Reducing the error in estimating annual production of benthic microflora: hourly to monthly rates, patchiness in space and time

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ABSTRACT: A formula based on the measured relation between solar radiation and photosynthetic rate was developed for computation of the annual productivity of benthic microflora. The saturating light level of 1260 μE m^{-2} s^{-1} obtained with intact sediment cores was > 5 × higher than reported in other studies. This, together with the invalid assumption that measurement periods were representative of average conditions, accounted for discrepancies among estimates when 3 formulas employed in other studies were applied to our data. The new formula developed in this study is likely to be the most reliable estimator of annual productivity, since it is based on measured relations between photosynthesis and irradiance and does not assume that measurement periods were representative of average conditions. Its drawback is that it requires laborious analysis. In addition to the annual study, intensive sampling was conducted during a single month (60 samples incubated every other day) to obtain an estimate closely approximating actual monthly production. By using this close-to-direct measurement of monthly production, we were able to assess quantitatively the sources of error likely to be introduced by converting the hourly rates to monthly production using two of the formulas. This error was compared to the error likely to be introduced by spatial heterogeneity and by temporal heterogeneity (i.e. different sampling rates). Results indicate that the error introduced by inadequate sampling in space and time outweighs the error introduced by the commonly used conversions from measured midday productivity to estimated monthly production. Compositing many small samples from a study area into each incubation chamber efficiently addressed the problem of spatial heterogeneity. Monthly production estimated from 4 sampling days per month agreed closely with monthly production determined from 16 sampling days (mean deviation 5 %). Estimates based on 1 or 2 sampling days mo^{-1} were not in close agreement with the 16 sampling day estimate (mean deviation 30 %).

INTRODUCTION

An array of assumptions is embedded in the estimation of annual productivity, since it is many steps removed from the hourly rates on which it is based. The assumptions made in taking these steps can have marked effects on the final estimate, regardless of the quality or quantity of the original measurements. These assumptions fall into 2 general categories: assumptions used to make the hourly rates representative of the entire day, and assumptions used to make the daily rates representative of the entire month.

In this paper we compute annual productivity using a series of 4 formulas of increasing complexity, where successively more is measured and less is assumed. By relating the behavior of the different estimators of productivity to conditions actually observed, we evaluate the probable reliability of the different methods. Our specific application pertained to benthic microfloral production in a southern California lagoon; however, the approach applies to all plants with high turnover rates.

By intensive sampling (e.g. incubating about 1000 samples during a single month), Shaffer & Cahoon (unpubl.) obtained bi-daily samples which encompassed the within-site variability of benthic microfloral productivity and standing crop. We use this study to

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compare the sources of error likely to be introduced by insufficient sampling in space and time with the error likely to be introduced by the different methods of conversion of hourly productivity to monthly production. From these results we offer suggestions on the experimental design which will yield a reliable estimate of annual production in the most parsimonious way.

METHODS

Annual study. Hourly gross primary productivity of benthic microflora was determined by incubating intact sediment cores in light and dark chambers at the sample sites and measuring changes in dissolved oxygen (Strickland & Parsons 1972), usually over 2 h periods around midday. Each month from June 1977 to July 1978, duplicate determinations were made at each of 17 permanent stations in the eastern arm of Mugu Lagoon, Ventura County, California, USA (34° 06' N, 119° 05' W) representing both intertidal and subtidal areas, and in different sedimentary regimes. Field work was conducted on 4 to 8 d preselected for each month, to eliminate conscious bias of weather conditions and to achieve presumably adequate representation of the average conditions of each month. Consult Shaffer & Onuf (1983) for a detailed description of sampling procedures and the site and an analysis of environmental factors influencing productivity.

Intensive 1 month study. For the intensive sampling during 1 mo, the incubation chambers (1 light, 1 dark) each contained 14 intact sediment cores (3.4 cm diameter, 0.5 m deep). The coring procedure caused little or no disturbance of the visible film of microflora on the cores. To obtain the 14 cores for each chamber, duplicate samples were taken (about 3 cm apart) at each of 14 sample sites located at predetermined distances along a 23 m long permanent transect. A 0.45 m² quadrat divided into 30 cells was set at each of the 14 sample sites. One of the 30 cells was randomly preselected for each day, no cell was sampled on more than 1 d. This sampling design assured that all samples from each of the 14 sites were discrete distances apart, yet eliminated the possibility that any sample taken late in the study had been disturbed by the removal of samples from the same site earlier.

These productivity measurements based on 28 cores were made at a subtidal site consisting of very poorly sorted sand (Folk 1968) and an intertidal site consisting of poorly sorted muddy sand. Along with the productivity measurements based on 28 cores, productivity was also measured using single 15 cm diameter intact cores similar to those used in the annual study. Both types of incubations were carried out at the intertidal and subtidal site every second day from September 6

through October 6, 1981. A more detailed description of the methodology and sample area is given in Shaffer & Cahoon (unpubl.).

Photosynthesis vs light intensity. Twelve *in situ* experiments were conducted from August 19 to November 15, 1979 to determine the relation between light intensity and photosynthetic rates of the benthic microflora. Individual sediment cores, from sites of variable location, elevations, and substrate composition were incubated under different fractions of full sunlight. Each core was incubated at 4 to 6 light levels in a randomized sequence. The productivity of each core at each light level was then expressed as a % of the maximum gross productivity measured for that core (which occurred at the highest 1 or 2 light intensities). This method of normalization, opposed to expressing the data in mgC [mg chl a]$^{-1}$ h^{-1}, was necessary because the chlorophyll a content in the finest sediments averaged 3 times as high as that in the coarsest sediments while both gross and net productivity followed the opposite trend (Shaffer & Onuf 1983). Light intensities ranging from 6 to 74 % of noon mid-summer sunlight (photon flux density in the photosynthetically active region [PAR] of approximately 2160 µE m^{-2} s^{-1}) were obtained by using neutral attenuation filters.

These filters were constructed by sandwiching from 1 to 15 sheets (each 1.0 µm thick) of frosted acetate in between transparent 3.2 mm (1/8 inch) thick plexiglass. The spectral transmission properties of the filters over the photosynthetically active region (PAR; 400 to 700 nm waveband) did not appreciably alter the spectral response of the quantum sensor. The only noteworthy deviation from neutral transmission occurred between 650 and 690 nm, where the filters overtransmitted by approximately 8.6 %.

Gross primary productivity was measured using the methodology described in Shaffer & Onuf (1983) except that the bottom and sides of the light chambers were made opaque and the cores incubated normal to the sun. These 2 differences in procedure restricted the light regime to direct incoming quanta. As a control, the same core was often incubated 2 or more times under the same filter; this allowed testing for changes in environmental conditions over the duration of the experiment as well as effects introduced by subjecting the cores to multiple incubations.

The photon flux density of PAR reaching the surface of the sediment was measured with a LI-COR model 185-A quantum meter The quantum sensor was fitted to a transparent plexiglass window (3.2 mm thick, 2.5 cm diameter) on the chamber bottom. Consequently, the quantum meter measured direct incoming light passing through the filter(s), the water inside the chamber and the window. Using this procedure light was measured prior to and directly following each

incubation. Changes from the beginning to the end of the incubations were negligible (mean change of $1.3 \pm 0.7 \%$; no change for 41 out of 46 incubations), because the experiments were conducted within 3 h of noon on cloudless days.

In order to estimate the annual production of the benthic community, it was necessary to determine the relation between hourly rates measured around noontime and rates during other periods of the day. To do so, oxygen evolution and uptake was monitored from sunrise to sunset in 3 light and 3 dark chambers on 4 cloudless days (Nov 18, 1978; Jul 12, 1979; May 26, Jul 12, 1980) replacing water at ca 2 h intervals. To determine whether multiple incubations altered productivity, incubations with additional cores were begun several hours after the experimental cores during each of the diel experiments. Since the control cores in all cases followed the same trends (increases or decreases) as the experimental cores, the trends were assumed real, not artifacts of multiple incubation. Mean daily solar radiation curves were calculated for each month from 365 d records obtained from the Climatology Department of the Pacific Missile Test Center located approximately 2 km from the study area.

A respiratory quotient of 1.0 was assumed to convert productivity measured by Winkler titration to g carbon (Strickland & Parsons 1972). Total benthic primary production of the study area was determined by weighting the stations according to the area that each station represented. This was based on substrate composition (J. P. Cermak, unpubl.) and variations in elevation (from a 22 transect bathymetry study, unpubl.).

Each monthly total for the entire lagoon was obtained by multiplying the area that each station represented by the monthly rate for that station, summing the station × area values and dividing by the total area. The annual estimate was obtained by summing the monthly values.

To assess the value of this laborious procedure, the hourly productivity measurements were also converted to annual estimates by applying 3 other formulas which have appeared in the literature (Table 1).

RESULTS

All routine productivity measurements were made around local noon. Since solar radiation is maximal at this time, and since photosynthesis depends upon solar radiation, it is likely that hourly productivity measured at midday is not equivalent to mean hourly productivity for the whole day. Also, unless measurements are made all days of a month, it is possible that conditions on measurement days are different from average conditions for all days of the month. Although these possibilities are intuitively obvious, it is not obvious how much of an error they will introduce into annual estimates. In the following, we apply a series of adjustments to hourly midday productivity measurements to extrapolate from measurement conditions to average conditions at midday and then from midday conditions to entire days. These adjustments were accomplished by determining a relation between photosynthetic rate and irradiance, measuring irradiance during incuba-

Table 1. Formulas for extrapolating hourly productivity to daily productivity

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Formula A (Marshall et al. 1971, Joint 1978)

Daily productivity = midday hourly production × hours of daylight

Formula B (Leach 1970, Riznyk et al. 1978)

$$\text{Daily productivity} = \frac{\text{production for the}}{\text{incubation period}} \times \frac{\text{solar radiation for the entire measurement day}}{\text{solar radiation for the incubation period}}$$

Formula C (Zedler et al. 1978, Zedler 1980)

$$\text{Daily productivity} = \frac{\text{midday hourly}}{\text{production}} \times 0.64 \times \frac{\text{mean daylength}}{\text{for the period}} \times \frac{\text{average daily solar radiation for the period}}{\text{daily solar radiation for the measurement day}}$$

Formula N (new, this study)

$$\text{Daily productivity} = A \cdot B \, \frac{C_1}{C_2} \cdot \frac{D_1}{D_2}$$

where A = hourly gross production obtained around noontime; B = average daylength (hours of sunlight) for the month; C_1 = solar radiation during incubation period (corrected for saturating light levels) for the average day of the month; C_2 = solar radiation during incubation period (corrected for saturating light levels) for the measurement day; D_1 = average percent maximum gross production for the entire day; D_2 = percent maximum gross production around noontime (D_1/D_2 is a constant for each of the 14 mo, Table 3)

tions, calculating mean daily solar radiation curves for each month, and adjusting the measured productivity values by conversion factors generated from all these sources as described below.

Photosynthesis *vs* light intensity

Photosynthetic rate increased as light intensity increased at low light intensities and was maximal over a wide range at high light intensities (Fig. 1). To determine the best fit between photosynthesis and light, 9 different mathematical formulations were
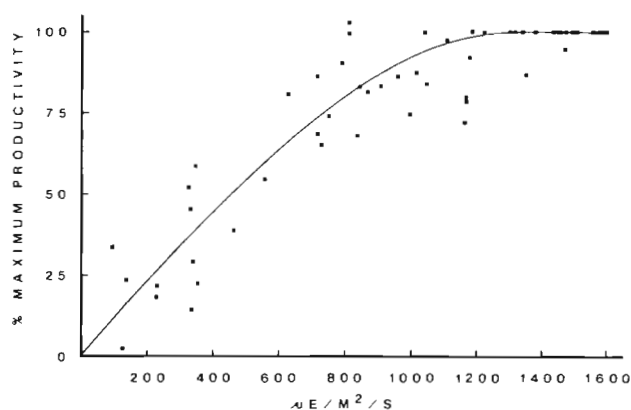

Fig. 1. Percent maximum gross productivity *vs* photon flux density conducted under natural conditions with unaltered benthic microfloral communities

employed, 8 of which were tested by Jassby & Platt (1976) for appropriateness in tracking this relation for phytoplankton communities. Of these, a linear regression (for the points below 900 $\mu E\ m^{-2}\ s^{-1}$) combined with a hyperbolic tangent function (Jassby & Platt 1976) proved the best fit, although even this combination produced portions of the curve which lay completely above all data points. Instead, a smooth curve was drawn by eye after applying the maximum entropy-moving average (ME-AR) technique (Ulrych & Bishop 1975, Ulrych & Clayton 1976). The saturating light level was determined to be 1260 $\mu E\ m^{-2}\ s^{-1}$ by averaging the points around the asymptote (Platt et al. 1975, Jassby & Platt 1976, Harding et al. 1980), or approximately 60 % of summer midday sunlight. The line was fit through the origin because the test for an intercept was not significant (t = 0.08, p < 0.44).

During these experiments, temperature increased by an average of 1.8 C° and a maximum of 4.1 C°. Assuming a 10 % $C^{°-1}$ increase in photosynthetic rate (Colijn & van Buurt 1975), the photosynthetic rate changed by an average of 3.8 % (± 0.9 %). The raw measurements changed by an average of 7.5 % (± 2.6 %).

Calculating annual production from hourly measurements

The relation of photosynthesis *vs* light intensity determined from intact sediment cores at Mugu Lagoon (Fig. 1) indicates that productivity measured once or a few times a month at midday cannot safely be assumed to represent average hourly productivity for entire days over the whole month. In fact, 'mean' midday conditions may be very rare, because the mean in southern California is usually a composite of many clear days, a few overcast or stormy days, and relatively few intermediate days, yet the mean is intermediate. Consequently, we adjusted measured midday hourly productivity to mean midday hourly productivity for each month by applying a solar radiation factor C_1/C_2, as defined in Table 1.

Mean midday hourly production was converted to average hourly production for the whole day by the correction factor D_1/D_2, as defined in Table 1. Both D_1 and D_2 were determined by using the curves of the solar radiation for the average day of the month and the measured relationship between photosynthesis and light intensity. The average incident radiation value ($\mu E\ m^{-2}\ s^{-1}$) for each time interval of the average day of the month was located on the photosynthesis *vs* photon flux density curve (Fig. 1) and the corresponding % maximum productivity value determined as in the example for May, 1978 (Table 2). This correction factor was determined for each of the 14 mo, ranging from 0.63 to 0.78, with generally lower values in winter and higher values in late spring and summer (Table 3).

To test the validity of this method, productivity was measured throughout the day on 4 occasions. The ratios of mean hourly productivity measured over the whole day to midday hourly productivity was compared to D_1/D_2 computed for the same days (Table 3). The close agreement between the conversion factor and the empirically determined productivity ratio suggests that the method is sound.

The effect and significance of the correction factors developed in this study are illustrated in Fig. 2. If sample periods fairly represented average midday conditions each month, there would be no need for correction factor C_1/C_2. In this study, sample periods did not fairly represent average midday conditions, even though sampling was conducted on 4 to 8 d each month. Sample periods were darker than average in 11 of 14 mo, in 1 mo by 50 % (Fig. 2a). Consequently, mean midday solar radiation (C_1) is greater than midday solar radiation on measurement days (C_2) in most cases, and the application of the correction factor C_1/C_2 increased estimated midday productivity substantially in most cases (Fig. 2c). The adjusted estimates ranged from −11 % (Jul 1978) to + 142 % (Feb 1978) of the

Table 2. Example of calculation of correction factor D_1/D_2 for adjusting midday hourly production to average hourly production for the average day of the month

Incubation period	Incident radiation ($\mu E\ m^{-2}\ s^{-1}$)	% maximum production	Minutes incubated
(1) Early morning	344	35	131
(2) Mid-morning	1011	93	120
(3) Late morning	1603	100	120
(4) Noontime	1832	100 (D_2)	120
(5) Early afternoon	1556	100	120
(6) Mid-afternoon	992	92	120
(7) Late afternoon	328	34	135
Total minutes of daylight			866

$$D_1 = \sum_{i=1}^{n} (\%\ \text{maximum production})_i (\text{proportion of day})_i \ \text{for incubation periods } i = 1 \text{ to } n$$

$$D_1 = .35 \times \frac{131}{866} + (.93 + 1.00 + 1.00 + 1.00 + .92) \times \frac{120}{866} + .34 \times \frac{135}{866} = 0.78$$

$$\frac{D_1}{D_2} = \frac{0.78}{1.00} = 0.78$$

Table 3. Correction factors D_1/D_2 for 14 mo from June 1977 through July 1978 calculated as described in 'Methods' and measured directly on 4 cloudless days

Month	D_1/D_2	Measured ratio	Predicted ratio[*]	Percent difference
Jun	0.68			
Jul	0.76			
Aug	0.72			
Sep	0.76			
Oct	0.68			
Nov	0.64	0.65	0.66	1.5
Dec	0.69			
Jan	0.63			
Feb	0.64			
Mar	0.66			
Apr	0.71			
May	0.78	0.72	0.76	5.3
Jun	0.74	0.78	0.78	0.0
Jul	0.74	0.76	0.79	3.8

[*] For the measurement day

measured values and averaged +13 % over the 14 mo study.

The discrepancies between measured and adjusted midday productivity values resulted from randomly sampling a highly variable variate and could have been different in magnitude and direction. The correction factor D_1/D_2, adjusting midday hourly productivity to average hourly productivity for the average solar day of each month, accounts for the systematic overestimates that would result from assuming that midday productivity applies to the whole day. As a result, this adjustment always reduces estimated hourly productivity (Fig. 2c, difference between dotted and solid lines), ranging from 22 % (May 1978) to 37 % (Jan 1978), and averaging 30 % over the 14 mo study.

Comparison of formulas

At least 3 other formulas besides the one developed in this study have been used in extrapolating hourly productivity to daily, monthly, and annual production (Table 1). Multiplying measured midday productivity by day length (Formula A) is subject to sampling error (measurement periods perhaps not representative of average conditions). Where light saturation occurs at low light intensities, Formula A introduces little systematic error. However, at Mugu Lagoon, light saturation occurred at high levels of solar radiation: 60 % of summer noon radiation. Annual productivity was 25 % higher calculated by Formula A than by Formula N. This discrepancy would have been greater still, except that the sampling error (corrected by C_1/C_2) and the systematic error (corrected by D_1/D_2) partially canceled in this study, as illustrated in Fig. 2c and described in the previous section. An extreme case occurred in February 1978 when ignoring average radiant energy resulted in a 6-fold underestimate in production. If measurement days are representative of average midday conditions, Formula A should overestimate daily production by $1 - D_1/D_2$, which averages 30 % and ranges from 22 to 37 %.
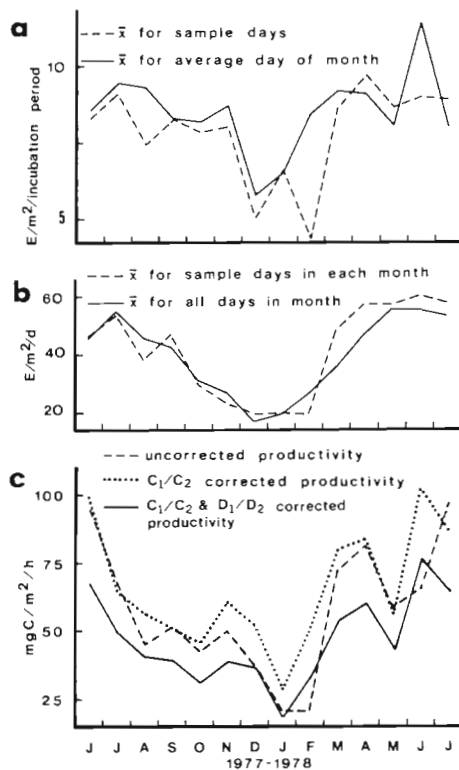
Fig. 2. Temporal variations measured for all stations combined. (a) Mean value of solar radiation for incubation periods of sample dates (dashed line) and for incubation periods of the average day of the month (solid line). (b) Mean value of solar radiation for days in which primary production was measured (dashed line) and for all days in each month (solid line). (c) Hourly gross productivity uncorrected (dashed line); corrected to average midday conditions for the month (dotted line); corrected to average hourly productivity for the average day of the month (solid line)

Fig. 3. Illustration of the defect in Formula B introduced by assuming that saturating light levels never occur

The fact that measurements were often conducted under darker conditions than average for the month resulted in both under- and overestimates when using Formula B (Table 1). Although a radiant energy factor is included, it considers only sample days (i.e. solar radiation for the measurement day/solar radiation for the incubation period). Underestimates were generated when both incubation periods and incubation days were darker than average for the month. Overestimates resulted when the incubation measurements occurred under darker conditions than average while incubation days were not nearly as dark and sometimes brighter than average for the month (compare the 2 curves in Fig. 2a with those in 2b). Both sources of error could have been remedied by replacing the numerator of the radiant energy factor with the solar radiation for the mean day of the month.

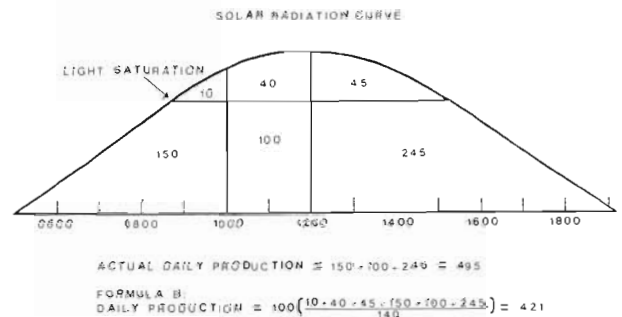Fig. 3 illustrates another defect of Formula B: the assumption that saturating light levels never occur will disproportionately decrease estimates of production for the rest of the day. Actual daily production will correspond to the area under the solar radiation curve below the saturating light level (i.e. 495 multiplied by some constant converting light to production). However, the relatively large portion of light above saturation during the incubation period will be applied to the rest of the day (i.e. 140 in the denominator instead of 100); resulting in an underestimate of 15 % for this example of the average day of the month during May, 1978. The discrepancy will be even greater for cloudless summer days and less in winter or for overcast incubation periods.

In comparison to Formulas A and B, Formula C (Table 1) decreases substantially the assumptions used to make the measured hourly rates representative of the entire day, and also the assumptions used to make the daily rates representative of the entire month. However, 3 sources of error will be incorporated into the annual estimate, 2 from the radiant energy factor used in the daily to monthly expansion, and 1 from the constant used in the hourly to daily expansion.

Firstly, although the radiant energy factor considers average weather conditions, it is a ratio of days, not incubation periods (i.e. solar radiation for the average day of the month/solar radiation for the measurement day). In this study, differences in insolation of entire sample days were often not representative of insolation during the incubation periods (compare Fig. 2a with Fig. 2b. On average, small differences occurred between daily solar radiation for measurement days and the mean day of the month, obscuring the large differences between the actual incubation periods and those for the average day of the month). Secondly, as in Formula B (Table 1), the radiant energy factor ignores saturating light levels, which may or may not cancel in the ratio. The third source of error is generated from the assumption that, throughout the year, the relation between hourly noontime productivity and average productivity for the entire day is constant. At Mugu Lagoon this is not the case. The correction factor of 0.64 worked well for the period in which it was measured

(winter), but underestimated productivity by 3 to 22 % for 11 of the 14 mo (Table 3). As a result, compounded with the previously discussed sources of error, Formula C yielded an annual estimate 22 % lower than the estimate derived from Formula N.

It should be apparent that all of the major sources of error inherent in Formulas A, B, and C stem from false assumptions about the relation between photosynthesis and light. For this reason, Formula N should yield a more reliable estimate of annual productivity, since it is based on the measured relation between photosynthesis and light intensity, computes the radiant energy factor (C_1/C_2) for the actual incubation period and relates midday hourly to daily average hourly productivity (D_1/D_2) for each month.

Spatial and temporal heterogeneity

The intensive month-long study of benthic microfloral production (Sep 6 to Oct 6, 1981) provided information on the sources of error likely to be incorporated into the estimate of monthly production due to (1) the formulas, (2) spatial patchiness, and (3) different temporal sampling rates. Fig. 4 displays the daily estimates of production based on Formulas A, N, and the best estimate of actual daily production. Actual daily production was obtained by multiplying hourly productivity by the product of day length and D_1/D_2 (to
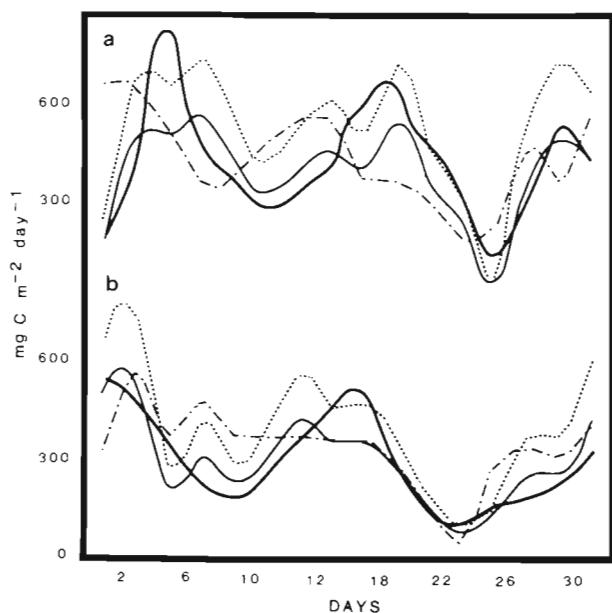

Fig. 4. Daily production from Sep 6 to Oct 6, 1981, for (a) intertidal muddy sand and (b) subtidal sand. 28-core measurements applying Formula A (dotted line), Formula N (thicker solid line) and actual production (thinner solid line); actual production for the 2-core measurements (dashed line)

make the noontime measurement representative of the entire day). It was not possible to use Formulas B and C because daily solar radiation curves were not available.

The average daily error caused by spatial patchiness can be seen in Fig. 4 by comparing the actual production curves for the measurements based on 28 cores with those based on only 2 cores. The 2 large cores did not adequately represent the highly variable distribution of standing crop at each location. The 28 cores taken at each location every sample day were more than enough to represent the different standing crop densities (Shaffer unpubl.). For example, on the first day of the study the 2-core productivity for the muddy sand site was greatly overestimated (Fig. 4), because the 2 large cores happened to contain a much denser and much healthier standing crop than the area as a whole (9.6 µg g^{-1} opposed to 4.5 µg g^{-1} with a pheophytin a to chlorophyll a ratio of 0.8 opposed to 1.4). The average daily error caused by spatial patchiness was 35 ± 8.5 % (standard error) for the sandy site and 63 ± 26.5 % for the muddy sand site.

The average error likely to be introduced in the monthly estimate attributable to the formulas was calculated for monthly sampling rates based on 1 d randomly selected, 2 d biweekly, 4 d randomly selected, 4 d weekly, and 4 d within 14 d periods. Each sampling rate was based on 10 trials. Each trial of the sampling rate based on 4 d within 14 d periods was obtained by randomly selecting the first day and then flipping a coin (when necessary) to determine forward or backward sampling: samples were drawn at 0, 4, 7, and 11 d.

Table 4 displays the mean error in the sand and muddy sand areas produced by the different sampling rates and Formulas A and N. A 3-way factorial ANOVA using Fixed Effects on sampling rate, formula type, and sediment type produced highly significant differences for all 3 main effects (F = 13.78, p < 0.001 for sampling rate, F = 30.00, p < 0.0001 for formula type, and F = 5.51, p < 0.02 for sediment type) and no significant interactions. Interestingly, sampling biweekly was no more effective than sampling 1 d mo^{-1} (the linear contrast producing F = 0.04, p < 0.84). The reason for this is that the trends in benthic microfloral production in Mugu Lagoon are regulated primarily by the tides (Shaffer & Cahoon unpubl.). Biweekly sampling maximizes the chances of obtaining 2 peaks (which occur during neap tides) or 2 troughs (which occur during spring tides) and negates the chance of obtaining a peak and a trough in production. The 3 sampling rates based on 4 d (Table 4) did not differ significantly from one another but were highly significantly different from 1 d and biweekly sampling (the linear contrast comparing 4 d random, 4 d weekly, and 4 d within 14 d

Table 4. Mean ($\bar{x}$), standard error (SE), range, and overestimate to underestimate ratio (O:U) for the average error introduced in the calculation of monthly production (Sep 6 to Oct 6, 1981) applying Formula A and Formula N to different sampling rates

	Percent error for different sampling strategies per month																			
	1 d random				2 d biweekly				4 d random				4 d weekly				4 d within 14 d			
	$\bar{x}$	SE	Range	O:U	$\bar{x}$	SE	Range	O:U	$\bar{x}$	SE	Range	O:U	$\bar{x}$	SE	Range	O:U	$\bar{x}$	SE	Range	O:U
Sand																				
Formula A	57	13	1–132	7:3	60	12	1–113	8:2	31	5	5–53	9:1	27	3	11–40	10:0	28	5	4–53	10:0
Formula N	42	9	3–87	5:5	46	6	11–70	5:5	16	2	4–30	4:6	11	2	2–19	5:5	11	3	1–36	6:4
Muddy sand																				
Formula A	41	7	15–84	8:2	37	8	7–70	8:2	31	6	7–61	10:0	31	5	11–54	10:0	30	4	8–45	10:0
Formula N	27	6	3–56	3:7	29	7	5–80	5:5	15	2	8–29	4:6	10	2	2–25	5:5	11	3	2–30	6:4

periods with 1 d and biweekly samples producing $F = 54.34$, $p < 0.0001$). The reduced error for weekly sampling compared to sampling 4 d at random (Table 4), though not significant, is likely to be real because a weekly sampling rate ensures either measuring near average production (with very little variation), or maximum and minimum production (with the highest variation), or representative increases and decreases in between the 2 extremes. This can best be seen by placing a ruler on the Formula N plots (Fig. 4) and looking at various combinations of weekly sampling. Sampling at 0, 4, 7, and 11 d within 14 d periods is about as accurate as sampling weekly (Table 4). In addition, this sampling rate will maximize the information about the environmental parameters governing productivity, because it maximizes the chances of measuring production on an increase, a decrease, a maximum, and a minimum (Shaffer & Cahoon unpubl.).

DISCUSSION

Productivity is usually measured around noontime and converted to a daily estimate by assuming a constant rate or by supplying a factor converting noontime productivity to average daily productivity. To accomplish the latter, it is necessary to generate a ratio of hourly noontime productivity to hourly productivity for the entire day, either empirically or by measuring an easily observable correlate of productivity and determining how it relates to productivity. Solar radiation is most amenable to this function, since it is relatively easily measured, and photosynthesis is directly dependent on light availability. Large amounts of error may be introduced, however, if solar radiation is used when its relation with productivity is assumed rather than measured. This problem is exemplified in analyses of Formulas A, B, and C, (Table 1) which make opposing assumptions. In Formula A, saturating light levels are assumed to occur essentially at sunrise and persist

until sunset, whereas Formulas B and C assume a direct linear relation between solar radiation and carbon fixation, ignoring saturating light levels.

Formula N (Table 1), however, relies on the measured relation between photosynthesis and light intensity. Of major consequence in this study is that the saturating light level of 1260 μE m^{-2} s^{-1} measured for the benthic microflora in Mugu Lagoon (Fig. 1) is approximately 5-fold higher than that measured for estuarine benthic microflora by most other investigators (Taylor 1964, Cadée & Hegeman 1974, Colijn & van Buurt 1975, Admiraal 1977) and from 2 to 8 times that measured by Williams (1962). There are 2 possible explanations for this discrepancy. First, it is likely that the saturation curves obtained from suspended benthic diatoms (Williams 1962, Taylor 1964, Colijn & van Buurt 1975), cultured diatoms (Colijn & van Buurt 1975, Admiraal 1977), or sediment cores which have been spread out (Cadée & Hegeman 1974) approximate *actual* saturation, rather than *ecological* saturation. The *ecological* saturation level is the incident radiation at the sediment surface required to saturate the integrated photosynthesizing population which encompasses the microflora occupying the surface layer (often several cells thick) to those 2 to 4 mm in the sediments (Taylor 1964, Fenchel & Staarup 1971).

The second explanation for the discrepancy between saturation values is that the intertidal sediments of the Ems-Dollard estuary and the Dutch Wadden Sea region, where much of this work was carried out (Cadée & Hegeman 1974, Colijn & van Buurt 1975, Admiraal 1977), characteristically contain a very low diversity of primarily pennate diatoms and, during submersion, a very turbid overlying water column (Colijn 1982). The water is generally so turbid that van Es (1982) assumed that there was no significant productivity as long as there was at least 10 cm of water over the sediments. In general, plants usually saturate just below the average maximum incident radiation level because, physiologically, decreasing the saturation level costs energy in terms of the initial increase in the content of

chlorophyll in the light harvesting complex of each cell and the continual maintenance of that higher level of chlorophyll. Perhaps in order to photosynthesize at all during submerged conditions, the benthic microflora must expend the extra energy required to maintain a very low saturating light level. Recent studies in the Ems-Dollard estuary provide evidence for the very low saturating light level. Admiraal & Peletier (1980) found that cell division rates of cultures incubated in the field at a high level mudflat were often similar to cell division rates of cultures incubated at a mid-level site which received much lower light levels. They concluded that the division rates of diatoms in the field are regulated primarily by temperature, not light. Admiraal et al. (1982) found that a light level of 70 µE $m^{-2} s^{-1}$ permitted cultures of benthic diatoms to achieve division rates similar to those grown under 1000 µE $m^{-2} s^{-1}$. Finally, van Es (1982) and Colijn & de Jonge (1984) found no correlation between benthic microfloral productivity and light in multi-year studies of the Ems-Dollard estuary.

While the low saturation level measured by previous investigators may be appropriate in certain wetland ecosystems (e.g. Ems-Dollard), there are several reasons to believe that the photosynthesis-light intensity relation observed in this study is more the rule than the exception. Most importantly, the experiments were conducted under natural conditions with essentially unaltered microfloral communities. Secondly, little or no correlation between productivity and incident radiation would be expected with the low saturating intensities measured by other investigators, yet many investigators have found light to be the principal parameter governing the productivity of estuarine benthic microflora (Pomeroy 1959, Williams 1962, Pamatmat 1968, Leach 1970, Marshall et al. 1971, Van Raalte et al. 1976, Zedler 1980). Thirdly, changes in productivity from sunrise to sunset determined from a daily solar radiation curve in conjunction with the photosynthesis vs light intensity curve match very closely with those actually measured in the field (Table 3).

Finally, under controlled laboratory conditions, using natural associations of intact benthic microflora from Mugu Lagoon, Holmes & Mahall (1982, and pers. comm.) obtained very similar results. Recently, Whitney & Darley (1983) at their summer creek bank site in Georgia, USA, obtained an even higher (1387 to 2044 µE $m^{-2} s^{-1}$) saturation level than the one obtained in this study. Their measurements differed from ours and others (Williams 1962, Taylor 1964, Colijn & van Buurt 1975, Holmes & Mahall 1982) in that photoinhibition occurred at most of their sites. One explanation for the photoinhibition observed by Whitney & Darley (1983) is that the measurements were conducted under exposed conditions; Holmes &

Mahall (1982) observed that subaerial exposure caused an initial increase in productivity, followed by a decrease caused by desiccation. We did not measure productivity under exposed conditions which occurred for less than 20 % of the time, integrated over our whole study area. Besides photoinhibition, Whitney & Darley (1983) observed a decrease (548 to 848 µE $m^{-2} s^{-1}$ for the creek bank site) in the light saturation level during winter. They attribute the low saturation and extreme photoinhibition during winter to acclimatization of the benthic microflora to several days of cloudy weather which preceded the sunny measurement days. We expect that a similar, though not as extreme, decrease occurs at Mugu Lagoon as well. Generally, during winter the coast of southern California has a higher proportion of cloudless skies than does the coast of Georgia.

If productivity were constant given constant light, Formula N would predict monthly production very accurately, based on a single measurement day. However, after the effects of light have been removed, fluctuations in productivity during a single month can approximate those over the entire year (e.g. corrected 28-core productivity curve in Shaffer & Cahoon unpubl.). Because other factors (e.g. tides, standing crop, temperature) influence productivity, a reliable estimate of monthly production requires measurements be made at each sample site several times during a month. If measurements were carried out during most or all of the days each month, the formula for actual production would yield the most accurate estimate of monthly production. Since this is rarely possible, measurements based on some subset must be made representative of the entire month. The results in Table 4 suggest that the error incorporated into the monthly estimate can be reduced to about 10 % by sampling as few as 4 d and applying Formula N. Using this method, the error introduced into the annual estimate would likely be about 5 %, because Formula N is an unbiased estimator (Table 4; the probability of overestimation and underestimation is the same). In contrast, Formula A has a strong tendency to overestimate monthly production (Table 4). For all 3 sampling schemes of 4 samples mo^{-1}, Formula A overestimated by about 30 %.

From these results we are able to make several suggestions on spatial and temporal sampling design. First, a large amount of error (an average of 49 ± 14 % for the 2 areas combined) due to within-site patchiness was greatly reduced by incubating a large number of cores in each chamber: it is far simpler to sample many times a day than many days a month. A recent study on the Gulf coast of the USA indicates that it is possible for 1 investigator to incubate 40 cores at each of 2 sample sites during a 6 h period (Shaffer unpubl.).

Secondly, no matter what formula is used to convert the hourly rates to monthly rates, sampling once or twice per month at each site is likely to introduce about 40 % error into the monthly estimate. It is necessary to conduct measurements at each sample site several times per month, because other factors besides light influence productivity. Formula N combined with a 4 d mo^{-1} sampling rate will likely reduce this error to about 10 %, and the error in the annual estimate to about 5 %. However, daily solar radiation curves may not be available, or the calculations based on Formula N may be prohibitively laborious. As an alternative, Formula A supplied with a correction factor of 0.70 (since this formula tends to overestimate by about 30 %) may suffice. These results strongly indicate, for a *given number* of days per month, that sampling at a few stations several times per month is much more informative than sampling at many stations once or twice a month. Presumably, by employing a 4 d mo^{-1} sampling rate, 2 investigators could obtain representative production estimates at 4 different sites, which would enable realistic monthly comparisons of both within and between site differences. At Mugu Lagoon, because the tides are responsible for much of the high turnover of benthic microfloral standing crop, sampling several times within 14 d periods is more informative than spreading the samples throughout the month. Similar high short-term variation has also been observed in the Gulf coast (Shaffer unpubl.) and the East coast (Williams 1962, Darley et al. 1981) of the USA, where processes other than tides may be primarily responsible. Therefore, sampling within 14 d periods may be appropriate for other areas as well. If measurements must be limited to once or twice a month, we believe the study is not warranted.

Our annual estimate of 168 to 170 g C m^{-2} for gross primary production is similar to measurements from similar studies on the west coast of the USA (Pamatmat 1968, Riznyk et al. 1978, Zedler et al. 1978, Zedler 1980) as well as the east coast (Pomeroy 1959, Marshall et al. 1971, Gallagher & Daiber 1974, Van Raalte et al. 1976) and Europe (Cadée & Hegeman 1974, 1977, Joint 1978, van Es 1982, Colijn & de Jonge 1984). However, we believe that the similarity of annual estimates from different regions is currently illusory. In all of the studies on annual production of the benthic microflora, few samples were incubated at each sample site during each sample day, and measurements were made at each site only once or at most twice each month. This includes our own study, where we measured production on 4 to 8 d each month, but only twice at each station. Furthermore, in each of the studies substantial error was embedded in the hourly to daily and daily to monthly conversions. The cumulative error introduced in the annual estimates by inappropriate sampling in

space and time, and inappropriate assumptions in converting the hourly rates to monthly rates could account fully for the total range of variation in the annual estimates. Therefore, the point is moot at this time whether or not benthic microfloral production is different between regions and will be moot until more complete studies are carried out.

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