17: Electrodes and chemicals

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I. Introduction

A. Purpose of the methods

Knowledge of the primary production rates of macroalgae is valuable not only from a descriptive point of view but also because it makes possible the rapid testing of hypotheses concerning the effects of environmental factors. Primary productivity is defined herein as the rate at which inorganic matter and free energy are converted to organic matter and bound energy per unit of algal material or unit of the earth's surface area. This is almost entirely due to photosynthesis, and sunlight is the energy source, as follows:

$$6H_2O + 6CO_2 \xrightarrow[\text{chlorophyll}]{\text{light}} C_6H_{12}O_6 + 6O_2 \qquad (1)$$

Equation 1 is essentially reversed for respiration. All parts of the equation potentially could be used to estimate primary production; however, this chapter focuses on CO_2 and O_2 fluxes. Some useful definitions are as follows:

Productivity = production per unit time

Gross primary production (GPP) = net photosynthesis (Ps) + respiration (R)

Net primary production (NPP) = GPP − R

NPP per day (24 h) = (NPP per daylight day) − (R per night)

Since all biological activity ultimately depends on net primary production, many ecologists require reliable methods for obtaining photosynthetic, respiratory, and other metabolic data relevant to their studies. Among the most widely used and credible (Czaplewski and Parker 1973) approaches to the assessment of productivity are those employing O_2 electrodes. The pH electrode technique is comparable but only half as sensitive (Marsh and Smith 1978) and not as frequently employed. The most time-tested dissolved-O_2 method is the modified Winkler chemical titration, which is quite labor intensive, comparatively inexpensive, and comparable in accuracy to the oxygen probe under most field conditions. An excellent

treatment of virtually all methods of O_2 measurement is available (Hitchman 1978) and will be of use to many readers.

B. Overview of incubation methods

Incubation methods involving any of the aforementioned techniques traditionally rely on the use of light and dark bottles (Gaarder and Gran 1927) deployed (1) in situ, (2) in sunlight incubators, or (3) in artificially illuminated chambers. Temperature control is achieved by water cooling, or more often by refrigeration/heating units in the last case. Metabolic studies in open ecosystems are dealt with by Kinsey (Chap. 21).

For in situ measurements, samples are incubated at or very near the same locations from which they were taken. This method has the advantage of most closely approximating natural conditions. However, site-specific and temporal variations of environmental parameters limit the degree to which the results can be generalized or compared with other habitats. This method requires much more effort and manpower than is needed when incubators are used.

Sunlight incubators are trays made of polycarbonate or other optically transparent materials that employ natural sunlight and colored filters (see Dring and Lüning 1977; Faust et al. 1982) to simulate the light at a given depth. This method approximates natural lighting somewhat, but local variations in conditions again limit the degree of generalization or comparison.

Artificially illuminated field incubators use combinations of fluorescent and incandescent lamps with color or neutral density filters in a portable environmentally controlled and mechanically stirred chamber (Doty and Oguri 1958; S. Blair, unpublished data). Environmental factors are kept consistent, so generalizations and comparisons among species or from one locality to another are more valid. However, these devices are by definition unnatural, and it is often difficult to extrapolate from data obtained in incubators to those that would pertain in the natural system. This method requires less manpower and effort, because incubations can be performed under a relatively controlled environment approximating that of the laboratory.

1. Laboratory incubation techniques. Laboratory experiments generally are conducted in large photoperiod incubators, typically maintained at or below ~200 $\mu E \cdot m^{-2} \cdot s^{-1}$ (~10,000 lux) by using cool-white fluorescent bulbs, which is well above saturation but below light inhibition for most macrophytes (King and Schramm 1976). Higher light energies of different spectra (Lüning 1981) are often obtained with slide projectors having 500-W incandescent bulbs. Both the

incubation water and associated equipment should be brought to the desired temperature before use to prevent the formation of small bubbles due to degassing on surfaces. All experimental material is collected submerged and immediately returned to the laboratory in insulated coolers.

a. Bottle experiments. Net photosynthesis is determined on selected whole plants by means of O_2 analyzers or pH meters and appropriate electrodes. Wide-mouthed, clamp-lid, rectangular canning jars (Snap Top Jars, Allied Trading Co.) serve well as incubation containers and can be readily and cheaply obtained nearly anywhere in the world. The seawater used during the incubations is taken at the time and place of algal collection, shaken vigorously to bring to O_2 saturation (Strickland and Parsons 1972), immediately filtered through a nannoplankton net (10-μm pore size) to remove most plankton organisms, and stored in the laboratory at the desired temperature in the dark. When individual bottles are being filled; they should be slowly submerged so as to exclude all air from the stocks of incubation water.

Comparative incubations are carried out between 0900 and 1500 h to reduce differences due to possible endogenous photosynthetic periodicities (e.g., see Hoffman and Dawes 1980). Incubation times for the larger macrophytes are usually restricted to about 2.0 h, since the representative thallus portions are much larger than those of smaller forms. During incubation, the water within the bottles (minimally four light and two dark replicates, since respiration is lower and less variable) is completely recirculated by means of magnetic stir bars and air- or water-driven magnetic stirrers (GFS Chemicals) at a minimum of 10-min intervals. Czaplewski and Parker (1973) described a useful technique for determining the required level of replication as a function of the confidence limits desired. Bottles are systematically rotated in position within the incubators to ensure uniform light conditions. A thin perforated acrylic sheet can be employed to increase the effectiveness of the stir bar and to keep it from contacting delicate algal material.

After the final O_2 or pH levels are recorded, individual thalli are carefully separated, spread, and photocopied or photographed; projected area determinations are made from each photocopy or print by overlaying a transparent gridwork of dots ($16 \cdot cm^{-2}$) and counting those intercepting the thalli. Care must be taken that the photocopier does not distort the specimens copied. If a uniform size change occurs, this is easily calculated and compensated. If a photocopier of sufficient quality is lacking, the plants can be spread and scored directly, although this slows the processing markedly and no permanent record is maintained. After photocopying, specimens are

dried at 80°C until they reach constant weight. All O_2 of pH values are converted to grams carbon fixed per square meter of thallus per hour and to milligrams fixed per gram dry weight per hour as outlined in Strickland (1960). For calcareous algae, ash-free dry weight may be used after 24 h of combustion at 500°C (see Brinkhuis, Chap. 22, for additional discussion). Other workers report their data as milligrams carbon per milligram chlorophyll *a* per hour after determining the pigment contents on fresh material (see extraction methods suggested in Duncan and Harrison 1982). For conversion of O_2 data to carbon units, a photosynthetic quotient (PQ = ratio of moles O_2 liberated to CO_2 taken up) and respiratory quotient (RQ = moles CO_2/MO_2) of 1.00 are usually assumed to facilitate interconversion with other data when different values are used. This assumption is unnecessary when pH measurements of CO_2 flux are determined directly. However, because calcification also influences CO_2 flux, the pH method must be supplemented with total alkalinity titrations in the case of calcareous algae. The differences among various experimental treatments can be examined statistically by single-factor analysis of variance or the Newman–Keuls Multiple Range Test (Sokal and Rohlf 1969).

b. Continuous-monitoring experiments. This design consists of simultaneously monitoring net photosynthesis via either O_2 or pH or both during controlled laboratory manipulations. Algal thalli are placed in specially constructed Plexiglas chambers, each fitted with pH and O_2 electrodes (Fig. 17–1; see Littler 1973a for details). Oxygen evolution can be read at the parts per 0.01 million level by means of a suitable O_2 analyzer and polarographic electrodes, whereas pH is monitored (to 0.001 pH unit) with a digital meter and combination electrodes. At the end of each experiment, thallus dry weights are determined as described earlier. The slopes of the curves for O_2 production and CO_2 uptake as a function of time are computed by regression statistics and compared for the different circumstances and measurement techniques. The incubation protocol is generally the same as that described for the bottle experiments, except that a cylindrical, magnet-driven stir bar under a perforated partition in each container is used to maintain a constant flow of medium past the alga and individual electrodes.

2. Specific field incubation techniques. Because our emphasis is on field O_2 studies, which we consider the method of choice in physiological–ecological work, we shall present the detailed step-by-step breakdown of the techniques developed by our group in the past decade. The emphasis will be on intertidal habitats, but the procedures are equally applicable subtidally (see Heine 1983).

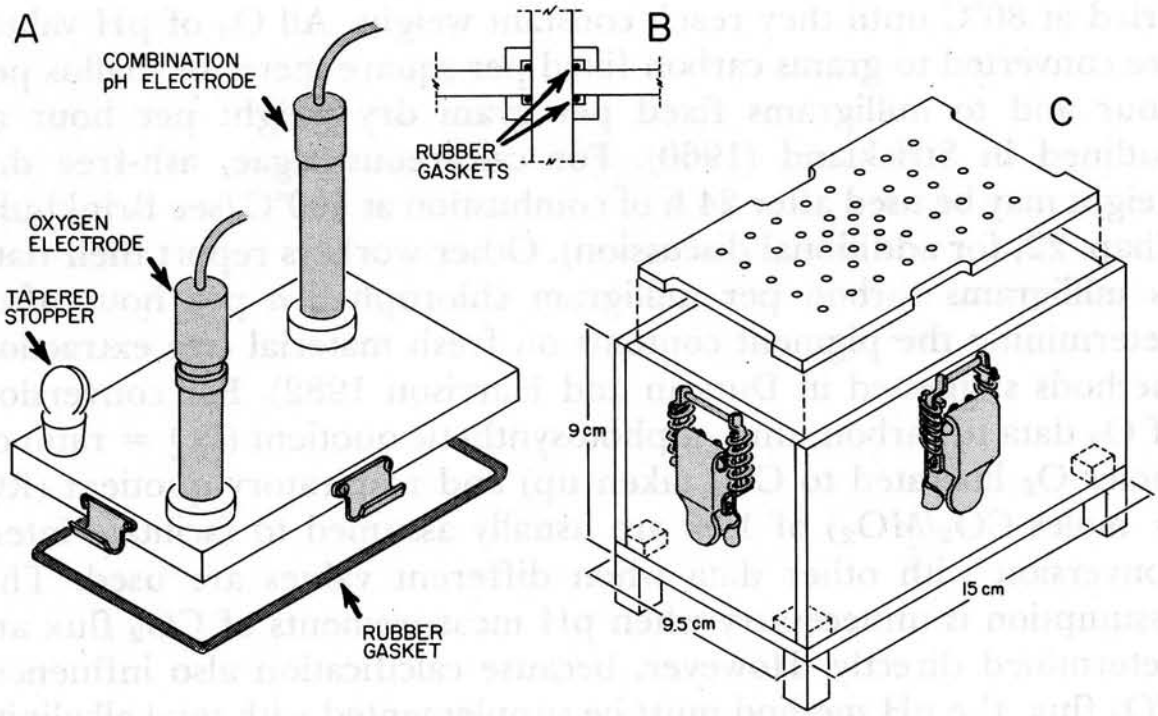

Fig. 17–1. Example of a gas-tight acrylic chamber used in photoperiod incubators for continuously monitoring CO_2 and O_2 flux. A, Design of the chamber lid with electrodes inserted; B, sectional view showing the design of the seals with an electrode inserted; C, view showing the component parts of the chamber.

a. Site selection. An area of the shoreline that is relatively flat and that receives direct sunlight during the complete incubation period (usually from 0900 to 1500) is selected as close as possible to the habitat from which collections are to be taken. The O_2 analyzer is set up in a shaded area out of direct sunlight. Bellows-type foot pumps (Zodiac boat inflators) and stirring racks are placed on hard level ground so as to prevent the contamination of the stirring turbines with particulate matter. If no hard substratum is present, flat pieces of plywood, cut to fit in the bottom of footlockers, are placed beneath the stirring apparatus.

b. Incubation water. Incubation jars are unpacked and placed upright in clear polycarbonate incubation trays (Curtin Matheson Scientific) filled with ambient seawater. The lids are opened, and stir bars, plastic partitions, and clean ambient seawater are added. The seawater is collected during early morning on the day of the experiments. Water from tide pools or that appears otherwise contaminated is not used.

c. Algal collection. All algal collections are made by diving or wading to obtain submerged material. Approximately twice as much algal material is collected as will be needed for incubation. Separate whole individual thalli should be used when possible; however, productivity measurements of the larger species are conducted on representative blades or branches. Care is taken to select individuals

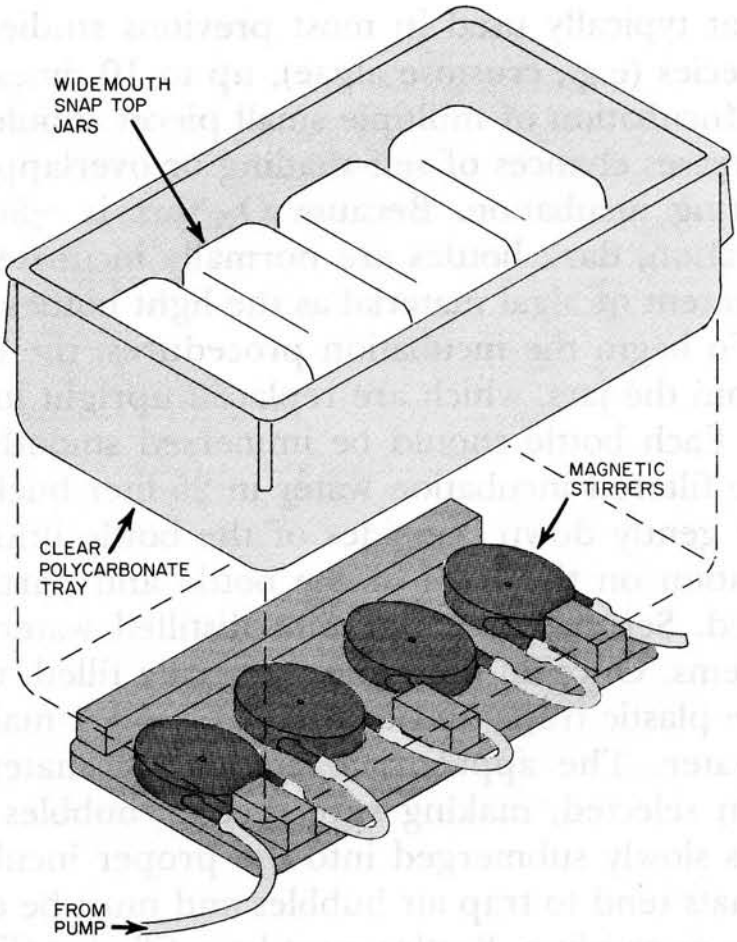

Fig. 17–2. Sunlight field incubation apparatus. It is important to connect all stir motors in series, and not in parallel, to provide uniformity and to reduce the volume of air or water required. The stir motor rack is placed inside the tray when water driven.

that are reasonably representative of populations within the system under study. If the specimens incubated are fertile, are wounded, or have unusual features, these observations are recorded. Macrophytes unduly exposed to air or to unnatural levels of sunlight or macrophytes otherwise injured are to be avoided. The collected plants are placed in separate, clear polycarbonate trays filled with ambient seawater and then hand-sorted and cleaned of epiphytes. Voucher samples can be taken from this material to provide taxonomic documentation (Tsuda and Abbott, Chap. 4). Identification notes, numbers, dates, substratum, depth, collector, and location should be included for all vouchers. Specimens are incubated at ambient water temperatures in approximately 0.5- to 4.0-ml wide-mouthed canning jars (volume is dependent on sizes and estimated productivity of the species), which are placed in clear polycarbonate trays (Fig. 17–2). Throughout the experiments, the average thallus dry weight per volume of water for a 2- to 4-h incubation period is maintained below the ratio of 0.04 g · liter^{-1} for high producers (e.g., sheetlike and filamentous forms). This is equal to about 0.2 to 0.4 g wet weight, which represents a very small quantity of material

relative to that typically used in most previous studies. For lower-producing species (e.g., crustose algae), up to 10 times this amount can be used. Incubation of multiple small pieces should be avoided, since this increases chances of self-shading or overlapping diffusion gradients during incubation. Because O_2 flux is ~50–90% lower during respiration, dark bottles are normally incubated with about double the content of algal material as the light bottles.

d. Setup. To begin the incubation procedures, the cooling water is emptied from the jars, which are replaced upright in the polycarbonate trays. Each bottle should be immersed smoothly below the surface of the filtered incubation water in 25-liter buckets, allowing water to flow gently down the sides of the bottle until nearly full. Bubble formation on the walls of the bottle and partition is to be strictly avoided. Scrubbing bottles with distilled water reduces degassing problems. Once all bottles are partially filled, they are kept upright in the plastic trays, and external cooling is maintained with ambient seawater. The appropriate amount of material for each species is then selected, making sure that no bubbles adhere, and each thallus is slowly submerged into the proper incubation bottle. Dense algal mats tend to trap air bubbles and must be eliminated by gentle shaking or swirling. Bottles must be refilled sufficiently below the surface so that the lids can be securely clamped without trapping air. Bubbles are often squeezed from the rubber washer into the bottles when the lids are clamped and should be released before the lid is secured. If all components are not at the same temperature, degassing on surfaces will occur. Once the first small bubble forms, degassing will accelerate (Littler 1979) since oxygen is more soluble in air than in seawater.

For every six bottles incubated, one initial bottle (i.e., without algal material) should also be included. These bottles are treated in the same way as those containing algae and analyzed for O_2 content before reading of the associated light and dark bottles. Additional initial bottles are retained to serve as checks for plankton or bacterial metabolism as well as possible electrode drift. All bottles should be completely submerged on their sides in the polycarbonate incubation trays. Trays should not shade each other, and personnel must be kept away from the trays while samples are incubating to prevent shading. Dark bottles, produced by wrapping and taping two layers of heavy-duty aluminum foil, are always incubated separately to prevent the impingement of reflected light on the initial and light bottles. It is important to ensure that hardware around the tops of the bottles does not puncture the foil. It normally takes 3–5 min to set up completely the minimum four replicate light bottles and two

replicate dark bottles per species. The "time in" recorded for each species is the time that the last bottle is completely set up.

e. Incubation. For studies designed to compare the performances of different species, sample incubation begins between 0900 and 1030 h, and final O_2 values are read between 1300 and 1500 h, with maximum incubation times lasting 3.5–4.5 h for light bottles and 5.0 h for dark bottles. Depending on the questions being asked, any midday depression in photosynthesis (Ramus and Rosenberg 1980) due to excessive light causing photoinhibition can be avoided by the reduction of the light with layers of neutral density screening (nylon window screening). Species that are high producers (e.g., filamentous or sheet forms) should be analyzed early to avoid bubble formation and nutrient depletion. At ~10-min intervals, each bottle is thoroughly mixed with stir bars and magnetic stirrers (Fig. 17–2) driven by an air pump or mixed continuously if an electric water pump (Pony Pump, Proven Pump Corporation) and gasoline generator are available. Our trays hold four 1-liter jars and fit onto racks with four appropriately placed motors for stirring. Cooling is accomplished by refilling the trays with ambient seawater at 10- to 15-min intervals or by the excess water from the electric pump.

f. Physical data. Physical measurements that are minimally required include light readings taken at 15-min intervals with three different sensors (Li-Cor model LI-185A, Lambda Instruments, Inc.): illumination (lux), photosynthetically active radiance (microeinsteins per square meter per second; $\mu E \cdot m^{-2} \cdot s^{-1}$), and power (watts per square centimeter; $W \cdot cm^{-2}$). The sensors are cosign-corrected and must be perfectly level during readings to ensure highest accuracy (see Ramus, Chap. 2). The sensors should not be shadowed by any object, particularly the measurer. When readings are taken during intermittent cloud cover, the approximate mean values occurring over 1-min intervals are recorded. A minimum of 30,000 lux is required for realistic natural field rates to ensure that measurements are conducted above light saturation (for typical light saturation values for marine macroalgae, see King and Schramm 1976; and Arnold and Murray 1980). Light approaching 120,000 lux is inhibitory for many seaweeds (see Lüning 1981), and the use of neutral density screening may be warranted in some cases. Air and water temperatures should be recorded to 0.2°C throughout the experimental run and out of direct sunlight, the time being noted. Periodically, the temperature of the incubation water should be checked and should not be allowed to rise more than 1.5°C above the ambient water temperature. Occasionally, dark bottles will be cooler than light bottles, but not by more than 1.0°C. If freshwater drainage is detected by

salinity measurements (to 0.5 ppt, American Optical Corporation, model 10412 salinity refractometer), the salinity of the ambient water should be monitored periodically.

We record all data on waterproof plastic paper (PolyPaper, Nalge Company) that is stored in an all-plastic three-ring binder. Essentially, three types of records must be maintained in the field: (1) incubation data, including species, time in, date, bottle number, time out, and dissolved O_2 values; (2) physical data, incorporating light measurements, water and air temperature, and salinity; and (3) general notes concerning algal collection data, weather conditions, or any other pertinent information. Three persons operating at a high level of field efficiency can incubate about 60 bottles per day; with completely mechanized cooling and stirring, two persons can handle up to 80 bottles per day.

g. Harvesting. Bottles are assigned their numerical order at the time they are harvested, and the final readings are taken. The light bottles are grouped by species and processed first with the time recorded for each. Each dark bottle should be unwrapped only just before being read. Aluminum foil develops light leaks at the corners of folds and must be discarded after each experimental run. When the sequence of O_2 readings is begun, all other bottles being incubated must continue to be stirred and cooled at frequent intervals.

h. Reading. In preparation for the dissolved-O_2 readings, the bottles to be read are placed in an upright position in a polycarbonate tray on a separate stir motor rack near the analyzer. Sufficient ambient seawater is replenished in the tray to provide proper temperature control. The clamp on the jar is unfastened and the suction seal is broken by pulling the rubber tab at right angles. The plastic partition is quickly removed, as is the specimen. The O_2 electrode in a no. 14 stopper is then inserted rapidly and smoothly into the bottle so that all of the air is displaced. Each algal specimen is retained without excess water in a labeled (species, date, location, and sequence number) Whirl-pak bag. For filamentous algal material that is too fine to remove quantitatively by forceps, the O_2 level is recorded first and the contents of the jar are then poured through a fine-mesh tea strainer and the thalli are scraped into a bag. The bottle, positioned over one of the stir motors, should be mixed at the maximum possible spin rate. If electric self-stirring electrodes capable of mixing the entire contents of the bottles are available, then the turbine stirrers and foot pump are not required during the reading process.

For measurement of the dissolved O_2, the analyzer is set to the appropriate scale and turned on after about 30 to 45 s of mixing. When making O_2 readings, one must take care to prevent differential

heating of the electrode or sample in direct sunlight. The O_2 readings in most light bottles will continue to change for ~2 min if stirring is at the maximal rate. It should be noted that the response becomes proportionately slower as the meter approaches the correct and final value. The final reading is recorded next to the sequence number of the bottle when the meter becomes stable.

i. Processing. Specimens should be stored in a dark, cool, and dry location. The samples are transported to the laboratory in an insulated cooler. Upon being returned to the laboratory, the samples are photocopied and weighed as soon as possible. We have found that materials can be left for no more than about 4 d, even under refrigeration, or they will begin to decompose partially. At humid, tropical field sites, we fix the specimens in 4% Formalin in buffered seawater, drain the excess liquid, and store them in the dark. The samples are sorted and processed in order of their incubation dates and times, the earlier materials being treated first.

Photocopying should be carried out by means of a rigorously standardized procedure. The contents of each Whirl-pak bag are shaken down, and the bag is cut with a razor blade near the bottom. The contents are placed onto the copier surface, and a forceps is used to spread the material to its natural configuration. The field label should be placed next to the specimen so that it appears legibly on the copy. The species numbers and identifications are recorded on the data sheet with the number of its tared aluminum foil container. After photocopying, the algae are dried to constant weight at 80°C in a drying oven. Final weighing to 0.001 g is generally done 2 or 3 d after the material has been dried and cooled in a desiccator.

Area determinations are made on the two-dimensional photocopies by placing a dot-matrix grid over the impression (see Brinkhuis, Chap. 22). The area is scored as cumulative hits (i.e., when the dots on the grid intercept the impression of the algal thallus), and the hits are directly converted to two-dimensional area. Lambda Instruments, Inc., also manufactures a leaf-area meter (model 3100-H.1) that is expensive but time saving in this application. Total surface area can be calculated for the appropriate geometric shapes involved (multiply two-dimensional area by 4 for spherical thallus portions, by $3.146 = \pi$ for cylinders, etc.). We reemphasize that care must be taken to ensure that distortion is negligible during the photocopying process. The point intercepts are calibrated to a specific area (e.g., $16 \cdot cm^{-2}$), and consequently hits are directly proportional to area.

j. Calculations. In calculating the photosynthetic data, we average the values for all initial bottles for a given species for the day of each experiment and contrast their mean with those of the light bottles, as well as with the dark bottle numbers, to obtain net photosynthesis

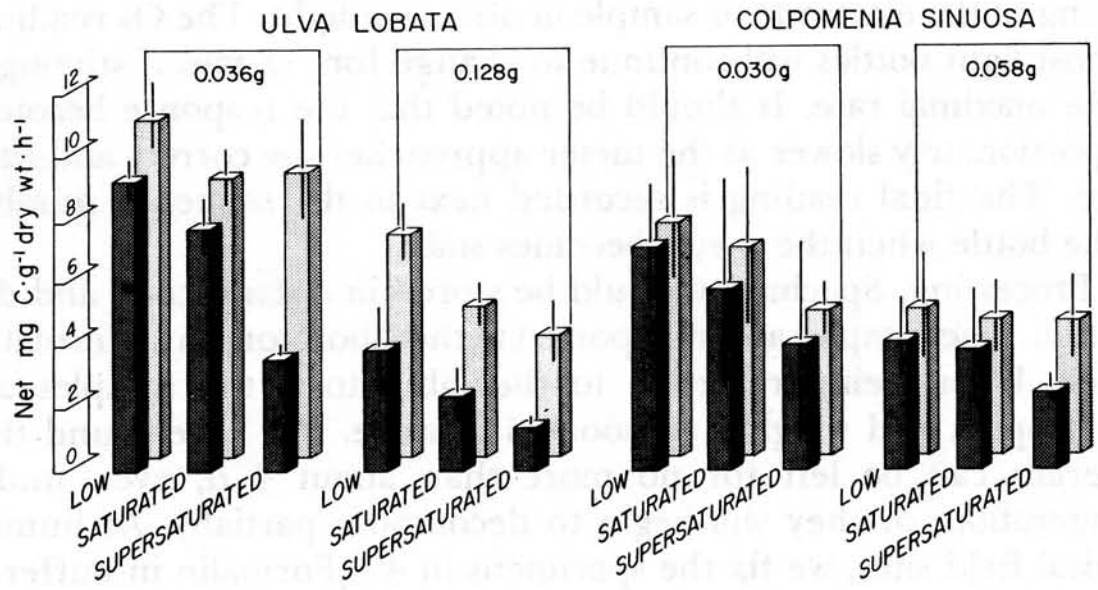

Fig. 17–3. Apparent photosynthesis per gram dry wt of *Ulva lobata* and *Colpomenia sinuosa* as a function of the interacting effects of bottle size, mean thallus weight, and initial dissolved O_2 tension (low, saturated, and supersaturated). The darker histograms are for 310-ml bottles, the lighter histograms for 1220-ml jars; ±95% confidence intervals are given by the straight lines at the top of each histogram. Modified from Littler (1979).

and respiration, respectively. After all calculations are completed, the average net productivities and respiration values, their standard deviations, 95% confidence intervals, and coefficients of variation are computed. These data are summarized on a separate data sheet and subsequently tabulated and plotted (Figs. 17–3 to 17–5). Calculations are as follows:

LB = O_2 content of the light bottle after incubation (milligrams O_2 per liter) × volume (liters)

DB = O_2 content of the dark bottle after incubation (milligrams O_2 per liter) × volume (liters)

IB = O_2 content of the water before incubation (milligrams O_2 per liter) × volume (liters)

t_x = incubation time

$$\text{NPP} \times t_x^{-1} = \text{LB} - \text{IB} \tag{2}$$

$$\text{GPP} \times t_x^{-1} = (\text{LB} - \text{IB}) + (\text{IB} - \text{DB}) \tag{3}$$

This provides a measure of net and gross photosynthesis and respiration for the period of incubation, expressed in units of dissolved O_2. To convert these O_2 units to units of assimilated carbon, that is, NPP or GPP, it is necessary to introduce not only PQs and RQs into the calculations, but also a factor to convert O_2 units to carbon units.

Dissolved O_2 is usually measured in units of milligrams O_2 per

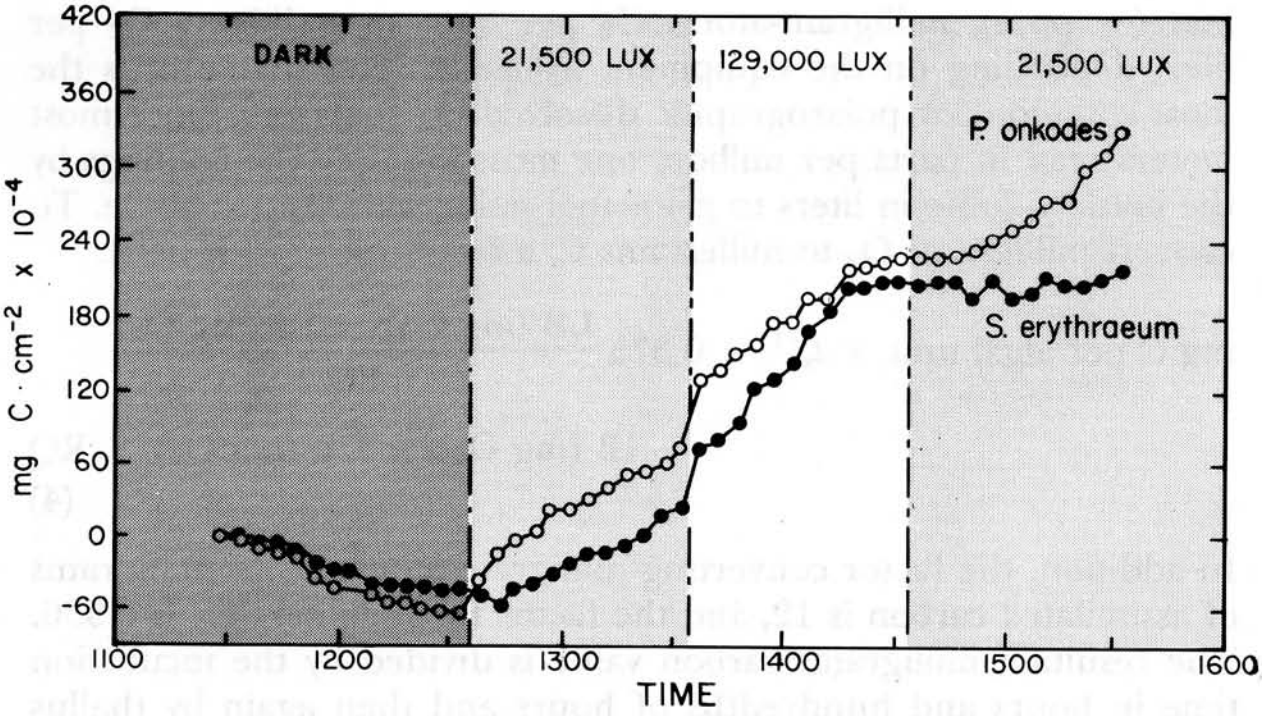

Fig. 17–4. Variations in the productivity of a light-adapted plant (*Porolithon onkodes*) and a shade-adapted macrophyte (*Sporolithon erythraeum*) to initial control, experimental, and final control light intensities.

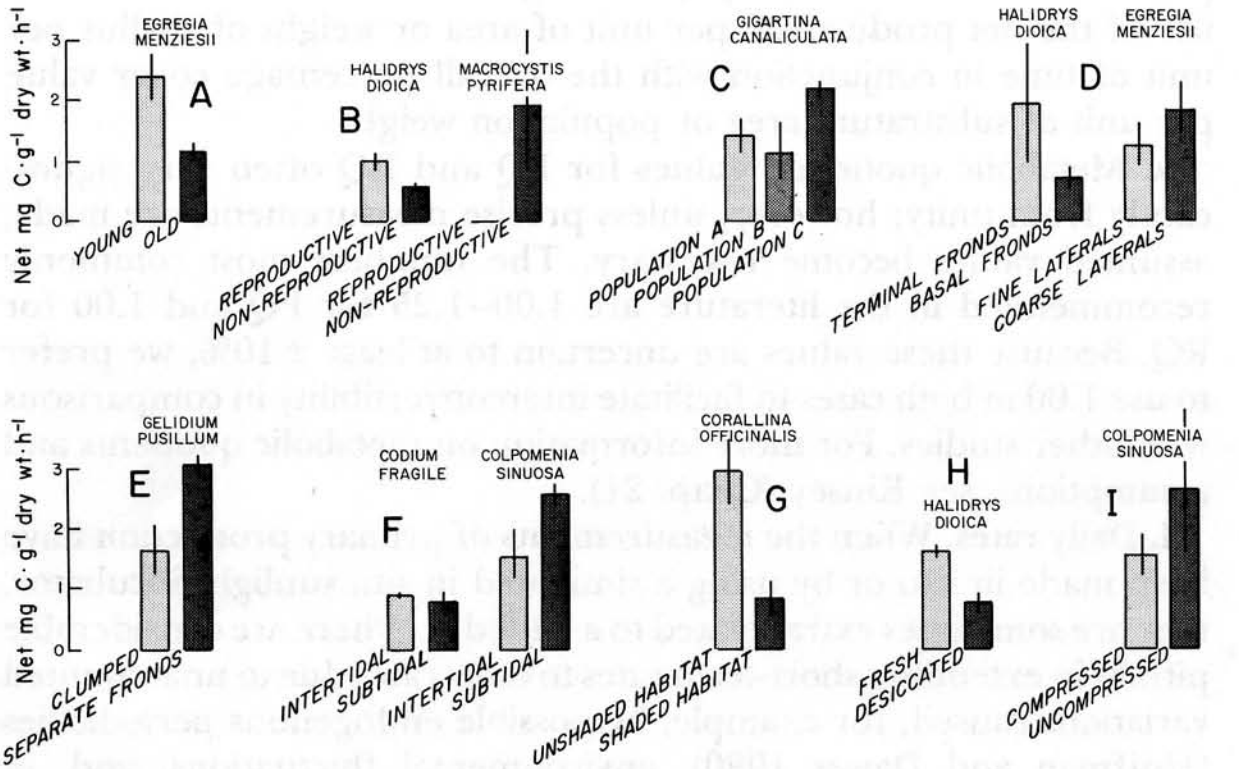

Fig. 17–5. Net productivity for (A) young vs. mature *Egregia,* (B) reproductive vs. nonreproductive *Halidrys* and *Macrocystis,* (C) wiry (populations A and B) vs. fleshy (population C) of *Gigartina,* (D) terminal vs. basal fronds of *Halidrys* and fine vs. coarse laterals of *Egregia,* (E) clumped vs. separate thalli of *Gelidium,* (F) intertidal vs. subtidal *Codium* and *Colpomenia,* (G) *Corallina* from unshaded vs. shaded habitats, (H) desiccated vs. fresh *Halidrys,* and (I) compressed vs. uncompressed *Colpomenia.* Modified from Littler and Arnold (1980).

liter (= ppm), milligram-atoms O_2 per liter, or milliliters O_2 per liter, depending on the equipment available. The first unit is the most common for polarographic dissolved-O_2 analyzers. Since most meters read in parts per million, one must multiply the readings by the bottle volume in liters to get actual milligrams O_2 per bottle. To convert milligrams O_2 to milligrams C, a factor of 0.375 is used:

$$\text{mg C per algal unit} \times t_x^{-1} = 0.375 \frac{\text{LB (mg O}_2\text{)} - \text{IB (mg O}_2\text{)}}{\text{PQ}} + \text{IB (mg O}_2\text{)} - \text{DB (mg O}_2\text{)} \times \text{RQ} \quad (4)$$

In addition, the factor converting milligram-atoms O_2 to milligrams of assimilated carbon is 12, and the factor for milliliters O_2 is 0.536. The resultant milligrams carbon value is divided by the incubation time in hours and hundredths of hours and then again by thallus weight, chlorophyll content, or area. One can convert milligrams carbon per square centimeter per hour to grams carbon per square meter per hour by multiplying by 10. If standing stock determinations have been made concomitantly with photosynthesis, the average net production for each macrophyte at a given site can be estimated by use of the net productivity per unit of area or weight of thallus per unit of time in conjunction with the overall percentage cover value per unit of substratum area or population weight.

k. Metabolic quotients. Values for PQ and RQ often vary significantly from unity; however, unless precise measurements are made, assumed values become necessary. The numbers most commonly recommended in the literature are 1.00–1.25 for PQ and 1.00 for RQ. Because these values are uncertain to at least ±10%, we prefer to use 1.00 in both cases to facilitate interconvertibility in comparisons with other studies. For more information on metabolic quotients and assumptions, see Kinsey (Chap. 21).

l. Daily rates. When the measurements of primary production have been made in situ or by using a simulated in situ sunlight incubator, they are sometimes extrapolated to a 24-h day. There are considerable pitfalls in extending short-term rates to daily rates due to unaccounted variations caused, for example, by possible endogenous periodicities (Hoffman and Dawes 1980), environmental fluctuations, and, in some cases, photoinhibition near midday (Ramus and Rosenberg 1980). If extrapolation is deemed necessary, respiration must be normalized to a 24-h day. The calculations would be as follows:

$$\text{NPP mg C per algal unit per 24-h day} = (\text{NPP} \times \text{h per daylight day} - (\text{R} \times \text{h per night}) \quad (5)$$

Short-term or even diurnal rates cannot be used to derive yearly rates adequately.

II. Equipment and materials

Analytical equipment for primary productivity measurements must be of the highest precision and accuracy available to detect the extremely small O_2 and CO_2 fluxes normally encountered. Precision is most critical because changes in parameters are of primary importance; however, if the instruments are not accurately calibrated initially, slope problems (Hitchman 1978) will occur. Consequently, instrumentation must be both accurate and capable of maximum precision (see Hitchman 1978; Polgreen and Coker 1981; Smith and Horner 1982).

A. Oxygen analyzers

1. Amplifiers. There are many analytical systems of sufficient quality, the best of which are polarographic instruments that can be precisely read at the 0.1- to 0.01-ppm level. We have had considerable experience with the Beckman Instruments Fieldlab oxygen analyzer, Yellow Springs Instruments model 57, and Orbisphere Laboratories model 2610. The last two are temperature-compensated systems and provide accuracy and precision at the required levels while being sufficiently reliable under rigorous field conditions. The O_2 electrode method exhibits close agreement with Winkler-determined O_2 values (Beckman Instruments, Inc. 1972; Hitchman 1978) and has a linear response throughout all dissolved-O_2 concentrations (including supersaturated levels). The Winkler method is much more labor intensive but is generally thought to provide increased sensitivity when used in the laboratory, although Orbisphere Laboratories manufactures an amplifier (model 2713) with a special flow-through electrode capable of indicating O_2 changes at the parts per hundred billion level. For both O_2 and pH, digital systems, such as the Orion digital pH analyzer (model 801) with the Orion digital printer (model 851) and the Orion automatic electrode switch (model 855), are preferred to analog meters because of the difficulty of reading a moving pointer due to boat motion and parallax. The best approach with both O_2 and pH analyzers is to record the millivolt output directly into a data logger. Also, an instrument interfacer (Analogue Devices) in combination with a microcomputer now makes it possible to program multiple electrodes for monitoring at selected intervals in the laboratory and to analyze, tabulate, and plot data in the absence of the investigator.

2. Membranes. Plastic (polyethylene) electrode membranes perform better than other materials because of their longer stability after calibration (Hitchman 1978). However, it should be noted that changing the membrane material from that specified by the electrode manufacturer will alter the thermal response characteristics, requiring appropriate compensation. Calibration of the O_2 electrode is required because the sensitivity of the analyzer depends on the tension in the sensor membrane. Typically, a plastic membrane relaxes during a period of about 1 h after mounting, with associated alterations in thickness and O_2 permeability. Therefore, it is essential to wait at least an hour before calibration. The sensitivities of different sensors, or the same sensor fitted with different membranes, lie within a range of $\pm 5.0\%$ around a mean. A newly refilled sensor should be immediately inspected for trapped air bubbles within the circumference of the sensor tip. Trapped air bubbles or folds in the membrane near the central area must be eliminated for satisfactory performance of the sensor at very low oxygen levels. Small folds in the membrane near the outer perimeter are unavoidable and have no influence. It is important to avoid touching the membrane of a calibrated sensor, since this or other disturbance can cause a change in membrane tension, which necessitates recalibration.

3. Cathodes. Electrodes with gold cathodes are preferable to platinum cathodes (Hitchman 1978) because of the resistance of gold to oxidation by free sulfide. Large cathodes and large electrolyte volumes are desirable because of their sensitivity; however, they tend to consume more O_2 than smaller units in closed systems. The latter have more rapid response times, which is important. The cathode is the most delicate part of the entire O_2-measuring system because it produces the signal that is eventually displayed and because it must maintain extreme sensitivity and selectivity for O_2 during the entire life of the system. It should never be touched by fingers or exposed to detergents or oily liquids.

4. Calibration. Calibration should preferably be conducted near the middle of the temperature range over which O_2 measurements are to be performed. Oxygen electrodes can be calibrated in water-saturated air or air-saturated distilled water, but it is slightly more precise to air-saturate the actual seawater to be incubated. The appropriate O_2 values for calibration are obtained from standard tables (Carpenter 1966) by interpolation from temperature, salinity, and barometric data. During calibration in air, a stable reading can be obtained only if the temperature of the air and that of the sensor are constant. Consequently, drafts should be avoided and the sensor

dried so that evaporative cooling does not occur. Agitation of the sample is not necessary during gas-phase measurements.

The temperature measurement detected by the sensor must be constant and agree with that given by a good-quality thermometer (within 0.2°C). About 2 min are required for stabilization of the display upon immersion of the sensor in water. Much longer periods are required for stabilization upon removing the sensor from water into air at a different temperature, due to the lower thermal conductivity of air. At first reading, the sensor will produce an anomalously high signal due to the consumption of the O_2 dissolved in the interior filling solution. The signal will stabilize within about 3 min to a steady value when read in air. Air-saturated water for calibration can be produced either by passing fine air bubbles through the water or by shaking with air. It is important to note that this equilibration process normally requires as much as 15 min. Most O_2 electrodes will require relatively high mixing rates in water, equivalent to about 50 cm $\cdot$ s^{-1} linear velocities. One should always check the adequacy of stirring by noting any effect of movement of the sensor that will cause the signal to rise if stirring is inadequate. The YSI and Orbisphere electrodes are available with built-in stirring devices that are quite advantageous because they eliminate the need for stir motors and other external means of mixing during the time of reading. Once the output becomes constant, it may be adjusted by means of the calibration control.

B. pH meters

A thorough discussion of the use of pH in metabolic studies has appeared (Smith and Kinsey 1978) and should be consulted if further methodological detail is required. The finest-quality instruments in terms of stability and sensitivity are essential for determining CO_2 flux in seawater. For field use, the unit must be readable to 0.001 pH unit and reliable to ± 0.005 pH unit; it should be battery-operated, or a field generator should be available. The combination pH electrodes (e.g., Broadly-James no. 9061-18S) can be calibrated in standard buffer solutions, but buffers near the salinity of the seawater to be measured are preferred. See Kinsey (Chap. 21) for a detailed discussion of pH electrodes and buffers.

For calcareous macroalgae, the pH method must be used in conjunction with total alkalinity titrations, because calcification as well as organic carbon flux affects the pH. This represents an advantage over O_2 methods, which cannot discriminate calcification, for such organisms. If total alkalinity is not determined, changes in pH are converted to changes in CO_2 concentration by means of the

standard procedures of Beyers (1970). This involves interpolating from a CO_2 versus pH function previously determined for a particular medium (i.e., by removing all CO_2 with bubbled N_2 and then titrating with CO_2-saturated distilled water to obtain a curve for pH as a function of millimolar CO_2) and then to grams carbon fixed per square meter of thallus, milligrams carbon per gram dry weight, or milligram carbon per milligram chlorophyll *a*. The CO_2/pH function is not linear and must be assessed for each different source of incubation water.

C. Winkler reagents and equipment

Winkler O_2 titration is a simple, accurate, nonautomated, low-cost, but labor intensive chemical means of dissolved-O_2 assessment. It requires only a limited amount of glassware including 300-ml biological oxygen demand (BOD) bottles, 150-ml Erlenmeyer flasks, reagent bottles, pipettes, a burette capable of reading to 0.01 ml, a stir bar, a stirring motor, and an incandescent light source. Special reagents required are manganous sulfate, sodium hydroxide, potassium iodide, soluble starch, sodium thiosulfate, hydrochloric acid, glacial acetic acid, sulfuric acid, sodium carbonate, carbon disulfide, and potassium iodate.

The reader is referred to Strickland and Parsons (1972) for basic detail, but for the sake of completeness, a working summary of the time-tested method is included here. Carpenter (1965a) described an improved version of the technique. A 1.0-ml manganous sulfate solution (480 g $MnSO_4^- \cdot H_2O$ per liter) is added to the BOD sample bottle with an automatic pipette, and the sample is restoppered and shaken. Then, 1.0 ml alkaline iodide solution (500 g NaOH per 0.5 liter + 300 g KI per 0.45 liter) (do not cross-contaminate pipettes) is added, and the sample contents are mixed thoroughly. After the precipitate settles slightly (2–3 min), it is mixed and resuspended. At this stage, the stoppered samples can be stored at constant temperature for up to 1 d if necessary.

After the precipitate again settles one-third of the way, 1.0 ml of concentrated sulfuric acid is added; the bottle is restoppered and then mixed until all of the precipitate dissolves. Within 1 h, 50.0 ml of this solution is pipetted into a flask and titrated at once over a mechanical stirrer with standard thiosulfate solution (145 g $Na_2S_2O_3 \cdot 5H_2O$ + 0.1 g Na_2CO_3 per liter + one drop CS_2) until a pale straw color remains. Five milliliters of starch indicator is added, and the titration is concluded to a colorless end point. A white background and good light source are needed to detect the end points accurately. The O_2 content is calculated from the following formula using the volume of the total titre (V) when a 50.0-ml aliquot is taken from a

300-ml BOD bottle:

$$\text{mg-atom } O_2 = 0.1006fV \quad (6)$$

To determine the f value, a 300-ml BOD bottle is filled with seawater and 1.0 ml of concentrated sulfuric acid added. The contents are mixed, 1.0 ml alkaline iodide solution is added, and the contents are stirred again. Finally, 1.0 ml of manganous sulfate solution is added and mixed. Fifty-milliliter aliquots are then withdrawn into two titration flasks, and 5.00 ml of 0.0100 N iodate is added to each of these flasks with a calibrated, clean, 5-ml pipette. After a 2- to 5-min delay, during which the two solutions are kept out of the direct sunlight, the iodine is titrated (using 5.0 ml of starch indicator) with the standard thiosulfate solution. Considering the mean volume V of both titers in milliliters, f is obtained by

$$f = 5.00/V \quad (7)$$

The milliliters of O_2 per liter in the water sample can be computed as follows:

$$\text{mg } O_2 \text{ per liter} = 16.00 \times \text{mg-atoms } O_2 \text{ per liter} \quad (8)$$

Carpenter's (1965b) modifications improve the accuracy of the Winkler technique, and microtechniques involving dispensers can be advantageous (Fox and Wingfield 1938; Duedall et al. 1971).

D. Calcification methods

The pH/alkalinity method (see Kinsey, Chap. 21) and the ^{14}C (Borowitzka 1979) and ^{45}Ca (Böhm 1978) isotopic–kinetic methods are precise enough that one can determine changes in calcification, the last two being most sensitive. Calcium electrodes do not provide the required level of sensitivity or precision to detect the small changes that take place during biological calcification in seawater (Littler 1973a).

III. Critical evaluation

A. Oxygen electrode, pH, and Winkler methods

In the field, the O_2 electrode technique is preferable to the pH electrode method because it is more reliable and nearly twice as sensitive (Marsh and Smith 1978). Also, compared with Winkler equipment and supplies, O_2 analyzers are less bulky, faster, and easier to use under rugged field conditions. No matter which of the various techniques is chosen for primary productivity studies, uncertainties will exist concerning precisely what is being measured. Much of the problem lies in the confusion regarding (1) excretion of

dissolved organic carbon and (2) whether respiration as measured by O_2 uptake in the dark is equivalent to that in the light, as well as the facts that (3) respiratory CO_2 or photosynthetic O_2 can be recycled during photosynthesis or respiration, respectively, without ever leaving the alga, (4) some plants can virtually "shut down" respiration in the dark or under nutrient deficiency, and (5) photorespiration, if present (Burris 1977; Kremer 1980), can involve the consumption of O_2 in the light during glycollate synthesis.

B. Units reported

We strongly suggest that productivity rates in ecological studies be reported in terms of area or weight of thallus (as opposed to pigment content) at saturating but not inhibiting light, because these are related to standing stocks and other more ecologically relevant factors. For example, space, light, and nutrients are known to be limiting resources in many benthic macrophyte communities (Dayton 1975), and algae compete for these by means of their surface area/cover. Biomass (organic dry weight) is also an ecologically significant parameter because it represents the standing stock or organically bound energy potentially available to higher trophic levels. Consequently, macrophyte cover and biomass are of primary ecological interest.

Furthermore, as Ramus et al. (1976, 1977) pointed out, the customary plot of photosynthetic performance versus chlorophyll content can be misleading. Only in optically thin plants can the chlorophyll concentration sometimes approximate linearity with O_2 production. A major problem with normalizing photosynthesis to the traditional parameter, chlorophyll *a* concentration, is that it brings into the calculations only one of several of the important light-harvesting pigments. Therefore, the expression of carbon flux per unit chlorophyll is not as appropriate in benthic ecology, as has been the convention in biological oceanography (mainly because phytoplankton biomass and area are relatively intractable parameters). Algae can also alter their pigment contents dramatically (Ramus et al. 1976; Ramus and van der Meer 1983) depending on the light environment, and this induces another source of variability. However, pigment data are certainly very appropriate for interpreting weight- and area-based photosynthetic differences or if questions concerning assimilation numbers and light-gathering capacities are posed.

C. Incubation conditions

Many of the studies on primary productivity of marine macrophytes have not adequately considered the effects of (1) incubation conditions, (2) antecedent environmental differences, and (3) intrinsic aspects of variation within the organisms themselves.

1. Thallus-weight/bottle-volume ratios. Generally speaking, ratios of thallus weight to bottle volume should be optimized while ensuring that the specimens used are representative of the organisms being investigated and that enough material is incubated to produce reliable, measurable metabolic changes (Fig. 17–3; Littler 1979). Even in relatively large containers, numerous small specimens can clump, shade each other, and result in overlapping diffusion gradients, a situation that will lead to markedly lower apparent production rates. For smaller algae, it is far better to incubate one individual in a small bottle than to use several or many thalli in a larger bottle. In the case of larger algae, the proportions of the container should be commensurate with the size and metabolic rate of an entire representative thallus, whenever practicable. Plastic bags and domes of various materials have occasionally been used (Towle and Pearse 1973; Hatcher 1977; Smith and Harrison 1977) as field incubation chambers. These are useful if provision is made for (1) adequate mixing, (2) prevention of gaseous exchange with the surrounding medium, and (3) adequate replication – all very difficult criteria to satisfy with large containers. In order that valid comparisons can be made with other studies, it is mandatory that ratios of thallus dry weight to bottle volume be reported.

2. Length of incubation. The length of the incubation period also should be optimized (Littler 1979), because this factor interacts with the thallus-weight/bottle-volume ratios. Too long an incubation period can be problematic in that autoinhibitory substances might accumulate (Curl and McLeod 1961), bacterial populations tend to increase on the surfaces within the bottles, or nutrients and inorganic carbon may be depleted. A short period can induce errors due to possible daily photosynthetic periodicities and transient CO_2 or O_2 carried over in response to previous holding conditions. Consequently, it is important to report the incubation interval so that the magnitude of changes in O_2, CO_2, and pH can be evaluated when comparisons are desired.

3. Continuous monitoring. Because the gas exchange rates are likely to change during the course of an experiment, the continuous-monitoring method, utilizing individual electrodes within chambers, has a definite advantage over bottle experiments in which only initial and final values are used to calculate rates. Continuous monitoring also permits the comparison of regression slopes (analysis of covariance; Sokal and Rohlf 1969) during initial control, experimental, and final recovery periods (Fig. 17–4) to assess the impact of environmental factors on the natural homeostatic capabilities of algae (Littler 1973b).

Table 17–1. *Slopes 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
Mean	4.20	15.04[a]	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
Mean	1.13	1.29	0.92	0.72

Note: Slopes expressed as milligrams carbon per gram dry weight each hour.
[a] Mean for stirring is significantly different from mean for no stirring at $P < .05$

4. Wounding. The use of cut disks or fragments, as well as rough handling, generally is to be avoided, depending on the nature of the experiment, because such phenomena as wound respiration and oxidation of organic exudates often result in unnaturally low net photosynthetic rates (Hatcher 1977; Dromgoole 1978).

5. Mixing. It has been shown consistently that some means of stirring is required to obtain realistic production rates in closed containers. However, mixing is much more critical for the more productive thallus forms having relatively high surface/weight ratios (Table 17–1). To ensure adequate mixing, a growth or NPP versus mixing rate curve can be generated to establish the water-motion saturation level (Santelices 1978). Since shading is difficult to avoid during hand shaking of bottles, we have found magnetic turbines operated by a water pump or a bellows-type air pump to be most effective for mixing. If shaking by hand is the only method available for agitation, we suggest that rectangular bottles be used, since rotating them results in the generation of considerable movement and momentum within the contained water mass.

6. Bottle type. The environmentally significant ultraviolet portion of the solar spectrum should be considered (Ohle 1958; Findenegg 1966) when incubation bottles are chosen. The bottles recommended here are made of the Wheaton "800"-type glass, which transmits less of the photosynthetically harmful ultraviolet spectrum than does Pyrex glass (Worrest et al. 1980). The rectangular versions are

preferable (Fig. 17–2) because of economy in packing, the flat bottle sides do not restrict stir bar action, and greater water motion can be produced by hand rotation than is the case with cylindrical jars. The insides of the jars should be cleaned in aqua regia, rinsed, and then aged in distilled water for 30 d before service. In our opinion, one should never expose incubation glassware to detergents, preservatives, chromic acid, or other toxic chemicals.

7. Metabolic quotients. We strongly recommend supplementing the O_2 electrode technique with some other method such as the pH electrode technique or ^{14}C labeling when experiments are to be conducted at high O_2 levels. Supplementation with the pH technique has the additional advantage of providing an estimate of the PQ and RQ. The PQ can be an extremely important parameter to measure because it provides a useful index of (1) the type of nutrition (e.g., NO_3^{2-} or NH_4^+ as nitrogen sources), (2) changes in physiological state (e.g., due to stress or changes in light energy; see Kindig and Littler 1980), and (3) the type of material stored (i.e., carbohydrates, proteins, or lipids). The RQ is influenced primarily by the compounds being metabolized and tends to increase at lower temperatures.

8. O_2 supersaturation. Procedures that might result in bubble formation must be avoided because this can be problematic depending on the bubble volume and the extent to which the gas and liquid phases are in equilibrium. Oxygen supersaturation poses problems (Burris 1977; Littler 1979; Kremer 1980) other than bubble formation in the measurement of productivity of marine algae in closed systems. Dromgoole (1978) has shown that macroalgae liberate O_2 more rapidly at low initial O_2 levels than at high levels. Estimates of productivity based on light and dark closed containers will vary as a function of the initial O_2 concentration and the relative sensitivities to O_2 tensions of photosynthesis and dark respiration in the organisms under study (Fig. 17–3; Littler 1979).

In remote field situations, it is not always possible to use N_2 or other gases to reduce the supersaturated levels of O_2 characteristically present near highly productive nearshore environments, and available CO_2 might also be decreased in the process. As a consequence, we recommend the vigorous pouring or shaking of a stock incubation batch for at least 15 min (Strickland 1960) until air saturation is obtained. Incubation water below O_2 saturation levels also can be obtained before sunrise when O_2 levels have been naturally reduced by night respiration. Depending on the objectives of the experiment, field comparisons can be made reasonably only under uniform conditions of initial dissolved O_2. The initial O_2 tensions of both the light and dark bottles must be given.

D. Intrinsic variability

Other important considerations relevant to many experimental designs are the intrinsic causes and ranges of photosynthetic variability (see Littler and Arnold 1980). Such variations can be quite pronounced owing to differences in season, age, reproductive condition, morphology, and thallus portion incubated, as well as to previous conditions of crowding, macrohabitat, microhabitat, desiccation, and physical stress (Fig. 17–5). These factors, unless controlled, can result in inordinate within-species discrepancies and certainly must be taken into consideration in the design of studies on marine macroalgal productivity. An understanding of sources of photosynthetic variation, such as those outlined earlier and documented in Figs. 17–3 and 17–5, is of paramount importance in order to (1) make accurate estimates of individual photosynthetic values, (2) determine seaweed contributions to marine productivity, and (3) analyze evolutionary strategies of carbon allocation. This knowledge is equally essential for both field and laboratory studies.

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