Agreement between the ^{14}C and oxygen methods of measuring phytoplankton production: reassessment of the photosynthetic quotient

Photosynthetic quotient
Primary production
Plankton photosynthesis
Carbon dioxide fixation
Oxygen production
Quotient photosynthétique
Production primaire
Photosynthèse planctonique
Fixation de CO^2
Production d'oxygène

P. J. leB. Williams, R. C. T. Raine[a], J. R. Bryan[b]
Department of Oceanography, University of Southampton, UK
[a]Present address: Department of Microbiology, University College, Galway, Ireland.
[b]Present address: Thames Water Authority, Rosebery Avenue, London.

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ABSTRACT

Field data showed close correlations between the rates of plankton photosynthesis measured by the ^{14}C and the oxygen techniques. The apparent photosynthetic quotients (PQ) of estuarine and coastal populations were observed to be in the region of 2.0; the high PQ was mainly attributed to nitrate acting as a nitrogen source. Calculations and observations suggest that rapidly dividing phytoplankton populations, using nitrate, will exhibit PQ values in the region of 1.5-1.8, and that the commonly adopted PQ value for marine phytoplankton of 1.25 will only be observed when ammonia (or a nitrogen source at a similar reduction level) is the predominant nitrogen source. It is argued that some of the reported earlier discrepancies between the results given by the ^{14}C and oxygen methods have arisen because no account was taken of the effect of the nitrogen source on the PQ.

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RÉSUMÉ

Concordance de la méthode au ^{14}C et de la méthode à l'oxygène pour la mesure de la production phytoplanctonique : reconsidération de la valeur du quotient photosynthétique apparent

Les résultats présentés montrent une corrélation étroite entre les vitesses de la photosynthèse planctonique mesurées par la méthode au ^{14}C et ceux obtenus par la technique de l'oxygène. Les quotients photosynthétiques apparents (PQ) des populations estuariénnes ou côtières se situent au voisinage de 2,0; les valeurs élevées sont principalement attribuées aux nitrates utilisés comme source d'azote. Les calculs et les observations suggèrent que les populations planctoniques se divisant rapidement en utilisant les nitrates doivent fournir des valeurs de PQ autour de 1,5-1,8 et que la valeur 1,25 communément adoptée pour le phytoplancton marin ne peut être observée que si l'ammoniac (ou des composés azotés au même degré d'oxydation) est la source d'azote prédominante. Les différences précédemment observées entre les résultats fournis par les deux méthodes ont donc probablement comme origine le fait qu'il n'a pas été tenu compte de l'effet de la source d'azote sur le PQ.

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INTRODUCTION

A general relationship exists between primary production of phytoplankton and the abundance of zooplankton and organisms higher up the marine food chain. Thus, models of this food chain (e. g. Ryther, 1969; Vinogradov *et al.*, 1972; Steele, 1974), commonly use the rate of primary production as a basis for calculation. For this, and other reasons, there is a continuing need for accurate estimates of the rate of primary plankton production of aquatic systems.

In marine work, plankton photosynthesis has been

measured by determining the rate of oxygen production and by the rate of carbon dioxide fixation. The latter method, which uses ^{14}C labelled bicarbonate as a tracer (Steemann Nielsen, 1952), has given rise to a large body of data from which most of our generalizations of the productivity of the ocean have been derived. Despite the widespread use of the ^{14}C-method, both in fresh water and marine studies, its overall accuracy still appears to be a matter of some uncertainty (see Fogg, 1975) and it is difficult to obtain from the literature an authoritative statement regarding the absolute accuracy of the ^{14}C-method. As an illustration of the problem, De Vooys (De Vooys, 1979) has recently produced a revised estimate of global oceanic phytoplankton production; the difference between his estimate of 43.5×10^{15} g C.yr^{-1} and the earlier one of 31×10^{15} g C.yr^{-1} produced by Platt and Subba Rao (1975) originates solely from corrections made for presumed systematic errors in the results of the ^{14}C-method. Some early studies suggested extensive discrepancies between the two methods (e. g. Ryther, 1954; McAllister *et al.*, 1964). To some extent these differences have been resolved as the accuracy of the ^{14}C-method has been improved with the use of liquid scintillation techniques for counting the samples and more especially for standardization of the isotope: Sournia (1971) has considered in the detail the errors present in the ^{14}C-method. There have been very few published comparisons of the results of the two methods in field studies, subsequent to these improvements in the methodology of the ^{14}C technique. Such comparisons have been hampered by the insensitivity of the conventional methods for determining the oxygen flux in the samples. Simple refinements to the Winkler method have made possible the precise determination of oxygen concentration on a routine basis (Bryan *et al.*, 1976). This has enabled comparisons of the two techniques to be made in inshore environments. When this is done, we have usually found a close correlation between the results given by the two methods (see Figs. 1 and 2). In such comparisons the implied photosynthetic quotient (PQ) is in the region of 2.0, significantly higher than we expected. We estimate that comparable implied PQ values were given by the results of earlier comparisons (McAllister *et al.*, 1964; Antia *et al.*, 1963; Thomas 1964; Ryther *et al.*, 1971), who concluded that the ^{14}C technique was underestimating production. In the present communication we argue that the apparently high PQ values we have observed can be approached when nitrate serves as a nitrogen source for photosynthesis and furthermore that much of the discrepancy between the oxygen and ^{14}C techniques in the studies cited above could be explained as an error resulting from the use of an inappropriate PQ value to compare the observed oxygen fluxes and carbon fluxes.

TERMINOLOGY, MATERIALS AND METHODS

It is important to specify the production terms used in the present communication. The terminology introduced by Strickland (1960) will be adopted for it is found that his distinction between net production and net primary production is essential when discussing the two methods of measuring plankton production. The definitions are repeated here with minor alteration.

Gross primary production rate

Gross rate of autosynthesis of the constituents of plant material in water. This will amount to the gross rate of algal photosynthesis and will include losses due to respiration and organic excretion. The use of this term has been criticised (Worthington, 1975) on the grounds that it cannot be measured in practice by either technique, for the dark bottle does not make allowance for photorespiration. Accordingly it was the recommendation of an I.B.P. committee that the term gross production be abandoned. Whilst recognizing this argument, we have chosen to use the term, despite the inherent difficulties, for if it is abandoned there is no basis for comparison of the two methods. It is however accepted that the present measurements of gross production by the oxygen technique will contain an error because no correction has been made for photorespiration.

Net primary production rate

Net rate of autosynthesis of the organic constituents of plant material in water. This term does not include respiration of organisms other than the primary producers. This is most likely to be net production, as measured by the ^{14}C technique; the oxygen technique cannot measure this process when active heterotrophic organisms occur in the sample, as will be the normal situation in the environment.

Net production rate

Net rate of production of plant organisms under the influence of all environmental factors. This will be net production, as measured by the oxygen technique. It cannot be measured in natural populations by the conventional ^{14}C technique.

These definitions make the point that with natural populations one should not look necessarily for agreement between *net production*, as measured by the oxygen technique, and the results of ^{14}C production measurements, for they measure very different overall processes. For example, net production at times will be negative, whereas the ^{14}C technique cannot give negative answers.

Photosynthetic quotient

This is taken as the molar ratio of the rate of oxygen production to the rate of carbon dioxide utilization.

Many details of the methodology are not needed for the present discussion and will only be dealt with in outline. Full details are given in Bryan (1979). The data for the seasonal profile of production (Fig. 1) were derived from 24 hours *in situ* incubations of samples taken from three depths from each of six stations, sited along the length of the estuary. The ^{14}C-samples were counted with a thin-window proportional counter, which was cross-calibrated with a liquid scintillation counter. The latter was used to standardize the NaH $^{14}CO_3$. Uniformly labelled ^{14}C-labelled hexadecane (Radiochemical Centre CFR 6) was used as a standard. The calibration of the NaH $^{14}CO_3$ was found to be by

far the most tricky step in the whole procedure. We encountered problems similar to those reported by Iverson *et al.* (1976); however, with the fluor used in the present study it was possible to establish that it was not a pH effect, but more likely exclusion of the aqueous phase from the gel. The solution to the problem was essentially the same as that used by Iverson *et al.* All the photosynthetic rates based on oxygen flux determination given in the present accounts are of gross production (i. e. the difference between the light and dark bottle). The dark bottle values have also been subtracted in the ^{14}C determinations.

RESULTS AND DISCUSSION

Figure 1 shows the results, in summary form, of a study of primary productivity in an estuary. The scaling of the axes, set to a molar $\Delta O_2 / -\Delta CO_2$ of 2, is only a matter of convenience. The correlation between the results is good (correlation coefficient = 0.96; degrees of freedom = 166); the mean observed molar relationship between the gross amount of oxygen produced to carbon dioxide consumed was 1.95. Similar close correlations between the two methods was found in time course experiments. Figure 2 shows the results of such an experiment on a water sample from a Scottish sea loch. Again the implied PQ is close to 2. The results show no significant change in PQ with time.

The reason for the apparently high PQ values was not immediately obvious. Initially we suspected a systematic error in the photosynthetic measurement. The oxygen method is unlikely to suffer from systematic errors due to the analytical technique. The calibration of the ^{14}C technique therefore was examined very carefully for a source of error. The measurement of the radioactivity of the samples was cross-checked by counting a selected number of filters both with liquid scintillation counter and an end-window counter. In neither case was there any sign of a significant systematic error. The exudation of photosynthetic products can give rise to an underestimation of production with the ^{14}C technique. This process was not measured as a matter of routine but spot determinations were made throughout the course of the study; the observed rates were generally less than 10% of total photosynthesis, this is in agreement with Thomas's (1971) observations in North American estuaries. Such rates, if ignored, would not account for the high observed PQ values.

A systematic difference between the two techniques will arise by virtue of the fact that oxygen flux measurements will relate to gross primary production whereas the ^{14}C method gives a value between gross primary production and net primary production (in Strickland's terminology). It is recognised that this is a complex issue but we conclude that it is not the prime cause of the high implied PQ for the following reasons. First, phytoplankton respiration, which gives rise to the difference between gross primary production and net primary production, probably lies in the region 10-20% of gross photosynthesis in actively growing populations (Steemann, Nielsen, Hansen, 1959; Hobson *et al.*, 1976; Laws, Wong, 1978). It has been argued (see e. g. Steemann Nielsen, 1963) that recycling of respiratory carbon dioxide will give a result with the ^{14}C technique, lying part-way between gross and net primary production. The recent study of Hobson *et al.* (1976) illustrates that after about 30 hours continuous illumination, radiochemical equilibrium is reached in the internal carbon dioxide pool, only from this point onwards will the ^{14}C technique measure net production. Without becoming too engrossed in the details of the argument, one may

Figure 1

Mean rate of photosynthetic production in Southampton Water, measured by the oxygen and ^{14}C-techniques. Each data point was normally derived from measurements on 18 samples, taken from various locations and depths in the estuary. For clarity only the oxygen data points have been connected up. Oxygen data: ⊙ ; ^{14}C-data: ×.

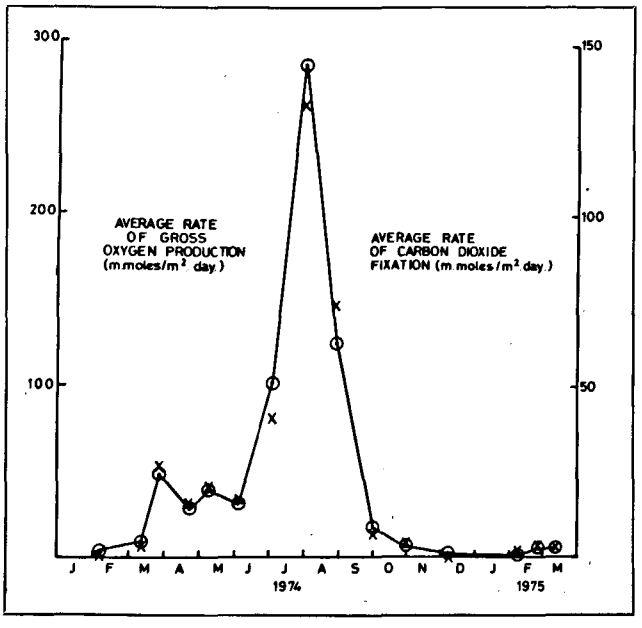
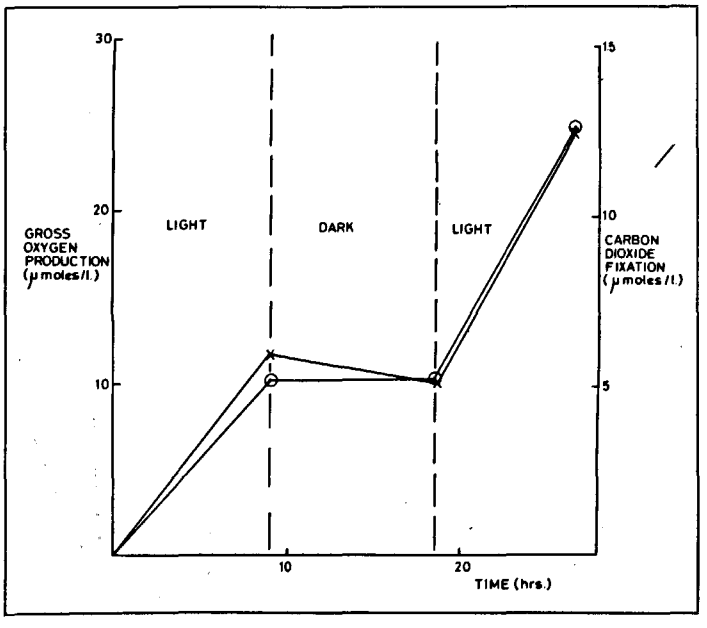


Figure 2

Photosynthetic production, measured by the two methods, through a light-dark-light cycle. The sample was taken from the DAFS experimental facility at Loch Thurnaig. Oxygen data: ⊙ ; ^{14}C-data: ×.



estimate that errors between 5-15% could commonly occur between oxygen and the ^{14}C method due to the uncertainty over whether the ^{14}C technique is measuring gross or net production and as such would be insufficient to be the principal cause of the high observed PQ values. Further support for this conclusion is given by the time course experiment (Fig. 2): the longer the incubation time the closer the ^{14}C technique approaches a measure of net production, thus any discrepancy between the two methods due to this should increase with time. There was however no evidence of a relative decrease in the amount of photosynthesis measured by the ^{14}C technique in the second light period (Fig. 2). Finally, the error due to the ^{14}C technique giving a value between that of gross and net primary production will also be present when the technique is used with algal cultures. With a culture of *Dunaliella* it was found that with ammonia as a nitrogen source "acceptable" PQ values of 1.1 and 1.25 were obtained whereas when nitrate was present the characteristically high PQ values were observed (Table 1).

We estimate that the systematic errors introduced into the ^{14}C measurements, as a result of ignoring phytoplankton organic exudations and the gross-net primary production problem in the present work, would probably lie in the range 5-20% and as such should not be the prime cause of the high PQ values.

The main explanation of the high observed PQ values appears to be associated with the nature of the inorganic nitrogen source. Cramer and Myers (1948), using manometric techniques, showed that the PQ of *Chlorella* increased when nitrate replaced ammonia as the nitrogen source for photosynthesis. We obtained similar results with *Dunaliella* using the O_2 and ^{14}C plankton productivity methods (Table 1). In both studies, the change from one nitrogen source to another mainly affected the rate of carbon dioxide fixation, which is to be expected if the rate of photosynthesis is energy (i. e. light) limited. Cramer and Myers calculated that the PQ values they observed with ammonia and nitrate as the nitrogen sources (1.06 and 1.47 respectively) were consistent with empirical equations based on the elemental composition of their cultures of *Chlorella*. Eppley and Sloan (1965), using a variety of methods, measured the PQ of cultures grown with nitrate as the major nitrogen source. The three diatoms they studied gave PQ values ranging from 1.49 to 1.89; a value of 1.6 was obtained with the coccolithophore *Syracosphaera elongata*. In contrast low PQ values were obtained with *Coccolithus huxleyi* (PQ=1.2) and *Peridinium trochoidem* (PQ=1.1). Although it is recognised that the PQ values for marine phytoplankton may range from 1.0 to 1.6 (Strickland, 1965; Parsons *et al.*, 1977), for reasons which never seemed to have been discussed, a value in the region of 1.25 is regarded to be a median one and is commonly used in calculations. In view of this, the apparently high PQ values for some cultures and natural populations obtained by ourselves and others need further consideration. It is convenient to separate the overall PQ into two components: a "carbon" PQ controlled by the state of reduction of the photosynthetic products (i. e. the relative amounts of carbohydrate, lipid and protein produced) and a "nitrogen" PQ which is dependent upon the state of reduction of the nitrogen source (i. e. whether nitrate or ammonia is used). The relative contribution of these two components to the overall PQ will depend upon the ratio of the rates of carbon and nitrogen assimilation, which will be reflected in the C : N ratio of the cell.

The "carbon" PQ may be determined from cultures grown on an ammonium based medium or calculated from elemental analysis. Typical values lay in the region 1.1 to 1.35 (Ryther, 1956) with a generally accepted median value of 1.25, and there seems no compelling reason to question this value. Cramer and Myer's early work illustrated that the nitrogen source will affect the overall PQ, although the implications of this work do not seem to have been fully recognised. This is unfortunate because as will be seen the "nitrogen" PQ can potentially have a much greater effect on the overall PQ than the "carbon" PQ. Utilization of nitrate, in place of ammonia, will result in the production of two extra molecules of oxygen per atom of nitrogen assimilated, if one assumes nitrate assimilation to obey the following stichiometric equation

$$NO_3^- + 2H_2O = NH_3 + OH^- + 2O_2.$$

The effect of the "nitrogen" PQ is illustrated in Table 2, where the overall PQ has been calculated for various C : N assimilation ratios, using the accepted "carbon" PQ value of 1.25.

The results illustrate very clearly that for growth on nitrate as a nitrogen source, the C : N assimilation ratio is the major factor determining the PQ. Antia *et al.* (1963) reported atomic C : N ratios of approximately 3 : 1 for a diatom crop growing vigorously in

Table 1

The effect of inorganic nitrogen source on the photosynthetic quotient of a culture of Dunaliella.

	Nitrogen source	Concentration (μmolar)	Specific rate of photosynthesis (μmoles/μg chlorophyll a. h.) Gross oxygen production	Carbon dioxide fixation	Observed molar PQ ($\Delta O_2/-\Delta CO_2$)
Experiment 1	Ammonia	200	0.227	0.204	1.10
	Nitrate	200	0.280	0.125	2.25
Experiment 2	Ammonia	50	0.143	0.113	1.26
	Nitrate	50	0.146	0.083	1.75

Table 2

The relationship between C : N assimilation ratio and the expected photosynthetic quotient for growth on nitrate. The "carbon" PQ (see text) is assumed to be 1.25.

Atomic C : N assimilation ratio	Calculated PQ
3 : 1	1.92
4 : 1	1.75
6 : 1	1.58
8 : 1	1.50
10 : 1	1.45
15 : 1	1.38

nitrate-rich water, which was the situation prevailing in the environments we studied. Such a C : N ratio would yield a PQ in the region of 1.8. Thus the PQ values observed by ourselves and Eppley and Sloan, although high, are by no means anomalous when nitrate is the inorganic nitrogen source. At the same time one is not attempting to argue that the methods do not still contain unresolved residual systematic errors, only that these are quite possibly minor in relation to the error of ignoring the effect of the nitrogen sources on the PQ. The nitrogen budget for Southampton Water, which was the site from which the data in Figure 1 were obtained, is dominated throughout the year by nitrate input from the rivers (Collins, 1978), this is consistent with our observations that the PQ is high and constant through the year. Ammonia is present at concentrations in the range of 1-10 μg-at/l; direct measurements of nitrate and ammonia changes in dark and light bottles (Chan, 1978) demonstrated that photo-assimilation of nitrate was not inhibited by ammonia at these concentrations. This is consistent with the observations of Bienfang (1975) but is at variance with other work (see e. g. McCarthy, Taylor, Taft, 1977) which has demonstrated that ammonia concentrations in excess of 1.0 μg-at N/l suppress nitrate utilization. Presently we can offer no explanation for these differences.

Carbon to nitrogen ratios as low as those observed by Antia and his co-workers are perhaps exceptional, although the low C : N ratios they observed during the nitrogen-rich phase of the bloom do stand up to critical examination (Banse, 1974). Studies of C : N ratios have been made by a variety of workers and it would appear that a spread in atomic ratios from 4 to 15 may be expected, lower values are encountered at high growth rates (Caperon, Meyer, 1972). Thus for rapidly dividing populations, growing on nitrate as a nitrogen source (e. g. a spring bloom in temperature seas or a bloom in upwelling waters), PQ values between 1.5 to 1.8 may be expected. As nitrate becomes exhausted, the growth rate will fall, the C : N ratio will increase and the PQ consequently should decrease to 1.35-1.45. At the same time ammonia, produced by bacterial and zooplankton respirations will begin to assume a major role as a nitrogen source, resulting in a further fall in the PQ value. It is only in this situation that the commonly adopted PQ value of 1.25 will be reached. Thus the notion of a typical PQ value for phytoplankton and particularly a value of 1.25 is misleading.

We have only considered the semi-long term mean PQ values. Over short periods of time (i. e. fractions of a day) a greater range of PQ values may be expected (see e. g. Strickland, 1960, p. 64). The lack of general recognition that PQ values approaching 2.0 can be expected for phytoplankton may have been largely responsible for some of the reported "discrepancies" between the ^{14}C and oxygen methods. As examples, in two well-known studies (Antia *et al.*, 1963; Ryther *et al.*, 1971), it was concluded that an unsatisfactory agreement had been obtained between the two methods. These conclusions were based on an expected PQ value of 1.2; the ratio between carbon flux (as determined by the ^{14}C method) and oxygen flux estimated from the data given in the papers was in the region of 2.0. The planktonic populations in both studies were diatoms growing in nitrate-rich water (10-20 μg at N/l): in the light of the present arguments it would be more reasonable to assume that both methods were giving substantially correct results and the main source of error lay in assuming an inappropriate PQ value.

It is a corollary of the present argument that the PQ value should be a reasonable sensitive indicator of whether nitrate is being used by the algae as a nitrogen source.

CONCLUSIONS

It is thus our conclusion that the agreement between the two methods of measuring plankton photosynthesis is potentially quite good, although this is in no way meant to imply that the methods are presently without problems. It has been our experience for example that, even when liquid scintillation techniques are used, neither the measurement of the radioactivity of the samples nor the calibration of the ^{14}C bicarbonate is at all straightforward and unless particular care is taken we would agree with Steemann Nielsen's generalization (Steemann Nielsen, 1975) that one should not consider the absolute accuracy of the measurement of primary production (by the ^{14}C method) to be better than $\pm$ 30%. The oxygen technique also possesses problems beyond the easily determined analytical one of precision. They mainly centre round the uncertainty of the magnitude of the error introduced by the present inability to correct for photorespiration. The present study has only established that it is possible to obtain some measure of agreement between the two techniques and it leaves open the very important question how accurately these methods determine *in vivo* rates. This one would presume was the point Ryther and his co-workers (Ryther *et al.*, 1971) were making when they concluded that "the ^{14}C technique, as used in routine oceanographic exploration, is useful as a relative index of organic production, but cannot be expected to provide accurate absolute values." For a variety of reasons it is not feasible to undertake a comparison of measured and observed rates of production using carbon as a basis. A major problem is the absence of a method based directly on carbon flux to measure the loss of organic material due to microbial and zooplankton respirations.

It would, however, appear to be quite feasible to do this with samples from inshore regions using oxygen as a parameter, now that oxygen fluxes due to plankton photosynthesis and respiration may be measured directly with adequate precision in coastal water.

Although it may be possible to achieve a close correlation between the two methods for measuring phytoplankton productivity, it should be noted that they measure different, although linked, processes. The ^{14}C-method ideally determines carbon flux, whereas the oxygen method gives results more associated with energy flux.

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