SAMPLING AND INTERPRETIVE CONSIDERATIONS IN THE MEASUREMENT OF MACROPHYTIC PRIMARY PRODUCTIVITY: AN OVERVIEW WITH RECOMMENDATIONS

by
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ELECTRODE TECHNIQUES FOR MEASURING MARINE MACROPHYTIC PRIMARY PRODUCTIVITY

Laboratory Incubation Techniques

Experiments in my laboratory are conducted in Percival photoperiod incubators maintained at 7500 lux (140 $\mu E/m^2/sec$) by cool-white fluorescent bulbs. Incubation temperatures approximating those in the field at the time of collection are used consistently, and the incubation equipment is cooled before use to prevent the formation of small bubbles by degassing on surfaces. All material is collected submerged and immediately returned to the laboratory in insulated ice chests for experimentation.

Bottle Experiments

Net photosynthesis is determined on randomly-selected whole plants using Beckman Field Lab or Yellow Springs Instruments O_2 analyzers and electrodes (calibrated in air or saturated distilled water) with 500-3000ml widemouthed clamp-lid canning jars as the incubation containers. The oxygen-electrode method exhibits close agreement with Winkler-determined O_2 values and has a linear response to dissolved O_2 concentrations (including supersaturated levels). The Winkler method is much more labor intensive but slightly more sensitive when used in the laboratory. The bottles and jars are cleaned in aqua-regia and aged in distilled water prior to use. The seawater used during the incubations is taken at the time and place of algal collection, shaken vigorously to bring it to O_2 saturation, and immediately filtered through a nanoplankton net (pore size 10 µm) to remove most planktonic organisms. Individual bottles are slowly submerged and filled, thus excluding all air from the stocks of incubation water.

Several whole thalli per bottle, rather than torn or cut fragments, are selected randomly to control for age or physiological differences. All incubations (2.0-6.0 h each) are carried out between 0900 and 1750 hrs. to reduce differences due to possible daily photosynthetic periodicities.

During incubation, the bottles (usually four light and two dark replicates) are stirred by means of egg-shaped stir bars and air-driven magnetic stirrers at 10.0 min. intervals and systematically rotated in position between the incubators to ensure equal light conditions during the 4.5 to 6.0 h experimental intervals. A thin perforated acrylic sheet is used to increase the effectiveness of the stir bar and to keep it from contacting the algal material.

After the O_2 levels are recorded, individual thalli are carefully separated, spread, and photocopied; projected area determinations are made from each photocopy by overlaying a transparent gridwork of dots ($1/cm^2$) and counting those intercepting the thallus. Care must be taken that the photocopies do not enlarge or reduce the specimens copied. Thalli are then dried at 60°C until they reach constant weight. All O_2 values are converted to g C fixed/m of thallus/h and to mg C fixed/g dry wt/h as outlined in Strickland (1960). For calcareous algae, ash-free dry weight is used following 24 h of combustion at 400°C. A photosynthetic quotient of 1.00 is usually assumed to enable easy interconvertability with other data where different PQ values are used. The effects of various experimental treatments are routinely examined statistically by single factor analysis of variance or the Newman-Keuls multiple range test (Sokal and Rohlf, 1969).

Continuous Monitoring Experiments

This design consists of simultaneously monitoring net photosynthesis via both O_2 and pH electrodes during controlled laboratory manipulations. Algal thalli are placed in specially constructed 870ml plexiglas chambers fitted with pH and O_2 electrodes (see Littler, 1973a for details). Oxygen evolution is measured using the O_2 analyzers; pH is measured with an Orion digital pH meter (Model 801), printer, automatic electrode switch and Bradley-James electrodes. At the end

each experiment, thallus dry weights are determined as above. Changes in pH are converted to changes in CO_2 concentration by the standard procedures developed by Beyers (1970), using a CO_2 vs. pH function previously determined for the medium (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 mM CO_2), and then to g C/m^2 of thallus of mg C fixed/g dry wt. The slopes of the lines for O_2 production and CO_2 uptake as a function of time are computed by regression statistics and compared for the different conditions and measurement techniques. The incubation conditions are generally the same as those described above for the bottle experiments, except cylindrical air-driven stir bars under a perforated partition in each container are used to maintain a constant flow of medium past the algae and electrodes.

Field Incubation Techniques

In the field, only the O_2-electrode technique is used because we have not been able to obtain a pH-meter of suitable sensitivity and reliability for field use. Also, compared to O_2 analyzers, Winkler equipment and supplies are inordinately bulky and difficult to use under primitive field conditions. Specimens are again incubated at ambient water temperatures in 500, 1220, 2000, or 3000 ml widemouthed canning jars (depending upon the size and productivity of the thallus) that are placed in clear polycarbonate trays near the water's edge in full sunlight. Throughout the experiments, the average thallus concentration per volume of water is maintained below the ratio of 0.03 g/l, except considerably more material (~ 2x) is often used in dark bottles to measure the relatively low respiration rates. 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 light and blank bottles. Care is taken to select individuals that are relatively representative of populations occurring within the system under study. Thalli are collected while submerged, placed in trays of ambient seawater, and gently cleaned of obvious epiphytes. Whole organisms are incubated whenever possible; measurements of the larger species are conducted on representative blades or branches. At approximately 10 min. intervals each bottle is thoroughly mixed with air-driven magnetic stirrers and stir bars driven by a bellows-type foot pump; a minimum of four light and two dark bottles are typically incubated per species. Our trays hold four 1220ml jars and fit onto racks with four appropriately placed motors for stirring. Cooling is accomplished by refilling the trays with ambient seawater at 10-15 min. intervals. Incubation periods vary from two to four hours' duration between the times of 0930 and 1500 hrs. Incubation times for the larger macrophytes are usually about 2-3 h, since the representative thallus portions are much larger than those for smaller forms. The water used is collected just prior to the experiments, filtered through a nannoplankton net (10 µm pore size), then shaken in a sealed, contaminant-free bucket to bring the oxygen level to ambient air saturation. During the incubation period, light intensity and quantum flux are measured at 15-30 min. intervals by a LI-COR Quantum/Photometer (Model LI 185) and optimally should not drop below the presumed saturation intensity of 20,000 lux. (See King and Schramm [1976a] for typical saturation values of macroalgae). When making oxygen readings, one must take care to prevent differential heating of the electrode or sample in direct sunlight. The total net production for each macrophyte at a given site can then be estimated using the net productivity per square meter of thallus per unit of time in conjunction with the overall percent cover value per square meter of substrate.

Littler M. M. 1980.

Electrode techniques for measuring marine macrophytic primary productivity.
In Abbott I. A., Foster M. S. and Eklund L. F. eds. Pacific seaweed aquaculture, pp. 186-7. California Sea Grant College Program, Institute of Marine Resources, University of California, La Jolla, California.