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THE EFFECTS OF BOTTLE VOLUME, THALLUS WEIGHT, OXYGEN SATURATION LEVELS, AND WATER MOVEMENT ON APPARENT PHOTOSYNTHETIC RATES IN MARINE ALGAE

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ABSTRACT

Littler, M.M., 1979. The effects of bottle volume, thallus weight, oxygen saturation levels, and water movement on apparent photosynthetic rates in marine algae. *Aquat. Bot.*, 7: 21—34.

The importance of container volume, thallus weight, O_2 tension, and water motion to measurements of photosynthetic rates in *Ulva lobata* (Kütz.) Setchel & Gardner (a species with a relatively high photosynthetic rate) and *Colpomenia sinuosa* (Roth) Derb. & Sol. (relatively low photosynthetic rate) were examined by means of O_2 changes in bottles and through pH and O_2 flux in continuously monitored chambers. The photosynthetic rate of *Ulva* averaged 36% lower in 310-ml bottles than in 1220-ml jars. Apparent photosynthesis varied as a function of (1) the initial dissolved O_2 levels (regardless of bottle size), and (2) the particular metabolic reactions of the individual producers to different O_2 tensions. Although similar trends were observed for *Colpomenia*, the effects of bottle size and initial O_2 concentration were not as pronounced as for *Ulva*. The larger thallus weights of both species resulted in lower photosynthetic values, even in the large containers, due to clumping and self-shading of individual thalli. Mixing had a greater effect on apparent photosynthesis than on dark respiration, and was more critical for *Ulva* than for *Colpomenia*. Specific recommendations are put forth to deal with problems arising from the variations in methodology examined.

INTRODUCTION

Photosynthesis studies of marine macroalgae have traditionally been made by enclosing the organisms in diverse sizes and shapes of containers (i.e., light and dark "bottles") and incubating them under varying times and conditions. However, the published information regarding the impact of such dissimilar treatments on measurements of macroalgal photosynthesis has not been critically evaluated. Consequently, it has become increasingly difficult to compare results from different studies or examine the validity of extrapolations to natural situations. For planktonic communities, the effects of enclosure have been shown (Verduin, 1959, 1960; Hepher, 1962) to reduce apparent photosynthetic rates; the bottle method has been demonstrated

(Wetzel, 1965; Hartman and Brown, 1967) to be inadequate for production measurements of aquatic vascular plants because of their internal retention of gaseous O_2. Others have commented briefly on the ramifications of thallus weight (e.g., Wood, 1968; Mantai, 1974; Buesa, 1977; Wallentinus, 1978), stirring rate (e.g., Wetzel, 1965; UNESCO, 1973; Buesa, 1977), length of incubation (e.g., Verduin, 1960; Hepher, 1962; Johnston, 1969; Buesa, 1977; Wallentinus, 1978), and bottle size (e.g., Johnston, 1969; UNESCO, 1973; Buesa, 1977). Apparent photosynthesis in some marine plants has been shown (Black et al., 1976; Downtown et al., 1976; Burris, 1977; Dromgoole, 1978a) to be sensitive to oxygen concentration.

This paper examines the degree to which variations in bottle volume, thallus weight, incubation time, oxygen tension, and mixing rates may interact to affect the assessment of primary productivity in marine macroalgae, and suggests some guidelines for dealing with the problems encountered.

METHODS AND MATERIALS

All photosynthesis experiments were conducted in the laboratory in two Percival photoperiod incubators maintained at 7500 lux (140 μE $m^{-2} s^{-1}$) by cool-white fluorescent bulbs. Incubation temperatures approximated those in the field at the time of collection (summer), i.e., 20° C for all experiments except the assessments of stirring effects which were done in the spring at 12.5° C. Comparisons were made between the saccate, cushion-form *Colpomenia sinuosa* (Roth) Derb. & Sol. and the sheet-like *Ulva lobata* (Kütz.) Setchell & Gardner, which have been documented by Littler and Murray (1974) as having low and high photosynthetic rates, respectively, relative to other morphological forms of marine macroalgae. Both were collected submerged and immediately returned to the laboratory for experimentation.

Bottle experiments

Net photosynthesis was determined on randomly-selected whole plants using Beckman Field Lab O_2 analyzers and electrodes, with 310-ml BOD bottles or 1220-ml wide-mouth clamp-lid canning jars as the incubation containers. The Beckman instrument exhibits close agreement with Winkler-determined O_2 values and has a linear response to dissolved O_2 concentrations (including supersaturated levels). The bottles and jars had been cleaned in aqua-regia and aged in distilled water. The sea water used during the incubations (33 ppt salinity) was taken at the time and place of algal collection (i.e., lower Newport Bay, California) and was immediately filtered through a nannoplankton net (pore size 10 μm) to remove most planktonic organisms. To test the effect of O_2 content on measurements of photosynthesis, the sea water was bubbled by means of an airstone with (1) N_2 for 15.0 min, (2) air for 5.0 min, and (3) O_2 for 45 s; these treatments provided low (0.7 ppm), saturated (6.6 ppm), and supersaturated (15.5 ppm) levels of O_2. Because very

TABLE I

The thallus mean dry weights, ranges, and dry weight/volume ratios for both bottle and continuous monitoring experiments

Species and experiment	Thallus weights (g)		Weight/volume ratios ($g\ l^{-1}$)		
	Mean	Range	Bottles (310 ml)	Jars (1220 ml)	Chambers (870 ml)
Ulva lobata					
Bottle experiment	0.036	0.015—0.059	0.116	0.030	
	0.128	0.061—0.307	0.413	0.105	
Continuous monitoring					
O_2 effects	0.065	0.053—0.075			0.075
Stirring effects	0.023	0.019—0.027			0.026
Colpomenia sinuosa					
Bottle experiment	0.030	0.015—0.061	0.097	0.025	
	0.058	0.039—0.084	0.187	0.048	
Continuous monitoring					
O_2 effects	0.095	0.077—0.113			0.109
Stirring effects	0.112	0.089—0.134			0.129

little "free" CO_2 exists at the pH of sea water (8.4), the bubbling procedure probably had a negligible effect on available carbon. Individual bottles were slowly submerged and filled, thus excluding all air from these stocks of incubation water. Several bottles were used to determine the initial dissolved O_2 content for each stock during every experiment following calibration in air.

Two thallus dry weight ranges were used for each of the species tested to yield the range of thallus weight/volume ratios given in Table I. Several whole thalli per bottle, rather than torn or cut fragments, were selected randomly to control for age or physiological differences; this accounts for the broad ranges (Table I) in individual thallus weights. All 317 incubations (4.5—6.0 h each) were carried out between 09.00 and 17.50 h to reduce differences due to possible daily photosynthetic periodicities.

During incubation, the bottles were stirred by means of air-driven magnetic stirrers at 10-min intervals and systematically rotated in position between the two incubators to ensure equal light conditions during the 4.5–6.0-h experimental intervals.

After the O_2 levels were recorded, individual thalli were carefully separated, spread, and photocopied; projected area determinations were made from each photocopy by means of a point-intercept method. Thalli were then dried at 60° C until they reached constant weight. All O_2 values were converted to g C fixed m^{-2} of thallus h^{-1} and to mg C fixed g dry $wt.^{-1}$ h^{-1} as outlined in Strickland (1960). A photosynthetic quotient of 1.00 was assumed to enable easy interconvertability with other data where different PQ values were used. The effects of the various treatments were examined statistically by single factor analysis of variance and the Newman—Keuls multiple range test (Sokal and Rohlf, 1969).

Continuous monitoring experiments

A second set of 23 incubations consisted of simultaneously monitoring net photosynthesis via both O_2 and pH at low, saturated, and supersaturated O_2 levels. Algal thalli (0.07 dry g l^{-1} *Ulva*, 0.11 dry g l^{-1} *Colpomenia*) were placed in specially constructed 870-ml plexiglas chambers fitted with pH and O_2 electrodes (see Littler, 1973 for details). Oxygen evolution was measured every 10 min using five Field-Lab analyzers; pH was measured at 6-min intervals with an Orion digital pH meter (Model 801), printer, automatic electrode switch, and Bradley—James electrodes. At the end of each experiment, thallus dry weights were determined as above. Changes in pH were converted to changes in CO_2 concentration by the standard procedures developed by Beyers (1970) using a CO_2 vs. pH function previously determined for the medium, and then to g C m^{-2} of thallus or mg C fixed g dry $wt.^{-1}$. The slopes of the O_2 production and CO_2 uptake regression lines were computed statistically and compared for the different conditions and measurement techniques. The incubation conditions were the same as those described above for the bottle experiments, except air-driven stir bars under a perforated partition in each container maintained a constant flow of medium past the algae and electrodes.

Additionally, a set of 21 incubations were used to compare stirred with unstirred chambers to assess the effects of mixing rate on both apparent photosynthesis and dark respiration determined by pH. Dissolved O_2 levels were initially at air saturation when the mixing experiments were begun. Because field temperatures were colder at the times of the mixing experiments, the incubations were carried out at 12.5°C and the mean thallus weight/volume ratios were 0.03 dry g l^{-1} for *Ulva* and 0.13 dry g l^{-1} for *Colpomenia*.

RESULTS

Bottle experiments

The synergistic effects of bottle volume, O_2 saturation levels, and thallus size on photosynthesis per g dry weight of *Ulva lobata* are summarized in Fig. 1. The photosynthetic rates measured in the 310-ml BOD bottles averaged about 64% of those in the 1220-ml jars and were consistently lower under all treatments. In the 310-ml bottles, apparent photosynthesis decreased as a function of increases in the amount of dissolved oxygen initially present for both thallus weight means examined (i.e., 0.036 and 0.128 g). However, the average rates for thalli with means of 0.128 g were 63% lower than determined for those with average weights of 0.036 g.

In the case of the larger jars, apparent photosynthesis of *Ulva lobata* decreased with increases in the initial levels of dissolved O_2 present for both ranges of thallus weights used (Fig. 1). The mean photosynthetic rates for the larger thalli were also lower (45%) than for the smaller thalli. Irrespective

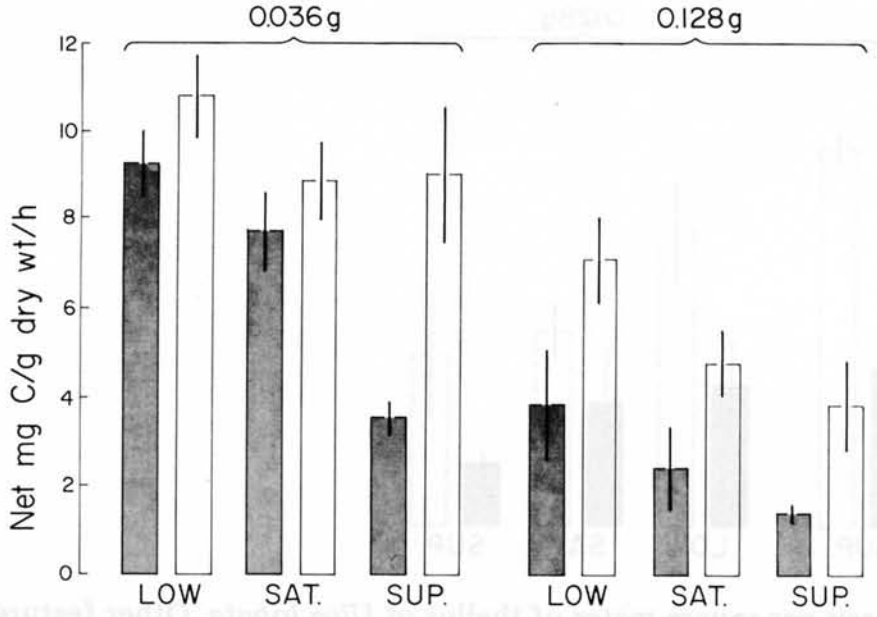

Fig. 1. Apparent photosynthesis per gram dry weight of *Ulva lobata* as a function of the interacting effects of bottle size, mean thallus weight, and initial dissolved O_2 tension (low, saturated, and supersaturated). The darker histograms are for the 310-ml bottles, the lighter histograms are for 1220-ml jars. ± 95% confidence intervals are given by the straight lines at the top of each histogram.

of bottle size or weight of thallus, the rates under the lowest initial O_2 levels were significantly ($P<0.05$) greater than those under the highest levels, except for the thalli averaging 0.036 g in the 1220-ml jars. For this last combination (Fig. 1), the quantity of O_2 initially present had no significant effect on apparent photosynthesis. When photosynthesis was calculated on the basis of thallus area for *U. lobata* (Fig. 2), patterns identical to those described above were obtained with only slight changes in the magnitude of the differences.

In *Colpomenia sinuosa* (Fig. 3), the differences were not as pronounced as for *Ulva lobata*. For example, the measured photosynthetic rates as a function of thallus dry weight in the 310-ml bottles averaged only 12% lower than in the 1220-ml jars. Even though the latter consistently yielded slightly higher values, none of the differences were statistically significant ($P<0.05$). Apparent photosynthesis in the 310-ml bottles decreased with increasing levels of initial dissolved O_2 for both ranges of thallus weights used; however, the rates for the smaller thalli averaged 39% higher than those for the larger thalli.

Photosynthetic rates of *Colpomenia sinuosa* in both the 1220-ml jars and 310-ml bottles (Fig. 3) decreased with increased initial levels of dissolved O_2. In the 1220-ml jars, photosynthetic rates of the larger thalli (mean = 0.058 dry g) averaged 29% lower than for the smaller thalli (mean = 0.030 g). For *C. sinuosa*, the effects of initial O_2 concentration were not as dramatic as in *Ulva lobata*, and most differences, irrespective of thallus weight or bottle size, were non-significant ($P > 0.05$).

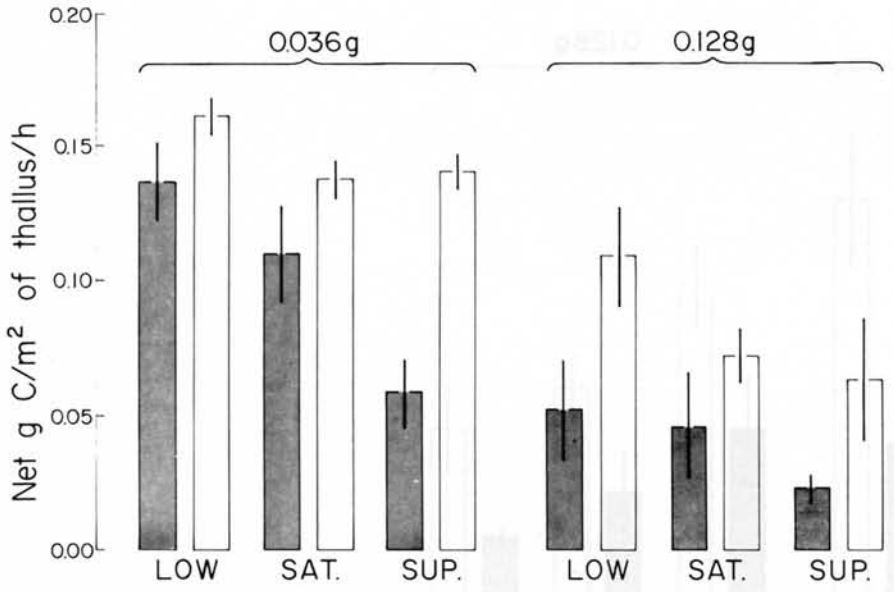

Fig. 2. Apparent photosynthesis per square meter of thallus of *Ulva lobata*. Other features are the same as in Fig. 1.

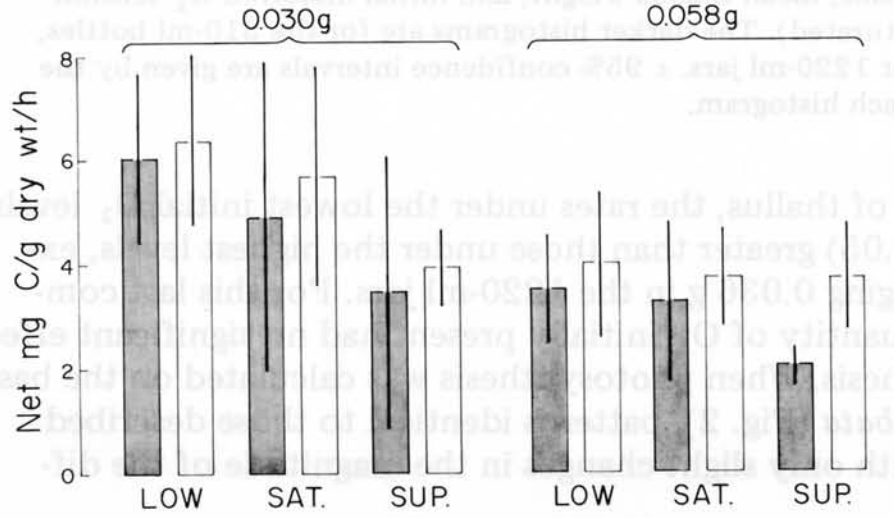

Fig. 3. Apparent photosynthesis per gram dry weight of *Colpomenia sinuosa*. Other features are the same as in Fig. 1.

Continuous monitoring experiments

A typical time series of photosynthesis measured by both pH and O_2 evolution under the three levels of initial O_2 concentration is presented for *Ulva lobata* in Fig. 4A, B, and C. The pH technique yielded rates that were somewhat lower (mean reduction of 24%) as the concentration of initial dissolved O_2 increased. However, more pronounced differences were shown by the O_2-electrode method. At low initial levels of O_2, the O_2 electrode and pH techniques gave identical rates; but at successively higher levels of dissolved O_2, the O_2 electrode yielded correspondingly lower values and severely underestimated net photosynthesis by a factor of four (as compared with that measured by pH) at supersaturation (Fig. 4C).

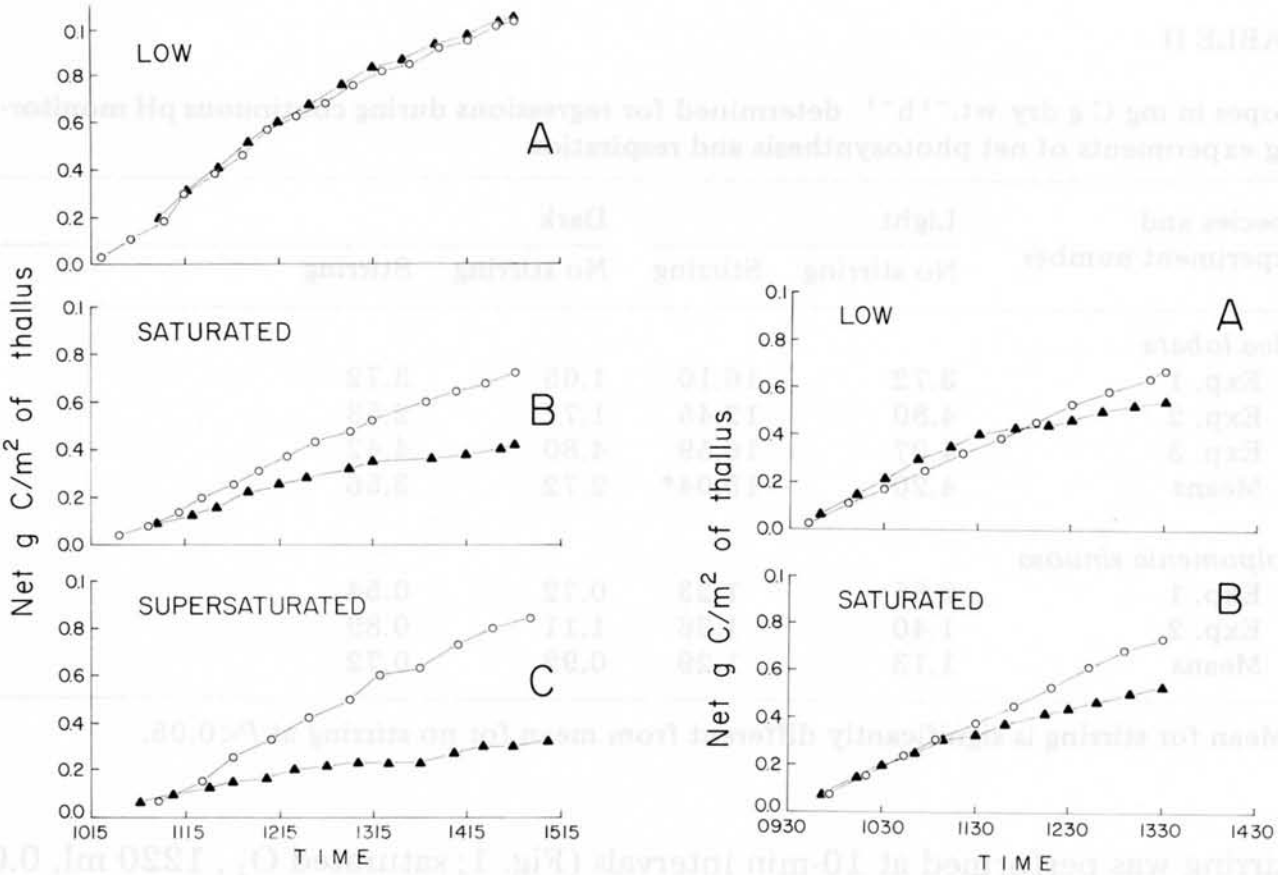

Fig. 4. Apparent photosynthesis of *Ulva lobata* as measured by both pH (○) and O_2 (▲) techniques at low (A), saturated (B), and supersaturated (C) initial levels of O_2.

Fig. 5. Apparent photosynthesis of *Colpomenia sinuosa* as measured by both pH (○) and O_2 (▲) techniques at low (A) and saturated (B) initial levels of O_2.

An example of continuous pH and dissolved O_2 monitoring for *Colpomenia sinuosa* under low and saturated levels of initial O_2 is given in Fig. 5A and B. As was the case for *Ulva lobata*, the pH method produced comparable apparent photosynthetic rates at both low and saturated O_2 levels (rates of 0.17 and 0.18 g C m^{-2} h^{-1}, respectively). The photosynthetic rate assessed by the O_2 electrode was consistent with the pH electrode during the first 2 h at low initial O_2 levels, but gave similar values for only the first hour and a half under saturated O_2 conditions (Fig. 5A and B). Also, the discrepancy between O_2 and pH measurements of apparent photosynthesis was more pronounced (~30% greater) at the end of the experiment under the higher initial O_2 levels. For both *Colpomenia* and *Ulva*, small gaseous bubbles (total volume <0.5 ml) appeared in the chambers in approximately equal amounts, irrespective of initial O_2 concentration.

The experiment to assess the effects of continuous stirring vs. non-stirring (Table II) resulted in a considerably larger effect on measurements of net photosynthesis than on dark respiration. Additionally, (*Ulva lobata*) showed a significant (3.6 fold) increase in apparent photosynthesis in stirred vs. unstirred chambers. When ~0.03 g l^{-1} of *U. lobata* was stirred continuously (Table II), its photosynthetic rate was about 1.6 times that obtained when

TABLE II

Slopes in mg C g dry wt.$^{-1}$ h^{-1} determined for regressions during continuous pH monitoring experiments of net photosynthesis and respiration

Species and experiment number	Light		Dark	
	No stirring	Stirring	No stirring	Stirring
Ulva lobata				
Exp. 1	3.72	16.10	1.65	3.72
Exp. 2	4.80	12.45	1.72	2.53
Exp. 3	4.07	16.59	4.80	4.42
Means	4.20	15.04*	2.72	3.56
Colpomenia sinuosa				
Exp. 1	0.85	1.23	0.72	0.54
Exp. 2	1.40	1.36	1.11	0.89
Means	1.13	1.29	0.92	0.72

*Mean for stirring is significantly different from mean for no stirring at $P<0.05$.

stirring was performed at 10-min intervals (Fig. 1; saturated O_2, 1220 ml, 0.036 g). There was no significant effect ($P > 0.05$) of mixing on either photosynthesis or dark respiration, relative to still water for *Colpomenia sinuosa* (Table II). When apparent photosynthesis was compared for similar weight/volume ratios (~0.13 g l^{-1}), continuous mixing (Table II) did not result in a photosynthetic increase over intermittent mixing (Fig. 3; saturated, 310 ml, 0.058 g).

DISCUSSION

The ratio of bottle volume to weight of algal material was shown to have a dramatic impact on measurements of apparent photosynthesis in marine macroalgae (Figs. 1—3) — the primary result being a considerable lowering of rates in the smaller bottles used. Much of this effect has been attributed to depletion of nutrients (e.g., Buesa, 1977), or excretion of auto-inhibitory substances has been suggested (Findenegg, 1965) with little supportive data. A more plausible explanation (see Dromgoole, 1978b) may be that carbon is depleted earlier at the higher weight to volume ratios.

Buesa (1977) examined the production rates of *Thalassia testudinum* Banks ex König and *Caulerpa cupressoides* (West) C. Ag. in 77, 286, 546, and 1214-ml bottles using mean thallus weights (0.16 g l^{-1} for *T. testudinum* and 0.12 g l^{-1} for *C. cupressoides*) within the range of those of the present study. Although the same ratio of plant material to sea water was consistently maintained regardless of bottle size, Buesa (1977) obtained net photosynthesis and dark respiration values in the 77—546 ml bottles that were only about one half those obtained in the 1214-ml bottles for both species. The cause of the bottle-size effect recorded by Buesa remains obscure and would not be easily

attributable to a surface bacteria effect (Hepher, 1962) because of the relatively large ratio of photosynthesis/respiration in marine macrophytes. Perhaps to maintain the constant algal weight/volume ratios in the smaller bottles, Buesa had to use torn or cut fragments of thalli which has been shown (UNESCO, 1973) to lower production rates in some marine algae. It is clear that studies in the laboratory using very small discs or segments cut from only a few thalli can lead to interpretative problems if such measurements are extrapolated to natural field conditions. For example, cut thalli had to be washed in sea water for several hours prior to measurements (Dromgoole, 1978a), or inordinately large dark respiration values would be obtained. Some of this effect may have been non-biological and due (Dromgoole, 1978a) to release of phenolic compounds and their subsequent oxidation.

During the present investigation, the lighter thalli in the small bottles outperformed the heavier thalli in the large jars (Figs. 1—3) at both low and saturated levels of initial O_2; performance at supersaturation was nearly equal in all cases. However, the lower weight/volume ratios should predictably have favored the larger jars (i.e., 0.105 g l^{-1} jars vs. 0.116 g l^{-1} bottles for *Ulva;* 0.048 g l^{-1} jars vs. 0.097 g l^{-1} bottles for *Colpomenia*). This finding is in marked contrast to the observation of Buesa (1977) and is attributed to competition for nutrients or CO_2 and the mutual self-shading of individual thalli even when surrounded by an ample volume of sea water.

The amount of thallus selected for incubation had a pronounced effect on measured photosynthetic rates (Figs. 1—3) for many of the same reasons that bottle volume was important. Wood (1968) credited the decreased values (2—10 fold) as a function of increased amounts of *Cladophora* (0.1—1.4 dry g l^{-1}) to light limitations due to self-shading. Adams and Stone (1973) used roughly double the amount of *Cladophora* per volume of water as did Wood with correspondingly lower photosynthetic rates. Also, a seven-fold decrease in photosynthesis of benthic *Cladophora* populations over a nine-fold range of thallus dry weights/100 cm^2 of substratum (0.02 dry g l^{-1} to 0.29 g l^{-1}) was reported by Pfeifer and McDiffett (1975). These workers felt that mutual shading of the algal filaments might be as important as nutrient competition or excretion concentration in reducing the production efficiency.

Buesa (1977) examined the effect of a range of thallus weights (0.004—3.80 dry g l^{-1} on apparent photosynthesis for 11 species of marine macrophytes using 1214-ml bottles. The results varied from species to species but the highest values for both photosynthesis and respiration were obtained when weight/volume ratios were between 0.020 and 0.195 g l^{-1}. Inhibition of primary productivity in *Ceramium tenuicorne* (Kütz.) Waern was recorded (Wallentinus, 1978) as a function of increasing length of incubation time and sample size incubated. For the largest thalli incubated (~0.6 g l^{-1}), productivity measured over a 3.0 h period was 50% lower than for the smaller thalli (~0.01 g l^{-1}). Thallus concentrations intermediate to these showed ~25% reductions in productivity for 6.0 h vs. 1.0 h incubation times. Johnston (1969) stated that if a tissue to incubation volume ratio of 0.1—0.3 dry g l^{-1}

was employed, no nutrient or carbon deficiency occurred in experiments lasting up to 24 h. In the present study (Figs. 1—3), highest rates of photosynthesis were obtained (over 4.5—6.0 h-periods) when average thallus weights did not exceed 0.048 g l^{-1} of sea water and when thallus areas were kept below 30 cm^2, irrespective of bottle volume.

The consequences of water movement on measurements of apparent photosynthesis were striking (Table II). The greatest effect was shown for *Ulva lobata* where continuous measurements made over a non-stirred period were considerably lower (73%) than over a period of vigorous stirring. I interpret the difference to a build-up of concentration gradients within the system; insufficient flow rate past the electrode is usually not a problem in pH measurements. Such differences are presumably representative of real reductions in photosynthesis due to the build-up of diffusion barriers at the plant/water boundary layer. Kanwisher (1966) observed a doubling of photosynthetic O_2 production following shaking during respirometer studies of marine macroalgae. Buesa (1977) also confirmed a similar response to mixing for marine macrophytes in his report of 34% photosynthetic reductions in non-shaken vs. shaken bottles. The rate of photosynthesis in the marine macroalga *Padina* was recorded (UNESCO, 1973) to be 61% lower in unstirred vs. stirred bottles. The effects of current flow (2.1 cm s^{-1}) on benthic *Cladophora* populations (Pfeifer and McDiffett, 1975) led to 12 and 33% increases in net photosynthesis and dark respiration, respectively, over values obtained in still water. Similarly (Whitford and Schumacher, 1961), the benthic alga *Oedogonium kurzii* Zeller had 70% greater dark respiration rates in shaken vs. non-shaken flasks. In the case of *Colpomenia sinuosa* (Table II), continuous mixing did not result in an increase in photosynthesis over that in still water or intermittent mixing. The difference is probably related to (1) the relatively low surface/weight ratio of *Colpomenia* (0.2 cm^2 mg^{-1} vs. 0.6 cm^2 mg^{-1} for *Ulva*) which would reduce its uptake and exchange potentials even in vigorous currents, and (2) its low productivity which would result in smaller diffusion gradients than in a high producer such as *Ulva*.

An abrupt and consistent pulse in dark respiration (lasting several minutes) occurred following the light treatments. Dromgoole (1978a) and Burris (1977) also recorded a transient respiratory increase when algal material was transferred to the dark following high light conditions. When CO_2 production at the time of darkening is greater than steady-state dark respiration, photorespiration is indicated (Tregunna et al., 1966; Jackson and Volk, 1970; Burris, 1977). No effect of stirring was evident during longer (1.0 h) measurements of dark respiration (Table II) following this post-illumination burst in CO_2 production.

Bubbles can result from degassing on surfaces (e.g., sides of the container, stir bars, partitions, algal thalli) at warmer temperatures than the sea water or by metabolic O_2 production at high levels of supersaturation. Bubble formation may have serious consequences depending on the volume of the gas and the extent to which the gas and liquid phases are in O_2-tension equilibrium.

However, the formation of small bubbles apparently had little appreciable effect on measurements of dissolved O_2 in the sea water medium because both pH- and O_2 -derived numbers were quite close (Figs. 4A and 5A). Buesa (1977) also reported a similar observation. He compared bottles containing 2.5 ml of air to those without air and found a slight reduction (8%) in the bottles with air, although the difference was not statistically significant ($P>0.9$). Buesa's findings could be due to low stirring or shaking which may not have maintained equilibrium between the gas and liquid phases thereby reducing the error. Because vigorous shaking or stirring is desirable for other reasons, bubble-formation should be avoided whenever possible.

The effect of variations in dissolved O_2 levels is complex (Figs. 4A, B, C, and 5A, B). There would seem to be several possible interpretations. First, it has been documented that apparent photosynthesis in marine plants (Black et al., 1976; Downtown et al., 1976; Dromgoole, 1978a) as well as dark respiration (e.g., Dromgoole, 1978a) are quite sensitive to O_2 concentration. This photosynthetic decrease under increased concentrations of O_2 — the Warburg effect — is considered (Jackson and Volk, 1970; Black et al., 1976; Burris, 1977) to be an indication of photorespiration. Secondly, the sensitivity, and thus accuracy, of the O_2 electrodes may decrease as a function of very high concentrations of O_2 (i.e., beyond saturation); this is not likely because the response has been shown (Beckman Instruments, Inc., 1972) to be linear at O_2 levels in sea water higher than those used in the present research. Thirdly, and related to (1) the assumption of a PQ of 1.00 may not be justified because of the possibility that PQ might alter with increased O_2 tension and this may also contribute to the discrepancy in the two methods (in addition to any electrode error). In general, O_2 evolution in marine macroalgae seems to be inhibited by high O_2 tensions (Downtown et al., 1976; Dromgoole, 1978a) as is ^{14}C uptake (Black et al., 1976; Burris, 1977); however, this may be highly species specific, since photosynthesis in the marine macroalga *Chaetomorph* sp. was highest under increased O_2 tension (Burris, 1977). Also, the impact of O_2 concentration was shown (Beardall et al., 1976) to be very species specific for five marine phytoplankton species. Over a pH range from 6.5 to 7.9, Dromgoole (1978a) found a consistent level of inhibition of net photosynthetic O_2 output as O_2 concentration increased up to 200% saturation for *Carpophyllum maschalocarpum* (Turn.) Grev. Dromgoole (1978a) suggested that the increased inhibition of apparent photosynthesis of *C. maschalocarpum* above levels of O_2 that were saturating for dark respiration (i.e., >100% saturation) indicated either a photorespiratory response or an O_2 inhibition of true photosynthesis. The decreases in photosynthetic rates for the macroalgae *Sargassum* and *Enteromorpha*, and the microalgae *Chlorella*, *Glenodinium*, and *Thalassiosira* at increased O_2 levels were attributed solely to photorespiration by Burris (1977). Nonetheless, the present data (Fig. 4A, B, C) clearly show not only a reduction in CO_2 uptake at the higher O_2 levels (mean decrease of ~24% as measured by pH) but a dramatic decline in photosynthetic O_2 production relative to CO_2 uptake at high O_2 levels in sealed containers.

SUMMARY

It would appear from the results of this study and others that thallus weight/volume ratios should be optimized — highest values were consistently obtained for ~0.030 g l^{-1} — while assuring that the specimens used are representative of the organism being investigated and that enough material is incubated to generate measurable metabolic responses. Even in relatively voluminous containers, small replicate specimens can clump and tend to compete with and shade each other, a situation which will surely lead to lowered apparent photosynthetic rates. Therefore, in the case of the small algae it would be far better to incubate one, or a few, individuals in a smaller bottle than to use many thalli in a larger bottle. In the case of large algal forms, the proportions of the container should be commensurate with the size and metabolic rate of an entire representative thallus (whenever practicable). For comparative purposes, it is imperative that thallus dry weight/volume ratios be reported. The length of the incubation period should be optimized because this factor interacts with the bottle volume/thallus weight ratios. Therefore, it is just as important to specify the incubation time so that the magnitude of changes in O_2, CO_2, and pH can be calculated in comparative studies.

Because the effects of changes in O_2, CO_2, and pH (and possibly nutrient depletion) are likely to vary with individual species, the continuous monitoring method, utilizing electrodes in chambers, has a distinct advantage over bottle experiments where only initial and final O_2 values are used to calculate photosynthetic rates.

The use of cut discs or segments (as well as rough handling) is to be avoided, depending on the nature of the experimental design, because such phenomena as "wound" respiration and exudation of organic matter often lead (Dromgoole, 1978a) to unnaturally low measurements of net photosynthesis.

The present data, and that previously published, consistently show that some means of agitation is required to obtain realistic production rates in closed containers. This requires that procedures leading to bubble formation be avoided as this can be problematical depending upon the bubble volume and the extent to which the gas and liquid phases are in equilibrium. However, mixing would appear to be more critical for highly productive forms having relatively high surface/weight ratios. Since shading is difficult to avoid during hand shaking of bottles, I have found air-driven magnetic stirrers operated by a foot pump to be most effective; also considerably more turbulent circulation is produced per unit time. Additionally, I strongly recommend supplementing of O_2-electrode data with some other method (e.g., pH, ^{14}C, Winkler) when experiments are to be conducted at high O_2 levels. Supplementation with the pH or ^{14}C technique would have the added advantage of providing an estimate of the PQ.

Oxygen supersaturation also poses other problems in measuring the productivity of marine algae in closed systems. Dromgoole's (1978a) data indicate that macroalgae can more rapidly effect equal changes in O_2 content at

low levels than at high levels of initial dissolved O_2. It is clear that estimations of productivity based on light/dark closed containers will vary as a function of (1) the O_2 concentration used as well as (2) the relative reactions of photosynthesis and dark respiration in the producers under study to different O_2 tensions. In remote field situations it is not always possible to use N_2 or other gases to reduce the supersaturated levels of O_2 typically present near highly-productive nearshore habitats and available CO_2 might also be decreased in the process. Instead, the vigorous pouring or shaking of a stock incubation aliquot is recommended (Strickland, 1960) until air saturation is attained. Alternatively, incubation water below saturation can be repetitively obtained from productive communities at a fixed time prior to sunrise when O_2 levels have been naturally reduced by night respiration. Depending on the nature of the experiment, reasonable field comparisons must be made under uniform conditions of initial dissolved O_2 and the initial tensions of the light and dark bottles should be given.

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