

REVIEW AND CRITIQUE OF PRIMARY PRODUCTIVITY MEASUREMENTS¹

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Introduction

This paper is concerned primarily with the methodology employed in primary productivity measurements and the interpretation of productivity data. I shall also discuss briefly the magnitude of productivity values throughout the world's oceans, without any attempt to give any comprehensive review of the literature.

There are many different methods which may be used to estimate the rate of photosynthesis by phytoplankton. Since ¹⁴C became available after the second world war, it has largely replaced most of the classical methods for estimating primary production. Many of the classical methods are still of value in certain types of environmental studies, but this report will discuss in detail only the methodology based on radiocarbon measurements.

Measurement of Photosynthesis

In studies of primary productivity the chief objective usually is to obtain quantitative data on the direct input of reduced carbon into the base of the food chain. The best approach therefore is to measure the reduction of CO₂ directly, although it is possible to estimate this rate of primary production by indirect methods as outlined below.

a) *pH*. During photosynthesis under favorable environmental conditions, the rate of CO₂ uptake may be 10 to 20 times the normal rate of respiratory CO₂ release. As the uptake of CO₂ (or any of the other species of inorganic carbon) will affect the hydrogen ion concentration, it is possible to estimate the rate of CO₂ uptake by measurement of the pH of the surrounding medium. This method is not very useful in the marine environment as the large reservoir of inorganic carbon (25–30 mg inorganic C/liter) makes the method relatively insensitive.

b) *Oxygen*. Before the advent of the radiocarbon method (Steemann Nielsen, 1952), most estimates of primary production were made by measurement of the amount of oxygen released in bottled samples. The drawbacks of this method have been discussed (Strickland, 1960), the main one being that the method lacks the sensitivity required to be used in open-ocean studies.

c) *Nutrient Uptake*. By measurement of the uptake of essential inorganic nutrients (e.g. nitrogen substrates and phosphate), coupled with assumptions regarding the carbon/nitrogen or carbon/phosphorus ratios in the phytoplankton cells, it is possible to estimate the rate of CO₂ reduction. This method cannot be very precise because of the relatively large variations in ratios of elements in cellular material, but it has been used to advantage in estimating large-scale productivity of water masses.

d) *Chlorophyll*. The basis for estimating productivity from chlorophyll measurements is that assimila-

tion values (the rate of CO₂ reduction per unit chlorophyll per unit time) are quite similar for phytoplankton in most parts of the oceans. With our better understanding of the effects of temperature (Eppley, 1972), light, and nutrients on the rate of photosynthesis, it is possible to estimate productivity even in those environments where conditions are not optimum for photosynthesis.

Lorenzen (1970) has demonstrated that there is reasonably good agreement between surface chlorophyll values and integrated productivity values for the entire euphotic zone. This promise of estimating primary production from surface chlorophyll measurements has recently led to the development of remote sensing devices which analyze the spectral signature of upwelled light from the upper portion of the euphotic zone. Arveson has discussed a convenient sensor which can operate from light aircraft, while NASA has developed the Scanning Imaging Spectroradiometer which operates from planes at high altitudes (>35,000 ft.) or from satellites. These remote sensors are likely to be of great help in temporal surveys of productivity on a worldwide basis.

e) *Measurement of Biomass*. In laboratory cultures it is feasible to estimate photosynthetic rates by measurement of various cell constituents such as carbon, nitrogen, pigments, ATP, etc. This is not practical for most field investigations, however, because of the large amounts of organic detrital material relative to phytoplankton biomass and also because of the low concentrations of phytoplankton, coupled with generation times ranging from one-half to many days.

f) *Uptake of Radiocarbon*. Although the estimation of primary productivity by assimilation of ¹⁴C-labeled bicarbonate (Steemann Nielsen, 1952) seems relatively simple and direct, there are some difficulties in the methodology which should be considered.

i) *Treatment of sample*. The conditions during preparation of the water sample and the conditions at which the samples are incubated are very important. The importance of wall effects and of composition and orientation of the bottles during incubation have been described by many workers, but the most drastic effects may be caused by changes in temperature and light intensity. The use of on-deck incubators to stimulate conditions in the water column suffers from many drawbacks. A much safer and more realistic approach is to use *in situ* techniques whenever possible. It is very important that care is taken to minimize physiological shocks, especially light, during sampling and preparation of the bottles. This is particularly true when working with populations sampled from dim light or from deep in the water column

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(Goldman *et al.*, 1963). A satisfactory way to minimize such effects is to do all sample preparation at night, and to incubate the samples *in situ* from dawn to sunset.

ii) *Filtration.* The amount of cellular material retained by the filter will depend upon the characteristics of the filter and the extent of cellular damage or lysis caused by physical stress during filtration. There are a variety of different filters in common use for productivity work, but most investigators use membrane filters of various types or glass fiber filters. The use of micro-fine glass fiber filters (Reeve Angel 984H) or Whatman GF/C fiber filters offers some advantages over membrane filters in regard to speed of filtration and ease of handling. The mean retention characteristic of these filters is less than 1.0 μ , so there is little or no danger of losing significant amounts of nanoplankton (Sheldon, 1972; Holm-Hansen, in preparation). The pressure at which the sample is filtered is important, as too great a differential pressure may result in cell lysis and loss of cellular material. Arthur and Rigler (1967) have reported significant losses of ^{14}C -labeled material which they attribute to such a lysis during filtration. This interpretation, however, does not agree with other data, which show a linear response between amount of sample filtered and amount of cellular material recovered such as chlorophyll or ATP (Sutcliffe *et al.*, in preparation). Nalewajko and Lean (1972) have suggested that the loss of material as reported by Arthur and Rigler may be due to retention of soluble materials by membrane filters.

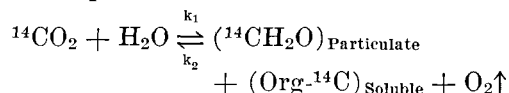
iii) *Treatment of filter.* After filtration of the sample, one wants to eliminate all inorganic carbon from the filter without causing any loss of labeled organic matter. Steemann Nielsen suggested fuming of the filter with HCl fumes, a practice which was adopted by many investigators. Some manuals on measurement of primary productivity, however, state that it is not necessary to fume the filter (Vollenweider, 1969; Strickland and Parsons, 1968). Some oceanographers, in fact, state that filters should not be fumed as such a treatment can cause significant losses of labeled organic material. In our own work, not only have we not found any such losses of organic material by fuming, but we have demonstrated the necessity to fume the filter (Williams *et al.*, 1972).

I usually fume the filter for a few minutes before placing it in a 5.0 disposable plastic scintillation vial, and then drying it in a desiccator with silica gel. Other investigators also use some alkali in the desiccator to help remove the CO_2 . Ward and Nakanishi (1971) have recently claimed, however, that drying of the filter causes losses up to 30% of the total fixed radiocarbon. On the basis of our knowledge of the assimilation routes of carbon via photosynthesis, such losses seem very unlikely to me. There are enough conflicting reports in the literature, though, that some controlled experiments are essential before we can have faith in the correctness of any one procedure.

iv) *Counting of ^{14}C .* Most investigators have used Geiger-Mueller tubes for counting of the incorporated radiocarbon. Not only is the efficiency of GM tubes

relatively low (usually less than 20%), but there are serious problems connected with self absorption and geometry. Scintillation counting is far more efficient (up to about 95%) and simpler, does not suffer from the above problems, and can also be used with wet filters if an appropriate fluor is used. Ward and Nakanishi (1971) have recently compared the results of counting samples by both these methods.

v) *Respiratory losses.* The ^{14}C technique as commonly used measures the increase with time of ^{14}C in the particulate fraction:



Respiration is continuing concomitantly with photosynthesis, however, so that some of the ^{14}C -photosynthate will be respired back to CO_2 and H_2O (rate k_2 above). This correction should be incorporated into the calculations for primary productivity, but the conventional ^{14}C technique does not yield any information to use for making this correction. The amount of heterotrophic ^{14}C fixation in dark bottles is not relevant to this question.

A further complication is caused by different pools of cellular metabolites within cells. During a 4–12 hour incubation with ^{14}C -bicarbonate, the specific activity of respiratory substrates may differ in the various organelles of the cell. It is extremely difficult, therefore, to estimate the amount of labeled carbon which has been lost through respiration during the incubation period, even if one had an accurate determination of the total rate of respiration.

vi) *Excretion of soluble organic compounds.* Laboratory and field data indicate that the amount of soluble ^{14}C -labeled organic material which is excreted by phytoplankton ranges from a few percent to 40% of the total amount of carbon which is reduced. Most studies on primary productivity ignore this soluble fraction, but it should be considered as it represents an input into the ecosystem of energy-rich material that can be used for heterotrophic growth processes. This soluble fraction is more difficult to measure than the particulate fraction, and there are many papers in the literature which must be viewed with caution due to the analytical techniques used. One of the problems in these studies involves the purity of the ^{14}C -bicarbonate solutions used during the incubation period. Any labeled organic contaminants in the preparation (which apparently are quite common) will be interpreted as "excreted carbon." We minimize this danger by exposing our sodium bicarbonate solutions to UV irradiation, which oxidizes all the organic material. To determine the amount of labeled organic materials in the filtrate after the incubation period, we first eliminate all inorganic carbon by acidifying and purging with nitrogen, after which we combust all organic material to CO_2 , which we transfer to hyamine solution and count by scintillation counting techniques (Williams *et al.*, 1972).

It should also be noted that Nalewajko and Lean (1972) have demonstrated retention of dissolved organic material by membrane filters.

vii) *Isotope effects.* There is an abundant literature on the biological discrimination between ^{12}C and ^{14}C (Strickland, 1960) which indicates a discrimination value of about 6%. The resulting error will usually be insignificant relative to other sources of error in the determination of primary productivity.

Productivity Profiles

I want to point out three interesting problems concerning the distribution and activity of phytoplankton cells in regard to productivity measurements.

a) Nearly all studies on primary productivity deal only with the "euphotic zone," the bottom of which is arbitrarily defined as that depth at which the light intensity is 1% of surface illumination. There is sufficient data in the literature (Kiefer *et al.*, 1972; Venrick *et al.*, 1973; Anderson, 1969) to indicate that healthy phytoplankton populations are found at depths much greater than this 1% light level and that they do show a net reduction of CO_2 . Further evidence for this view is the fact that one can grow many algae in the laboratory at light intensities considerably less than 1% of average surface illumination. Halldal (1968) has also shown a net oxygen production with symbiotic algae when exposed to light intensities of about 1×10^{-5} that of sunlight. On the basis of work such as the above, I think our interests in photobiology in natural waters should extend to at least 500 m. Concomitant with such studies, it is important to improve on the light-measuring devices available for field work, and to measure energy in terms of light quanta and wavelength, and not in terms of photometric units which are related to sensitivity of the human retina.

b) Although most productivity studies are concerned with particulate carbon, one should also consider the energy content of the cellular material. The only photochemical reactions of photosynthesis involve the formation of reducing substances (NADH and NADPH) and ATP, which are used in dark reactions to reduce CO_2 and to drive most of the energy-requiring synthetic reactions. Photosynthesis is thought to be a two-step photochemical process, photosystem II serving to produce electrons from water with the evolution of oxygen, and photosystem I which is involved with formation of reducing power and high-energy phosphate bonds (Rabinowitch and Govindjee, 1969). Photosystems I and II can be experimentally isolated and may also be physiologically independent in certain cells. During cyclic photophosphorylation by photosystem I ATP is formed from ADP and inorganic phosphate. This ATP may then be used as an energy source for the cells, or it may be used to "upgrade" the caloric content of the reduced carbon. I do not know of any field studies designed to test this suggestion, but it is possible that such photophosphorylation reactions may be involved in sustaining life in deep-water populations.

c) Photoinhibition of photosynthesis is very commonly invoked to explain the relatively low concentrations of phytoplankton in surface waters, as compared to higher biomasses at greater depths. The basis for this suggestion lies in data from laboratory work

demonstrating that there is inhibition of photosynthesis beyond a certain light level. This physiological response to high light intensities, however, is very dependent upon many physical and biological factors. The point at which high light intensities become inhibiting is not characteristic of any particular species, but depends upon the conditions of the experiment and the immediate history of the cells.

Although one can not dismiss the possibility of photoinhibition of photosynthesis in surface waters, it is likely that the distribution of chlorophyll is a function of nutrient concentrations and temperature, as well as of light intensity. There is a considerable amount of field data to indicate that chlorophyll concentrations can often be related to the concentration of inorganic nutrients, particularly nitrate. In Lake Tahoe, for instance, the chlorophyll maximum is at about 100 m, which also corresponds to the depth where nitrate concentration starts to increase with increasing depth, but the low light levels below 100 m apparently then become limiting for phytoplankton growth. In nutrient-rich waters, on the other hand, phytoplankton biomass often is highest at the surface and decreases with depth, indicating that at least some phytoplankton populations are not being seriously inhibited by high light intensities.

If productivity data are expressed as micrograms of carbon fixed by a microgram of chlorophyll-a per hour for samples from the surface down to the depth where the light intensity equals the saturating intensity for photosynthesis there is generally no indication of photoinhibition in the surface waters. Assimilation numbers commonly are either fairly uniform throughout the upper portion of the water column or they increase slightly toward the surface.

The reduced phytoplankton biomass in surface waters may also reflect greater grazing pressures at these levels. Most of the total biomass estimates based on measurements of ATP instead of chlorophyll indicate greatest biomass in surface waters. One interpretation of these data is that this increase in biomass in surface waters reflects increased populations of heterotrophic cells. We do not have enough data at present to know if this suggestion is correct.

Although I am suggesting that photoinhibition of photosynthesis may not be very important in the ocean, I certainly do visualize that high light intensities may have pronounced effects on phytoplankton fine structure and function, as well as on distribution of organelles. Dale Kiefer, in our laboratories, is studying the fluorescence characteristics of phytoplankton as related to cell structure, CO_2 reduction, light scattering, and light absorption under varying light intensities. I am hopeful that such studies, in conjunction with further field observations, will clarify this nebulous area of physiological effects of high light intensities.

World-Wide Productivity Data

I had intended to give a quick review and discussion of productivity on a global scale, but I think that would require more time than is available today. Instead I will refer to the excellent article by Ryther

(1969) in which he estimates the productivity in the major marine provinces (Table 1). Although it is difficult to estimate the yearly production because year-round data generally are not available for most oceanic stations, the data in Table 1 most likely convey a realistic appraisal of primary production.

TABLE 1
Primary productivity in the major ocean province
(data from Ryther, 1969)

Province	Per-centage of Ocean	g C/m ² /yr	mg C/m ² /day	Total Productivity (10 ⁹ tons C/yr)
Open ocean.....	90	50	140	16.3
Coastal.....	9.9	100	280	3.6
Upwelling.....	0.1	300	820	0.1

Strickland (1960) has also compiled many references concerning primary productivity in the world's oceans. The figures of Ryther agree quite well with most reports in the literature. Thus, Zeitschel (1969) has reported an average value of 382 mg C/m²/day for the Gulf of California, Menzel and Ryther (1960) reports a value of 72 g C/m²/year for the Sargasso Sea, Jitts (1969) shows about 48 g C/m²/year for the Indian Ocean, and El-Sayed (1970), on the basis of extensive, multiyear programs, shows figures of 100 to 500 mg C/m²/day for the Antarctic Ocean. The one major oceanic area for which we have very little data is the Arctic Ocean. On the basis of available data from T-3 Ice Island which indicates about 5 to 10 mg C/m²/day (for only 6-8 weeks, however), Hopkins (1969) has suggested that primary productivity can not sustain the zooplankton respiratory requirements. It is obvious that we need more information from the Arctic Ocean.

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