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Gallagher home

METHODS FOR ESTIMATING PRIMARY PRODUCTION USING THE ^{14}C & O_2 METHODS

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Assignment

Discussion Topic

Why and how can primary production be estimated using the ^{14}C and O_2 methods?

Required Readings

Comment

Just skim the Peterson (1980) article. It does provide a nice overview of the great debate over gyre production, but we'll be covering that in a future class.

Peterson, B. 1980. Aquatic primary productivity and the ^{14}C-CO_2 method: a history of the productivity problem. Ann. Rev. Ecol. Syst. *11*: 359-385.

Supplemental

Falkowski, P. G., E. A. Laws, R. T. Barber, and J. Murray. 2003. Phytoplankton and their role in primary, new, and export production. pp 99-121 in M. J.. R. Fasham, ed., Ocean Biogeochemistry: The Role of the Ocean Carbon Cycle in Global Change, Springer, Berlin. *[These authors summarize production patterns, especially rates of new and export production, from sites around the world studied as part of the Joint Global Ocean Flux (JGOFS) study.A pdf of this chapter can be found here:* ***http://www.ocean.washington.edu/2004/academics/options/chemical/faculty/MurrayJ/papers/Falko.pdf****]*

Fogg, G. E. 1980. Phytoplanktonic primary production. Pp. 24-45 *in* R. S. K. Barnes and K. H. Mann, *eds*., Fundamentals of Aquatic Ecosystems. Blackwell, Oxford. *[An easy-to-read summary of primary production. I use this as a reading for lectures to undergraduate classes on primary production.]*

Gallegos, C. L. and W. N. Vant. 1996. An incubation procedure for estimating carbon-to-chlorophyll ratios and growth irradiance relationships of estuarine phytoplankton. Mar. Ecol. Prog. Ser. *138*: 275-291. *[The C:Chl a ratio is difficult to determine (see* ***Redalje & Laws 1981****). During balanced growth, C and Chl a are produced proportionate to C:Chl a. Incubations performed here using the dilution method, to reduce grazing effects (and grazer biomass). Grazers lead to overestimates of C:Chl a]*

Harrison, W. G. and T. Platt. 1980. Variations in assimilation number of coastal marine phytoplankton: effects of environmental co-variates. J. Plankton Research *2*: 249-260. *[Confirms Platt & Jassby (1976) that temperature is the dominant environmental covariate of assimilation number, accounting for 40% of the observed variation, but this correlation may be due to seasonal phytoplankton succession and changes in C:Chl ratio (p.257)]*

Parsons, T. R., Y. Maita, and C. M. Lalli. 1984a. A manual for chemical and biological methods for seawater analysis. Pergamon Press, Oxford. *["Uptake of Radioactive Carbon" Pp. 115-120]*

Miller, C. B. 2004. Biological Oceanography. Blackwell Science, Malden MA. 402 pp. Chapter 3, especially pp. 46-51.

Parsons, T. R., Takahashi, and Hargrave.1984b. Biological Oceanographic Processes, 3rd Edition. Pergamon Press. Pp. 61-66.

Pregnall, A. M. 1991. Photosynthesis/Translocation: Aquatic. Pp. 53-75 *in* D. C. Coleman and B. Fry, eds., Carbon Isotope Techniques. Academic Press, San Diego. *[Marshall Pregnall presents a brief, concise summary of how to estimate primary production using the ^{14}C-CO_2 method. Pregnall includes a nice section on the calculations necessary to compute production and estimate DOC production]*

Comments on the readings

Bruce Peterson (1980) provides an excellent summary of the uses and limitations of the ^{14}C technique for estimating primary production. He presents the history behind biological oceanography's "Great Debate" over the rate of global primary production.

Falkowski & Raven (1997, Chapter 9) provides a superb recent overview on the measurement of primary production using the ^{14}C method. Read through the material below on the definitions of gross and net production and my overview of the methods. Then, read Falkowski & Raven for the details. I also have detailed slides incorporating many of the key figures from Falkowski & Raven (1997) that I'll post in Prometheus.

Pregnall (1991) provides a concise description of how to estimate primary production using the ^{14}C technique. **Parsons *et al.* (1984a)** also describes the ^{14}C technique with less explanation than Falkowski & Raven (1997) and Pregnall (1991) but with more description of the protocols. Refer to this article for 'recipes' for the reagents, recommendations on incubations, and formulae for calculating primary production. **Gallegos & Vant (1996)** provides a recent analysis of the effects of grazing during productivity incubations.

The ^{14}C & O_2 techniques

A review of the light-dark bottle O_2 method will help us to understand the principles and problems of the ^{14}C method. **Mills (1989, p. 153)** attributes the 1899 discovery of the light-dark bottle O_2 method to Whipple, head of the Boston Water Works and Gordon McKay Professor of Sanitary Engineering at Harvard (Do you think that chair still exists?), and the Norwegians Gaarder & Gran. Whipple suspended phytoplankton samples in the water to estimate production. The Norwegians Gaarder & Gran were the first oceanographers to use the light-bottle, dark-bottle

Box 1. Steps in estimating phytoplankton production using the light and dark bottle O_2 method.

1. Obtain samples from the appropriate depths and light conditions. This is best done at dawn. Avoid exposing samples to direct sunlight even for seconds to avoid photoinhibition.
2. Split samples equally between light and dark bottles. Measure the initial oxygen concentrations in the bottles.
3. Incubate the paired bottles *in situ* (preferred) or using the simulated *in situ* methods. The latter attempts to mimic *in situ* light intensity (and quality), usually using neutral density filters.
4. Incubate for 2 to 24 hours. Twenty-four hour dawn-to dawn-incubations gives the least ambiguous results.
5. Calculate gross and net community production:
   a. Light bottle O_2 minus initial O_2 concentration is net community production. In the absence of heterotrophs, the difference is net primary production.
   b. Dark bottle final O_2 concentration minus initial O_2 concentration is respiration
   c. Light bottle minus dark bottle is gross community production.

O_2 method in 1916, publishing their results in English in 1927. Box 1 outlines the steps in the light & dark bottle O_2 method. Note that the standard O_2 method only estimates gross and net primary production if there are no heterotrophs in the incubation. If significant photorespiration were occurring, the standard O_2 method would underestimate respiration due to phytoplankton and would underestimate gross production. The estimate of net production (light-initial would include the effects of photorespiration.

Box 2. Steps in estimating phytoplankton production using the ^{14}C method.

1. Prepare a $H^{14}CO_3^-$ solution of known activity
2. Obtain samples from the appropriate depth and light conditions. Don't expose samples to direct sunlight.
3. Split samples between experimental and control bottles. Add ^{14}C spike to both experimental and control bottles. A variety of controls or blanks have been used: Time-0, dark-bottle, DCMU, DCMU & dark-bottle
4. Incubate using *in situ* (preferred) or simulated *in situ* methods for 2 to 24 hours
5. Gently filter the particulate matter for later laboratory analysis (a 0.4-µm filter is now common). A sample of the medium can be obtained to estimate DOC production.
6. Determine radioactivity of POC (& DOC) and the amount of Chl *a* in the sample bottles
   a. Estimate or measure the specific activity of the DIC in the incubation bottle [dpm/g DIC].
   b. Measure the ^{14}C activity in the particulate (and optionally the dissolved) organic phases. The filtered samples or liquid samples (for DOC) are first acidified to drive off unfixed ^{14}C, then the sample's radioactivity is determined by **liquid scintillation counting**.
7. Carbon fixed per sample bottle is obtained by:

$$\frac{dC}{dt} = \frac{1.05\ A^*}{I^*\ T}.$$

$$\text{where, } A^* = \text{activity of } ^{14}C \text{ in sample POC (\& DOC)} \left[\frac{dpm}{sample}\right].$$

$$I^* = \text{specific activity of DIC in bottle} \left[\frac{dpm}{gC\ DIC}\right].$$

$$T = \text{Incubation time } [h].$$

$$1.05 = \text{Isotopic discrimination factor.}$$

8. The control ^{14}C uptake (time 0, DCMU control, dark bottle) should be subtracted from A*
9. Chl *a* concentration to estimate of Chl *a*-specific production should be determined from the time-0 and ^{14}C-spiked bottle to obtain initial and final estimates of Chl *a*.

The steps involved in performing a ^{14}C incubation are shown in Box 2. There are a variety of different ways of carrying out ^{14}C incubations. The "Great Debate" in biological oceanography is whether the ^{14}C technique underestimates primary production in the open ocean. The basic technique hasn't changed much since it was invented by Steeman-Nielsen in 1952. Great care is now taken in obtaining healthy phytoplankton samples. Clean bottles are used and care is taken to ensure that the ^{14}C spike solution isn't contaminated with heavy metals. Starting in the late 1970s, samples of seawater from the incubation vessels was taken to estimate the amount of primary production converted to dissolved organic carbon. Usually the easiest variable to measure is the specific activity of the DIC pool in the incubation bottle after the spike is added. Usually the DIC concentration can be estimated (it doesn't change much), or better yet it can be determined back in the laboratory. In certain situations, the DIC concentration can change significantly during an incubation. This can occur when applying the ^{14}C method to estimating benthic diatom production. Samples to estimate DIC should be taken before and after the incubation.

It is crucial that the filtration step uses low filtration pressures to avoid rupturing the phytoplankton cells. Usually, the ^{14}C technique is used to obtain P *vs.* I curves, requiring an accurate estimate of Chl *a* in the bottle. The variable measured in P *vs.* I incubations is Chl *a* -specific production. One common procedure for estimating Chl *a* concentration is to take a sample before adding the ^{14}C spike. This avoids having to deal with radioactive contamination when measuring Chl *a*

spectrophotometrically or fluorometrically back at the laboratory. However, if the Chl *a* concentration increases during the incubation, Chl *a*-specific production will be overestimated using only a Time 0 Chl *a* estimate.

One of the real problem areas for the ^{14}C method is that while it is assumed that the ^{14}C estimate estimates something between gross and net production, there is no theory to determine what percentage of net production is being estimated. In theory, the ability of the O_2 method to estimate respiration gives it a distinct advantage over the ^{14}C method. While the O_2 method provides an estimate of gross production, the ^{14}C technique estimates something between gross and net. **Peterson (1980, p. 365)** presents Steeman-Nielsen argument that if 60% of respired CO_2 is refixed by phytoplankton and if respiration is 10% of gross photosynthesis, then the ^{14}C method should give rates comparable to 94% of gross photosynthesis. At a 20% respiration rate, the ^{14}C technique would be 86% of gross production. The O_2 method measures both primary production in the form of particulate and dissolved organic carbon:

$$\mathbf{n}\,CO_2 \xrightarrow{light} \underset{\mathbf{1}}{\textit{Particulate organic C}} + \underset{\mathbf{2}}{\textit{Dissolved organic C}} + \underset{\mathbf{3}}{\mathbf{n}\,O_2}. \qquad \textbf{(1)}$$

$$\textit{O}_2 \textit{ method measures } \textit{O}_2 \textit{ production, equivalent to measuring } \mathbf{1} + \mathbf{2}.$$

$$^{14}\textit{C method measures only } \mathbf{1} \textit{ if only filtered POC is counted.}$$

These advantages of the O_2 method are more than offset by some of the problems, chief being that the standard Winkler method for determining O_2 concentrations isn't sensitive enough to measure low rates of primary production (either in low productivity areas or in low light or with short incubations). A second huge limitation is that the currency of biological oceanography, for good or ill, is carbon, not oxygen. In order to convert primary production from units of ΔO_2 per m^2, the oceanographer must assume a photosynthetic quotient (PQ=moles O_2 produced: moles C fixation). The PQ is not a constant. A final limitation of the O_2 method is that size fractionated productivity measurements are impossible. Such measurements are often very valuable in determining the production of different phytoplankton size groups. Filtering phytoplankton through mesh sieves prior to doing an O_2 analysis would severely damage the cells. Using the ^{14}C method, the POC in the incubation can be performed after the incubation.

GRAZING WITHIN THE BOTTLE

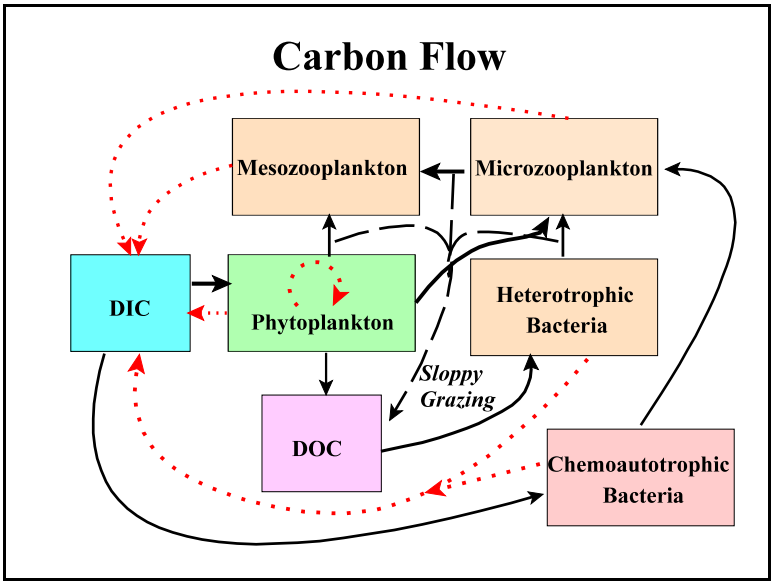

Figure 1. Flow diagram showing the path of labeled carbon in a ^{14}Cincubation. Within the phytoplankton cell, much of the CO_2 produced by respiration will be refixed by RUBPCO before leaving the cell. Photorespiring cyanobacteria leak copious amounts of glycolate, but such leakage may be low in nature (**Colman 1989**).
The chemoautotrophic nitrifying bacteria fix DIC using RUBPCO, but they are inhibited by light.

Grazing within the bottle complicates the interpretation of both the O_2 and ^{14}C methods. It is impossible to exclude grazers on the basis of size. Some protocols recommend using a screen to remove the mesozooplankton, however often the most important grazers in bottles are the microzooplankton (ciliates and heterotrophic nanoflagellates) which cannot be separated from the phytoplankton by size. Figure 1 shows some of the many pathways for labeled carbon in a typical ^{14}C incubation. With short incubations, the ^{14}C method should be more likely to estimate gross primary production, because less of the label should appear as DOC and as organic carbon in heterotrophic organisms. The DOC pool represents a major product of primary production but was not routinely estimated until the 1980s. Most grazers on phytoplankton and heterotrophic bacteria are "sloppy", losing a considerable amount of the fixed carbon into the surrounding water.

While it is impossible to filter only the grazers out of a bottle, it is possible to dilute their effects. As we will discuss later in the semester when covering zooplankton grazing, grazing rates are density-dependent. By diluting out the phytoplankton in a sample, the effects of grazing can be greatly reduced. Of course, in many areas of the ocean and in lakes, grazing is tightly coupled to primary production. Removing the grazers can produce highly inaccurate estimates of the "real" primary production rate.

PHOTORESPIRATION

Photorespiration is one of the major problems faced by all photosynthetic organisms. It poses a major problem in interpreting data produced from the ^{14}C-productivity method too. The most abundant enzyme in the world is undoubtedly ribulose-bisphosphate carboxylase/oxygenase, called RuBPCO or more commonly called "Rubisco." In its simplest terms, autotrophy is fixation of carbon by Rubisco. Not all autotrophs are photoautotrophs, nitrifying bacteria & sulfide-oxidizing bacteria also use Rubisco, but with reduced inorganic compounds as the energy source. Rubisco combines one carbon molecule from CO_2 to the five-carbon molecule ribulose bisphosphate to produce a 6-carbon molecule used to produce all other biomolecules. The

Calvin cycle is the set of coupled reactions that produces the 5-carbon ribulose biphosphate substrate for Rubisco. All autotrophs use the Calvin cycle and Rubisco. The fixation of carbon requires energy. Photoautotrophs get the energy (reducing power) from light; chemoautotrophs get the energy from reduced inorganic compounds.

However, Rubisco can function as an oxygenase. If O_2 binds to the active site of Rubisco instead of CO_2, the enzyme doesn't create the 6-carbon molecule needed for growth. Instead Rubisco splits the 5-carbon ribulose bisphosphate into a 3-carbon phosphoglycerate and a 2-carbon phosphoglycolate molecule. Rubisco encountering O_2 is the biochemical equivalent of "Go to Jail & Do Not Pass Go" in Monopoly. The inhibitory effect of O_2 on photosynthesis due to photorespiration is called the **Warburg effect**. The 3-carbon molecule produced by photorespiration can immediately reenter the Calvin cycle to be built up to a 5-carbon ribulose biphosphate. Two glycolate molecules must be condensed to scavenge a single 3-carbon pyruvate that can again participate in the Calvin cycle. The lost CO_2 is called photorespiration. As the name implies, photorespiration occurs only in the light and is not assessed with the dark bottle in the O_2 method.

The affinity of Rubisco for CO_2 is very poor. Some have argued that the poor affinity of Rubisco for its primary task of fixing carbon is due to a quirk of evolutionary history. The Rubisco molecule evolved early in the history of the earth at a time when CO_2 concentrations were more than twice what they are today and there was no oxygen. The first photoautotrophs were the cyanobacteria. They lack the scavenge pathway needed to condense two 2-carbon molecules to form the 3-carbon pyruvate. Cyanobacteria in which Rubisco is acting as an oxygenase produce large amounts of glycolate which is excreted from the cells. For a cyanobacterium fixing CO_2 two billion years ago, maintaining a high CO_2:O_2 concentration at the Rubisco active site was not much of a problem. Over geologic time, external O_2 concentrations increased and CO_2 concentrations decreased. Photorespiration which is directly related to the O_2:CO_2 ratios at the Rubisco active site increased.

Minimizing photorespiration must have been an adaptive trait under strong selection. There were four major evolutionary advances for maintaining high CO_2:O_2 ratios at the Rubisco active site. First, phytoplankton evolved CO_2 concentrating mechanisms, the most important being the bicarbonate pump. Phytoplankton actively transport bicarbonate into the cell, expending energy in the process. The intracellular bicarbonate is converted to CO_2 near the site of Rubisco by the enzyme bicarbonate anhydrase. Cyanobacteria and most phytoplankton have active bicarbonate pump systems, but the pump is shut down until needed. In the laboratory, cyanobacteria grown with high CO_2 partial pressures will photorespire, leaking glycolate, at a high rate for a short time after being transferred to a low CO_2 partial pressure. The photorespiration rate declines as the cells physiologically adapt to the low CO_2 conditions.

The second major evolutionary advance was the evolution of a more efficient Rubisco molecule. Higher algae, like the diatoms and green algae, evolved a more efficient Rubisco molecule but its affinity for substrate is still dismal. The half-saturation constant (K_m) for *in vitro* carbon fixation by eukaryotic ribulose biphosphate carboxylase/oxygenase (RuBPCO) is relatively high ($K_m \approx$ 6-20 μM CO_2 (aqu), **Prins & Elzenga 1989**). This K_m is slightly less than the ambient

concentration of CO_2 (aqu) in seawater (pH 7.8-8.2). At a pH of 7.5 freshwater contains about 12 µM CO2 . By using a bicarbonate pump mechanism, **Beardall (1991)** found that phytoplankton can reduce the effect of K_m of Rubisco to 3 µM CO_2. This is still a poor affinity for substrate. By contrast, the K_s for nitrate, ammonium and phosphate uptake may be less than 0.3 µM in oligotrophic phytoplankton species. Under slightly alkaline conditions (>pH 8.2), with no CO_2 concentrating mechanism at the active site of the RuBPCO, eukaryotic phytoplankton photosynthesis rate could be strongly controlled by the availability of CO_2. The affinity of cyanobacterial RuBPCO for CO_2 is much lower than eukaryotic RuBPCO. The in vitro K_m for cyanobacterial RuBPCO, which is structurally similar to eukaryotic RuBPCO, is a very large 200-350 µM (**Colman 1989**). In the past ten years, it has been found that the dinoflagellates evolved with yet a third major type of photoautotrophic Rubisco. Orellana & Perry found that antibodies produced to diatom Rubisco will react strongly with Rubisco ranging from green algae to corn, they do not bind strongly with dinoflagellate Rubisco.

The third major evolutionary advance was the development of the scavenge pathway to convert glycolate to pyruvate. This pathway isn't found in cyanobacteria, but it is found in most other plants. One of the ways of estimating photorespiration rates is to add inhibitors of the C_2 scavenge pathway, such as aminooxyacetate AOA (**Tolbert *et al.* 1985**). After AOA addition, photorespiration results in direct excretion of glycolate with relatively high ^{14}C activity. In essence, AOA turns eukaryotic phytoplankters photorespiration physiology into that of a cyanobacterium.

A fourth major evolutionary advance in photosynthesis was the evolution of C-4 metabolism. C-4 metabolism is a term coined by **Morris (1980)** and includes true C-4 photosynthesis, which is found in a subset of multicellular plants and Crassulacean acid metabolism (CAM). True C-4 plants partition the incorporation of atmospheric CO_2 into C-4 compounds and the fixation of CO_2 by Rubisco into different cell types. In true C-4 photosynthesis, CO_2 is added to the 3-carbon molecule phosphoenolpyruvate (PEP) to form a 4-carbon molecule by the efficient enzyme PEP carboxykinase (or other β-carboxylases). Since the carbon is added at the 2nd or β carbon molecule in the 3-carbon chain, this class of enzymes is known as the β carboxylases. In desert plants, this energy-requiring β carboxylation reactions occur mainly at night when the stomata (openings to the atmosphere) in the bundle sheath cells are open. During the day, the stomata are closed limiting water loss. Photosynthesis occurs as CO_2 is cleaved from the 4-carbon storage compounds at the Rubisco active sites in the mesophyll cells. In Crassulacean Acid Metabolism (CAM), the CO_2 is fixed first into C-4 compounds and then CO_2 is cleaved from the 4-carbon storage compounds in the same cells. In CAM metabolism, significant amounts of carbon are stored as C-4 compounds at night.

It isn't known how important C-4 like metabolism is to phytoplankton. **DesColas-Gros & Fontugne (1985, p. 3)** found no PEP carboxylase activity in marine diatoms, but high activities of PEP carboxykinase (EC 4.1.1.49). PEP carboxylase and PEP carboxykinase both produce C-4 compounds from C-3 compounds and CO_2. **Glover & Morris (1979)** surveyed phytoplankton in the Bigelow marine phytoplankton type culture collection, analyzing the β-carboxylase:Rubisco ratios. Table 1 shows that the diatoms have very high ratios of β carboxylase:Rubisco and display very little inhibition of photosynthesis by oxygen (the Warburg effect). The diatoms have adaptations that allow them to photosynthesize even under very low CO_2 and high O_2 environments. The presence of high β-carboxylase activities in the diatoms doesn't mean that they are using C-4 photosynthesis. The diatoms may merely be storing nitrogen. One of the

major pathways for incorporating nitrogen in phytoplankton cells is to incorporate NH_4^+ into C-4 skeletons produced by PEP carboxylases (**Guy *et al.* 1989**, **Vanlerberghe *et al.* 1990**).

Table 1. Rubisco:PEPCase activity in selected marine phytoplankton and sensitivity to inhibition by oxygen (Warburg effect). Table 2 from **Morris (1980)**.

	Group	**RuBPCO:PEPCase**	**% inhibition**
Dunaliella	Green flagellate	29	17
Synechococcus sp.	Cyanobacterium	15	18
Thalassiosira pseudonana	diatom	7-14	0
Phaeodactylum tricornutum	diatom	8	4.5
Skeletonema costatum	centric diatom	0.5	3
Surirella ovata	diatom	2.9	0
Amphipora paludosa	pennate diatom	6.6	0
Navicula pelliculosa	pennate diatom	2.3	0

Photorespiration looms as a huge issue in interpreting ^{14}C results. Recent work now indicates that photorespiration is nil in physiologically healthy algae. **Weger *et al.* (1989)** used ^{18}O to estimate dark respiration and photorespiration in healthy diatoms in the laboratory and found that the latter was virtually unmeasurable. **Colman (1989)** argues that photorespiration represents only a small portion of gross production in healthy cyanobacteria. Obviously, natural selection has led to efficient mechanisms for phytoplankton to cope with their inefficient Rubisco molecules. However, if the mechanism for maintaining high CO_2:O_2 activities at the Rubisco active sites involves C-4 metabolism, the ^{14}C technique could underestimate production. For example, if phytoplankton cells produce C-4 storage products at night to fuel photosynthesis during the following day, then short-term ^{14}C incubations would underestimate production. Rubisco would be fixing unlabeled carbon taken in prior to the incubation. In Eppley's classic studies of oligotrophic gyre production, he always performed 24-hour incubations. In theory, **Peterson (1980)** argued that many short incubations should produce a summed production estimate much greater than one long incubation. In a long incubation, the phytoplankton carbon would be grazed and respired. However, when Redalje put this idea to the test, he found that long incubations produced higher production estimates than summed short-term incubations. Temporal decoupling of carbon acquisition and fixation by Rubisco could account for this pattern.

DCMU, Time Zero blanks, & the role of the dark bottle

Since 1989, a major problem has emerged in the interpretation of the dark bottle in the ^{14}C technique. The dark bottle is not used in the standard ^{14}C technique. While biological oceanographers often performed parallel ^{14}C incubations with dark bottles, they rarely incorporated the dark bottle uptake in their calculations. There shouldn't be much if any incorporation of ^{14}C into particulate matter in the dark bottle. While **Morris (1980)** had clearly indicated that C-4 metabolism was possible in marine phytoplankton, most biological oceanographers believed that phytoplankton, as true C-3 plants, don't fix carbon in the dark.

Starting in 1989, biological oceanographers received a wake-up call: dark-bottle uptake of ^{14}C was shown to be very significant. Dr. Karl Banse (1989 ASLO meeting presentation in Alaska), **Harris *et al.* (1989)**, and **Prakash *et al.* (1991)** recommend strongly that the dark bottle uptake values be subtracted from the light bottle. **Harris *et al.* (1989)** found that the dark-bottle ^{14}C uptake in open ocean areas in low light could be nearly the same as the light bottle uptake. **Li & Dickie (1991)** provide evidence that much of the dark-bottle CO_2 uptake, especially in dimly lit water, may be due to chemoautotrophic bacteria like the marine nitrifiers. Failure to subtract the value of the dark bottle, especially in analyses of oceanic phytoplankton production, can produce assimilation numbers which far exceed the presumed physiological maximum for photosynthesis.

Often, the herbicide DCMU, which blocks electron transfer in Photosystem II, is used instead of the dark bottle or with a dark bottle. DCMU will instantly stop all fixation of CO_2 by the Calvin cycle. However, DCMU also blocks several of the key enzymes used to assimilate bicarbonate via C-4 metabolism. The DCMU blank might reveal a low uptake of ^{14}C, but the difference in ^{14}C uptake between the DCMU blank and the light incorporation of ^{14}C could represent the activity of β carboxylases and Rubisco.

The Time-zero blank used in some ^{14}C incubations estimates the amount of short-term abiotic incorporation of ^{14}C into abiotic particulate phases in the incubation bottle. Its value is usually low.

O_2 or ^{14}C?

Which technique is better: ^{14}C or O_2? The ^{14}C method replaced the O_2 method because it was more sensitive. Now, with more sensitive techniques for measuring O_2 concentrations, this is not a major consideration. It was never a good reason for choosing the O_2 method over the ^{14}C method in eutrophic coastal waters. The O_2 method provides estimates of both gross and net production; the ^{14}C technique does not.

The ^{14}C method has several advantages other than sensitivity over the O_2 method. First, production is usually expressed using carbon. The conversion of production, measured as $\frac{dO_2}{dt}$ to $\frac{dC}{dt}$ requires an assumed photosynthetic quotient. Second, the ^{14}C method can be used to

provide size-fractionated estimates of production. The amount of production by the microplankton can be distinguished from that of the picoplankton. Finally, the ^{14}C method can be combined with estimates of ^{14}C activity in photosynthetic pigments to provide taxon-specific production and specific growth rates (**Redalje & Laws 1981**, **Redalje 1983**, **Gieskes & Kraay 1989**, **Strom & Welschmeyer 1991**, **Goericke &Welschmeyer 1993b**).

ALTERNATE TECHNIQUES FOR MEASURING PRIMARY PRODUCTION

Change in seawater properties

Primary production can be measured crudely by increased water-column O_2 concentration, particulate organic carbon, Chl *a* (requiring an assumed C:Chl *a* ratio), or decreases in essential nutrients (N or P). **Mills (1989)** reviews how oceanographers from the Plymouth biological station, led by Harvey, used changes in phosphorus and seawater pH to estimate the areal primary production of the North Sea.

In two weeks, we will discuss **Ryther *et al.* (1971)**, who estimated primary production in upwelling systems using both changes in seawater properties and the ^{14}C technique.

Stable isotopes

$^{18}O_2$ production from ^{18}O-labeled H_2O has recently been used to great effect in measuring primary production rates in the field (**Grande *et al.* 1989**). The major drawback to this technique is that it requires a mass spectrometer to measure the production of the stable isotope ^{18}O. The following equation shows how the method works:

$$H_2{}^{18}O + CO_2 \xrightarrow{(light)} CH_2O + {}^{18}O\,{}^{16}O\,.$$

Like the original light and dark bottle method, to convert $^{18}O^{16}O$ production to carbon fixation requires an estimate of the photosynthetic quotient. By assuming a PQ of 1.25, **Grande *et al.* (1989)** found that the ^{14}C method estimated between 60% to 100% of ^{18}O gross production.

Weger *et al.* (1989) used a mass spectrometer with a membrane inlet and ^{18}O to measure O_2 production and respiration in the lab.

Falkowski's flash-probe fluorescence

Falkowski has developed a method for estimating photosynthetic rate by measuring the fluorescence produced using rapidly paired flashes of light. The first flash of light produces a fluorescent signal identical to the one used by **Lorenzen's (1966)** *in situ* fluorescence method. This signal provides an estimate of the amount of Chl *a* in the cell (or more precisely, the amount of Chl *a* associated with Photosystem II). When a photon of light is absorbed by a phytoplankton cell, there are three possible fates for it: it can fluoresce, be converted to chemical energy, or be

converted to heat. The conversion to chemical energy is what we want to measure. Falkowski's method relies on the fact that it takes a few milliseconds for the electron transport systems to recharge after processing a photon of light. By exciting the phytoplankton cell with a second flash of light immediately after the first, the activity of electron transport in Photosystem II can be determined. The difference in the fluorescence yield produced by the first and second flash of light is directly related to the photosynthetic rate of the cell. A senescent phytoplankton cell, one treated with DCMU, or one whose photosystem II was photoinhibited by exposure to bright light would produce the same fluorescence yield with both flashes. An actively photosynthesizing cell would have a large difference in fluorescence signal between flashes.

Falkowski, and others, have developed algorithms to produce P *vs.* I curves using the flash-probe fluorescence technique. It does not replace the standard ^{14}C method, because the algorithms were developed so that the flash-probe fluorescence measurements produce similar estimates to the ^{14}C technique. Just as **Lorenzen's (1966)** *in situ* fluorescence technique must be calibrated relative to the more accurate laboratory spectrophotometric and fluorometric approaches, the Falkowski flash-probe method must be calibrated against standard ^{14}C incubations. The advantage of this method is that it allows rapid, broad-scale synoptic estimates of the parameters of the P *vs.* I curve without need for incubations.

Natural fluorescence

Kiefer *et al.* (1989) estimate primary production from natural solar-induced fluorescence. As phytoplankton cells absorb natural sunlight, they fluoresce. Kiefer's detectors, now sold by Biospherical Instruments, detect this fluorescence signal, and from it determine the primary production rate. **Kiefer *et al.* (1989)** estimated that 2 atoms of carbon are fixed for every photon of natural light emitted as fluorescence. The 2:1 empirical ratio shouldn't be confused with the quantum yield of photosynthesis. **Laws *et al.* (1990)** state that the minimum number of photons that must be absorbed by photosynthetic pigments to fix one atom of carbon is eight. This ratio is called the **quantum requirement** and its inverse the **quantum yield**. **Kiefer *et al.* (1989)** estimated a quantum yield of 0.07 (quantum requirement=14.3) in the South Pacific gyre..

This method was introduced to biological oceanography at about the same time as Falkowski's flash-probe method. The method has not been used extensively.

Satellite remote sensing

Oceanographers are now estimating primary production by satellite. We will devote a discussion section to the burgeoning field of "satellite remote sensing." At present, satellites can be used to estimate changes in Chl *a* in seawater and, from this, presumed minimal rates of primary production. Such calculations require assumptions of the C:Chl *a* ratio. Trevor Platt and co-workers have showed how estimates of light and sea-surface Chl *a* concentrations can be combined with estimates of the subsurface Chl *a* distribution and P vs I characteristics to estimate primary production. Behrenfeld & Falkowski (1997) produced models that allow the subsurface productivity profiles to be estimated from surface Chl *a*, temperature, and light.

Terms & Concepts

Avogadro's number: 6.022×10^{23} mol^{-1}

β emitter, β decay: ^{14}C, ^{32}P, and ^{3}H are all β emitters and their activity is measured using the liquid scintillation counter.

carboxysome site of RuBPCO in cyanobacteria

carotenoid- an accessory pigment (along with the phycobilins); long poly isoprenoid molecules having conjugated double bonds (p. 597 Lehninger) 2 major classes: carotenes [no oxygen] and xanthophylls [contain oxygen]

DCMU A metabolic inhibitor of photosytem II see Appendix of Terms

fluorescence efficiency η, = the probability that the absorption of a photon at the excitation wavelength λ_{ex} will produce a photon at the emission wavelength λ_{em}

light reaction see Handout 2

photosynthetic quotient- See **Append1-def.pdf**

phycobilin- red algae have relatively little chlorophyll a but a lot of phycoerythrobylin (Lehninger, p. 597), a red phycobilin the protein conjugate of phycoerythrobilin is phycoerythrin; phycocyanin is the analogous conjugate of phycoerythrin in the cyanobacteria.

pyrenoid site of RuBPCO in chloroplasts *cf.*, carboxysome

quantum yield- see Appendix of terms. Moles of CO_2 fixed by one mole-photon of light absorbed by pigments.

Liquid scintillation counting ^{14}C activity is almost always determined using liquid scintillation counting. ^{14}C is a β-emitting radioisotope, as is ^{3}H and ^{32}P. The SI unit for radioactivity is the Becquerel (Bq), equal to 1 disintegration per second. The Curie is equivalent to 3.7×10^{10} disintegrations per second. One μCi (micro Curie) is equivalent to 37kBq. The specific activity is the amount of radioactivity present (in Curies or dpm) per unit of weight (g or moles).

To detect the number of β decays is a 4-step process (Peng, p. 8):

i. absorption of energy by the solvent
ii. formation of the solvent excited state
iii. energy transfer from solvent to solute
iv. fluorescence emission by the solute (radiative transition from its first excited singlet state).

This reaction usually takes place in a counting or **scintillation vial**, composed of glass or plastic.

Quenching is the general reduction of maximum light output of the scintillation system. A combination of primary and secondary solutes used to produce photons is called a **fluor** or scintillation. A fluor is a light transducer, converting nuclear energy into light photons. Photocathodes are used to detect the emission of photons of light. Usually coincident photocathodes are used to reduce background light emission. With coincident detection, photons must be detected at 2 photocathodes simultaneously.

simulated *in situ* incubations: Incubations done out of the water, usually using neutral-density filters to simulate *in situ* light levels.

Strickland & Parsons equations to estimate production:

$W=12{,}000 \times A \times F_t$

W= Weight of one mole of Carbon in mg.

A = total carbonate alkalinity in milliequivalents per liter

F_t = Table nine≈ .95

Radiocarbon measured photosynthesis $\left[\frac{mgC}{m^3 h}\right] = (R_s - R_b) \times W \times 1.05/(R \times N)$

R_s is	the normalized radioactivity of the sample planchette
R_b is	the normalized radioactivity of a blank (*e.g.*, blackened BOD bottle or DCMU treated sample)
R	is the total activity added to the bottle
W	DIC in the bottle
N	time (h or d)
1.05	The kinetic isotope fractionation, estimating the reduction of ^{14}C fixation relative to ^{12}C

thylakoid membranes (Parsons *et al.* (1984) p. 62)- Lehninger(p. 590) flattened membrane sacks or vesicles within the chloroplast, which occur in stacked arrangements called grana. Contain the photosynthetic pigments as well as the enzymes for the light-dependent reactions.

Outlines

REQUIRED PAPERS

Peterson, B. 1980. Aquatic primary productivity and the ^{14}C-CO_2 method: a history of the productivity problem. Ann. Rev. Ecol. Syst. *11*: 359-385.

1. Introduction
   - a. Steeman-Nielsen introduced the technique in 1952
   - b. Few oceanographers aware of the assumptions used in estimating primary production using the ^{14}C-CO_2 method
   - c. Overview of the technique to be presented.
     - i. technical pitfalls
     - ii. inconsistent results
2. **The ^{14}C-CO_2 METHOD**
   - a. *The Basic Technique*
     - i. Strickland *et al.*, provides method.
     - ii. Marshall Pregnall provides a slightly different version of the method
     - iii. a dark bottle is used. *[What is the purpose of the dark bottle?]*

Equation 1 (p. 360).

Carbon uptake = counts in particulate & DOM/(Total counts added) x available inorganic carbon x 1.05

- iv. dark bottle subtracted, recorded separately, or ignored
- v. Methods: How incubations are performed.
  - (1) Incubations performed for 0.5 - 24 hours under appropriate conditions.
    - Long incubations can be very important for estimating respiration costs.
  - (2) What size filter should be used for filtering phytoplankton samples?
    - The smaller the better, if damaging cells is not a problem.

Does this equation give us estimates of μ, the specific growth rate of phytoplankton?

Problems with the carbon to chlorophyll level.

- vi. Purposes of the dark bottles in incubations.

(1) abiotic formation of labeled particulate carbon + active dark uptake of CO2
(2) Does not measure respiration directly
(3) often replaced with a DCMU blank, as recommended by Legendre
(4) *[Banse 1989 (ASLO abstract) argues that it must be included]*

b. *Technical Problems*
i. OBTAINING THE SAMPLE
(1) Toxic materials
(a) metals
(b) Tygon or rubber tubing inside go-flow bottles.
(2) photoinhibition
(3) [Evidence for problems]
(a) change in cell numbers
(b) change in species composition
(c) change in DCMU-induced fluorescence (Cullen)
ii. INOCULATING AND INCUBATING
(1) simulated *in situ* incubations
(2) how can turbulence by assessed?
(3) Is the DIC pool depleted during the experiment?
if the DIC pool is depleted (*e.g.*, freshwater, *[microphytobenthic production]* then equ. 1 is inappropriate)
iii. SAMPLE PREPARATION FOR COUNTING.
(1) filter is crucial AA MiliporeTM filters recommended by Parsons *et al.*
(2) get activity of filter pore water.
(3) Schindler's improvement (p. 362, left): acidify the sample to pH 4, bubble with N_2, Add a subsample (or concentrate the DOC and POC) for counting.
(4) Estimates of the total inorganic carbon pool:
(a) alkalinity titration, the standard technique.
(b) infrared gas analysis, after acidification of the sample.
(c) gas chromatography.
iv. COUNTING THE SAMPLE

3. **The early years and the great debate**
a. Steeman Nielsen 1.5 $X10^{10}$ tons of global production
b. Earlier measurements, based on O_2 production, 15.5 x 10^{10} tons (published by Rabinowitz)
c. Some argued that the low estimates using the O_2 methods were due to a bacteriocidal effect
d. Steeman Nielsen suggested antibiotics were released in the light.
e. Steeman Nielsen found that C-14 compared exactly to gross photosynthesis. approximately 94%
*[Check **Grande et al., 1989** for recent estimates]*
f. Ryther & Vacarro found the two techniques similar if short incubations used.
Postulated respired ^{14}C-CO_2 being refixed.
g. (page 365) Steeman Nielsen argued that if 60% of respired CO_2 is refixed, and if respiration is 10% of gross photosynthesis then the C-14 method should give rates comparable to 94% of gross photosynthesis. At a 20% respiration rate than the correction factor would be 14%.

Figure 1. Steeman Nielsen's method for estimating Net & Gross photosynthesis.

h. Rodhe of Sweden was the first to show that many short incubations give superior results to one long incubation.
i. (note that Gieskes & Kraay observed different results)
ii. *[Eppley noted that long incubations needed for 24-h respiration rates.]*
iii. Martin *et al.* 1988 use very long, several day incubations.

4. Second generation studies:
a. Antia *et al.*, observed that because **35-40% of the organic matter was excreted**, the gross oxygen production was much higher than 14-C uptake. *"In these coastal waters during diatom blooms the 14-C method measures the net production of particulate matter whereas the oxygen method measures the gross total production of organic material."*
b. Photosynthetic quotient, Peterson page 368 1.0 for hexose sugars and 1.4 for fat production
Williams *et al.* (1983) found that in nitrogen rich waters a PQ of 2.0 was most appropriate.

5. Third generation: challenging the method.
a. simulated *in situ* incubations
b. Redfield ratios: 276:106:16:1 (by atoms) O:C:N:P

c. Coulter counters used to estimate production (net)
d. How productive are the open ocean areas? Sheldon and Sutcliffe found that Sargasso sea water may have high rates of production.
e. **Sieburth** noted that the C-14 primary production estimates may be too low to support the high rates of heterotrophic activity in the open ocean areas.
f. **Gieskes** observed that small bottles result in high mortality of open ocean species.
g. p. 372. Lake ecologists have the advantage of being able to directly compare O_2 and ^{14}C
   i. *[oceanographers face problems because of the low sensitivity of Winkler titrations]*
   ii. *[Grande's method allows direct comparisons]*

6. Photoinhibition and photorespiration
   a. photoinhibition due to:
      i. rapid increase in respiration, decline in net photosynthesis
      ii. a slower decline in gross photosynthesis that paralleled the decrease in chlorophyll fluorescence
   b. Static light bottle incubations may result in underestimates of 20-80% in production. Algal cells are normally mixed. Reviewed by Falkowski (1984)
   c. Photorespiration (page 374): refers to the oxidation of glycolate, a recent product of the oxygenase activity of RuBPCO. With concomitant consumption of O_2 and release of CO_2 in the light. As much as 50% of the fixed CO_2 can be respired in C3 plants. A normal incubation would underestimate production because within a few minutes the ^{14}C would be released back in the water. In the dark bottle, photorespiration ceases almost immediately.
   d. The importance of photorespiration has not been documented.
7. Carbon flow models.
8. Summary
   a. Lack of respiration measurements a big drawback
   b. ^{14}C-uptake underestimates production for unknown reasons.
   c. Better carbon flow models needed

Falkowski, P. G. and J. A. Raven. 1997. Aquatic Photosynthesis. Blackwell Science, Malden MA. 375 pp. *[Read Chapter 9, **Read pp. 263-276, 282-288** on fast repetition rate fluorescence and nonphotochemical quenching; skim the rest of the chapter.]* {}

SUPPLEMENTAL

Parsons, Takahashi, and Hargrave. 1984. Biological Oceanographic Processes, 3rd Edition. Pergamon Press. Pp. 61-66.

1 **THE PRIMARY FORMATION OF PARTICULATE MATERIALS**
   1.1 AUTOTROPHIC PROCESSES
      1.1.1.1 definitions of:
         1.1.1.1.1 autotrophy: don't require organic materials as a source of energy
         1.1.1.1.2 autotrophs are primary producers of autochthonous material.
      1.1.2 Basic Photosynthetic Reactions:

$$n\ CO_2 + 2n\ H_2A \xrightarrow{(light)} n\ (CH_2O) + 2n\ A + n\ H_2O. \qquad (27)$$

where, H_2O, H_2, H_2S, $H_2S_2O_3$, and some organic compounds can be used as the H-donor in H_2A but only light is used as the energy source.
Three step process
      1.1.2.1 capture light
      1.1.2.2 change energy into another chemical form
      1.1.2.3 fix CO_2 using ATP and NADPH
[See Appendix1-def.pdf for a fuller equation]

Light absorbed in the visible region 300 nm to 720 nm, 112 kcal of energy for every mole of carbohydrate formed.

Fig. 26 Light absorption of intact cells, and photosynthetic action spectra
Chl. absorption peak above 680 nm
Chl *a* fluoresces at 730 nm from light absorbed at longer wave lengths
Chl *a* fluorescence at 684 nm and 695 nm from light absorbed at shorter wavelengths
1.2 Energy absorbed at the longer wavelength Chl *a* 680 is used directly for photochemical reactions or emitted as fluorescence (Fl 730), but energy absorbed at the shorter wavelengths is transferred to Chl *a* 670 before being used or emitted as fluorescence (at 684 and 695 nm)

Fig. 27. Photosystems I and II.
Photosystem I fluoresces at 730 nm
Energy absorbed at shorter wavelengths is transferred by the accessory pigments to Chl *a* 670 before being used or emitted as fluorescence at 684 nm and 695 nm. Photosystem II liberates oxygen from water and transfers electrons to plastoquinone. This series of reactions is carried out in the light. **DCMU**, a herbicide, blocks electron transport beyond the Photosystem II trap and light absorbed is released as fluorescence. The relative fluorescence after DCMU addition has been proposed as a measure of photosynthetic capacity [used effectively by Cullen *et al.*]

2 Dark reaction: Calvin-Benson cycle (p. 63)
Hatch-Slack pathway is an alternative.

Quantum yield: see HO2
Action spectra: light utilization formed by combining the light absorption with the quantum yield.
Photosynthetic Quotient: ratio of evolved O_2 to fixed CO_2. 1 for carbohydrates, 1.25 for proteins and 1.43 for lipids.
Dark Respiration: 10% of P_{max}

Photorespiration $^{18}O_2$ can be used to estimate algal respiration.
additional mitochondrial respiration and photorespiration [Weger *et al.*, 1989L & O.]
Two steps:

Fig. 30. Photosynthetic carbon fixation pathway. Oxidation of C5 compound to produce **phosphoglyceric acid** (C3) and phosphoglycolic acid (C_2) from ribulose diphosphate (C_5). High O_2, low CO_2 high light intensity, high temperature and high pH all favor photorespiration. Furthermore algae do not lose much CO_2 by photorespiration since they refix the CO_2 by photosynthesis.

> *"Photorespiration is sensitive to red and white light but insensitive to blue light which is the most predominant in the marine environment. Furthermore, algae don't lose much CO_2 during photorespiration because they refix the CO_2 by photosynthesis (Tolbert 1974)"*

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Jesperson, A.-M., J. Nielsen, B. Riemann, and M. Søndergaard. 1992. Carbon-specific phytoplankton growth rates: a comparison of methods. J. Plankton Res. *14*: 637-648. *[Axenic cultures used. Problems in all methods.* ***Redalje-Laws (1981)*** *^{14}C method labels Chl* a *higher than total algal C, producing too high an estimate of μ]*

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Laws, E. A., D. J. Redalje, L. W. Haas, P. K. Bienfang, R. W. Eppley, W. G. Harrison, D. M. Karl and J. Marra. 1984. High phytoplankton growth and production rates in oligotrophic Hawaiian coastal waters. Limnol. Oceanogr. *29*: 1161-1169.

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Redalje, D. G. and E. A. Laws. 1981. A new method for estimating phytoplankton growth rates and carbon biomass. Marine Biology *62:* 73-79. *[A landmark paper describing the Chl a-specific labeling procedure for estimating μ]* {**2**, **12**, **19**, **22**}

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Taguchi, S., G. R. DiTullio and E. A. Laws. 1988. Physiological characteristics and production of mixed layer and chlorophyll maximum phytoplankton populations in the Caribbean Sea and western Atlantic Ocean. Deep-Sea Res. *35*: 1363-1377. *[Chl a and DiTullio's protein specific labeling are used to estimate absolute and relative growth rates, respectively, using clean techniques. Surprisingly, 23% of inorganic carbon fixation occurred at night and by phytoplankton. Taguchi et al. attribute this to the incorporation of DOM produced during the day, but fixation via C4-type pathways may provide an alternate explanation. The chl maximum is neither a biomass nor production maximum]*

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Welschmeyer, N. A. and C. J. Lorenzen. 1984. Carbon-14 labeling of phytoplankton carbon and chlorophyll a carbon: determination of specific growth rates. Limnol. Oceanogr. *29*: 135-145.{?}

Welschmeyer, N.A., R. Goericke, S. Strom and W. Peterson. 1991. Phytoplankton growth and herbivory in the subarctic Pacific: A chemotaxonomic analysis. Limnol. Oceanogr. *36*: 1631-1649

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Aizawa, K. and S. Miyachi. 1986. Carbonic anhydrase and CO_2 concentrating mechanisms in microalgae and cyanobacteria. FEMS Microbiology Rev. *39*: 215-233. *[An excellent review of the role of intracellular and extracellular CA activity in freshwater and marine microalgae.]*

Anderson, A. E., J. J. Childress and J. A. Favuzzi. 1987. Net uptake of CO_2 driven by sulphide and thiosulphate oxidation in the bacterial symbiont-containing clam *Solemya reidi*. J. exp. Biol. *133*: 1-31.

Anderson et al. 1989. Crystal structure of the active site of ribulose-biphosphate carboxylase. Nature 337: 229. *[RUBISCO isolated from Rhodospirillum rubrum is a dimer of 2 identical L chains with 30% amino acid sequence identity with higher plants. Higher plants have 8 L chains, and 8 smaller chains.]*

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Badger, M. R. 1980. Kinetic properties of RuP_2carboxylase from *Anabena variabilis*. Arch. Biochem. Biophys. *201*: 247-254. *[The Michaelis-Menten half-saturation constant, K_M for this blue-green algal enzyme is 290 μM CO_2. Badger acknowledges that this is 10 times earlier estimates. Prins and Elzenga review the literature that shows eukaryotic RuBPCO has a K_m of 6-20 μM CO_2]*

Badger, M. r., A. Kaplan, and J.A. Berry. 1980. Internal inorganic carbon pool of Chlamydomonas reinhardtii. Evidence for a carbon dioxide concentrating mechanism. Plant Physiol. *66*: 407-413.

Badger, M. R. and T. J. Andrews. 1982. Photosynthesis and inorganic carbon usage by the marine cyanobacterium *Synechococcus* sp. Plant Physiol. *70*: 517-523. *[An HCO_3^- concentrating mechanism is proposed]*

Badger, M. R., M. Bassett and H. N. Comins. 1985. A model of HCO_3- accumulation and photosynthesis in the cyanobacterium *Synechococcus* sp. Plant Physiol. *77*: 465-471. *[^{18}O labeling used to show that cells must leak CO_2: carbon uptake 30% higher than net photosynthesis]*

Bailly, J. and J. R. Coleman. 1985. Effect of [CO_2] on protein biosynthesis and carbonic anhydrase expression in Chlamydomonas reinhardtii. Pl. Physiol. *87*: 833-840. *[Carbonic anhydrase is synthesized at high pH]*

Bazzaz, F. A. and K. Garbatt. 1988. The response of annuals in competitive neighborhoods: effects of elevated CO_2 . Ecology 69: 937-946. *[At higher CO_2 concentrations, C_3 plants outcompete C_4 plants. There was no consistent relationship between CO_2 and production]*

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Beardall, J. 1991. Effects of photon flux density on the 'CO_2-concentrating mechanism of the cyanobacterium *Anabena variabilis*. J. Plankton Res. *13* Suppl: 133-141. *[Good review. Freshwater at pH 7.5 contains 12 µM CO_2 and 180 µM HCO_3^-. Chlorophyte RuBPCO K_m ≈30 µM, Cyanobacterial RuBPCO K_m ≈ 100 µM. Here the 'observed' K_m is 3.2 µM. Good discussion of the quantum requirements of the carbon-pump mechanism]*

Beardall, J., D. Mukerji, H. E. Glover and I. Morris. 1976. The path of carbon in photosynthesis by marine phytoplankton. J. Phycol. *12*: 409-417. *["It is suggested that photosynthesis in marine diatoms depends on an active PEPCase utilizing bicarbonate as a substrate and that a less active RuDPCase utilizes CO_2". As noted on p. 415, high fixation of bicarbonate by PEPCase may only occur at high diatom densities, as in spring blooms. This would be consistent with isotopically heavier $\delta^{13}C$ values during bloom periods.]*

Beardall, J. and J. A. Raven 1981. Transport of inorganic carbon and the 'CO_2 concentrating mechanism in *Chlorella emersonii* (Chlorphyceae). J. Phycol. *17*: 371-373. *[Reanalyzed by Gehl et al., 1990]*

Beardall, J., H. Griffiths, and J. A. Raven. 1982. Carbon isotope discrimination and the CO_2 accumulating mechanism in *Chlorella emersonii*. J. Exp. Bot. *33*: 729-738. *[HCO_3^- uptake induced by nitrogen limitation. High HCO_3^- utilization produces high intracellular CO_2, reducing the loss of NH_3 resulting from the conversion of glycine into serine in the photorespiratory carbon-oxidation cycle.]*

Bedu, S., G. Peltier, and F. Joset. 1989. Correlation between carbonic anhydrase activity and inorganic carbon internal pool in strain *Synechocystis* PCC 6174. Plant Physiol. *90*: 470-474.

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Bidwell, R. G. S. and J. McLachlan. 1985. Carbon nutrition of seaweeds: photosynthesis, photorespiration and respiration. J. exp. Mar. Biol. Ecol. *86*: 15-46.

Brewer, P. G. and J. C. Goldman. 1976. Alkalinity changes generated by phytoplankton growth. Limnol. Oceanogr. *21*: 108-117. *[Uptake of nitrate does increase alkalinity, and uptake of NH_4^+ reduces alkalinity, but not as much as the Stumm-Morgan relationship predicts. There is an acidic shift.]*

Burnell, J. N. 1990. Immunological study of carbonic anhydrase in C_3 and C_4 plants using antibodies to maize cytosolic and spinach chloroplast carbonic anhydrase. Plant Cell Physiol. *31*: 423-427. *[Western blots of CA concentration]*

Burns, B. D. and J. Beardall. 1987. Utilization of inorganic carbon by marine microalgae. J. exp. Mar. Biol. Ecol. *107*: 75-86. *[DIC concentration by diatoms]*

Caperon, J. and D. F. Smith. 1978. Photosynthetic rates of marine algae as a function of inorganic carbon concentration. Limnol. Oceanogr. *23*: 704-708. *[The ^{14}C fixation of 3 axenic cultures and 3 field phytoplankton samples was estimated after changing total DIC. The relationship fit the Monod relationship with K_s values of 0.8 -3 mg/l DIC for the lab cultures and 4.3 to 5.3 mg DIC/l (.36 to .44 mM) for the field cultures. The diatom Phaeodactylum tricornutum had a K_s of .83 mg/l, Chlorella vulgaris 1.3 mg/l, and Amphidinium carteri 2.8 mg DIC/l. Seawater typically has 23 mg DIC/l]*.

Cavanaugh, C. M. 1983. Symbiotic chemo-autotrophic bacteria in marine invertebrates from sulphide-rich habitats. Nature *302*: 58-61.

Cavanaugh, C. M. 1985. Symbioses of chemoautotrophic bacteria and marine invertebrates from hydrothermal vents and reducing sediments. Biol. Soc. Wash. Bull. *6*: 373-388.

Cavanaugh, C. M., P. R. Leving, J. S. Makin, R. Mitchell, and M. E. Lidstrom. 1987. Symbiosis of methylotrophic bacteria and deep-sea mussels. Nature *325*: 346-348.

Colman, B. and C. Rotatore. 1988. Uptake and accumulation of inorganic carbon by a marine diatom. J. Exp. Bot. *39*: 1025-1032.

Colman, B. 1989. Photosynthetic carbon assimilation and the suppression of photorespiration in the cyanobacteria. Aquat. Bot. *34*: 211-231. *[A superb review of the biochemistry of DIC uptake, photosynthesis, and photorespiration in cyanobacteria. Cyanobacteria are not like other eukaryotic microalgae. They actively take up HCO_3^- and exhibit little photorespiration or O_2 inhibition.]*

Cook, C. M. , T. Lanaras and B. Colman. 1986. Evidence for bicarbonate transport in species of red and brown macrophytic marine algae. J. Exp. Bot. *37*: 977-984.

Crawford, D. W. and D. A. Purdie. 1997. Increase of PCO_2 during blooms of Emiliana huxleyi: theoretical considerations on the asymmetry between acquisition of HCO_3^- and respiration of free CO_2. Limnol. Oceanogr. *42*: 365-372. *[Emiliania uses HCO_3^- for calcification and photosynthesis. A model produced here to show that CO_2 could increase]*

Descolas-Gros, C. and M. R. Fontugne. 1985. Carbon fixation in marine phytoplankton: carboxylase activities and stable carbon-isotope ratios; physiological and paleoclimatological aspects. Marine Biology *87*: 1-6. *[They measured 3 carboxylase: RuBP carboxylase, PEP carboxylase, and PEP carboxykinase. The $\delta^{13}C$ values of Skeletonema in culture became isotopically heavy as the culture aged (-21 to -11). The C_3 pathway was by far the most important.]*

Descolas-Gros, C. and G. de Billy. 1987. Temperature adaptation of RuBP carboxylase: kinetic properties in marine Antarctic diatoms. J. exp. mar. Biol. Ecol. *108*: 147-158.

Dixon, G. K. and M. J. Merrett. 1988. Bicarbonate utilization by the marine diatom *Phaeodactylum tricornutum* Bohlin. New Phytol. *109*: 47-51. *[HCO_3^-may be the carbonate species crossing the plasmalemma. Earlier, Morris (1980) had found this species had clear C_4-type metabolism.]*

Dugdale, R. C. and F. P. Wilkerson. 1991. Low specific nitrate uptake: a common feature of high-nutrient low-chlorophyll marine ecosystems. Limnol. Oceanogr. *36*: 1678-1688.

Duker, C. S., R. W. Litaker, and J. Ramus. 1987. Seasonal variation in RuBPCase activity and N allocation in the chlorophyte seaweeds *Ulva curvata* (Kutz) De Ton and *Codium decorticatum* (Woodw.) Howe. J. exp. Mar. Biol. Ecol. *112*: 145-164.

Falkowski, P. G., Y. Fujita A. Ley and D. Mauzerall. 1986. Evidence for cyclic electron flow around photosystem II in *Chlorella pyrenoidosa*. Plant Physiol *81*: 310-312. *[A cyclic PSII-driven electron flow at saturating light intensities involving 15% of the total electron flow, cited by Canaani]*

Fogg, G. E. 1977. Excretion of organic matter by phytoplankton - a comment. Limnol. Oceanogr. *22*: 576-577.

Fogg, G. E. 1986. Picoplankton. Proc. R. Soc. Lond. B. *228*: 1-30. *[This superb review discusses why small cells must leak due to short diffusion path lengths]*

Foyer, C. H. 1984. Photosynthesis. Wiley-Interscience. *[A nice textbook summary]*

Fukuzawa, H., S. Fujiwara, Y. Yamamoto, M. L. Dionisio-Ses, and S. Miyachi. 1990. cDNA cloning, sequence, and expression of carbonic anhydrase in *Chlamydomonas reinhardtii*: regulation by environmental CO_2 concentration. Proc. Natl. Acad. Sci. USA *87*: 4383-4387.

Gavis, J. and J. F. Ferguson. 1975. Kinetics of carbon dioxide uptake by phytoplankton at high pH. Limnol. Oceanogr. *20*: 211-221. *[At high pH, dehydroxylation of HCO_3^- to CO_2 inadequate to maintain CO_2 [aqu] concentrations around algal cells. Cited by Rasmussen as evidence for HCO_3^- uptake by mesopsammic microalgae.]*

Gehl, K. A., B. Colman, and L. M.. Sposato. 1990. Mechanisms of inorganic carbon uptake in *Chlorella saccharaophila*: the lack of involvement of carbonic anhydrase. J. Exp. bot. *41*: 1385-1391. *[Acid-tolerant green alga accumulates intracellular pools of DIC, which is unaffected by AZA. External CA found in this green alga and many of these green algae, invalidating kinetic methods. External CA plays no role in carbon fixation. RuBPCO for this species has a K_m of 64 mmol $m^{3\ (p.\ 1388)}$. No photorespiration observed (Gehl et al., 1987) CO_2 is suggested to be the principal carbonate species crossing the chloroplast membrane, and not HCO_3^- as suggested by Beardall (1981). Growth on high CO_2 may suppress specific bicarbonate transporters (Badger et al., 1980). Rate of fixation of CO_2 greater than could be supported by spontaneous breakdown of HCO_3^-. Active uptake of CO_2 against an acidic pH and CO_2 gradient indicated. External CA may be required to maintain equilibrial concentrations of CO_2 for the CO_2 symporter.]*

Geraghty, A. M. J. C. Anderson, and M. H. Spalding. 1990. A 26-kilodalton limiting-CO_2 induced polypeptide of *Chlamydomonas* is distinct from the 37 kilodalton periplasmic carbonic anhydrase. Plat Physiol. *93*: 116-121 *[Low CO_2 induced cells have low photorespiration, little Warburg effect. CHI inhibitors used]*

Giordano, M. and S. C. Maberly. 1989. Distribution of carbonic anhydrase in British marine macroalgae. Oecologia *81*: 534-539 *[CA found in Rhodophyta save Chondrus, absent in Codium, Enteromorpha sp. and Monostroma (greens), and Phaeophyta. External CA present, but constitutes only 2% of total CA. Thus, HCO_3^- is the species usually crossing the plasmalemma. Species from the high intertidal and low-light subtidal had significantly higher activity than species from the mid and low intertidal, rockpools or high-light region of the subtidal. No strong relationship between CA and ability to use HCO_3^-.]*

Glover, H. E. 1983. Measurement of chemoautotrophic CO_2 assimilation in marine nitrifying bacteria: an enzymatic approach. Marine Biology *74*: 295-300. *[Measurement of RuBPCase activity]*

Glover, H. and I. Morris. 1979. Photosynthetic carboxylating enzymes in marine phytoplankton. Limnol. Oceanogr. *24*: 510-519. *[RuBPCase and PEPCase measured. RuBPCase: PEPCase ratio is a poor predictor of production. The RuBPCO:PEPCase ratio is low for diatoms, especially those in stationary phase (low CO_2). Thalassiosira pseudonana 13-1 has a RUBPCASE to PEPCase ratio of 14.4, clone 3-H has a ratio of only 6.6]*

Glover, H. E and C. H. E Smith. 1988. Diel patterns of carbon incorporation into biochemical constituents of *Synechococcus* spp. and larger species in the Northeast Atlantic. Marine Biology *97*: 259-267.

Glover, H. E., B. B. Prezelin, L. Campbell, M. Wyman, and C. Garside. 1988. A nitrate-dependent *Synechococcus* bloom in surface Sargasso sea water. Nature *331*: 161-163. *[A 3-d bloom is documented after a rainfall event]*

Goldman, J. C. 1973. Carbon dioxide and pH: effect on species succession. Science *182*: 307 {With rebuttal by J. Shapiro}

Goldman, J. C. 1999. Inorganic carbon availability and the growth of large marine diatoms. Mar. Ecol. Prog. Ser. *180*: 81-91. *[Highly unlikely that CO_2 is ever the limiting factor for phytoplankton growth]*

Goldman, J. C., D. B. Porcella, E. J. Middlebrooks, and D. F. Toerien. 1972. The effects of carbon on algal growth. Wat. Res. *6*: 637-679. *[Cited by **Harris 1986** to state that phytoplankton are never carbon limited; Harris disagrees.]*

Goldman, J. C., W. J. Oswald, and D. Jenkins. 1974. The kinetics of inorganic carbon limited algal growth. J. Water Poll. Cont. Fed. *46*: 554-574. *[Only the Monod expression is needed to model carbon-limited phytoplankton growth; Droop's k_q:Q_m is nearly 1. Cited by Harris 1986 to state that phytoplankton are never carbon limited; Harris disagrees.]*

Goldman, J. C. and M. R. Dennett. 1983. Effect of nitrogen source on short-term light and dark CO_2 uptake by a marine diatom. Marine Biology *76*: 7-15.

Graham, D., M. L. Reed. B. D. Patterson, and D. H. Hockley. 1984. Chemical properties, distribution and physiology of plant and algal carbonic anhydrases. Ann. NY Acacd. Sci. *429*: 222-237.

Grande, K. D., J. Marra, C. Langdon, K. Heinemann and M. L. Bender. 1989. Rates of respiration in the light measured in marine phytoplankton using ^{18}O isotope-labelling technique. J. exp. Mar. Biol. Ecol. *129*: 95-120. *[^{18}O labeling can separate light from dark respiration and can be used to estimate gross photosynthesis. The ^{14}C technique produces production estimates less than gross, but greater than community O_2 production. In incubations performed with N_2 bubbling, rates of production were twice as high as when incubations were performed in situ. This result would be consistent with Gould and Gallagher's (1990) hypothesis that stirring is necessary to limit the diffusional constraints on production. The bubbled simulated in situ incubators produce diffusional boundaries around the cells that are probably more like field conditions.]*

Guy, Vanlerberghe, and Turpin. 1989. Significance of phosphoenolpyruvate carboxylase during ammonium assimilation, carbon isotope discrimination in plants, and respiration by the N-limited green alga Selenestrum minutum. Plant Physiol. *89*: 1150-1157 *[70% of C incorporated through PEPCase during nitrogen assimilation. Anapleurotic metabolism using TCA cycle]*

Harris, G. P. 1986. Phytoplankton ecology. Chapman and Hall, London. *[The effect of CO_2 limitation on production and species composition is briefly reviewed, mainly for freshwater, on pp. 73-78. Goldman et al. (**1972**, **1974**) that CO_2 should not limit production. Harris concludes that high pH and low CO_2 can lead to daily decreases in production and changes in species composition to species which utilize HCO_3^-. {lec18}]*

Harris, G. P, B. Griffiths, and D. P. Thomas. 1989. Light and dark uptake and loss of ^{14}C: methodological problems with productivity measurements in oceanic waters. Hydrobiologia *173*: 95-105. *[Dark incorporation ≈ light incorporation in oligotrophic waters. Rapid bacterial growth was noted. 60% of light incorporation is lost at night in oligotrophic waters. Failure to subtract the dark uptake has produced gross overestimates of production, with assimilation numbers exceeding physiological optima (6.2 mgC $Chl^{-1}h^{-1}$). Shaking produces greatly enhanced bacterial growth and dark incorporation rates.]*

Harrison, W. G. and L. J. E. Wood. 1988. Inorganic nitrogen uptake by marine picoplankton: evidence for size partitioning. Limnol. Oceanogr. *33*: 468-475. *[Tests and confirms Malone's (1980) prediction that phytoplankton >20 µm fix mainly NO_3^-, while picoplankton fix mainly NH_4^+. The picoplankton also includes heterotrophic bacteria, which may take up the majority of NH_4^+ in coastal waters.]*

Hatch, M. D. and C. F. Slack. 1970. Photosynthetic CO_2 fixation pathways. Ann. Rev. Plant Physiol. *21*: 141-162. *[A description of true C_4 photosynthesis]*

Hatch, M. D. and J. N. Burnell. 1990. Carbonic anhydrase activity in leaves and its role in the first step of C_4 photosynthesis. Plant Physiol. *93*: 825-828. *[The first step in C_4 photosynthesis is the conversion of CO_2 to HCO_3^- in the mesophyll cells. The rate of this reaction far exceeds DIC demands, but produces just enough HCO_3^- so that the PEP is working at or near its K_m. The PEP carboxylation step may be rate limiting for C_4 photosynthesis]*

Holbrook , G. P., S. Beer, W. E. Sinar, J. B. Reiskind, J. S. Davis and G. Bowes. 1988. Photosynthesis in marine macroalgae: evidence for carbon limitation. Can. J. Bot. *66*: 577-582. *[Inorganic carbon is seawater may be a nutrient limiting the photosynthesis and productivity of certain macroalgae. Radiolabeled bicarbonate was added to artificial bicarbonate free sea-water {see Beer and Eshel 1983} to estimate the DIC vs PP curve. Altering pH with 100-fold changes in CO_2 did not noticeably affect PP, indicating bicarbonate uptake as the major source of DIC for photosynthesis. Dark increases in malate levels occurred in one species, but not at levels indicative of CAM metabolism]*

Hough, R. A. and M. D. Fornwall. 1988. Interactions of inorganic carbon and light availability as controlling factors in aquatic macrophyte distribution and productivity. Limnol. Oceanogr. *33*: 1202-1208. *[Bicarbonate utilizer loses in competition to CO_2 only plant in low light]*

Husic, H. D., M. Kitayama, R. K. Tagashi, J. V. Moroney, K. L. Morris, and N. E. Tolbert. 1989. Identification of intracellular carbonic anhydrase in *Chlamydomonas reinhardtii* which is distinct from the periplasmic form of the enzyme. Plant Physiol. *89*: 904-909.

Ibelings, B. W. and S. C. Maberly. 1998. Photoinhibition and the availability of inorganic carbon restrict photosynthesis by surface blooms of cyanobacteria. Limnol. Oceanogr. *43*: 408-419. *[English lake district]*

Jackson, G. A. 1987. Physical and chemical properties of aquatic environments. P. 213-233 *in* M. Fletcher, T. R. G. Gray, and J. G. Jones, *ed*s. Ecology of microbial communities. Cambridge University Press, Cambridge.

Jaworsky, G. H. M, J. F. Talling, and S. I. Heaney. 1981. The influence of carbon-dioxide depletion on growth and sinking rate of two planktonic diatoms in culture. Br. Phycol. J. *16*: 395-410. *[CO_2 can be limiting in culture. Cited by Cullen (pers. comm.) and Harris (1986)]*

Kaplan, A., M. R. Badger, and J. A. Berry. 1980. Photosynthesis and the intracellular inorganic carbon pool in the blue-green alga Anabena variabilis: response to external CO_2 concentration. Planta *149*: 219-226. *[Supposedly no CA in freshwater Anabena, but see Coleman (1989) for evidence for CA]*

Kaplan, A., D. Zenvirth, L. Reinhold, and J. A. Berry. 1982. Involvement of a primary electrogenic pump in the mechanism for HCO_3^- uptake by the cyanobacterium *Anabena variabilis*. Plant Physiol. *69*: 978-982.

Keeley, J. E. and G. Busch. 1984. Carbon assimilation characteristics of the aquatic CAM plant, *Isoetes howellii*. Plant Physiol. *76*: 525-530. *[$\delta^{13}C$ is not a good indicator of the photosynthetic pathway. This species fixes respired CO_2]*

Keeley, J. E., L. O. Sternberg, and M. J. Deniro. 1986. The use of stable isotopes in the study of photosynthesis in freshwater plants. Aquatic Botany *26*: 213-223. *[Even true C-3 plants can look like C-4 plants if there are constraints on the diffusion of CO_2]*

Kerby, N. W. and J. A. Raven. 1985. Transport and fixation of inorganic carbon by marine algae. Adv. Bot. Res. *11*: 71-123. *[A superb review of DIC transport and photosynthesis]*

Laing, W. A., W. L. Ogren, and R. H. Hageman. 1974. Regulation of soybean net photosynthesis by the interaction of CO_2, O_2, and ribulose 1,5-biphosphate carboxylase. Plant Physiol. *54*: 678-685.

Laws, E. A, P. A. Thompson, B. N. Popp. and R. A. Bidigare. 1998. Sources of inorganic carbon for marine microalgal photosynthesis: a reassessment of $\delta^{13}C$ data from batch culture studies of Thalassiosira pseudonana and Emiliania huxleyi. Limnol. Oceanogr. *43*: 136-142. *[Thompson & Calvert (1994, 1995) had concluded that HCO_3^- was the major DIC source using the Rayleigh distribution equation. Correct usage indicates that CO_2 could also be the DIC source]*

Legendre, L, S. Demers, C. M. Yentsch, and C. S. Yentsch. 1983. The ^{14}C method: patterns of dark CO_2 fixation and DCMU correction to replace the dark bottle. Limnol. Oceanogr. *28*: 996-1003. *[Using cultures of Dunaliella primolecta, they found that DCMU inhibited the active dark uptake of CO_2. The rate of dark uptake increases linearly with cell density (their Fig. 8). It was not noted by them, but this pattern might be an adaptation to carbon limitation in phytoplankton grown at high density leading to carbon storage in the dark (C_4-type metabolism)]*

Li, W. K. W., H, E. Glover, and I. Morris. 1980 Physiology of carbon photoassimilation by *Oscillatoria thiebautii* in the Caribbean Sea. Limnol. Oceanogr. *25*: 447-456.

Lüttge, U. 1987. Carbon dioxide and water demand: Crassulacean acid metabolism (CAM), a versatile ecological adaptation exemplifying the need for integration in ecophysiological work. New Phytologist *106*: 593-630.

MacDonald, F. D. and B. B. Buchanan. 1987. Carbon dioxide assimilation. Pp. 175-198 *in* J. Amesz *ed*. Photosynthesis, New Comprehensive Biochemistry. Vol. 15. Elsevier Publishers, Amsterdam. 355 pp.

MacIntyre, H. L. and R. J. Geider. 1996. Regulation of Rubisco activity and its potential effect on phytosynthesis during mixing in a turbid estuary. Mar. Ecol. Prog. Ser. *144*: *247-264. [Rubisco activity changes in response to irradiance, see* ***Pichard*** **et al.** ***1996****, MacIntyre and Geider produce and apply a model of Rubisco induction to shallow San Antonio Bay and deeper Delaware Bay]*

Madsen, T. V and D. Sand-Jensen. 1987. Photosynthetic capacity, bicarbonate affinity and growth of *Elodea canadensis* exposed to different concentrations of inorganic carbon. Oikos *50*: 176-182.

Manaduri, A. and A. Melis. 1984. Photochemical apparatus organization in Anacystis nidulans (Cyanophyceae. Effect of CO_2 concentration during cell growth. Plant Physiol. *74*: 67-71. *[Higher Ψ to PSII noted in low CO_2 adapted cells; no mention of light quality]*

Mayo, W. P., I. R. Elrifi, and D. H. Turpin. 1989. The relationship between ribulose biphosphate concentration, dissolved inorganic carbon (DIC) transport and DIC-limited photosynthesis in the cyanobacterium *Synechococcus leopoliensis* grown at different concentrations of inorganic carbon. Plant. Physiol *90*: 720-727.

Michel, C., L. Legendre, S. Demers, and J. -C. Therriault. 1988. Photoadaptation of sea-ice microalgae in springtime photosynthesis and carboxylating enzymes. Mar. Ecol. Prog. Ser. *50*: 177-185. *[RuBPC, PEPC and PEPCK assayed, with the first being dominant]*

Miller, A. G. and B. Colman. 1980. Evidence for HCO_3^- transport by the blue green alga (cyanobacterium) *Coccochloris peniocystis*. Plant Physiol *65*: 397-402. *[A clever centrifugation technique into silicone is described to do pulsed radiolabel additions. The half saturation constant for uptake of DIC in culture was approximately 0.16 μM CO_2 , which is about 100 times less than the half-saturation coefficient for RuBPCase. Therefore, this species must be using bicarbonate. CA activity also measured.]*

Moroney, J. V., H. D. Husic, and N. E. Tolbert. 1985. Effect of carbonic anhydrase inhibitors on inorganic carbon accumulation by *Chlamydomonas reinhardtii*. Plant Physiol. *79*: 177-183. *[An external and internal CA facilitate the transport of CO_2 across the plasmalemma]*

Mortain-Bertrand, A., C. Descolas-Gros, and J. Jupin. 1987. Simulating effect of light-to-dark transitions of carbon assimilation by a marine diatom. J. exp. mar. Biol. Ecol. *112*: 11-26.

Mortain-Bertrand, A., C. Descolas-Gros, and J. Jupin. 1987. Short-term ^{14}C incorporation in *Skeletonema costatum* (Greville) Cleve (Bacillariophyceae) as a function of light regime. Phycologia *26*: 262-269. *[Patterns of carbon fixation vary depending on the light:dark regime. C-4 metabolism occurs, but in contrast to earlier work by others, they conclude that the C-3 pathway is dominant.]*

Morris, I. 1980. Paths of carbon assimilation in marine phytoplankton. Pp. 139-159 *in* P. G. Falkowski, ed., Primary Productivity in the Sea. Plenum Press, New York. *[A tremendous review]*

Mukerji, D. and I. Morris. 1976. Photosynthetic carboxylating enzymes in *Phaoedactylum tricornutum*: assay methods and properties. Marine Biology *36*: 199-206.

Mukerji, D., H. E. Glover, and I. Morris. 1978. Diversity in the mechanism of carbon dioxide fixation in *Dunaliella tertiolecta* (Chlorophyceae). J. Phycol. *14*: 137-142.

Mustardy, L, F. X. Cunningham, and E. Gantt. 1990. Localization and quantitation of chloroplast enzymes and light-harvesting components using immunocytochemical methods. Plant Physiol. *94*: 334-340. *[7 photosynthetic proteins localized by immunolabeling with colloidal gold on log phase cells grown under red green and white light.. RuBPCO only in pyrenoid. Red-light grown cells had increased labeling per thylakoid length for polypeptide of PSII.???, conversely green light cells had a decreased density of PSII proteins PSII to Ψ sizes are highest under red light, lowest under green and intermediate with white light. Cultures grown with CO_2 enrichment.]*

Nara, M., Y. Shiraiwa, and T. Hirokawa 1989. Changes in the carbonic anhydrase activity and the rate of photosynthetic O_2 evolution during the cell cycle of Chlorella ellpsoidea C-27. Plant Cell Physiol. *30*: 267-275. *[cited by Nara et al. (1990). CA activity peaks at the same time as O_2 evolution: 8h after the start of the light cycle]*

Nara, M., Y. Shiraiwa and T. Hirokawa. 1990. Enzymatic inactivation of extracellular carbonic anhydrase and its effect on $K_{1/2}$ (CO_2) for photosynthesis in *Chlorella ellipsoidea* C-27. Plant Cell Physiol. *31*: 961-967. *[The ratio of extra- to intracellular CA is about 1. Pronase P inactivates about half of extracellular CA,* ***without affecting photosynthetic activity,*** *but doubling K_m for CO_2 fixation. The external CA is bound tightly to the cell wall. HCO_3^- is probably converted to CO_2 for fixation (see also CA inhibitors below)]*

Ode, D. J., L. L. Tieszen and J. C. Lerman. 1980. The seasonal contribution of C_3 and C_4 plant species to primary production in a mixed prairie. Ecology *61*: 1304-1311. *[A very good paper. Seasonal shifts to the C_3 plant {$\delta^{13}C$=-26.7} from the C4 plant ($\delta^{13}C$=-12.9} in spring and fall]*

Ogawa, T., T. Omata, A. Miyano, and Y. Inoue. 1985. Photosynthetic reactions involved in the CO_2-concentrating mechanism in the cyanobacterium Anacystis nidulans. Pp., 287-304 *in* Lucas and Berry, *eds*., Inorganic carbon uptake by aquatic photosynthetic organisms. *[DCMU inhibits carbon uptake by aquatic photosynthetic organisms; DIC accumulation driven by Ψ.]*

Okabe, K. S.-Y. Yang, M. Tsuzuki and S. Miyachi. 1984. Carbonic anhydrase: its content in spinach leaves and its taxonomic diversity studied with anti-spinach leaf carbonic anhydrase antibody. Plant. Sci. Lett. *3*: 145-153. *[Miyachi produced a monospecific Ab, see also Burnell 1990]*

Osmond, C. B., N. Valaane, S. M. Haslam, P. Uotila, and Z. Roksandii. 1981. Comparisons of $\delta^{13}C$ values in leaves of aquatic macrophytes from different habitats in Britain and Finland; some implications for photosynthetic processes in aquatic plants. Oecologia *50*: 117-124. *[If CO_2 is limiting, plants look more like the source inorganic carbon]*

Osmond, C. B., K. Winter. and H. Ziegler. 1981/2. Functional significance of different pathway of CO_2 fixation in photosynthesis. Pp. 479-547 *in* O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler, *eds*., Physiological Plant Ecology II, Water Relations and Carbon Assimilation.

Paerl, H. W. 1988. Nuisance phytoplankton blooms in coastal, estuarine and inland waters. Limnol. Oceanogr. *33*: 823-847. *[Contains a superb discussion of the factors controlling nuisance blooms of cyanobacteria and dinoflagellates in freshwater and marine systems. On pages 827-828, he discusses the important role played by DIC limitation in maintaining cyanobacterial blooms and scums. They are restricted to the surface (partially) by the need for atmospheric CO_2 input. Colman (1989) provides a nice discussion of the advantages of cyanobacterial metabolism under conditions of high O_2 and low DIC.]*

Pichard, S. L, L. Campbell, J. B. Kang, F. R. Tabita and J. H. Paul. 1996. Regulation of ribulose biphosphate carboxylase gene expression in natural phytoplankton communities. I. Diel rhythms. Mar. Ecol. Prog. Ser. *139*: 257-265. *[RuBPCO synthesized with an endogenous diel periodicity to peak with high light intensities in* Prochlorococcus*]*

Pierce, J. and T. Omata. 1988. Uptake and utilization of inorganic carbon by cyanobacteria. Photosynth. Res. *16*: 141-154.

Prins, H. B. A. and J. T. M. Elzenga. 1989. Bicarbonate utilization: function and mechanism. Aquatic Botany *34*: 59-83. *[A superb review of the literature on bicarbonate utilization. "Seen as a black box, a HCO_3^- utilizing plant is not very different from another very efficient CO_2 scrubber: a C_4 plant"(p. 63). There are 3 ways to demonstrate HCO_3^- utilization: Primary production (PP) as a function of pH (with constant ΣCO_2; little change in PP probably indicates HCO_3^- utilization), slow equilibration between HCO_3^- and CO_2, and isotopic discrimination. The pH drift technique is described in detail for demonstrating HCO_3^- utilization. Using pH microelectrodes, the layer around a C_3 plant reaches only 9, while an HCO_3^- user produces higher pH (>10). Interestingly, the presence of C_3 metabolism can be assessed by checking the lag in the incorporation of ^{14}C-labeled bicarbonate as opposed to ^{14}C-labeled CO_2. The rate of photosynthesis can be compared with the kinetic rate of conversion of HCO_3^- to CO_2. (see Miller and Colman 1980)]*

Rau, G. H., T. Takahashi, and D. J. Des Marais. 1989. Latitudinal variations in plankton $\delta^{13}C$: implications for CO_2 and productivity in past oceans. Nature *341*: 516-517. *[Due to temperature dependence in the Henry's Law coefficient relating pCO_2 (aqu) to CO_2(aq), CO_2[aqu] is far more abundant in cold Antarctic waters than in the tropics. There is an inverse relationship between CO_2 (aq) concentration and $\delta^{13}C$ ratio of the phytoplankton. Rau et al. (1979) confirm model predictions that the CO_2 concentrations in the Cretaceous atmosphere may have been over twice that of even today's fossil-fuel enriched pCO_2 (atm) concentrations.]*

Rau, G. H., J.-L. Teyssie, F. Rassoulzadegan, and S. W. Fowler. 1990. $^{13}C/^{12}C$ and $^{15}N/^{14}N$ variations among size-fractionated marine particles: implications for their origin and trophic relationships. Mar. Ecol. Prog. Ser. *59*: 33-38. *[Smaller particles have lower $\delta^{13}C$ and $\delta^{15}N$. Interpretation is fuzzy because detritus, heterotrophs and autotrophs are all analyzed within each size class]*

Raven, J. A. 1970. Exogenous inorganic carbon sources in plant photosynthesis. Biol. Rev. 45: 167-221.

Raven, J. A. 1974. Carbon dioxide fixation. Pp. 434-455 *in* W. D. Stewart, *ed.*, Algal Physiology and Biochemistry. Blackwell.

Raven, J. A. 1985. The CO_2 concentrating mechanism. Pp. 67-82 *in* W. J. Lucas and J. A. Berry, eds, Inorganic carbon uptake by aquatic photosynthetic organisms. Am Soc. Plant Physiol., Rockville Md. *[HCO_3^- utilization reduces photorespiration by increasing intracellular CO_2]*

Raven, J. A. 1986. Physiological consequences of extremely small size for autotrophic organisms in the sea. Can. Bull. Fish. Aquat. Sci. *214*: 1-70.

Raven, J. A. and J. Beardall. 1981. Respiration and photorespiration. Can. Bull. Fish. Aq. Sci. *210*: 55-82. *[Many species of phytoplankton have a bicarbonate pump in the cell wall. Cited by Harris 1986]*

Raven, J. A. and W. J. Lucas. 1985. Energy costs of carbon acquisition. Pp. 305-324 *in* W. J. Lucas and J. A. Berry, eds., Inorganic carbon uptake by photosynthetic organisms. Amer. Soc. Plant Physiol., Rockville MD.

Raven, J. A. and A. M. Johnston. 1991. Mechanisms of inorganic carbon acquisition in marine phytoplankton and their implications for the use of other resources. Limnol. Oceanogr. *36*: 1701-1714. *[Part of the ASLO Geritol symposium; not much chance for DIC limitation]*

Raven, J. A. and C. B. Osmond. 1992. Inorganic carbon acquisition processes and their ecological significance in inter and subtidal macroalgae of North Carolina. Functional Ecology *6*: 41-47.

Reinfelder, J. R., A. M. L. Kraeplel, and F. M. M. Morel 2000. Unicellular C_4 photosynthesis in a marine diatom. Nature 6807: 996-999. [Cited by **Cloern et al. 2002**]

Ruby, E. G., H. W. Jannasch and W. G. Deuser. 1987. Fractionation of stable carbon isotopes during chemoautotrophic growth of sulfur oxidizing bacteria. Appl. Env. Micro. *53*: 1940-1943.

Rundel, P. W., J. R. Ehrlinger, K. A. Nagy, eds., 1989. Stable isotopes in ecological research. Springer-Verlag.

Sakamoto, M. 1971. Chemical factors involved in the control of phytoplankton production in the experimental lakes area, Northeastern Ontario. J. Fish. Res. Bd. Can. 28: 123-128.

Schindler, D. W., G. j. Brunskill, S. Emerson, W. B. Broecker, and T. H. Peng. 1972. Atmospheric carbon dioxide: its role in maintaining phytoplankton standing crops. Science *177*: 1192-1194.

Schuller, K. A., W. C. Plaxton, and D. H. Turpin. 1990. Regulation of phosphoenolpyruvate carboxylase from the green alga *Selenestrum minutum*. Properties associated with replenishment of TCA cycle intermediates during amino acid biosynthesis. Plant Physiol. *93*: 1303-1311.

Serle, J. B., J. S. Lustgarten, E. A. Lippa, C. B. Camras, D. L. Panebianco and S. M. Poolos. 1990. MK-927, a topical carbonic anhydrase inhibitor. Arch. Opth. *108*: 838-841.

Sharp, J. H. 1977. Excretion of organic matter by phytoplankton: do healthy cells do it? Limnol. Oceanogr. *22*: 447-455.

Smith, F. A. and N. A. Walker. 1980. Photosynthesis by aquatic plants: effects of unstirred layers in relation to assimilation of CO_2 and HCO_3^- and to carbon isotopic discrimination. New Phytol *86*: 245-259.

Spalding. Photosynthesis and photorespiration in freshwater green algae. Aquat. Bot. *34*: 181-209.

Surif, M. B. and J. A Raven. 1989. Exogenous inorganic carbon sources for photosynthesis in seawater by members of the Fucales and Laminariales (Phaeophyta): ecological and taxonomic implications. Oecologia 78: 97-105. *[CA may facilitate the supply of CO_2 from the atmosphere to the thallus when photosynthesizing in air]*

Talling, J. F. 1976. The depletion of carbon dioxide from lake water by phytoplankton. J. Ecol. *64*: 79-121.

Talling, J. F. 1985. Inorganic carbon reserves of natural waters and ecophysiological consequences of their photosynthetic depletion. Pp. 403-420 *in* W. J. Lucas and J. A. Berry, *eds*., Inorganic carbon uptake by photosynthetic organisms. Amer. Soc. Plant Physiol., Rockville MD.

Thompson, P. A. and S. E. Calvert. 1994. Carbon isotope fractionation by a marine diatom: the influence of irradiance, daylength, pH, and nitrogen source. Limnol. Oceanogr. *39*: 1835-1844. [Data reanalyzed by Laws *et al.* 1998]

Thompson, P. A. and S. E. Calvert. 1995. Carbon isotope fractionation by Emiliania huxleyi. Limnol. Oceanogr. *40*: 1835-1844. [Data reanalyzed by Laws *et al.* 1998]

Tolbert, N. E. 1974. Photorespiration. Pp. 474-504 *in* W. D. Stewart, *ed*. Algal physiology and biochemistry. University of California Press.

Tolbert, N. E., H. D. Husic, J. V. Moroney, and B. J. Wilson. 1985. Relationship of glycolate excretion to the DIC pool in microalgae. Pp. 211-227 *in* W. J. Lucas and J. A. Berry, *eds*., Inorganic carbon uptake by photosynthetic organisms. Amer. Soc. Plant Physiol., Rockville MD. *[Discusses the cellular processes leading to glycolate excretion in Chlamydomonas and cyanobacteria. Glycolate appears to be actively transported out of cells, to which an inhibitor of C_2 metabolism is added. Glycolate excretion in nature is trivial.]*

Troughton, J. H. 1979. $\delta^{13}C$ as an indicator of carboxylation reactions. pp. 140-147 in M. Gibbs and C. Lalzko, eds, Encyclopedia of Plant Physiology, Photosynthesis II Vol 6. Springer-Verlag, Berlin.

Tu, C. K., M. Acevado-Duncan, G. C. Wynns and D. Silverman. 1986. Oxygen-18 exchange as a measure of accessibility of CO_2 and HCO_3^- to carbonic anhydrase in *Chlorella vulgaris* (UTEX 263). Plant Physiol. *80*: 997-1001. *[$C^{18}O^{18}O$ is added and the conversion to $H_{218}O$ is directly related to intracellular CA. In practice, internal CA is measured by disappearance of $C^{18}O^{18}O$ using a mass spec with a membrane port. DIC concentrations are set so that depletion via fixation is negligible. The membrane appears relatively impermeable to HCO_3^-, but relatively permeable to CO_2]*

Turpin, D. H., A. G. Miller, and D. T. Canvin. 1985. Chemostats in the study of inorganic carbon metabolism in microalgae. Pp. 437-448 *in* W. J. Lucas and J. A. Berry, eds., Inorganic carbon uptake by photosynthetic organisms. Amer. Soc. Plant Physiol., Rockville MD.

Turpin, D. H., A. G. Miller, J. S. Parslow, I. R. Elrifi, and D. T. Canvin. 1985. HCO_3^-limited photosynthesis and growth in the cyanobacterium *Synechococcus leopoliensis*. Pp. 449-458 *in* W. J. Lucas and J. A. Berry, *eds*., Inorganic carbon uptake by photosynthetic organisms. Amer. Soc. Plant Physiol., Rockville MD.

Vanlerberghe, G. S, K. A. Schuller, R. G. Smith, R. Feil, W. C. Plaxton and D. H. Turpin. 1990. Relationship between NH_4^+ assimilation rate and *in vivo* phosphoenolpyruvate carboxylase activity. Plant Physiol. *94*: 284-290. *[PEPC plays a key Anapleurotic function. NH_4^+ assimilation requires carbon skeletons from TCA cycle intermediates. These TCA components are replaced by the carboxylation of PEP to OAA by PEPC.]*

Vitousek, P. M. 1994. Beyond global warming: ecology and global change. Ecology *75*: 1861-1876. *[Vitousek in this MacArthur award-winning lecture cites 3 major causes of global change: 1) Atmospheric increase of CO_2 {will coral reefs dissolve?} {He doesn't extend the atmospheric CO_2 graph back to the Cretaceous to see that the present levels are not 'unique'. He reviews Bazzazz's work on the effects of CO_2 on terrestrial plants, 2) Changes in Nitrogen biogeochemistry caused by fertilizer production, and 3) Land-use change. He also briefly alludes to DDT, overharvesting of fisheries, and biological invasions and introduction of exotic species.]*

Wada, E., M. Terazaki, Y. Kabuza, and T. Nemoto. 1987. ^{15}N and ^{13}C abundances in the Antarctic Ocean with emphasis on the biogeochemical structure of the food web. Deep-Sea Res. 34: 829-841. *[The $\delta^{13}C$ (-26.9) and $\delta^{15}N$ (+0.5) values are very low for Antarctic phytoplankton indicating maximal kinetic isotope effects]*

Weger, H. G., R. Herzig, P. G. Falkowski and D. H. Turpin. 1989. Respiratory losses in the Limnol. Oceanogr. ? 1153-1161. *[^{18}O is used to estimate both dark and photorespiration, the latter is nil]*

Yang, S.-Y., M. Tsuzuki, and S. Miyachi. 1985. Carbonic anhydrase of *Chlamydomonas*: purification and studies on its induction using antiserum against *Chlamydomonas* carbonic anhydrase. Plant Cell Physiol. *26*: 25-34.

EFFECTS OF TOXIC SUBSTANCES (ESPECIALLY METALS) AND CONTAINMENT ON PRIMARY PRODUCTION ESTIMATES

Comment

It is ironic that trace metal contamination (*e.g.*, copper) may have led to poisoning of gyre productivity samples, but Martin and others believe many areas of the world's oceans, including gyres, may be limited by trace metal concentrations (*e.g.*, Fe^{3+} (ferric ion)).

Cullen, J. J., Z. Mingyuan, and D. C. Pierson. 1986. A technique to assess the harmful effects of sampling and containment for determination of primary production. Limnol. Oceanogr. *31*: 1364-1372. *[This may be a definitive refutation of the idea that metal contamination plagues current open-ocean estimates of primary production. See if you can find flaws; I couldn't.]*

Fitzwater, S. E., G. A. Knauer, and J. H. Martin. 1982. Metal contamination and its effect on primary production estimates. Limnol. Oceanogr. *27*: 544-551. *[Small concentrations of metals (200 ng/l) were shown to have drastic effects on production estimates. The major source appeared to be `dirty' sampling procedures and contaminated stock solutions of chemicals.]*[?]

Gieskes, W. W. and G. W. Kraay. 1984. State-of-the-art in the measurement of primary production. Pp. 171-190 *in* M.J.R. Fasham, ed., Flows of energy and materials in marine ecosystems, Plenum. *[G. and K. take great care to decontaminate the glass bottles used for incubations and the metal contamination of the ^{14}C spike.]*

Jackson, G. A. and J. J. Morgan. 1978. Trace metal chelator interactions and phytoplankton growth in seawater media: theoretical analysis and comparison with reported observations. Limnol. Oceanogr. *23*: 268-282. *[EDTA can have 2 effects: binding toxic metals (e.g., Cu) or providing essential micronutrients (e.g., Fe). Free ferric ion is at exceptionally low concentration in their model $10^{-19.9}$, but 100 times more Fe^{3+} is bound to EDTA. They assume a 10 µm diffusive boundary. They find that EDTA does not enhance Fe diffusion rates, since the flux of $Fe(OH)^{2-}$ is so large.]*

Marra, J. and K. Heinemann. 1984. A comparison between noncontaminating and conventional incubation procedures in primary production measurements. Limnol. Oceanogr. *29*: 389-392. *[They found few problems, but they used exceptionally clean stock solutions for their `dirty' conditions.]*

Price, N. M., P. J. Harrison, M. R. Landry, F. Azam, and K. J. F. Hall. 1986. Toxic effects of latex and Tygon tubing on marine phytoplankton, zooplankton and bacteria. Mar. Ecol. Prog. Ser. *34*: 41-49. *[95% of phytoplankton were killed in 4 days by unwashed latex. Tygon was toxic but less so.]*

Steeman Nielson, E. and S. Wium-Andersen. 1970. Copper ions as poison in the sea and fresh water. Marine Biology *6*: 93.

Sunda, W. and R. R. L. Guillard. 1976. The relationship between cupric ion activity and toxicity of copper to phytoplankton. J. Mar. Res. *34*: 511-529.

Williams, P. J. LeB. and J. I. Robertson. 1989. A serious inhibition problem from a Niskin sampler during plankton productivity studies. Limnol. Oceanogr. *34*: 1300-1304. *[The center rubber cord of Teflon lined go-flows was the culprit]*

MISCELLANEOUS

Kiefer, D. A., W. S. Chamberlin, and C. R. Booth. 1989. Natural fluorescence and chlorophyll a: relationships to photosynthesis and chlorophyll concentrations in the western South Pacific gyre. Limnol. Oceanogr. *34*: 868-881. *[Natural solar-induced fluorescence measured]*

Lorenzen, C. J. 1966. A method for the continuous measurement of *in vivo* chlorophyll concentration. Deep-Sea Res. *13*: 223-227. *[The classic paper describing the use of pumped water through a Turner Model III fluorometer with excitation peak at 445 nm and emission peak at >645 nm.]*

Ryther, J. H., D. W. Menzel, E. M. Hulburt, C. J. Lorenzen and N. Corwin. 1971. The production and utilization of organic matter in the Peru Coastal current. Inv. Pesq. *35*: 43-59. *[A parcel of water is followed & biological & chemical properties surveyed — the classic study of upwelling]*

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