18: The carbon-14 method for measuring primary productivity

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I. Introduction

A critical evaluation of the ^{14}C method (Steemann-Nielsen 1952) as applied to the measurement of benthic macroalgal photosynthesis (primary productivity) is given in this chapter. At present, there are no standard, universally applicable isotopic techniques for measuring the photosynthesis of all marine macroalgae, because this approach has had only limited application to seaweeds in the past decade. Before attempting any experimentation, one must have a thorough understanding of the photosynthetic metabolism of macroalgae, as well as a familiarity with radiotracer techniques and safety measures.

Several reviews (Harris 1978; Peterson 1980; Dring and Jewson 1982) have discussed the technical pitfalls of this method in the measurement of planktonic primary productivity, as well as the problems associated with the interpretation of whether this technique actually measures net or gross photosynthesis. Many of these practical and theoretical problems are also relevant to the measurement of macroalgal photosynthesis (Kremer 1981a). On a theoretical basis, gross or "real" photosynthesis is measured by the ^{14}C method only if the following conditions are met (Thomas 1963). (1) ^{14}C is assimilated at the same rate as ^{12}C; (2) no ^{14}C is incorporated into cellular material by nonmetabolic processes; (3) no ^{14}C is lost by dark respiration and/or photorespiration (Burris 1977), which may accompany photosynthesis; and (4) no ^{14}C is lost by excretion. In reality, none of these conditions is completely satisfied; therefore, with the ^{14}C method, one measures (Peterson 1980) something between net and gross photosynthesis.

A discrimination factor of 1.05 (see Peterson 1980) is often used to correct for differences in the metabolism of ^{14}C compared with that of ^{12}C (^{12}C is incorporated 1.05 times faster than ^{14}C).

Estimates of ^{14}C incorporation by processes other than photosynthesis are often obtained from specimens incubated in the dark. Dark ^{14}C fixation (DCF) in macroalgae is significant, and it has been demonstrated (see Kremer 1979, 1981a,b) that active ^{14}C fixation in the dark occurs via carboxylating enzymes, primarily phosphoenol-

pyruvate carboxykinase (EC 4.1.1.32) and, to a lesser extent, phosphoenolpyruvate carboxylase (EC 4.11.31). The amount of nonmetabolic uptake via adsorption, absorption, and isotopic exchange is thought to be small in comparison with that via DCF, except for calcareous macroalgae (Littler 1973). In the past, values of DCF have been subtracted from the light carbon fixation (LCF) rates, recorded separately, or omitted entirely.

Direct loss of recent photosynthetically fixed ^{14}C can occur either through active excretion (as well as wounding) or from respiratory processes that occur in the light. Rates of organic ^{14}C excretion are normally low in marine macroalgae and represent less than 5% of the LCF rates (Brylinsky 1977). The rate of refixation of respired (dark mitochondrial respired and/or photorespired) $^{14}CO_2$ that occurs in the light cannot be accurately estimated. This point proves to be the major (Peterson 1980) theoretical question concerning this method.

Depending on the question(s) being asked and the level of accuracy of information needed, the choice of using the ^{14}C method rather than the conventional (Gaarder and Gran 1927) O_2 light and dark bottle techniques should be carefully evaluated (see Littler and Arnold, Chap. 17). Although the general equation for photosynthesis seems relatively simple, the light-dependent incorporation of ^{14}C into photosynthetic products is an extremely complex process. Researchers who attempt to use this method, under either field or laboratory conditions, must be aware of the theoretical and practical difficulties involved or enormous errors in the estimation of macroalgal photosynthesis will occur. Reviews on macroalgal photosynthesis (Kremer 1978, 1981a; Ramus 1981) and radiotracer techniques (Neame and Homewood 1974; Peng 1981) should be consulted for appropriate background information.

II. Equipment and materials

A general list of equipment and supplies is given in Table 18–1. The following sections provide an overview of the types of equipment and supplies that have been used in past studies and the limitations that various macroalgae and incubation conditions impose on the use of such items.

A. Incubation equipment

1. Incubation vessels. Thallus size, metabolic activity, and incubation time are important criteria for the selection of optimal incubation vessel size. Preliminary linearity runs for *each* algal species should be conducted before experimentation to avoid "bottle effects" (see Littler

Table 18–1. *General list of equipment and supplies for measuring the productivity of marine benthic macroalgae by the ^{14}C method*

I. *Incubation*
   A. Incubation vessels: canning jars (variable sizes), BOD bottles, custom plexiglass chambers, polyethylene or polyamide–polyethylene bags
   B. Stirring: air-driven magnetic stirrers, foot pump, Teflon-coated magnetic stir bars, battery-operated electric stirrers or submersible electric pumps
   C. Incubation trays: clear polycarbonate animal cages
   D. Miscellaneous: hand-held 10-μm plankton net, large-volume buckets, thermometers, salinity refractometer, aluminum foil, water-resistant tape, water-resistant labels, plastic bags, ice chest, quick-freeze Freon, analytical balance

II. *^{14}C and accessories*
   A. ^{14}C: working stock solution (550 $\mu Ci \cdot ml^{-1}$), syringes, micropipettes with disposable tips, rubber gloves, radioactive identification labels, metal waste disposal can
   B. Activity determination: scintillation vials, liquid scintillation counter, CO_2-trapping agent, saltwater-compatible LSC cocktail, quenched standards
   C. Alkalinity determination: 250-ml plastic jars, pH meter, standardized HCl, 50-ml pipettes, buffers
   D. Tissue extraction: 80% ethanol, 6 *N* HCl, 30% H_2O_2, NCS (Amersham), variable-temperature (up to 80°C) water bath, other solvents for fractionation

and Arnold, Chap. 17). Most commonly, seaweeds have been enclosed in biological oxygen demand (BOD) bottles of either 300- or 500-ml capacity (Wassman and Ramus 1973; Bach and Josselyn 1979; Arnold and Murray 1980) or wide-mouthed canning jars, the size of which ranges from 0.5 to 4.0 liters (Littler and Arnold 1980, 1982; Heine 1983). Specially constructed plexiglass or polyvinyl chloride chambers with accessible sampling ports have been used for both in situ (Brylinsky 1977; Hatcher 1977; Rogers and Salesky 1981) and laboratory (Littler 1973; Darley et al. 1976) experiments. For in situ experiments on large kelps (Towle and Pearse 1973; Lobban 1978) or rockweeds (Guterstam 1976), polyethylene or polyamide–polyethylene plastic bags of various sizes can be used. The O_2 and CO_2 permeability properties of these bags should be known before experimentation.

2. Mixing. Adequate mixing during incubation is necessary to ensure that photosynthesis is not diffusion-limited. The method of stirring is dependent on the type of incubation vessel chosen and the species incubated. For in situ experiments employing plexiglass chambers (Brylinsky 1977) or jars (Littler and Arnold 1980; Heine 1983), air-

driven magnetic stirrers (A. H. Thomas Company) have been used with propulsion coming from compressed air tanks or manually operated foot pumps. For subtidal incubations (Heine 1983) it is important to consider whether the emitted bubbles interfere with the incident light field. Battery-operated stirrers (Bittaker and Iverson 1976; Marker 1976) and water pumps (Hatcher 1977; Rogers and Salesky 1981) have also been used successfully. Stirring within plastic bags by the buffeting action of oceanic swells is thought to be adequate (R. Zimmerman, personal communication); however, this probably varies with specific incubation conditions.

B. Incubation materials

Radioactive ^{14}C is normally obtained in the bicarbonate form ($NaH^{14}CO_3$) as a dry solid or as a sterile aqueous solution in a sealed ampoule (New England Nuclear or Amersham). Specific activities are variable, but for productivity studies they should be ~5–50 mCi · $mmol^{-1}$. The powdered $NaH^{14}CO_3$ is recommended, and stock solutions are prepared by adding an appropriate volume of sterile filtered (0.2-μm pore size) seawater or a synthetic substitute (Strickland and Parsons 1972). The stock solution should be adjusted to pH 9.5 (Peterson 1980) to prevent loss of activity due to $^{14}CO_2$ blowoff that occurs at a lower pH. Working solutions of small volume (5 ml) can be prepared from the concentrated stock. The stock and working solutions should be stored in a freezer in separate sterile glass vials until used (see Neame and Homewood 1974).

III. Methods

A. Incubation of sample

An incubation vessel of appropriate size is chosen and filled with filtered ambient seawater (10-μm plankton net) to exclude air, and a defined amount (usually 1–10 μCi per liter of incubation water) of radioactivity from the ^{14}C working solution is introduced. This can be done by injection of the contents of a syringe through rubber serum caps installed in plexiglass chambers, polyethylene bags, or the glass lids of canning jars. For BOD bottles, the activity can be easily added directly through the aperture with a fixed-volume pipette. Subtidal incubations are most difficult, and Drew (1973) and Smith (1981) give an overview of the methods of collecting and incubating seaweeds without bringing them to the surface. If subtidal specimens are brought to the surface so that they can be more easily placed in incubation vessels and cleaned, they should not be exposed to air or unnatural levels of sunlight. After introduction of the ^{14}C, the incubation medium should be stirred for at least 2 min, and then

three 0.5-ml subsamples (the volume depends on the specific activity and seawater capacity of the cocktail) are taken for initial activity. It is best to introduce the alga into the vessel at this point, rather than before the label is added as is necessary in most subtidal incubations (Drew 1973; Towle and Pearse 1973). Initial activity samples should always be taken, since the activity of working ^{14}C solutions can change during storage (Neame and Homewood 1974). Great care should be taken at this point to prevent pipette error, since any inaccurate estimates of initial activity translate into inaccuracies in the estimate of productivity. Initial activity samples should be immediately mixed with liquid scintillation counting (LSC) cocktail and stored in a cool, dark place until counted.

The LSC system should be compatible with seawater; it should not show strong color or chemical quench, nor cause acidic-induced changes in the chemical CO_2 equilibria of the sample. Iverson et al. (1976) found that Aquasol II (New England Nuclear) behaves as a Lewis acid and causes loss of activity as $^{14}CO_2$ gas, which escapes from solution into the head space of the vial, with the result that initial counts are lowered. This is a time-dependent process and can cause a significant reduction in the count rate of initial activity samples. With acidic cocktails such as Aquasol II, a CO_2-trapping agent such as phenethylamine (Iverson et al. 1976), ethanolamine, or hyamine is added to prevent lowered count rates. Cheaper alternatives to expensive commercial cocktails can be easily made in the laboratory (Waite et al. 1973). Whatever LSC cocktail is chosen, it should be tested for its capacity to mix with seawater and to retain inorganic ^{14}C (HCO_3^- and CO_2). K. E. Arnold and S. Manley (unpublished data) routinely use a cocktail of 10 ml of Aquasol II and 3.0 ml of NCS (Amersham) for each 0.5-ml initial water sample. The trapping agent is added first, followed by the seawater sample, and the vial closed and shaken vigorously. The cocktail is then added with further mixing.

Initial incubation water samples are also taken for determination of total "CO_2," the volume of which depends on the technique used (Strickland and Parsons 1972). Smith and Kinsey (1978) give a thorough guide to the practical problems of measuring pH and carbonate alkalinity in seawater.

B. Dark carbon fixation

Traditionally, incubation of experimental thalli in the dark provided a "blank" or a control for nonbiological mechanisms of ^{14}C uptake (Thomas 1963). More recent studies (for a review see Kremer 1981a) have shown that DCF is associated with the activity of carboxylating

enzymes other than ribulose-1,5-bisphosphate carboxylase-oxygenase.

Dark carbon fixation rates can be determined using the same general incubation procedures as outlined earlier. Rates of DCF for Rhodophyta and Chlorophyta are almost always (Kremer 1979, 1981a,b) less than 3% of the corresponding LCF rates. In Phaeophyta, especially the kelps and rockweeks, DCF rates can range upward to 30 to 50% of LCF. Highest DCF rates are associated (Kremer 1981a) with the young meristematic growing regions (transition zone) of the laminae. Most DCF occurs primarily through the activities of the anapleurotic enzyme phosphoenolpyruvate carboxykinase, and the distribution of its activity has been found (Kremer 1981a) to exhibit longitudinal profiles similar to those of DCF along kelp blades.

Since DCF is much lower in the red and green seaweeds, greater specific activities of $H^{14}CO_3^-$ are needed in the incubation water to obtain measurable rates. Generally up to 50 $\mu Ci \cdot liter^{-1}$ are needed, depending on the incubation time and metabolic activity. Patterns of ^{14}C fractionation into end products of fixation are likely to be much different from those found with LCF experiments. In young growing kelp tissues, for instance, there is a much higher dark incorporation of ^{14}C into the insoluble fraction in short-term experiments (1–3 h) (Willenbrink et al. 1979). It should be emphasized that all incubation manipulations must be performed in the strict absence of light; otherwise, inaccurate rates of DCF will be obtained. This obviously gives rise to very special problems for field incubations, and for this reason in situ DCF experiments have been largely ignored.

C. Extraction of experimental thalli

Upon termination of incubations, the algal thalli are carefully removed and rinsed for several minutes in unlabeled seawater. Material should *not* be rinsed in acidified seawater, as some have done in the past (Wassman and Ramus 1973), because this may begin the extraction process prematurely. Thalli are blotted and dried, and their fresh weights are determined (fresh-weight/dry-weight ratios are determined on similar thalli). This can be done in the field with a battery-operated balance (see Brinkhuis, Chap. 22). Thalli are then placed immediately into an extraction solvent [volume is variable, but normally 10–20 times (milliliters) the fresh weight (grams) is sufficient] and stored in the dark until processed. The solvent routinely used (Brinkhuis and Jones 1974; Brinkhuis 1977a,b) is 80% ethanol acidified to pH ~2.0 with concentrated HCl; this removes any residual cell-wall-bound $H^{14}CO_3^-$ (Willenbrink et al. 1979). Depending on the experimental design, the use of other

solvents may be more appropriate. For larger thalli such as kelps, productivity can be estimated from tissue subsamples taken down the longitudinal axis (Towle and Pearse 1973; Johnson et al. 1977; Küppers and Kremer 1978; Arnold 1980) of the lamina and from "representative" subsamples of stipe and holdfast. If extraction cannot be accomplished in the field, whole thalli or tissue subsamples can be quick-frozen on dry ice (Penhale and Smith 1977) or with liquid Freon (Brinkhuis and Jones 1974) and stored until processed for LSC. Long time intervals between termination of incubation and fixation of the algal tissue should be avoided.

D. Excretion of ^{14}C-labeled dissolved organic matter

Final water samples for estimating the quantity of ^{14}C excreted as dissolved organic matter (DOM) may also be taken upon termination of incubation. Sample size can range from 1 to 100 ml depending on the initial activity, the metabolic rate, and the percentage of LCF excreted (normally less than 5%). These samples should be prefiltered (0.2 μm) and kept on ice (to avoid bacterial degradation) until processed. It may also be worthwhile to dissolve and count the prefilters after an acid wash to assess possible particulate ^{14}C loss (Fankboner and de Burgh 1977).

E. Precautions and waste disposal

In general, all containers in contact with ^{14}C should be clearly labeled with radioactivity stickers. Most academic institutions have specific health and safety regulations concerning the handling and disposal of radioactivity, and these should be consulted before experimentation. Disposal of radioactivity is one of the most costly aspects of this type of research, principally because of the large amount of activity remaining in the incubation water. The remaining $H^{14}CO_3^-$ can be precipitated with barium hydroxide, and this effectively reduces the total volume of activity to be disposed.

F. Sample work-up and calculations

General aspects of LSC procedures can be found in the monographs by Neame and Homewood (1974) and Peng (1981). A familiarity with these techniques is mandatory for accurate measurements of ^{14}C. Only specific aspects of these procedures as they apply to ^{14}C productivity measurements are discussed here.

1. Initial activity. Initial activity samples should be dark-adapted for at least 24 h (as should all LSC samples) to avoid problems of chemical luminescence and photoluminescence (Peng 1981). These samples should then be counted as soon as possible thereafter, since most

LSC cocktails will evaporate with time, thus changing the ratio of water sample to cocktail and enhancing the formation of precipitates. A separate quench curve obtained by one of the three major (Peng 1981) methods – internal standard, channels ratio, and external standard channels ratio (ESCR) – should be constructed for water samples. The simplest and least costly is the ESCR method, which necessitates having a liquid scintillation counter with an external standard (most commercial counters have this feature). A consistent procedure should be adopted on the basis of preliminary experiments designed to provide information on the optimal sample/cocktail volume ratio, total cocktail–sample volume, and need for CO_2-trapping agents. All of these variables influence the amount of quench that occurs in each sample, and once established, they should not be altered because this will change the quench characteristics.

2. *Release of ^{14}C-labeled DOM.* The technique employed by Smith (1975) for measuring excreted ^{14}C-labeled DOM by phytoplankton is easily modified for examining macroalgal excretion rates. After incubation, a known volume (10–100 ml) of the filtered incubation medium is placed in a special apparatus (see Smith 1975) and acidified to pH 2.5 to 3.0 by the addition of concentrated HCl or H_3PO_4. The volume added (usually less than 1 ml) will depend on the sample size and acid strength. Upon acidification, the solution is bubbled with air for at least 10 min. The $^{14}CO_2$ that is driven off is collected in an NaOH trap. A functional apparatus can be easily designed from a pair of side-arm filtration flasks, Tygon tubing, stoppers, and Pasteur pipettes (Fig. 18–1). Other possible designs can be found in the papers by Gachter and Mares (1979) and Wessels and Birnbaum (1979). The activity remaining in solution represents the amount of excreted (acid-stable) organic carbon. For low excretion rates, higher sample/cocktail volume ratios can be used to give significant counts above background. For accurate measurements, a separate quench curve should be prepared. Blanks prepared by the addition of a known amount of working stock $NaH^{14}CO_3$ solution to sterile filtered seawater and acidified to pH 2.5 to 3.0 should also be run to test the effectiveness of the method and to control for any possible contamination (Williams et al. 1972) of the original stock activity with acid-stable ^{14}C.

3. *Sample extraction and solubilization.* Information regarding the fractionation of photosynthetically fixed ^{14}C into different products can be conveniently obtained from laboratory or field ^{14}C fixation experiments. Studies involving the measurement of flow of carbon into major end products suggest that the resultant patterns of carbon

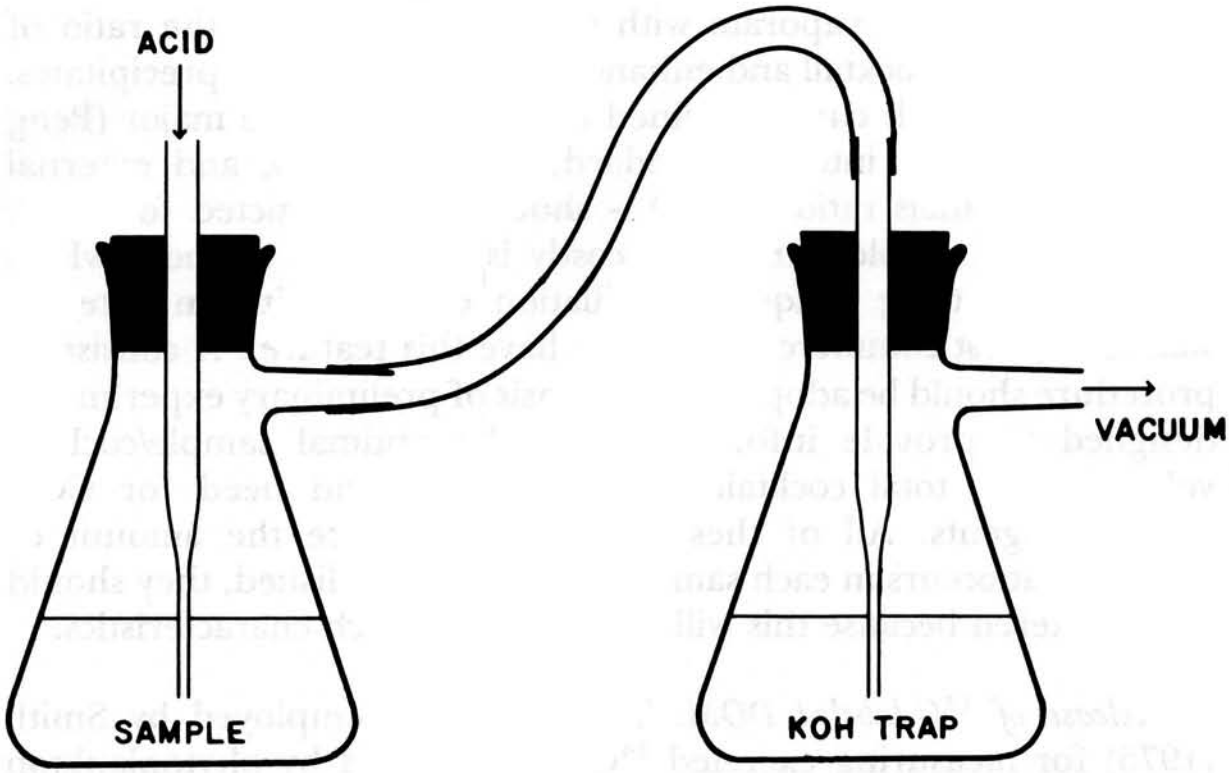

Fig. 18–1. Apparatus for the production of acid-stable ^{14}C-labeled dissolved organic matter.

allocation are altered dramatically by such factors as light intensity (Kirst 1981), light quality (Bird et al. 1981), O_2 levels (Burris 1977; Kirst 1981), and nitrogen supply (Bird et al. 1981). Fractionation studies require significantly more bench work, and a good general discussion of these procedures can be found in Howard et al. (1975) and Kremer (1978) as well as in a paper on phytoplankton fractionation by Hitchcock (1983). Generally, when incorporating a fractionation design into productivity studies, one must employ higher activities of $H^{14}CO_3^-$ and in the initial sample fixation step, use a solvent that is compatible with the chosen fractionation scheme.

For routine productivity measurements, a convenient fractionation scheme involving ethanol-soluble and ethanol-insoluble fractions (Brinkhuis and Jones 1974) or acetone-soluble and acetone-insoluble fractionation (Wassman and Ramus 1973) is sufficient. In highly productive and fast-growing seaweeds such as *Ulva* and *Enteromorpha* (Brinkhuis 1977b; K. E. Arnold, unpublished data), ^{14}C uptake into ethanol-insoluble fractions may approach 50% or more of the total uptake, whereas in rockweeds and kelps (Brinkhuis 1977a,b; Buggeln 1979; K. E. Arnold and S. Manley, unpublished data), the proportion of the ethanol-insoluble activity is normally less than 15%. The ratio of extraction volume to sample weight should be optimized such that extraction is efficient and yet the count rate per milliliter of extract remains at least 1000 disintegrations per minute (dpm) above background.

Techniques routinely used for solubilization of the insoluble material are given by Lobban (1974) and Gagne et al. (1979). These techniques are relatively simple and inexpensive and can be used to solubilize unextracted tissues as well. Loss of some activity by oxidation to $^{14}CO_2$ remains a problem, and the extent appears to vary (Gagne et al. 1979) with different species. Routinely (K. E. Arnold and S. Manley unpublished data), 7–9% of the label is lost when kelp tissues are solubilized directly by either technique. The method of Gagne et al. (1979) is preferred (K. E. Arnold and S. Manley, unpublished data) because it produces less quench, produces lower chemiluminescence, and offers more complete digestion. Samples with a dry weight of up to 25 mg can be solubilized in a scintillation vial by the addition of 0.25 ml of 30% H_2O. The vial is capped and kept at 50°C for 18 h in the dark, followed by the addition of 4 ml NCS (Amersham) at 50°C for an additional 16 h in the dark. A solubilization technique used for aquatic angiosperms (Beer et al. 1982) makes it possible to measure both ^{14}C-labeled photosynthate and chlorophyll *a* on the same tissue sample. This technique might prove useful for macroalgal tissues as well. An alternative to solubilization involves the combustive oxidation of the total tissue to CO_2 (Brylinsky 1977; Penhale and Smith 1977; Kremer 1978; Adkin and Ho 1981), followed by trapping in an organic base. Wassman and Ramus (1973) suggested drying the tissue and grinding it into a fine powder followed by suspension into a gelled fluor. In this case, quench correction must be accomplished (Neame and Homewood 1974) by means of the channels ratio method.

4. Calculations. Rates of primary production are normalized to some parameter of biomass (fresh weight, dry weight, ash-free dry weight, or chlorophyll *a* content) or photosynthetic area (unit area of thallus surface or square meters of intertidal surface). The normalization parameter will depend on the particular ecological or physiological question(s) being asked. The most easily obtained biomass parameter is dry weight. For comparison purposes, we suggest that, even if other parameters are used, a conversion factor to productivity per unit dry weight be provided. Productivity per unit thallus area is also easily obtained for most sheetlike forms (e.g., *Ulva, Porphyra,* and *Laminaria*), and area may be expressed as square meters of one (Littler and Arnold 1980) or both (Wheeler 1980) sides of a thallus. Ramus (1978) suggested that seaweed photosynthesis, normalized to chlorophyll *a*, will often be incorrect, leading to gross over- or underestimation of net primary productivity (for a more complete discussion of this, see Littler and Arnold, Chap. 17).

Equation 1 can be used for the calculation of net particulate primary productivity on an hourly basis:

$$P_n = \frac{S_{dpm} \times CO_2 \times V \times 1.05}{W_{dpm} \times B \times H} \quad (1)$$

where P_n is the net particulate primary productivity, expressed as milligrams carbon per gram dry weight each hour or grams carbon per square meter each hour. S_{dpm} is the total corrected (including quench and background) activity (disintegrations per minute) in the sample, including both the activities from the ethanol-soluble and -insoluble fractions; CO_2 is the total carbon available for photosynthesis in terms of milligrams carbon per liter, calculated from pH and total alkalinity data; V is the volume of the incubation vessel in liters; 1.05 is the isotopic discrimination quotient; W_{dpm} is the total corrected activity (disintegrations per minute) in the incubation water; B is biomass (grams dry weight) or photosynthetic area (square meters of thallus or of intertidal surface); and H is the length of incubation in hours.

A sample calculation for the net particulate primary productivity of a juvenile sporophyte of *Eisenia arborea* (from Arnold 1980) is as follows:

$$P_n = 2.20 \text{ mg C} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$$

$$= \frac{1.50 \times 10^5 \text{ dpm} \times 26.4 \text{ mg C} \cdot \text{liter}^{-1} \times 1.22 \text{ liters} \times 1.05}{1.89 \times 10^7 \text{ dpm} \times 0.035 \text{ g} \times 3.48 \text{ h}} \quad (2)$$

If estimates of excretion of ^{14}C-labeled DOM are obtained, they should be reported separately and not added to the total sample activity, since by definition (Peterson 1980) the ^{14}C technique approximates net particulate primary productivity. These rates are normally presented as a percentage of the corresponding LCF rates.

Similarly, rates of DCF are also reported separately as milligrams carbon per gram each hour or grams carbon per square meter each hour or as a percentage of the LCF values. Kremer (1981b) suggests that DCF rates be included in gross productivity calculations, yet the theoretical reasons for this remain unclear.

In the initial design of ^{14}C experiments, it is wise to use calculations such as those just presented to estimate the initial activity necessary. This calculation is based on an approximate value for the expected productivity (this might be estimated from literature values) and the minimum value of activity needed in the tissue samples. These generated estimates are determined for a specific volume of incubation water, weight of tissue, and incubation time interval.

IV. Critical evaluation

A. Application considerations

With proper application, the ^{14}C technique offers very important advantages over the traditional O_2 light and dark bottle technique, particularly (1) when within-plant photosynthetic differences attributed to age, development, and reproduction are to be examined without the problems associated with excision of isolated tissue plugs from the parental thallus (Hatcher 1977; Arnold 1980; K. E. Arnold and S. Manley, unpublished data); (2) when greater sensitivity is needed for the measurement of photosynthesis on algal thalli that are either small and difficult to manipulate (such as laminarian gametophytes; Kremer and Markham 1979) or that form complex turf associations (Borowitzka et al. 1978) or when low metabolic rates are expected such as observed for many algal crusts (Littler and Arnold 1982); (3) when greater insight is needed with respect to the photosynthetic strategies of carbon allocation and how carbon flow into various end products varies with ontogenetic and environmental factors; and (4) when rates of photosynthesis in air (Kremer 1978; Darley et al. 1976) are to be compared with rates of photosynthesis under water. Alone or along with uptake of ^{45}Ca, the incorporation of $H^{14}CO_3^-$ can be used to estimate the rates of calcification in marine macroalgae. A review of methods of calcification rate measurement can be found in Borowitzka (1977).

The employment of the ^{14}C technique should be carefully weighed according to the questions being asked, since some applications, such as primary productivity measurements of large algal thalli and community productivity studies, may be unnecessarily laborious. Furthermore, it should be emphasized that specific techniques that work for one algal species may have to be modified for another, and the key to success is to conduct several preliminary experiments to ensure that label losses are kept to a minimum. The ^{14}C technique does, however, have some serious disadvantages as compared with other physiologically based (O_2 evolution) techniques. As pointed out in the introduction, this method measures something between net and gross photosynthesis. Furthermore, because dark respiration cannot be determined or accurately estimated (Steemann-Nielsen and Hansen 1959) by this technique, 24-h net primary productivity rates (see Littler and Arnold, Chap. 17) cannot be calculated. Economically, the method is much more costly, in terms of both initial equipment outlay and the inordinately large quantities of supplies needed for counting ^{14}C activity. In addition, it is much more labor intensive than either the O_2 or pH electrode technique, particularly when whole-plant rates are to be obtained on moderately large thalli.

B. Dark carbon fixation

The measurement of $^{14}CO_2$ uptake in the dark, as outlined earlier, will probably yield values that seriously underestimate the true rate of DCF, since simultaneously occurring respiratory processes are giving off CO_2. Some of the CO_2 is refixed by phosphoenolpyruvate carboxykinase while the rest leaves the thallus surface and enters solution. Kremer (1981b) found an overall 25% increase in DCF rates in kelp frond samples in which the action of the Krebs cycle (respiration) had been inhibited with monofluoroacetate. Since DCF partially compensates for dark respiratory release of CO_2, Kremer (1981b) believes that the inclusion of DCF rates in calculations of gross productivity is justified. Because DCF represents a more special case involving mainly members of the Phaeophyta (particularly kelps), we believe that it is inappropriate at this time to include DCF rates in productivity calculations for marine macroalgae in general. Furthermore, we suggest that, when such measurements are taken, they be reported separately. The real applicability of these analyses, however, lies in the ability (Arnold 1980) to fingerprint the actively growing sink (Lobban 1978) tissues within fronds of kelps.

C. Release of ^{14}C-labeled dissolved organic matter

Release of ^{14}C-labeled DOM can occur through active excretion such as that associated with photorespiratory release of glycolate (Fogg 1981) or passive leakage due to wounding or release of mucilage. Estimates of the release rates of ^{14}C-labeled DOM by marine macroalgae are important to the understanding of both the dynamics of carbon flow within plants and the flow of carbon between algae and other biotic components of the community. We therefore recommend that ^{14}C-labeled DOM release data be routinely taken during ^{14}C experiments.

Numerous interpretive errors, however, are associated with the estimation of DOM release by the methods outlined in this chapter. The analysis of the rate of release of ^{14}C acid-stable label into solution probably underestimates the true release of DOM, because in short-term incubations, released products have a low specific activity although the results of time course experiments (Brylinsky 1977) of release of acid-stable label and pulse–chase experiments suggest that, in the algae examined (*Acanthophora, Chondria, Dictyota,* and *Sargassum*), release rates were not seriously underestimated. This is likely, however, to vary with the species incubated. In seaweeds with low metabolic turnover of the photosynthetic products such as in kelps, release of mucilages (which are metabolically of greater distance from the site of fixation than the primary products) of very low

specific activity occurs (K. E. Arnold, personal observation). Thus, in this case, the release of DOM would be dramatically underestimated.

Wounding of the thallus material before or during incubation causes significant release of DOM. In fact, the frequently cited work of Khailov and Burlakova (1969) on DOM release by macrophytes gives very high rates because the experimental thalli were in various stages of death and decomposition. In the incubation of thalli, great care should be taken to prevent wounding. Furthermore, experiments conducted on representative tissue plugs or thallus parts (Drew et al. 1982) should include an assessment of the effects of wounding and loss of ^{14}C-labeled DOM: otherwise, the results must be viewed with caution. Another complication of estimating DOM release arises from possible bacterial uptake (Brylinsky 1977) and metabolism of the released compounds back into $^{14}CO_2$.

D. Carbon budgets of kelps

Estimates of the primary productivity of large seaweeds such as kelps are difficult to obtain with ^{14}C techniques. In the past (Drew 1973; Towle and Pearse 1973; Johnston et al. 1977; Drew et al. 1982), isolated thallus parts or representative segments were incubated and the total plant productivity calculated from these rates after correction for the distribution of the various plant parts within the thallus. In the case of complex kelps such as *Macrocystis,* accurate estimates of whole frond productivity are thus difficult to obtain without elaborate subsampling regimes. Time and cost are key factors that detract from the use of ^{14}C methods on large, morphologically complex algae.

E. Comparisons with other methods

Remarkably few simultaneous measurements of O_2 evolution and ^{14}C uptake have been conducted on marine macroalgae. Calculated values of net particulate carbon fixation are almost always (Arnold 1980) lower than the corresponding rates of net photosynthesis calculated from O_2 experiments in which a PQ (photosynthetic quotient; ratio of O_2 evolved to CO_2 fixed) of unity is assumed. Good agreement between the two methods was found by Arnold (1980 and unpublished data) for photosynthetic measurements on *Enteromorpha intestinalis* and young sporophytes of *Eisenia arborea.* An average PQ of 1.14 (range, 0.96–1.32) was found in short-term experiments (2.5 h) for *Eisenia. Enteromorpha* collected from low-nitrogen habitats had higher PQ values (average of 1.91) than thalli collected from higher nitrogen habitats (average of 1.32). Hoffman and Dawes (1980) found PQs that ranged from 1.2 to 1.3 for *Gracilaria verrucosa*

and 1.2 to 1.4 for *Bostrychia binderi*. Despite the uncertainties intrinsic in the ^{14}C and O_2 techniques, it is interesting to note how close these values are to the value of 1.25, suggested by Ryther (1956) for the conversion of O_2 values to carbon equivalents. Other values for PQs of benthic macroalgae can be found in a paper by Buesa (1980). Given the great sources of potential error in photosynthetic measurements, PQ values should be interpreted with great caution.

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