THE PRODUCTIVITY OF HAWAIIAN FRINGING-REEF CRUSTOSE CORALLINACEAE AND AN EXPERIMENTAL EVALUATION OF PRODUCTION METHODOLOGY[1]

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ABSTRACT

Estimates of the mean net total contribution to the Waikiki reef by *Porolithon onkodes*, *P. gardineri*, and *Sporolithon erythraeum* are quite close. *Hydrolithon reinboldii* and melobesioid "C" contribute 5 and 3 times more. The net contribution to the total reef system by fringing-reef crustose Corallinaceae is 5.7 g C m^{-2} day^{-1}. Crustose coralline algae are comparable, as producers, to other photosynthetic reef organisms.

Several measurements of productivity were used in replicate on the same thalli. The ^{14}C data, although somewhat higher, were not significantly different from the O_2 and pH electrode data; rates determined by all three techniques showed the same serial order, by species, from highest to lowest producer. The pH and O_2 electrode methods are more useful and reliable than the ^{14}C data where high sensitivity is not critical.

The Winkler oxygen method has been classically applied to light and dark incubated bottles for studying benthic productivity. Its sensitivity permits significant determinations where O_2 evolution is as low as 0.15 mg $liter^{-1}$. Where O_2 evolution exceeds 0.5 mg $liter^{-1}$, polarographic O_2 analyzers are useful. Variations in CO_2 have also been used to measure photosynthesis, and changes in pH (Beyers et al. 1963) have proven to be reasonably sensitive indices to CO_2 uptake. The ^{14}C technique is the most sensitive, widely accepted, and routinely applied of the methods.

Such workers as McAllister et al. (1961) and Thomas (1964) have compared the ^{14}C method, Winkler O_2 (light-dark bottle), and pH measurements for determining the productivity of natural and cultured phytoplankton populations. Müller and Knöpp (1971) evaluated ^{14}C, O_2 electrode, and Winkler analyses in a study of primary productivity in flowing waters.

In my study, all of the above techniques in addition to others (gas chromatography, Ca electrodes, ^{45}Ca, and atomic absorption spectrophotometry) were used on the same thalli simultaneously, to measure the productivity of reef-building coralline algae.

Previous workers (e.g. Setchell 1926, 1928; Crossland 1938) have debated the relative importance of calcareous organisms in the reef environment. Biologists have classically stressed the importance of standing stock (usually in subjective terms) and geologists have emphasized sediment measurements. Few approaches have considered the functional roles played by reef-building organisms or the correlation between standing stock and the skeletal components that remain after grazing, export, and resolution. As Goreau (1963) pointed out, the missing quantity is a calcification-rate parameter. Among the three studies to date of the functional importance of crustose Corallinaceae, that of Sargent and Austin (1954) assumed from the density of crustose coralline algae on the seaward face of the reef at Rongelap Atoll that the area had high photosynthesis. They measured net oxygen production of various reef organisms in jars set in the flowing interisland waters: three measurements of a single *Porolithon* thallus yielded rates comparable to those of corals (based on sample wet weight). Goreau (1963) first measured carbon fixation and calcium carbonate deposition rates for various reef-building algae by radioisotope techniques (^{14}C and ^{45}Ca simultaneously). He reported the uptake

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rates in terms of milligram moles of C or Ca fixed per milligram N per hour (an index of cell protein), which is difficult to relate either to the standing stock of calcareous organisms or to sedimentological studies.

Marsh (1970) found the productivity of unidentified crustose Corallinaceae from Eniwetok Atoll and Kaneohe Bay, Hawaii, measured with a specially constructed dissolved-oxygen electrode, to be within the same order of magnitude but lower than that of other photosynthetic reef organisms. However Smith (1973) compared the productivity at Eniwetok and obtained results that may be contradictory to those of Marsh. Both a transect dominated (visually) by a mixture of corals and coralline algae and one dominated by a coralline algal turf (*Porolithon* and *Jania*) calcified at 4,000 g $CaCO_3$ m^{-2} yr^{-1}, but the coralline algal community showed a higher net production (24-hr day) rate than did the coral-algal community (7.20 vs. 1.56 g C m^{-2} day^{-1}). It may be that organisms not visually prominent are relatively more important metabolically than the calcifying organisms, since total community metabolism was the parameter measured.

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METHODS AND MATERIALS

The productivity of crustose Corallinaceae as measured includes the rate of fixation of particulate and dissolved organic carbon (primary and secondary productivity) as well as the rate of fixation of particulate inorganic carbon (calcification). The experimental chambers used (Fig. 1) are modifications of that of Doty (1967). The metal screws were moved outside the internal cavity to avoid contact with the culture medium. Three double-O-ring apertures were constructed in the top to seal three electrodes (pH, O_2, and Ca) airtight and a tapered stopper was fitted in one corner of the top to facilitate filling and expelling air from the chamber. The chambers hold 600 ml rather than 300 and contain a rack to support the crustose thalli on monofilament line during incubation.

The portion of each species incubated consisted of approximately equal fragments of the same thallus or similar thallus chipped from the same square meter patch of the Waikiki fringing reef at 0930 hours. Each species was sampled in the area of its peak of abundance (Littler 1973). Three to five small fragments of the freshly collected algae were placed horizontally on a support of monofilament line in each of two duplicate chambers containing Millipore-filtered seawater; a third chamber (without electrodes and covered with aluminum foil) was used as a blank to correct for dark uptake by the algae. The lids, with the electrodes inserted, were placed on the chambers and secured, and the chambers were completely filled so that no air bubbles remained. The two chambers were placed in a water bath over identical magnetic stirring motors, modified to run cooler, in a photoperiod incubator, at 24.0°C, above the light saturation intensities of the coralline algae (10,800–21,500 lux). The rate of stirring was adjusted to a standard setting and the chambers were allowed to equilibrate for about 1 hr.

During the equilibration period, initial samples were taken. A 300-ml sample of the water used had reagents added for later Winkler analysis. Two ampoules of 10 ml each were labeled and sealed for measurement (by procedures in Strickland and Parsons 1968) of initial dissolved organic carbon and calcium. All seawater was from one 40.0-liter Millipore-filtered (0.45-μm pore size) batch that was collected from just inside the algal ridge area of the Waikiki reef, filtered immediately and stored in the dark in a sterilized polyethylene carboy.

Data recording began at 1130 each day to minimize any effects of periodicity. Dissolved O_2 and pH were monitored for a 1-hr dark period, at the end of which the chambers (including the dark control)

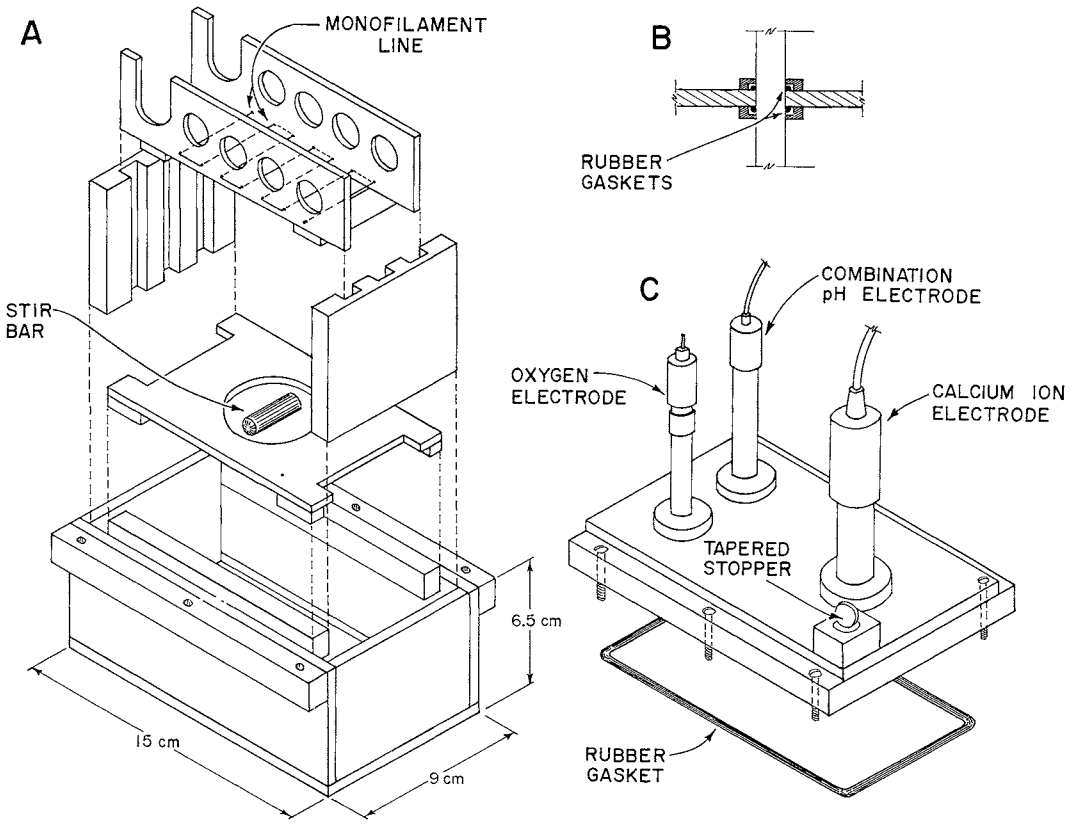

Fig. 1. One of the gastight experimental chambers used in the photoperiod incubators for determining the productivity of crustose Corallinaceae. A—an exploded view showing the component parts of the chamber; B—a sectional view showing the design of the seals with an electrode inserted; C—the design of the chamber lid with electrodes inserted.

were inoculated with ^{45}Ca and ^{14}C and pH and O_2 monitored for a 2-hr light period. Considerable care was taken (*see* Littler 1973) to ensure that the natural homeostatic capabilities of the organisms were not exceeded during the experimental exposure.

After the 3-hr incubation the algal thalli were removed from the chambers in a darkened room, rinsed with filtered seawater, and stored inside labeled envelopes in an aluminum desiccator. Water samples corresponding to the initial ones described above were taken from each chamber. The area of the algal fragments used was calculated by photographing the thalli and measuring the projected area with a planimeter. I also used the method of Marsh (1970) to estimate absolute area, but since earlier work (Littler 1973) indicated a closer correlation between primary productivity and unit of projected area the latter is used here.

Dissolved O_2 readings were converted to changes in carbon per unit of time by standard methods (Strickland and Parsons 1968), assuming a photosynthetic quotient of 1.00. Because pH changes reflect both photosynthesis-respiration reactions and calcification they were considered to be indicative of the total organic and inorganic carbon fixed. The method for conversion of pH changes to changes in carbon dioxide concentrations was described by Beyers et al. (1963). The radioisotope techniques were standard (Strickland and Parsons 1968) and a wet-ashing separation de-

Table 1. The mean gross productivity (above light saturation) of the important corallines on the Waikiki fringing reef in mg Ca or C cm^{-2} (of thallus) $min^{-1} \times 10^{-4}$; determined by the ^{14}C, ^{45}Ca, O_2- and pH-electrode techniques simultaneously. Confidence limits are given at the $P = 0.05$ level and the number of experiments is in parentheses

	^{14}C					
	Inorg. C	Org. C	Total C	^{45}Ca	pH	O_2
Melobesioid "C"	(2) 1.10 ± 1.21	(2) 0.43 ± 0.38	(2) 1.52 ± 0.59	-	(2) 1.76 ± 3.88	(2) 1.57 ± 2.54
Sporolithon erythraeum	(4) 1.48 ± 0.41	(4) 0.97 ± 0.35	(4) 2.45 ± 0.42	(4) 1.40 ± 1.01	(10) 2.72 ± 0.38	(9) 2.25 ± 0.39
Hydrolithon reinboldii	(8) 2.22 ± 0.33	(8) 1.64 ± 0.33	(8) 3.86 ± 0.51	(8) 0.93 ± 0.50	-	-
Porolithon onkodes	(12) 3.22 ± 0.64	(12) 0.70 ± 0.17	(12) 3.92 ± 0.62	(12) 1.86 ± 0.90	(15) 3.28 ± 0.36	(12) 3.23 ± 0.47
Porolithon gardineri	(2) 4.56 ± 0.44	(2) 1.60 ± 0.19	(2) 6.15 ± 0.64	(2) 1.89 ± 1.65	(2) 4.90 ± 0.95	(2) 3.75 ± 1.37

scribed by Doty (1967) was used to isolate organic carbon, inorganic carbon, and calcium carbonate activity. Daily net production rates were calculated from hourly rates by multiplying by the total hours per day when the light intensity was above the saturation intensity, minus the hourly respiration rate times the total dark hours.

RESULTS AND DISCUSSION

The rates determined by all three techniques are summarized in Table 1 and show the same serial order, by species, from highest to lowest producer. Since most experiments were performed on *Porolithon onkodes* and *Sporolithon erythraeum*, these show narrower confidence limits for all of the methods. The ^{14}C technique yielded data reasonably close to the O_2 and pH electrode data although the ^{14}C data were usually higher. Müller and Knöpp (1971) in their laboratory study found that the two oxygen methods coincided closely but were not correlated as well with the ^{14}C technique. My results agree with those of McAllister et al. (1961) and Thomas (1964), who, although not continuously following changes in productivity, did note that values obtained by ^{14}C, O_2, and pH were about equivalent.

The advantages of the isotopic technique are that inorganic carbon, organic carbon, and calcium fixation can be simultaneously measured. The disadvantages are that the equipment is relatively expensive and the chemical partitioning methods are comparatively time consuming. An inherent problem is the high rate of dark fixation of ^{14}C by coralline algae ($\sim$ 10–30% of light uptake), probably owing to absorption, adsorption, and isotopic exchange with skeletal materials. This introduces a further source of variability when the light uptake rates are corrected for dark uptake rates. Morris et al. (1971) recommend that the dark fixation values be ignored in phytoplankton work until more is known about the mechanism of dark fixation. The Corallinaceae are problematical in this respect and, because of their calcification, indeed represent a special case. However, if the dark rates were ignored, the ^{14}C figures for total carbon fixed (Table 1) would be considerably higher than those from either the O_2 or pH electrodes. Intercalibration work with noncalcareous algae might be more informative since the skeletal exchange problems would be diminished.

I found Winkler determinations to calibrate the O_2 electrodes to be unnecessary

because calibration in air or saturated seawater served the same purpose and was quicker. The electrode data show close agreement between replicates (Table 1) and there is essentially no significant ($P > 0.05$) difference between productivities determined by the pH or the O_2 electrodes for *P. onkodes* and *S. erythraeum*. Because the electronic techniques permit continuous monitoring of a given experiment and can provide instant-readout capabilities the electrode data may be more useful than the isotope data where extreme sensitivity is not required.

The ^{45}Ca uptake rates (Table 1) are highly variable. A similar high degree of variance was encountered by Goreau (1963) who presented evidence that the scatter of the data was caused by fluctuations in the nature of the algae themselves and not by the analytical technique. Because the calcium-ion electrode had a maximum sensitivity of plus or minus only about 3% of the total calcium in solution, it was insensitive to the changes in dissolved calcium of this study. The gas chromatographic analyses of the initial and final ampoule samples, although indicating that some dissolved organic carbon was being produced during the short experimental runs, were also so insensitive as to be unreliable quantitatively. The atomic-absorption measurements likewise proved insensitive to the slight uptake rates of dissolved calcium under the experimental conditions used. Although inconsistent, there was some indication that the calcium content of the water did decrease by several parts per million during most experiments.

Both respiration and photosynthesis (measured by monitoring O_2 and CO_2 changes) were linear with time during all of the experiments. Respiration accounts for about 20% of gross production, in agreement with the estimate (22–25%) of Marsh (1970). The mean gross production rates above light saturation (in mg C cm^{-2} min^{-1} $\times 10^{-4}$), computed as the average of the pH and O_2 data, were: melobesioid "C," 1.66 ± 0.50; *S. erythraeum*, 2.39 ± 0.26; *P. onkodes*, 3.43 ± 0.25; *P. gardineri*, 4.32 ± 1.30. I calculated the mean gross productivity for the 32 samples of coralline algae measured by Marsh (1970) from his figure of 0.048 mg O_2 cm^{-2} hr^{-1}, using the relationship he cited where 1 mg $O_2 \cong 0.3$ mg C. This yielded a mean production rate of 2.40 mg C cm^{-2} $min^{-1} \times 10^{-4}$, which is intermediate in the range of values for Hawaiian Corallinaceae (Table 1).

Calcifying systems are only poorly known and both the radioisotope and pH data are more complicated than would be the case in noncalcifying systems. The numbers for $CaCO_3$ dictate that the weight ratio for Ca to inorganic C uptake be about 3.3 to 1.0. The values in Table 1 show a mean measured fixation ratio of 1.0 to 1.7. Two potential reasons for part of the observed discrepancy, aside from possible errors due to ionic exchange or chemical separations before counting, are the possibility of a physiological uptake mechanism that allows the deposition of carbonate in some form other than $CaCO_3$ with a periodicity independent of photosynthesis or a large internal exchange of inorganic and organic carbon. Relevant to the first point is the finding (Moberly 1968) that the ratio of $CaCO_3$ to $MgCO_3$ deposition in coralline algae is directly related to the growth rate, with as much as 50% molar substitution of $MgCO_3$ during brief periods of growth. This alters the expected ratio to 1.7:1.0, an improvement but still far from the measured values. Perhaps bearing on the discrepancy are my observations and those of P. S. Dixon (personal communication) that various articulated Corallinaceae, while continuing to grow for months in laboratory cultures, take on a noncalcified fleshy texture even though $CaCO_3$ is supplied in the medium.

A second issue concerns the use of pH to estimate organic production in calcifying systems. The pH of the water surrounding calcifying algae changes in response not only to photosynthesis-respiration reactions but also to calcification-solution reactions (Ryther 1956). In organisms containing about as much inorganic as organic carbon it seems likely that inorganic carbon fix-

Table 2. The contribution of various populations and communities in net (N) and gross (G) g C m^{-2} day^{-1} to the total productivity of their respective reef systems

	N	G
Intertidal blue-green algae at Eniwetok (Bakus 1967)	0.65 to 2.15	-
Fourteen species of corals from Florida keys (Kanwisher and Wainwright 1967)	-	2.7 to 10.2
Entire reef communities (Sargent and Austin 1949, Odum and Odum 1955, Kohn and Helfrich 1957, Qasim and Sankaranarayanan 1970)	-	4 to 8.3
Two reef communities (Smith 1973)	1.56 and 7.20	3.00 and 8.64
Unidentified crustose Corallinaceae from Eniwetok and Hawaii (Marsh 1970)	0.66	1.5
Five species of Hawaiian crustose Corallinaceae (this study)	0.5 to 2.6	0.6 to 3.4

ation should be a significant fraction of the total carbon fixation. The agreement between pH and O_2 numbers (Table 1) suggests that most of the CO_2 change under my experimental conditions is due to photosynthesis rather than calcification. This again might imply a peculiar physiological periodicity in $CaCO_3$ deposition (not coupled to photosynthesis) to explain the values obtained. Another alternative has been suggested by S. V. Smith (personal communication) involving two sources of error that calcification imposes on the Beyers et al. (1963) pH/CO_2 curve: the slope of the curve would be steepened, so that pH would overestimate both net production and respiration, and the entire curve displaced lower on the graph by changes in the total alkalinity, thus partially compensating for the first effect on net production but enhancing the error on respiration. In looking at net production, one might therefore generate an empirically satisfactory relationship between pH changes and O_2 productivity that would in reality be deceptive in terms of function. However, if this were occurring here then pH and O_2 numbers for respiration should differ, with those derived from pH data considerably higher. This still does not explain my data, as there was no significant difference ($P > 0.05$) between the pH and O_2 respiration values; so something peculiar seems to have been taking place with regard to $CaCO_3$ metabolism in these algae.

My data, although statistically reproducible, admittedly lead to problems of interpretation and for this reason must be considered somewhat preliminary. However, they are useful in showing the inadequacy of our present knowledge of the physiology of calcifying systems and suggesting areas for further investigation. For instance, one might attempt to partition the pH changes observed into those due to calcification and those due to photosynthesis by following changes in the total alkalinity, as was effectively done by Smith (1973). Studies of periodicity and stress effects on $CaCO_3$ deposition rates might prove rewarding.

With the above unresolved problems in mind, I calculated the net productivity of each crustose alga (average of pH and O_2 data) in conjunction with its cover (Littler 1973) on a square meter basis to evaluate the role of crustose Corallinaceae and their contribution to total reef production at Waikiki. Although production rates vary between species and within species, depending on environmental conditions (Littler 1973), the estimates of the mean total contribution of *P. onkodes*, *P. gardineri*, and *S. erythraeum* are quite close, about 0.5 g C m^{-2} (of reef) day^{-1}. *Hydrolithon reinboldii* and melobesioid "C," because of their dominance in cover (10.6% and 17.0%), contribute considerably more to the overall fringing-reef production, 2.6 and 1.5 g C m^{-2} (of reef) day^{-1}.

Table 2 compares the contribution to total reef production of Hawaiian crustose Corallinaceae with that of other reef primary producers. The productivity of Hawaiian species lies within the range reported (in terms of carbon fixed) for most of the organisms in Table 2. The rates reported by Marsh (1970) for coralline

algal contributions to certain reef tops at Eniwetok are somewhat lower, but one would expect to find differences in carbon incorporation rates of the species themselves since they were taken from different environments. Goreau (1963) also reported differences that ranged from 3.5 to 60.0 mg moles of C mg N^{-1} hr^{-1} for coralline algae from Jamaica. The total contribution calculated for Hawaiian fringing-reef crustose Corallinaceae taken together, 5.7 g C m^{-2} (of reef) day^{-1}, lies within the range of values previously reported (Table 1) and the productivity values of the individual species indicate that they are comparable to other photosynthetic reef organisms in their role as primary producers.

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