enriched with PES medium (Provasoli 1966) (20 ml l^{-1} seawater). More than 60 individuals [fresh weight 25 g, dry weight (85 °C, 24 h) 14 g, carbonate-free dry weight 2.8 g] were placed in each vessel with 1 liter of culture medium. The culture medium was renewed at the beginning of each light and each dark period. Calcification was determined from the change in concentration of Ca^{2+} by sampling 1 ml of the medium every 30 min. Ca^{2+} was analyzed automatically by means of a high performance ion-chromatography unit (Shimadzu HIC-6, CDD-6A, SIL-6A). Dissolved inorganic carbon (DIC) in the seawater was measured by infrared analysis using a Shimadzu total organic carbon analysis unit (TOC-5000). The pH in the culture medium was measured and automatically recorded with a Hanna pH meter (HI8418).

Experiment 2

For the experiment on the effects of DIC concentrations on photosynthesis and calcification, the DIC concentration of the seawater was modified by first removing the DIC by acidification below pH 3 with HCl, and then bubbling CO_2-free air through the water for 5 h to remove gaseous CO_2. The pH was then again increased to 8.2–8.4 with the addition of NaOH under CO_2-free air. Inorganic carbon was added in the form of $NaHCO_3$, and the pH was finally adjusted to 8.22 ± 0.01 with NaOH or HCl. The calculated DIC concentrations were checked by infrared gas analysis using the total organic carbon analysis unit. The total carbon fixed by photosynthesis and calcification was determined from the difference in the DIC in the sealed tubes (50 ml) with and without algal samples. Simultaneously, the concentration of Ca^{2+} in the seawater was measured and losses due to calcification were determined. During the incubation period (20 min, since longer incubation resulted in decreased calcification) at 20 °C and 300 μmol photons $m^{-2}\ s^{-1}$ in an incubator, three tubes each containing 3 to 4 samples (1.0 to 1.2 g fresh wt) and one tube without samples (control) were agitated on a shaker simultaneously. Photosynthesis of *Corallina pilulifera* was saturated at 300 μmol photons $m^{-2}\ s^{-1}$ (Gao 1992). Since calcification is a chemical combination of Ca^{2+}- and $CO_3{}^{2-}$-forming $CaCO_3$, in which the atomic ratio of Ca to C is 1:1, the calcification rate as nmol Ca min^{-1} is equal to the rate as nmol C min^{-1}. The rate of photosynthetic inorganic carbon fixation was obtained by subtracting the calcification rate from the rate of total inorganic carbon fixation.

Experiment 3

The $CaCO_3$ and $MgCO_3$ contents of the algal thalli were determined by placing the dried (85 °C, 24 h) samples into an HCl solution, liquefying the carbonates, adjusting the pH to 7–9, and measuring the Ca^{2+} and Mg^{2+} concentrations with a high-performance ion-chromatograph unit. The average organic carbon content of $CaCO_3$-free dried organic matter was 24.5% in the articulated calcareous red algae *Amphiroa foliacea* and *Galaxaura rugosa* (Atkinson and Smith 1983). Taking this value to be valid for *Corallina pilulifera* also, the molar ratio of inorganic carbon to organic carbon of the algal thalli was estimated on this basis.

Results

Experiment 1

In the low-CO_2 culture of *Corallina pilulifera*, pH increased and Ca^{2+} concentration decreased over time during the light period; Ca^{2+} concentration also decreased slightly during the dark period, despite a decrease in pH (Fig. 1). In the high-CO_2 culture, pH decreased to a constant level in the renewed culture medium at the beginning of the light period; no decrease in Ca^{2+} concentration was observed. A slight increase in the Ca^{2+} concentration occurred in the dark period. The rate of decrease in the Ca^{2+} concentration of the low-CO_2 culture could represent the rate of calcification by the algal thalli, since no crystallization of $CaCO_3$ was observed when the pH of the culture medium rose. Linear regression analysis revealed that the decrease in the Ca^{2+} concentration over time was significant in the low ($R^2 = 0.86$) but not in the high ($R = 0.01$) CO_2 culture media during the light period. The estimated calcification rate in the low-CO_2 culture during the light period was 972 nmol Ca or C g (dry wt)$^{-1}$ min^{-1}, about twice that during the dark period.

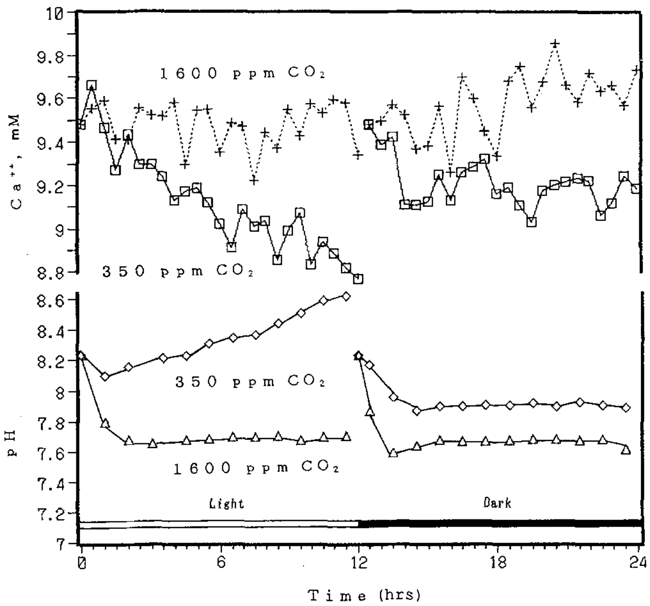

Fig. 1. *Corallina pilulifera.* Diurnal variations in pH and Ca^{2+} concentration in cultures aerated with air (350 ppm CO_2) or with air +1250 ppm CO_2 (1600 ppm CO_2). Culture media were renewed at beginning of light and beginning of dark period

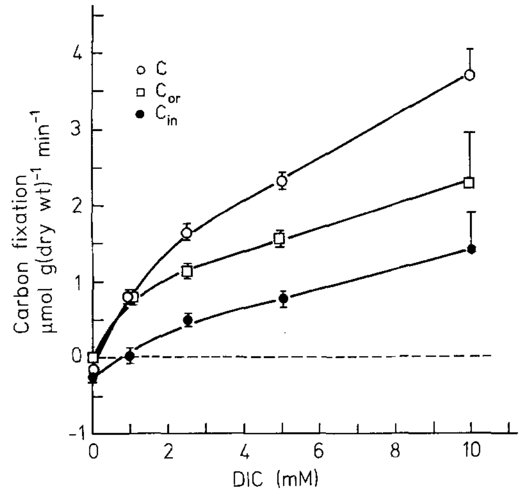

Fig. 2. *Corallina pilulifera.* Rates of calcification (•), photosynthetic carbon fixation (□) and their summed rate (o) as a function of dissolved inorganic carbon (DIC) concentration of the seawater. Measured at 20 °C, 300 μmol photons $m^{-2}\ s^{-1}$, in sealed tubes. Means of 10 to 15 samples +SD

Experiment 2

The measured rate of total inorganic carbon fixation due to photosynthesis and calcification increased with increasing DIC concentration in *Corallina pilulifera* (Fig. 2). The rates of calcification and of photosynthetic fixation of inorganic carbon also increased with increasing DIC concentration. Total inorganic carbon fixation, photosynthesis and calcification were not saturated in seawater with elevated DIC concentrations up to 10 m*M*. The DIC compensation point for algal calcification was ≃ 1 m*M*.

Experiment 3

Carbonate contents (% dry wt) were $\simeq$70% for $CaCO_3$ and 10% for $MgCO_3$ in *Corallina pilulifera* (Table 1). The estimated molar ratio of inorganic to organic carbon was 2.0.

Table 1. *Corallina pilulifera.* Content (% dry wt) of carbonates ($CaCO_3$, $MgCO_3$) and molar ratio of inorganic carbon to organic carbon (C_{in}:C_{or}). Means±SD of five plants

$CaCO_3$ (a)	$MgCO_3$ (b)	Total (a+b)	C_{in}:C_{or}
7.0±1.3	9.6±0.6	(79.6±1.5)	2.0±0.2

Table 2. Dissolved inorganic carbon concentration (DIC) and CO_3^{2-} in culture medium containing *Corallina pilulifera*; equilibrated with 350 and 1600 ppm CO_2 at 20 °C

CO_2 (ppm)	pH	DIC (m*M*)	CO_3^{2-} (%)
350	8.2	1.95	9.4
1600	7.6	2.12	2.5

Discussion

When CO_2 is added to a culture to effect aeration, part of the CO_2 is dissolved in seawater, forming carbonic acid, which dissociates to bicarbonate and then to carbonate and attains a new equilibrium; this results in an increase of H^+ ions in the seawater. Therefore, addition of CO_2 lowers the pH of the culture medium. In the present study, the increased pH in the low-CO_2 culture of *Corallina pilulifera* during the light period may have been attributable to the photosynthetic utilization of HCO_3^- by *C. pilulifera*, as shown previously for the non-calcareous red alga *Porphyra yezoensis* (Gao et al. 1991). In the high-CO_2 culture, CO_2 dissolution might have exceeded photosynthetic "CO_2" removal during the light period, so that pH decreased to a constant level at which the two processes (photosynthetic "CO_2" removal and CO_2 dissolution) may have been balanced. In the dark, algal respiration and the addition of CO_2 reduced the pH. During the light period, Ca^{2+} concentration decreased as pH increased in the low-CO_2 culture, while the Ca^{2+} concentration did not change as pH decreased in the high-CO_2 culture. Therefore, calcification in *C. pilulifera* can be considered to be a pH-dependent process. Borowitzka (1981) reported that the rate of calcification in the articulated coralline red algae *Amphiroa anceps* (Lamarck) Decaisne and *A. foliacea* Lamouroux increased proportionally to increased pH due to photosynthesis in the pH 7.0 to 9.0 range, possibly stimulated by increased CO_3^{2-} under the increased pH. In the present study, neither calcification nor photosynthesis of *C. pilulifera* reached their maximum rates at DIC concentrations up to 10 m*M*, suggesting that photosynthesis and calcification are limited in *C. pilulifera* by the amount of inorganic carbon in the surrounding seawater.

The CO_3^{2-} concentration of seawater equilibrated with 350 and 1600 ppm CO_2 was calculated according to the methods recommended by UNESCO (1987) (Table 2). High pH results in high CO_3^{2-} concentrations which promote precipitation of $CaCO_3$. Consequently, the increased calcification by *Corallina pilulifera* concomitant with a rise in the pH of the low-CO_2 culture could have resulted from an increase in the CO_3^{2-} concentration arising from a rise in pH. On the other hand, elevated DIC levels in the seawater at constant pH (Fig. 2) result in increased calcification as well as photosynthesis of *C. pilulifera*. This implies that addition of free CO_2, by lowering the pH, does not enhance calcification but that the addition of carbonate and bicarbonate do enhance this process.

Smith and Roth (1979) studied the effect of the addition of CO_2 on the calcification of *Bossiella orbigniana* (Decaisne) Silva by measuring ^{45}Ca incorporation into the crystalline matrix, and reported that the maximum calcification rate was between 1100 and 10 500 ppm CO_2. The discrepancy in regard to the effect of high CO_2 on the calcification of coralline red algae between Smith and Roth's study and the present study probably arises from the different techniques used. In Smith and Roth's experiments, the solution and precipitation of $CaCO_3$ may have proceeded simultaneously in the buffered medium under conditions of high CO_2, and the measured incorporation of ^{45}Ca could have resulted from exchange of the isotopes. Specific differences between *B. orbigniana* and *Corallina pilulifera* could also partially account for the discrepancy.

In the present study, *Corallina pilulifera* displayed a diurnal variation in calcification, rates being higher during the light period than during the dark period. While caution is warranted in extrapolating short-term experiments in the laboratory to conditions in the sea, it can be reasonably hypothesized that calcification of *C. pilulifera* proceeds faster during the daytime than during the nighttime, since photosynthesis increases pH in the daytime and respiration lowers it at night around the calcification site in the alga. The day-night reversion of pH (pH increasing during the daytime and decreasing at night) has been confirmed by data for Takahama, Wakasa Bay, in the Sea of Japan (Komatsu 1989), from which area *C. pilulifera* was collected for use in the present study.

The estimated molar ratio of inorganic carbon to organic carbon in *Corallina pilulifera* was $\simeq$2.0, indicating that the incorporation of inorganic carbon into $CaCO_3$ is higher than the incorporation by photosynthesis. However, the measured rate of calcification was lower than the estimated fixation rate of inorganic carbon by photosynthesis (Fig. 2). A possible reason is that photosynthetic carbon fixation only takes place in the daytime, whereas calcification can occur both day and night, and the organic matter produced in the daytime is consumed at night. That is, photosynthetically fixed carbon is more likely to be recycled through respiration than is carbon fixed by calcification.

Literature cited

Akioka, H., Masaki, T., Johansen, H. W. (1981). The structure of *Alatocladia modesta*, an articulated coralline (Rhodophyta) endemic to Japan. Bull. Fac. Fish. Hokkaido Univ. 32: 39–51

Atkinson, M. J., Smith, S. V. (1983). C:N:P ratios of benthic marine plants. Limnol. Oceanogr. 28: 568–574

Baba, M., Johansen, H. W., Masaki, T. (1988). The segregation of three species of *Corallina* (Corallinales, Rhodophyta) based on morphology and seasonality in northern Japan. Botanica mar. 31: 15–22

Ballesteros, E. (1991). Seasonality of growth and production of a deep-water population of *Halimeda tuna* (Chlorophyceae, Caulerpales) in the north-western Mediterranean. Botanica mar. 34: 291–301

Borowitzka, M. A. (1981). Photosynthesis and calcification in the articulated coralline red algae *Amphiroa anceps* and *A. foliacea*. Mar. Biol. 62: 17–23

Borowitzka, M. A., Larkum, A. W. D. (1976a). Calcification in the green alga *Halimeda*. II. The exchange of Ca^{2+} and the occurrence of age gradients in calcification and photosynthesis. J. exp. Bot. 27: 864–878

Borowitzka, M. A., Larkum, A. W. D. (1976b). Calcification in the green alga *Halimeda*. III. The sources of inorganic carbon for photosynthesis and calcification and a model of the mechanism of calcification. J. exp. Bot. 27: 879–893

Borowitzka, M. A., Larkum, A. W. D. (1976c). Calcification in the green alga *Halimeda*. IV. The action of metabolic inhibitors on photosynthesis and photosynthesis and calcification. J. exp. Bot. 27: 894–907

Borowitzka, M. A., Larkum, A. W. D. (1977). Calcification in the green alga *Halimeda*. I. An ultrastructure study of thallus development. J. Phycol. 13: 6–16

Buesa, R. J. (1977). Photosynthesis and respiration of some tropical marine plants. Aquat. Bot. 3: 203–216

Digby, P. S. B. (1977). Photosynthesis and respiration in the coralline algae, *Clathromorphum circumscriptum* and *Corallina officinalis* and the metabolic basis of calcification. J. mar. biol. Ass. U.K. 57: 1111–1124

Gao, K. (1992). Studies on the CO_2 fixation by seaweeds. [in Jap.] Kansai Environmental Engineering Center, Osaka (Tech. Rep.)

Gao, K., Aruga, Y., Asada, K., Ishihara, T., Akano, T., Kiyohara, M. (1991). Enhanced growth of the red alga *Porphyra yezoensis* Ueda in high CO_2 concentrations. J. appl. Phycol. 3: 355–362

Jensen, P. R., Gibson, R. A., Littler, M. M., Littler, D. S. (1985). Photosynthesis and calcification in four deep-water *Halimeda* species (Chlorophyceae, Caulerpales). Deep-Sea Res. 32: 451–464

Komatsu, T. (1989). Day-night reversion in the horizontal distributions of dissolved oxygen content and pH in a *Sargassum* forest. J. oceanogr. Soc. Japan 45: 106–115

Littler, M. M., Littler, D. S., Taylor, R. R. (1983). Evolutionary strategies in a tropical barrier-reef system: functional form groups of marine macroalgae. J. Phycol. 19: 229–237

Okazaki, M., Ichikawa, K., Furuya, K. (1982). Studies on the calcium carbonate deposition of algae. IV. Initial calcification site of calcareous red alga *Galaxaura fastigiata* Decaisne. Botanica mar. 25: 511–517

Payri, C. E. (1988). *Halimeda* contribution to organic and inorganic production in a Tahitian reef system. Coral Reefs 2: 251–262

Provasoli, L. (1966). Media and prospects for the cultivation of marine algae. In: Watanabe A., Hattori A. (eds.) Cultures and collections of algae. (Proceedings of U.S. - Japan Conference, Hakone, September 1966) Japanese Society of Plant Physiology, Tokyo, p. 63–75

Quay, P. K., Tilbrook, B., Wong, C. S. (1992). Oceanic uptake of fossil fuel CO_2: carbon-13 evidence. Science, N.Y. 256: 74–79

Smith, A. D., Roth, A. A. (1979). Effect of carbon dioxide concentration on calcification in the red coralline alga *Bossiella orbigniana*. Mar. Biol. 52: 217–225

Stark, L. M., Almodovar, L., Krauss, R. W. (1969). Factors affecting the rate of calcification of *Halimeda opuntia* (L.) Lamouroux and *Halimeda discoidea* Decaisne. J. Phycol. 5: 305–312

Stumm, W., Morgan, J. J. (1981). Aquatic chemistry. Wiley, New York

UNESCO (1987). Thermodynamics of the carbon dioxide system in seawater. Report by the carbon dioxide sub-panel of the joint panel on oceanographic tables and standards. UNESCO tech. Pap. mar. Sci. 51: 1–35

Communicated by M. G. Hadfield, Honolulu