ECOS 630
Biol. Ocean. Processes
Chapter 7
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Gallagher home

FUNDAMENTAL UNITS OF BIOLOGICAL OCEANOGRAPHY: μ, B, & P

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Assignment

TOPICS

- What are biomass, production, and specific growth rate, and how are the measured?
- What are the major groups of phytoplankton and pigments?
- Why do phytoplankton fluoresce, and how is fluorescence used to estimate Chl *a*?

One of the major purposes of the discussion is to discuss the nomenclature and equations used to measure phytoplankton growth in the sea.

Does temperature control global rates of primary production, or does temperature merely affect phytoplankton species composition? Read **Eppley (1972)**, a classic, for the class discussion. Even though most oceanographers believe that global rates of primary production are not temperature-dependent, one should not conclude that temperature can be ignored. Indeed, temperature is usually highly correlated with parameters of the P *vs.* I curve (especially the maximum photosynthetic rate) and may drive phytoplankton succession. Usually, the correlation between P *vs.* I parameters and temperature is stronger than with nutrients (**Harrison & Platt 1980**).

REQUIRED READING

These readings are available on the UMB E-reserve system. The password was provided in class. Email Eugene.Gallagher@umb.edu if you've forgotten the password.
http://docutek.lib.umb.edu

Eppley, R. W. 1972. Temperature and phytoplankton growth in the sea. Fish. Bull. *70*: 1063-1085.

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.]*

SUPPLEMENTAL

Ahlgren, G. 1987. Temperature functions in biology and their application to algal growth constants. Oikos *49*: 177-190. *[A lake study: measured assimilation numbers, C:Chl a ratios, and estimated μ; provides equations relating these variables and shows that high assimilation numbers can be associated with low specific growth rates]*

Furnas, M. J. 1990. *In situ* growth rates of marine phytoplankton: approaches to measurement, community and specific growth rates. J. Plankton Research *12*: 1117-1151.

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 dilution method is used to estimate C:Chl a ratios.]*

Goldman, J. C. and E. J. Carpenter. 1974. A kinetic approach to the effect of temperature on algal growth. Limnol. Oceanogr. *19*: 756-766. *[Reviews growth rates estimated from chemostats.* ***Eppley (1972)*** *was based on batch cultures. Maximum growth predicted by:* $\mu_m = (1.80 \times 10^{10})\, e^{\frac{-6842}{°Kelvin}}$. *Proposes mechanisms whereby temperature can affect the outcome of competition. Tilman has confirmed and modeled such mechanisms.]*

Miller, C. B. 2004. Biological Oceanography. Blackwell Science, Malden MA. 402 pp. Chapter 2.

Rhee, G.-Yull. 1982. Effects of environmental factors and their interactions on phytoplankton growth. Adv. Microb. Ecol. *6*: 33-74. *[A comprehensive review of factors controlling phytoplankton growth. Some conclusions have changed in the 15 years since this review]*

Case Studies

CASE STUDY 1.1 THE EFFECTS OF TEMPERATURE ON SPECIFIC GROWTH

Eppley (1972) described the functional relationship between temperature and the specific growth rate of phytoplankton. **Goldman & Carpenter (1974)** performed a similar analysis of temperature effects on μ for phytoplankton grown in chemostat culture. We will discuss chemostats in class 5. At the time these papers were written, virtually all biological oceanographers thought that phytoplankton populations in nature were growing at a small fraction of μ_{max}. In class 5, we will read **Goldman (1980)**, who provides additional evidence for an argument he first made in **Goldman *et al.* (1979)**, that phytoplankton populations in the field may be growing at or near μ_{max}. **Ahlgren (1987)** provides a scholarly review of the history behind equations to explain the empirical relationship between μ and temperature. From 18 measurements, he fits a new empirical estimate of μ and compares it to **Eppley's (1972)** relationship and Goldman & Carpenter's relationship.

Eppley (**1972**, pp. 1072-1079) discusses the effects of temperature on assimilation number and temperature. This topic was addressed for Canadian waters by **Harrison & Platt (1980)**, in a paper that we'll cover next week. We will refer back to these sections after discussing light and phytoplankton growth in the next class. Pay particular attention to Eppley's brief section on simulation modeling. This paper serves as one of the foundations for both the **Steele & Frost (1977)** and **Kremer & Nixon (1978)** approach to modeling phytoplankton growth rates. Modelers often use Eppley's equation to predict the maximum possible specific growth rate for phytoplankton at a given temperature, and then reduce the rate due to light and nutrient limitation.

Temperature changes on a longer time scale than does light. Phytoplankton populations respond to environmental factors with short characteristic frequencies like diel light intensity change through physiological adaptation. Temperature changes on a timescale that is long relative to phytoplankton doubling times and is more likely to be a process leading to successional changes in the phytoplankton community.

CASE STUDY 1.2: FLUORESCENCE AND CHLOROPHYLL A STANDING STOCK

Lorenzen's (1966) paper describing *in situ* fluorescence measurements of Chl *a* led to a revolution in how oceanographers measure phytoplankton standing stock. No longer did oceanographers have to take samples with bottles, filter them, and measure Chl *a* spectrophotometrically back at the laboratory. Such measurements are still required in Lorenzen's method in order to determine the fluorescence yield for the phytoplankton populations in order to convert fluorescence units to grams of Chl *a* per m^{-3}. With the introduction of Lorenzen's method, biological oceanographers could obtain a detailed look at the fine structure of phytoplankton patchiness. **Harris (1986)** reviews much of this late 1960s and early 1970s literature on the characteristic scales of phytoplankton patchiness. The take-home message from most of these studies is that the patch structure of phytoplankton in the ocean is largely controlled by physical mixing processes.

In local waters, *in situ* fluorometry was used extensively by the MWRA contractors studying phytoplankton distributions in MA Bay. In Lorenzen's method, water had to be pumped on-board ship and passed through the light source of a Turner fluorometer in order to estimate fluorescence. Now small fluorescence detectors can be mounted on CTDs for vertical tows through the water column. Battelle New England had a torpedo-like batfish with a fluorometer that they used for mapping the fluorescence of Chl *a* in MA Bay.

Furnas (1990) provides a review of how to estimates μ and summaries of estimate specific growth rates from the ocean. Somewhat surprisingly, it is possible to estimate production without ever having to estimate the specific growth rate of phytoplankton. That is fortunate, since estimating μ from field populations is a notoriously difficult problem.

Fundamental units of biological oceanography: μ, B, & P

The Greek letter μ, pronounced 'μ', is the biological oceanographer's designation for specific growth rate. The word 'specific' does **not** mean the growth of a biological species. Specific merely means that the variable has units of [Time^{-1}]. μ is the same as the ecologist's instantaneous or per capita growth rate, r (or sometimes called 'little r'). Both μ and r have units of inverse time.

You must note the time unit for μ. Bacterial specific growth rates may have units of h^{-1}; most planktonic specific growth rates have units of d^{-1}; and fish specific growth rates may have units of y^{-1}. Unless otherwise noted, most specific growth rates in this course are d^{-1}.

Scientists usually reserve Greek letters for parameters, not variables. The terms are not synonymous. In departure from scientific convention, the biological oceanographer's μ is a variable, not a parameter. A parameter is an entity that is relatively unchanging over space and time. For example, the population mean in statistics is a parameter, μ as it turns out. This population parameter is estimated using a sample statistic, the sample mean or $\bar{X}$. Similarly, the population variance is a parameter, σ^2, estimated by a sample statistic, s^2, the sample variance. Although μ is a variable, μ_{max} is a parameter. μ_{max} is the maximum specific growth rate that a phytoplankton population can attain given unlimited resources.

Ecologists call the maximum instantaneous or per capita growth rate for a population r_{max}. r_{max} and is also known as the Malthusian parameter, honoring Thomas Malthus. Malthus in the 19th century stressed the importance of exponential population growth. As noted by Malthus and especially Darwin, an ungrazed population cannot grow at its maximum population growth rates for long without depleting its resource supply. In the Origin of the species, Darwin calculated how long it would take for the world to be covered with elephants with an exponential growth rate. With grazing, a population can grow at or near μ_{max} forever.

With enough resources (light and nutrients) and with no density-dependent self limitation, the specific growth rate of a population, μ, will be constant. If the population is growing at less than its physiological maximum rate, then the constant is called μ. If the population is growing at its maximum physiological rate, then the specific growth rate is called μ_{max}.

$$\begin{aligned} \frac{dN}{dt} &= \mu\, N. \\ \mu &= \frac{1}{N}\frac{dN}{dt}. \\ \frac{dN}{dt} &= \mu_{max}\, N, \textit{ with no resource limitation.} \\ \mu_{max} &= \frac{1}{N}\frac{dN}{dt}. \end{aligned} \qquad \textbf{(1)}$$

$$\frac{dN}{dt} = r\ N.$$
$$\frac{dN}{dt} = r_{max}\ N,\ \textit{with no resource limitation..} \qquad \textbf{(2)}$$

For a given set of environmental conditions, there is a maximum specific growth rate, μ_{max}, and **Goldman (1980)** calls the ratio $\frac{\mu}{\mu_{max}}$ the relative growth rate. As noted by Malthus in the early 19th century, the number of individuals in the population will increase exponentially unless cropped by predators or unless the specific growth rate declines as the population density increases. Integration of differential equations (1) and (2) produces the familiar equations for exponential growth:

$$N_t = N_0\ e^{\mu_{max} t}. \qquad \textbf{(3)}$$

$$N_t = N_0\ e^{r_{max} t}. \qquad \textbf{(4)}$$

where, ***e*** is the base of the Naperian logarithms (≈2.718), N_o is the number of individuals at time 0, and N_t is the number of individuals at time t. Please note that whenever equations are written, they must be dimensionally homogeneous. That means, if the units on the left side of the equal sign are "individuals", then the units on the right side of the equal sign must also be "individuals." The units in the variables in an exponent, such as $e^{x\,y}$, must be dimensionless. In Equ. 3 and 4, the dimensions for μ_{max} and t, $\frac{1}{T}$ and T, cancel producing the desired dimensionless exponent.

A little algebra allows us to write 2 equations analogous to (3) and (4):

$$ln\left(\frac{N_t}{N_0}\right) = \mu_{max}\ t. \qquad \textbf{(5)}$$

$$ln\left(\frac{N_t}{N_0}\right) = r_{max}\ t. \qquad \textbf{(6)}$$

where, *ln* stands for natural logarithm or log to the base e. Equations 5 and 6 are written with μ_{max} and r_{max}, which are the parameters for a population growing at the maximum possible rate. For populations growing with reduced growth rates due to resource limitation, the appropriate parameters are μ and r. Since Thomas Malthus was the first to describe the effects of exponential population growth, r_{max} is often called the Malthusian parameter for the population or the intrinsic population growth rate. "r", which can be substantially below r_{max}, can be called the per

capita growth rate or instantaneous growth rate (or the jargon phrase is "little r"). With these two equations, we can write equations to solve for the population doubling time, t_d, the time taken for $\frac{N_t}{N_0}$ to equal 2:

$$ln\left(\frac{N_{t_d}}{N_0}\right) = ln(2) = \mu\, t_d.$$
$$t_d = \frac{ln(2)}{\mu} \approx \frac{0.693}{\mu}. \quad \textbf{(7)}$$

$$ln\left(\frac{N_{t_d}}{N_0}\right) = ln(2) = r\, t_d.$$
$$t_d = \frac{ln(2)}{r} \approx \frac{0.693}{r}. \quad \textbf{(8)}$$
$$where,\ t_d = Doubling\ time.$$

Please note that the equations are dimensionally consistent since t_d has dimensions of [Time] and μ has units of $[\frac{1}{TIME}]$ = [TIME^{-1}]. The doubling time of a phytoplankton population in Massachusetts Bay is about 1 day. The doubling time for *E. coli* in your large intestine may be only 40 minutes.

It is now conventional to express specific growth rates only using inverse time, usually d^{-1}. However, many older oceanographic papers, for example **Eppley (1972)**, express μ with dimensions of [doublings per day]. 'Doublings per day' is equivalent to the inverse of doubling time ($1/t_d$). Thus,

$$Specific\ growth\ rate\left[\frac{doublings}{day}\right] = \frac{1}{t_d}.$$
$$= \frac{\mu}{ln(2)}. \quad \textbf{(9)}$$
$$\approx \frac{\mu}{0.693}.$$

Equation 9 says that you must multiply each of **Eppley's (1972)** μ estimates (in doublings per day) to obtain our current μ, expressed as d^{-1}. In ecology, there is one final designation for growth rate, the finite rate of population growth, λ. λ, pronounced 'lambda', is equal to $e^{r\,t}$, where t is equal to one unit of time. A non-growing population has an per capita growth rate, r, equal to 0, but its finite growth rate is 1.

BIOMASS (B, C OR CHL *A*)

Biomass is a scalar quantity, expressing the amount of living organic material present per unit area or volume. Most biological oceanographers express biomass as the mass of carbon per unit volume or area. Conventional units would be g C m^{-2} or g C m^{-3}. As we will soon discover, it is very difficult to estimate the amount of phytoplankton or bacterial carbon present in marine ecosystems. It is easier to estimate the amount of Chl *a* present. Chl *a* is found in all photoautotrophs. Biological oceanographers use Chl *a* to estimate phytoplankton biomass. Converting Chl *a* to carbon requires an estimate of the carbon to chlorophyll *a* ratio (C:Chl *a*). This ratio is always expressed on a weight-to-weight basis: $\frac{g\,C}{g\,Chl\,a}$. A typical C:Chl *a* ratio for fast-growing coastal phytoplankton might be 30, but the C:Chl *a* ratio can be more than 300. **Eppley (1972)** concluded that C:Chl *a* ratios in upwelling systems were 30-40 and 90-100 in well-lit low nutrient tropical waters. **Ahlgren (1987)** estimated C:Chl *a* concentrations from lake phytoplankton and found C:Chl *a* ratios up to **390.** Healthy shade adapted phytoplankton tend to have low C:Chl *a*, and nutrient-starved or senescent phytoplankton have very high C:Chl *a* ratios.

PRODUCTION

Production is the amount of new biomass produced per unit time in a given area or space. Production can be viewed as the change in biomass per unit time, or $\frac{dB}{dt}$, if all of the predation and other processes that reduce biomass were eliminated. In nature, much of the production can be eaten resulting in no net change in biomass.

Oceanographers usually define production as change in carbon per unit area or volume per unit time, or
dC/dt, with units of $gCm^{-2}d^{-1}$ or $gCm^{-3}h^{-1}$. Production per unit area is called **areal production**. A typical primary production rate for Massachusetts Bay in the spring might be 1 or 2 $gCm^{-2}d^{-1}$. During the summer production might drop to 0.3 $gCm^{-2}d^{-1}$.

As defined on Appendix 1-Definitions (posted on Prometheus), **primary production** is the production due to autotrophs. The most important group of autotrophs are the photoautotrophs, which fix carbon via photosynthesis. Photosynthesis uses sunlight as the energy source to assimilate dissolved inorganic carbon (**DIC**) to produce organic carbon compounds. Photosynthesis produces O_2.

Chemoautotrophs are also primary producers, but their production is usually far less than that of photoautotrophs in euphotic waters. Chemoautotrophs use reduced inorganic compounds as the energy source for the assimilation of DIC and production of organic compounds. Marine nitrifying bacteria, which use the reduced nitrogen compounds ammonia and nitrite as energy sources, are important marine chemoautotrophs. Sulfide-oxidizing bacteria provide most of the primary production for deep-sea hydrothermal vent ecosystems. Both photoautotrophs and chemoautotrophs use the enzyme ribulose biphosphate carboxylase, RuBPCO, to fix CO_2.

Net primary production is defined as **gross production** minus **respiration** by the phytoplankton themselves. Net and gross primary production have the same units, usually $gCm^{-2}d^{-1}$. One of the major advantages of the O_2 method over the ^{14}C method is that it can provides an estimate of respiration losses. **Peterson (1980)** discusses the difficulty in converting ^{14}C production estimates to gross production, a conversion requiring an estimate of the photosynthetic quotient (moles O_2 evolved to CO_2 fixed).

Primary production is usually estimated by incubating phytoplankton samples in bottles and measuring either the amount of DIC fixed or the amount of O_2 produced. Production estimates from bottle incubations are expressed as $mgCm^{-3}h^{-1}$, $mgCl^{-1}h^{-1}$, $mgO_2m^{-3}h^{-1}$, $mgO_2l^{-1}h^{-1}$. These volumetric production estimates change greatly with depth in the ocean and these depth-related changes strongly affect the conversion of volumetric to areal production estimates.

Specific production is another name for specific growth rate and is equal to:

$$\begin{aligned}\mu &= \textit{specific production}. \\ &= \frac{\textit{production}}{\textit{biomass}} = \frac{P}{B}. \\ &= \frac{\frac{dC}{dt}}{B}.\end{aligned} \qquad \textbf{(10)}$$

where, B is biomass usually in units of gCm^{-2} or gCm^{-3} and production must be in dimensions of $\frac{B}{TIME}$.

You must not confuse biomass with production. A crystal-clear tropical water-column may have the same areal production as a bright green mudflat. Low biomass is a characteristic of many tropical water columns. The Great Debate in biological oceanography, reviewed by **Peterson (1980)**, is over whether this low biomass is consistent with very high production (>1 $gCm^{-2}d^{-1}$) It is also possible to have high production with very low specific growth rates if the biomass is high enough.

Primary production is easier to estimate than phytoplankton specific growth rate. The major problem in applying Equation 10 to estimate μ is that it is difficult to estimate phytoplankton biomass. However, it is not necessary to estimate phytoplankton biomass to estimate production. The amount of carbon fixed or O_2 produced per unit volume or unit area can be estimated and provides a direct estimate of production. Alternatively, the amount of carbon fixed per unit Chl *a* can be estimated and then this estimate can be converted to production. The amount of carbon fixed per Chl *a* per unit time is called either the **Chl *a* specific-production** or the **assimilation number** and has units of $mgC(mg\ chl\ a)^{-1}\ h^{-1}$.

Primary production can be expressed as either $gCm^{-2}d^{-1}$ or $gO_2m^{-2}d^{-1}$. To convert from O_2 to carbon, one must estimate or assume a **photosynthetic quotient** [PQ]. PQ is the ratio of evolved O_2 to CO_2 incorporation. The photosynthetic quotient varies as a function of the physiological

status of the phytoplankton cells, especially the form of nitrogen being used for growth. In nitrate-rich coastal waters, **Williams *et al.* (1983)** recommend a PQ of 2.0, but in nitrate-depleted oceanic water, they recommend a PQ of 1.25. **Williams & Roberson (1991)** reviewed estimates of PQ ranging from 0.5 to 3.5 and concluded that the true oceanic range, after correcting errors, is from 1.0 to 1.36. This is near the range of PQ that **Stumm & Morgan's (1981, p. 194)** simplified photosynthetic equations predict:

With nitrate:

$$106\,CO_2 + 16\,NO_3^- + HPO_4^- + 122\,H_2O + 18\,H^+ \overset{light}{\rightleftharpoons} (CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 138\,O_2. \quad \textbf{(11)}$$

With ammonia:

$$106\,CO_2 + 16\,NH_4^+ + HPO_4^- + 108\,H_2O \overset{light}{\rightleftharpoons} (CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 107\,O_2 + 14\,H^+. \quad \textbf{(12)}$$

The ratios of C:N:P of 106:16:1 is known as the Redfield ratio, or sometimes as the Redfield-Ketchum-Richards ratio.

TECHNIQUES TO ESTIMATE SPECIFIC GROWTH RATE (MODIFIED FROM FURNAS 1990)

Estimating primary production is tough; estimating the specific growth rate, μ, of phytoplankton is tougher still. Furnas (**1990**, **1991**) reviews the four basic methods for estimating μ: frequency of dividing cells, biochemical indices, model approaches, and incubations without grazers.

Frequency of dividing cells

The basis of this technique is that if cell division occurs as a discrete part of the cell cycle, and the percentage of cells actively dividing can be determined, μ can be calculated. **McDuff & Chisholm (1982)** provide an analysis of equations for converting the frequency of dividing cells to specific growth rate, and recommend the following equation:

$$\mu = t_m^{-1}\, ln(\,1 + f\,).$$
$$\textit{where, } t_m = \textit{time division is evident (e.g., mitosis time).} \quad \textbf{(13)}$$
$$f = \textit{frequency of ind. in pop. dividing}.$$

Note that this equation is dimensionally correct; μ is in units of inverse time and *f* is dimensionless.

Rivkin (*e.g.*, **1986**, **Rivkin & Seliger 1981**, **Rivkin & Voytek 1986**) has applied this technique often in recent years to estimating eukaryotic algal specific growth rates. Rivkin adds radiolabeled thymidine to natural phytoplankton samples, performs the incubation, and then isolates individual phytoplankton cells of different species. By looking at the distribution of counts per cell, one can determine the frequency of diatom cells that are in the process of division (nearly twice the basal amount of DNA). Rivkin has also applied the frequency of dividing cells approach using radiolabeled Germanium, and replacement for silica in the diatom frustule and radiolabeled amino acids. This approach is very time consuming. Since the radioactivity taken up by single cells is relatively low, each cell must be examined for a long time in a liquid scintillation counter. It is difficult to convert species-specific μ values to system primary production.

Other cell-cycle markers include the frequency of paired cells and the frequency of cells containing higher DNA content. The latter marker can be detected using DNA-specific fluorescent dyes. One of the potential major advantages of the frequency of dividing cells approach is that it can allow an incubation-free method for estimating μ if the dividing cells can be identified from natural samples without requiring an incubation. Rivkin's use of the frequency of dividing cells requires an incubation, but identification of the DNA content per cell can be done on natural samples without requiring an incubation.

Biochemical indices

Redalje-Laws Chl *a*-specific labeling to estimate μ & C:Chl *a*
Redalje & Laws' (1981) Chl *a* specific labeling procedure is a very clever method for estimating two of the three major growth variables: μ and the C:Chl *a* ration. **Laws (1984)** provides one mathematical and biochemical model for the method.

Table 1 shows the variables used in the calculation of the specific growth rate, using the Redalje-Laws procedure.

Table 1. Definitions and variables used in applying the Redalje-Laws approach (from **Gould & Gallagher 1990**)

Variable	Units	Description
ΔC	$\frac{\mu gC}{sample \times time}$	C fixed during incubation
μ	$\frac{1}{h}$	specific growth rate
1.05	Dimensionless	Factor to account for isotope discrimination
A^*	$\frac{dpm}{sample}$	^{14}C activity of total particulate matter
C_p	$\frac{\mu gC}{sample}$	Microalgal C at the end of the incubation
I^*	$\frac{dpm}{\mu gC}$	Specific activity of DIC
R^*_{chl}	$\frac{dpm}{\mu gC}$	Specific activity of C in Chl *a* molecule
t	h	duration of incubation in hours.

A standard ^{14}C incubation is run, just as if the goal were merely to measure primary production. Usually the phytoplankton population would be split into two fractions after the incubation. One half of the sample would be used to estimate the radioactivity of the total phytoplankton fraction, called A^* in Table 1. With A^* and I^*, the specific activity of the dissolved inorganic carbon (DIC) in the incubation vessel, the amount of primary production can be determined using Equation 14:

$$\Delta C = \frac{1.05\ A^*}{I^* t}. \qquad \textbf{(14)}$$

Equation 14 is the same equation used for a standard ^{14}C incubation to estimate primary production (covered in the next class). To estimate μ, the radioactivity in the Chl *a* molecule must be determined. This was first done by using thin-layer chromatography of the photosynthetic pigments, but now the pigments are separated using HPLC. The Chl *a* fraction is removed, its mass estimated, and the amount of radioactivity is determined to estimate R^*. The amount of microalgal carbon at the end of the incubation can be determined from Equ 15.

$$C_p = \frac{A^*}{R^*}. \qquad \textbf{(15)}$$

The specific growth rate assuming a 12-h photic period to convert the hourly incubation timescale can be estimated using Equ. 16:

Box 1 Derivation of the Redalje-Laws equation to estimate μ as h^{-1}. The equation multiplies the hourly rate by 12 to obtain the daily specific growth rate.

$$\mu = \frac{1}{C_p}\frac{dC}{dt}.$$

$$C_p = C_o\, e^{\mu t}.$$

$$ln\left(\frac{C_p}{C_o}\right) = \mu t.$$

$$\mu = \frac{-ln\left(\frac{C_o}{C_p}\right)}{t}.$$

$$\mu = \frac{-ln\left(\frac{C_p - \Delta C\, t}{C_p}\right)}{t}.$$

$$\mu = \frac{-ln\left(1 - \frac{\Delta C\, t}{C_p}\right)}{t}.$$

$$\mu = \frac{-ln\left(1 - \frac{\left(\frac{1.05\,A^*}{I^*}\right)t}{\frac{A^*}{R^*}}\right)}{t}.$$

$$\mu = \frac{-ln\left(1 - \frac{1.05\,R^*}{I^*}\right)}{t}.$$

$$\mu = \frac{-ln\left(1 - \left(\frac{1.05\,R^*}{I^*}\right)\right)}{t} \times 12\,. \qquad \textbf{(16)}$$

Equation 16 certainly looks daunting. It is based on the balanced growth assumption, which states that new Chl *a* is being labelled at the same rate as the total microalgal carbon pool. **Redalje (1983)** and **Goericke & Welschmeyer (1993a)** tested this assumption, finding it is not true if the microalgae are switched from low to high light, or when sun-adapted microalgae are switched to lower light. Shade adapted algae, with a low C:Chl *a* ratio, drastically curtail the synthesis of Chl *a* when placed in high light and the Redalje-Laws method can grossly underestimate μ. If the balanced growth assumption is true, then the amount of microalgal carbon in the incubation vessel, C_p, can be estimated from Equ. 15. With this estimate of C_p, Equation 16 can be readily derived from **Equation 1**. This derivation is shown in the text box to the left.

Redalje & Laws (**1981**, **Laws 1984**) derived the equation originally under the assumption that the Chl *a* molecule turns over rapidly so that Chl *a* would have the same specific activity as the total phytoplankton carbon pool. **Welschmeyer & Lorenzen (1984)** derived a mathematically equivalent expression, under the less restrictive assumption that the new Chl *a* that is synthesized during an incubation matches the specific activity of the total pool of organic carbon synthesized during the incubation. This assumption is called the balanced growth assumption. If this 'balanced growth' assumption is met, the Redalje-Laws method can estimate μ, phytoplankton carbon content, and the C:Chl *a* ratio. **Goericke & Welschmeyer (1993a)** experimentally showed that the Chl *a* molecule does not turn over at a significant rate during the course of an incubation, invalidating one of the original assumptions of the method, but not the equation (Equation 16). They derived a more complicated expression for estimating μ using the Chl *a* labeling method. Their major recommendation is to use long incubations (24 h) to estimate μ with Chl *a*-specific labeling. **Jesperson *et al.* (1992)** found that the Chl *a* molecule was labeled at a higher rate than the total phytoplankton carbon pool. Thus Equation 16 would produce an overestimate of the phytoplankton specific growth rate.

The Redalje-Laws labeling method has been used by many investigators, most notably **Welschmeyer & Lorenzen (1984)** and Gieskes & Kraay (**1986**, **1989**). **Laws *et al.* (1987)** used the technique to show that phytoplankton in the oligotrophic open ocean were growing at very high specific growth rates. **Gould & Gallagher (1990)** adapted the Redalje-Laws Chl *a* labeling procedure to obtain the first field estimates of the specific growth rates for benthic diatoms.

Taxon-specific μ

Gieskes & Kraay (1989) modified the Redalje-Laws method to estimate the specific growth rate of different phytoplankton groups (*e.g.*, diatoms *vs.* cyanobacteria) by estimating the radioactivity in ^{14}C incorporation into different carotenoids. This technique was used to great effect by **Strom & Welschmeyer (1991)** who estimated the specific growth rate of diatoms in the subarctic Pacific. **Goericke & Welschmeyer (1993b)** provide more details on this labeling method.

Estimating relative specific growth

DiTullio & Laws (1986) developed a method to assess the amount of radioactivity taken up into the protein fraction of phytoplankton cells. From this estimate, they can derive the microalgal C:N ratio. Goldman showed that there was a predictable relationship between the C:N ratio in phytoplankton and the relative specific growth rate $\frac{\mu}{\mu_{max}}$. Using the Redalje-Laws and DiTullio-Laws method, **Laws *et al.* (1987)** showed that oligotrophic gyre phytoplankton, not only were growing at a high specific growth rate, but that this specific growth rate was close to $\mu_{max.}$

Model approaches

Conversion from assimilation number

Following **Eppley (1972)**, the chlorophyll-specific production or assimilation number can be combined with an estimates of C:Chl *a* ratio to estimate μ. **Eppley (1972)** had the equations slightly wrong, and when **Eppley (1972)** wrote his paper assimilation number hadn't been established as meaning the asymptote of the P *vs.* I curve. Here are the appropriate equations relating the C:Chl *a* ratio, μ, and assimilation number:

$$\mu_{max} = \frac{Assimilation\ number}{C{:}Chl\ \underline{a}} - specific\ respiration.$$
$$\mu = \frac{Chl\ \underline{a}\text{-}specific\ prod.}{C{:}Chl\ \underline{a}} - specific\ respiration. \qquad (17)$$

The first equation estimates μ_{max} only if nutrients are not limiting, since the assimilation number is the maximum Chl *a*-specific production for a given temperature and nutrient regime.

Other model approaches

Phytoplankton specific growth (μ) or relative growth (μ/μ_{max}) can be estimated, albeit crudely, using RNA/DNA ratios, adenylate charge ratios, and a variety of other approaches.

The Dilution method: Changes in mass, number, or volume after reduction of grazing

Minimal estimates of μ can be obtained by estimating the change in cell number, biomass or volume through time and dividing by biomass. That is, μ=1/C dC/dt, μ=1/N dN/dt or μ=1/V dV/dt, for mass [=carbon], number, or volume respectively. Unfortunately, change in phytoplankton biomass, numbers or volume provides a poor indication of μ because of grazing and other sources of mortality. If grazing mortality is expressed as a specific rate, that is [time^{-1}], then one can readily see why primary production can not be estimated from change in biomass alone:

$$\begin{aligned} \frac{change\ in\ biomass}{time} &= net\ primary\ production - grazing - other\ losses. \\ \frac{dC}{dt} &= C\,(\,\mu - G - m\,). \\ where,\ C &= biomass\ [\ gCm^{-2}\ or\ gCm^{-3}\ ]. \\ \mu &= specific\ growth\ rate\ [\ \frac{1}{time}\ ]. \\ G &= specific\ grazing\ rate\ [\frac{1}{time}\ ]. \\ m &= specific\ non\text{-}grazing\ loss\ (e.g.,\ DOM\ loss,\ sinking)\ [\ \frac{1}{time}\ ]. \end{aligned} \qquad \textbf{(18)}$$

Integration of Equation 18 yields:

$$\begin{aligned} C_t &= C_0\ e^{(\,\mu - G - m\,)\,t}. \\ ln\left(\frac{C_t}{C_0}\right) &= (\,\mu - G - m\,)\,t. \\ where,\ C_t &= biomass\ at\ time\ t. \\ C_0 &= biomass\ at\ time\ 0. \end{aligned} \qquad \textbf{(19)}$$

If specific growth rate is balanced by losses, Equ. 19 shows that phytoplankton biomass can remain constant despite high μ and grazing losses. If the grazers could be eliminated, then change in biomass could be used to provide an estimate of μ. Grazing mortality cannot be reduced by simply sieving out the grazers because they overlap in size with the primary producers. Grazing rate is known to be dependent on phytoplankton biomass. If a phytoplankton suspension is diluted, the rate of encounter between predator and prey can be greatly diminished, reducing G.

Mike Landry diluted phytoplankton samples with grazer-free water. Many others have now adopted this dilution method for estimating μ. **Furnas (1991)** reduced G by transferring phytoplankton cells to grazer-free incubation chambers that he suspended in the water column. The change in phytoplankton biomass in the absence of grazers provides a minimum estimate of μ.

Further discussion topics

Here are some issues to think about prior to the class discussion:

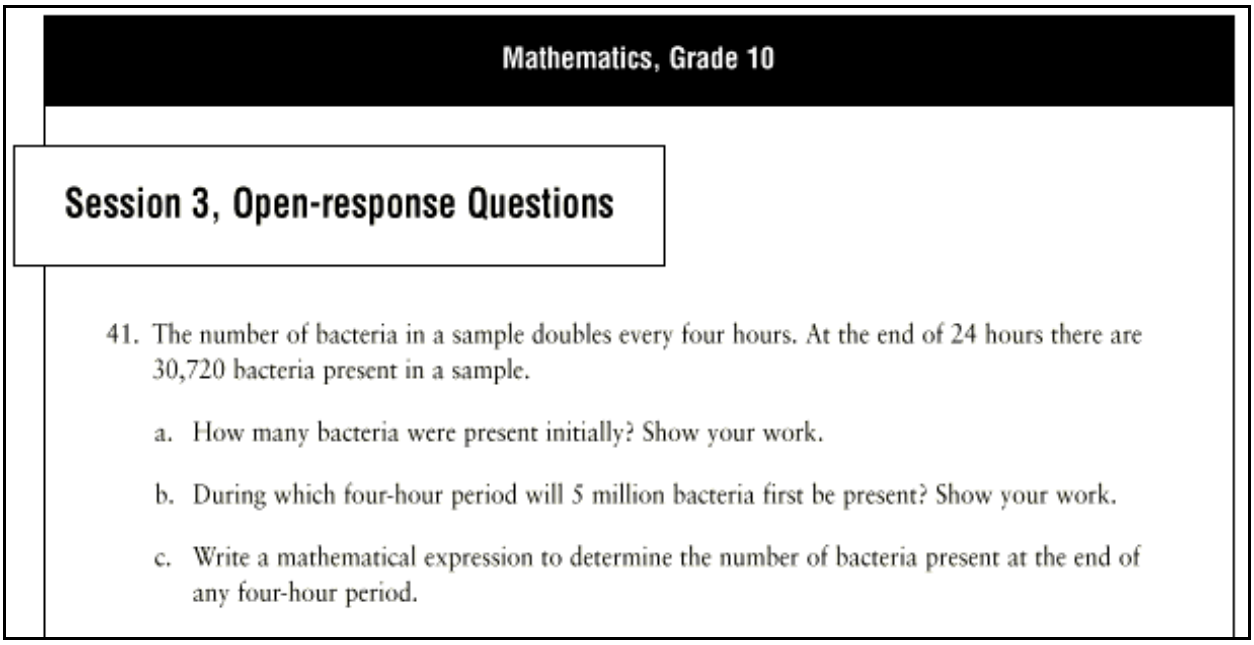
Problems

CONCEPTUAL PROBLEMS

1. Does Eppley's temperature equation allow an investigator to predict specific growth rate in field populations?
2. How does measurement of μ differ from measurement of production?
3. Why is it more difficult to estimate μ in the field than production?
4. Is it possible for specific growth rate in the field to increase while production remains constant?
5. Is it possible for specific growth rate in the field to increase while biomass is decreasing?
6. How does Eppley's discussion of the ^{14}C technique compare with Peterson's?

QUANTITATIVE PROBLEMS

1. Answer the MCAS test question shown in Figure 1.



Mathematics, Grade 10

Session 3, Open-response Questions

41. The number of bacteria in a sample doubles every four hours. At the end of 24 hours there are 30,720 bacteria present in a sample.

a. How many bacteria were present initially? Show your work.

b. During which four-hour period will 5 million bacteria first be present? Show your work.

c. Write a mathematical expression to determine the number of bacteria present at the end of any four-hour period.

Figure 1. A question from the 10th grade MCAS Math test

2. Estimate how long it would take for a *Synechococcus*, obeying exponential growth at 20º C, to fill the world's oceans. Use the following facts: *Synechococcus* has a diameter of about 1 micrometer, the distance from the equator to the pole is 10,000 km, the mean depth of the ocean is 4200m and 75% of the world's surface area is ocean.

Outlines of papers

ASSIGNED

Eppley, R. W. 1972. Temperature and phytoplankton growth in the sea. Fish. Bull. *70*: 1063-1065. [2, 3, 4, 7, 8, 14, 21, 24]

I. **Introduction:**
- A. Two questions:
  1. Why is temperature of so little importance?
  2. Why would anyone want to write a review on temperature and phytoplankton growth in the oceans?
- B. Answers:
  1. to suggest maximum growth and photosynthetic rates which might be reasonably expected for marine phytoplankton
  2. to point out the interrelationship among growth rate, photosynthetic assimilation number, and carbon to chlorophyll a ratios in the phytoplankton.

II. **Variation in specific Growth rate (μ) with temperature in laboratory cultures of unicellular algae.**
- A. **Figure 1** taken from Hoogenhout & Amesz (1965), chosen so that temperature would be limiting
  1. Variation due to
     a. cell size
     b. concentrations of photosynthetic pigments.
  2. General trends:
     a. exponential gradual increase up to about 40ºC

b. A smooth curve can be drawn through the 130 points.

$$\log_{10} \mu = 0.0275\ T - 0.070 \qquad \textbf{(1)}$$

[It is very important to note that Eppley expresses μ in divisions per day or doublings per day. Thus a population that doubles daily has a μ=1 doubling d^{-1}. In recent years, most biological oceanographers have expressed μ using inverse time. A population that doubles once a day has a specific growth rate of approximately 0.693, the ln (2). All of Eppley's μ values should be multiplied by ln (2) to convert to accepted common usage.]

3. Q_{10} for growth of 1.88
4. van't Hoff formula *[See **Ahlgren 1987, p. 177-178**: "Eppley based his function on data from batch cultures of laboratory algae, using in fact Berthelot's equation from 1862 (incorrectly named as Van't Hoff's rule by Eppley."]*

$$\mu = \text{`}\mu\text{'} = 0.851\ (1.066)^T$$

5. Belehradek function:

$$\mu = -2.46 \times 10^{-6}\ (T - [-40])^{3.45}$$

B. **Figure 2. Growth rate versus temperature for 5 different algae.**
   1. algae show temperature optima
   2. dependent slightly on salt concentrations

C. **Figure 3. Temp. vs growth curves for *Dunaliela tertiolecta*** at 5 different salt concentrations.
   1. 5 doublings per day max
   2. Useful in setting maximum dilution rate in chemostat culture.
      G. E. Hutchinson's concept of the niche.

III. **Estimates of the specific growth rates of phytoplankton in the sea.**

A. Methods
   1. Rates of increase of cell concentration or chlorophyll
      a. Nutrient depletion a problem
      b. self shading in rich water
   2. ^{15}N-nitrogen assimilation rates.
      a. Underestimated because of trypton
      b. [N enhancement effect]
   3. Carbon assimilation rates.

B. Solution: *"What is needed is an instantaneous method not confounded by the complexities of long incubations either in situ, in enclosed vessels, or in shipboard cultures. Unfortunately, no such method is in view."*

C. Eppley's cell volume method
   1. Carbon:volume ratios established
   2. [Coulter counter can be used]

D. ATP method
   1. problems
   2. Assumes a C/ATP ratio of 250

E. Coulter counter method

F. Percentage of paired dinoflagellate cells in cultures.

IV. **Results of growth rate measurements in the natural phytoplankton at different temperatures.**

A. **Table 2. Some estimates of the average specific growth rate of phytoplankton in the euphotic zone for various regions.**

Area	Doublings/day
Oligotrophic	
Sargasso sea	0.26
Fla. strait	0.45
Off Carolinas	0.37
Off Montauk	0.35
Off S. California (July 1970)	0.25-0.4
S. California (April-Sept. 67)	0.7 avg
Nutrient-rich	
Peru current	0.7
April	0.67
June	0.73
Off SW Africa	1.0
Western Arabian Sea	>1.0

B. **Table 3. Comparison of average growth rate using different methods**
1. ATP
2. Carbon
3. ^{15}N

V. **Relationship between phytoplankton growth rate and assimilation number.**
A. Assimilation number: chl specific production
B. How simple life would be if there was a constant C:Chl a ratio.
C. C:Chl ratio changes from 30-40 (upwelling) to 90-100 (low nutrient surface waters)

$$\mu=1/\delta t \log_2 [(C:Chla +\delta C/Chla)/C:Chl\ a] \quad (5)$$

Fig. 6. Photosynthetic rate (assimilation number/day) vs the specific growth rate of the phytoplankton from Equation (5)
Fig. 7. Photosynthetic rate (assimilation number/hour) vs the specific growth rate of the phytoplankton from Equation (5)

VI. **The Variation of assimilation number with temperature in the sea.**
A. **Figure 8.** The variation in maximum expected rate of photosynthesis(assimilation number) with temperature.
B. **Low temperature reduces the assimilation number and promotes increased carbon/chl ratios.**
C. Size Changes in phytoplankton size can have a major role.
1. Nanoplankton show higher assimilation numbers than do net plankton.
2. Increasing insolation in the spring results in increased water temperatures and often in stratification. Nutrients are depleted

3. Stratification reduces large cells
   a. sinking
   b. less effective at N uptake
4. seasonal increases in temp and ratio of nano- to net plankton increase assimilation numbers.

VII. **Implications for simulation models of phytoplankton production.**
A. Should P_{max} be a function of temperature?
B. Start out with a realistic estimate of maximum growth rate and then determine the effects of nutrient limitation (this is done in Kremer and Nixon)

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 fluorometer]*

I. Materials and methods
A. Turner model III
B. blue fluorescent filter with maximum transmission at 430 mµ
C. secondary filter opaque at light at wavelengths shorter than 645 mµ and reaching maximum transmission at 650 mµ

II. Discussion
A. Can not distinguish between Chl *a* and phaeophytin, which is normally not found in phytoplankton (p. 226)
B. In solution Chl. fluorescence decreases with increasing temperature at the rate of 0.3% per 1°C in the range of 0-35°C. *in vivo* fluorescence affected to a greater degree of 1.4%°C $^{-1}$

SUPPLEMENTAL

Ahlgren, G. 1987. Temperature functions in biology and their application to algal growth constants. Oikos *49*: 177-190. *[The relationships of algal growth constants such as $\hat{\mu}$ (maximum specific growth rate)m q_o (minimum nutrient content of algae), Y (yield coefficient), and K_s (half-saturation constant for growth) with temperature were investigated. Equations of the Belehrádek type are found to be appropriate]*.

I. Abstract

"Various kinds of temperature rules have been proposed for biological use, but reasons for choosing one before another have seldom been given. Arguments for such choices should include both theoretical and mathematical-statistical aspects. In this paper the relationships of algal growth constants such as $\hat{\mu}$ (maximum specific growth rate)m q_o (minimum nutrient content of algae), Y (yield coefficient), and K_s (half-saturation constant for growth) with temperature were investigated."

II. Introduction
Reviews existing analyses.
1. "**Eppley** based his function on data from batch cultures of laboratory algae, using in fact Berthelot's equation from 1862 (incorrectly named as Van't Hoff's rule by Eppley."
2. **Goldman & Carpenter (1974)** used Arrhenius' (1889) equation.

III. Temperature rules used in biology
A. Belehrádek (1935) division of temperature formulae
1. Group I (Rules of thermal sums)
   a. Burkhardt's equation
      $v=k(t-\alpha)$ (4)
   b. a linear form where the Y-intercept = -k and α = -Y intercept/k or α =X intercept.
   c. Equations are mathematical forms of the "rule of thermal sums"
2. Group II (Exponential equations)
   a. Berthelot (1862)
   b. Van't Hoff (1884)
   c. Arrhenius (1889)
3. Group III (built on physical processes)
   a. Belehrádek (1926) (11)
   b. Later modified $v=a(t-\alpha)^b$ (13)

c. "Belehrádek based his rule on the view that many biological processes are probably not controlled by chemical processes by physical processes such as diffusion and viscosity."

IV. Methods
- A. Very interesting: fits his data using the 2-parameter Verhulst (1838) logistic rather than the geometric J curve.
- B. Growth constants estimated by fitting suitable equations to the data using the SAS program NLIN (SAS users guide 1979)

V. Results
- A. Batch experiments
- B. Chemostat experiments
  1. fit Droop's (1973) q_o
  2. fit $\hat{\mu}$

Fig. 1. Results of P-limited chemostat experiments with *Scenedesmus auadricauda.*

- C. ^{14}C experiments
  1. Assimilation number estimated.
     a. Both AZ and P/B ratios increased with temperature (Table 7)

Table 7. Results of the ^{14}C experiments (AZ_{opt} ranges from 0.098 to 6.47 C Chl^{-1} h^{-1} {field population from L. Norrviken (1980), 20.7 °C with a Carbon:Chlorophyll ratio of 390 for Cryptomonads, small monads)

  b. Note that he uses the simple equation that μ=Assimilation number/(C:Chl *a*) ratio

- D. Estimation of biological zero
- E. Growth constants in relation to temperature.
  1. Belehrádek and Burckhardt-Harvey gave almost equally good fits to μ vs. t

$$\hat{\mu} = 0.0307\,(t - 0.18)^{1.13} \quad (r=0.945,\ n = 18) \qquad (15)$$

  2. Assimilation numbers, AZ, and P/B ratios (≈μ) based on incubator experiments with monocultures at light optima also showed good relationships with temperature (Fig. 3)

Fig. 3. Assimilation number (AZ) and primary production/biomass as functions of temperature for 3 species of algae.

  a. Chl:C ratio decreases with temperature.

Fig. 4. Assimilation number (AZ) and primary production/biomass as functions of temperature.

VI. Discussion
- A. Maximum specific growth rate, μ
  1. Belehrádek equation fits best
  2. Comparison with **Eppley (1972)** and **Goldman & Carpenter (1974)**

Fig. 5. Comparison of Ahlgren's new data with the **Eppley** and **Goldman & Carpenter** curves.

  3. "P/B values (≈ μ) multiplied by the factor 10 to get growth rates per day, agree fairly well with these earlier data from batch experiments."

- B. Yield coefficient, Y
- C. Minimum cell quota
- D. Half-saturation constant, K_s
- E. K_s for *Scenedesmus* independent of temperature.
- F. Interesting hypothesis:

"It is also reasonable that K_s should have the character of a real constant (Fig. 6). By definition K_s is the concentration which will support half the maximum growth rate. As the maximum growth rate increases with temperature, half the maximum growth rate should aslo increase with temperature, thus giving curves which separate immediately at the origin. A constant K_s leads to lower competition advantages with decreasing temperature. the initial gradient recommended by Talling (1979), or the ratio $\hat{\mu}/K_s$ (Healey 1980), used to characterize the competition status of an alga, would then be a useful tool to compare different algal species. A difference in that ratio, if necessary scaled to the same biological zero, for two species estimated in the laboratory at one temperature would be valid for all temperature conditions in the field. Is that too simple to be true?" p. 188

VII. Summarizing conclusion: Belehrádek best

Furnas, M. J. 1990. *In situ* growth rates of marine phytoplankton: approaches ot measurement, community and specific growth rates. J. Plankton Research *12*: 1117-1151.

Abstract: Maximal growth from 3 to 3.6 doublings d^{-1}

I. Introduction.
  A. aim: bring together published data on *in situ* growth on phytoplankton species and functional groups.
  B. Compare these rates with *in situ* growth of phytoplankton communities

II. **Approaches to measuring or estimating *in situ* growth rates of phytoplankton**
  A. Cytological or biochemical cell cycle markers.
    frequency of dividing cells
  B. Biochemical rate measurements and relative growth rate indices
    1. Redalje-Laws
    2. Redfield ratio and μ/μ_{max}
    3. **Gieskes & Kraay (1989)** pigment labeling of specific phytoplankton groups.
  C. Cage and bottle incubations
  D. Mathematical models of phytoplankton growth

III. The data set

Table I Growth rates of intact and size-fractionated phytoplankton in tropical and sub-tropical waters.

Table II Growth rates of intact and size-fractionated phytoplankton in temperate and polar marine waters.

Table III Maximum and mean *in situ* growth rates measured for centric diatom species and comparable maximum growth rates from culture

Table IV Maximum and mean *in situ* growth rates measured for pennate diatom species and comparable maximum growth rates from culture

Table V Maximum and mean *in situ* growth rates measured for dinoflagellate species and comparable maximum growth rates from culture

Table VI Maximum and mean *in situ* growth rates measured for microflagellate and ultraplankton species and comparable maximum growth rates from culture

IV. In situ growth rates of phytoplankton species and groups

V. Discussion.

Goldman, J. C. and E. J. Carpenter. 1974. A kinetic approach to the effect of temperature on algal growth. Limnol. Oceanogr. *19*: 756-766. *[Reviews growth rates estimated from chemostats. Maximum growth predicted by:* $\mu_m=(1.80 \times 10^{10})e^{(-6842/\text{Kelvin Temp})}$. *Proposes mechanisms whereby temperature can affect the outcome of competition. Tilman has confirmed and modeled such mechanisms.]*

1. uses Michaelis-Menten kinetics
2. Used Arrhenius equation:

$$\mu = A\, e^{-E/RT} \qquad (2)$$

where, A=constant, day^{-1}
E= activation energy, cal $mole^{-1}$
R = Universal gas constant, cal $^oK^-$ $mole^{-1}$
T = temperature, Kelvin scale

$$\mu = A\, e^{(-E/RT)}\, [S/(K_s + S) \qquad (3)$$

3. Assumes the half-saturation constant is temperature dependent.

$$\mu = A\, e^{(-E/RT)}\, [S/(K_s(T) + S] \qquad (4)$$

4. Reviews maximum growth rates for algae in doublings per day. (0.4 to 5.65)
5. *"Through application of equation (2) we can show graphically how temperature can play a role in species succession."*
6. Multiplicative growth model proposed.

Rhee, G.-Yull. 1982. Effects of environmental factors and their interactions on phytoplankton growth. Adv. microb. Ecol. *6*: 33-74.

I Temperature adaptation
- I.A poorly understood
- I.B at high temperature, the ratio of unsaturated to saturated fatty acids in the membrane commonly decreases, whereas at low temperature the opposite takes place. This ensures the fluidity and thus the stability of the membrane.

II Interactions with nutrients.
- II.A the maximum growth is set by temperature, but is rarely achieved.
- II.B temperature interacts with other environmental stresses to modify growth kinetics in a complex manner
- II.C temperature affects both the maximum growth and the half-saturation constant (p. 61)
- II.D can also affect the minimum cell quota
- II.E combined effects of nutrient limitation and temperature stress are grater than the sum of individual effects.

III Temperature-light relations.
- III.A photochemical reactions are insensitive to temperature. P vs I curves are not affected when light is limiting.
- III.B dark reactions are temperature sensitive.
- III.C therefore saturation light intensity decreases with temperature but not the slope of the P-I curve.
- III.D at the deeper layers of the euphotic zone, the effects of temperature on primary production may be minimal

Web Resources

Table 1. Web resources on phytoplankton

URL	Site	Description
http://www.soton.ac.uk/~ibg/	Ivo Grigorov Research Student (School of Ocean & Earth Sciences) Southampton Oceanography Centre	Marine Diatom index, including many SEM images

References

On Temperature Effects

Ahlgren, G. 1987. Temperature functions in biology and their application to algal growth constants. Oikos *49*: 177-190. *[A superb, review. The relationships of algal growth constants such as $\hat{\mu}$ (maximum specific growth rate)m q_o (minimum nutrient content of algae), Y (yield coefficient), and K_s (half-saturation constant for growth) with temperature investigated using batch culture, P-limited chemostats, and ^{14}C incubations. Note that Ahlgren uses the 2-parameter logistic to fit $\hat{\mu}$, not the standard equation for geometric growth. He obtained assimilation numbers of 6.47 mg C mg Chl $a^{-1}h^{-1}$ for a field phytoplankton population with a C:Chl ratio of 390 {his Table 7}. Equations of the Belehrádek type are found to be most appropriate].*

Goldman, J. C. 1977. Temperature effects on phytoplankton growth in continuous culture. Limnol. Oceanogr. *22*: 932-936.

Goldman, J. C. and E. J. Carpenter. 1974. A kinetic approach to the effect of temperature on algal growth. Limnol. Oceanogr. *19*: 756-766. *[Proposes mechanisms whereby temperature can affect the outcome of competition. Tilman has confirmed and modeled such mechanisms.]*

Harrison, W. G. and T. Platt. 1986. Photosynthesis-irradiance relationships in polar and temperate phytoplankton populations. Polar Biology *5*: 153-164. *[Further discussions of the covariation of temperature and assimilation number in field populations]*

Harris, G. P. 1986. Phytoplankton ecology. Chapman & Hall, London. *[Temperature effects are reviewed on pp. 188-190.]*

Harrison, W. G. and T. Platt. 1986. Photosynthesis-irradiance relationships in polar and temperate phytoplankton populations. Polar Biology *5*: 153-164. *[In contrast to **Eppley (1972)**, nutrients are unimportant and temperature is the second most important covariate, after depth, of P vs I parameters. Temperature is highly correlated with P^B_m in P vs I curves]*

Platt, T. and A. D. Jassby. 1976. The relationship between photosynthesis and light for natural assemblages of coastal marine phytoplankton. J. Phycol *12*: 421-430. *[The asymptote of the P vs I curve is strongly correlated with temperature.]*

Rhee, G.-Yull. 1982. Effects of environmental factors and their interactions on phytoplankton growth. Adv. microb. Ecol. *6*: 33-74. *[Temperature is covered on pp. 57-63. Rhee discusses the complex interaction between temperature and nutrient limitation.]*

Rhee, G.-Y. and I. J. Gotham. 1981. The effect of environmental factors on phytoplankton growth: light and the interactions of light with nutrient limitation. Limnol. Oceanogr. *26*: 635-648. *[The combined effects were greater than the individual effects and were not multiplicative.]*

Steemann Nielsen, E. 1975. Marine photosynthesis. Elsevier, Amsterdam. *[Temperature effects on photosynthesis are treated in chapter 11.]*

Tilman, D, M. Mattson, and S. Langer. 1981. Competition and nutrient kinetics along a temperature gradient: an experimental test of a mechanistic approach to niche theory. Limnol. Oceanogr. *26*: 1020-1033. *[The species with the lowest resource requirements, measured as R*, at a given temperature displaces all others.]*

Tilman, D. 1982. Resource Competition and Community Structure. Monographs in Population Biology, Vol. 17. Princeton University Press, Princeton, N.J. *[Tilman includes a section on the effects of temperature on the outcome of competition.]*

On estimating primary production & specific growth rate

(see also **Redalje-Laws ^{14}C-labeling technique**)

Behrenfeld, M. J. and P. G. Falkowski. 1997. A consumer's guide to phytoplankton productivity models. Limnol. Oceanogr. 42: 1479-1491. *[Reviews the major classes of P vs. I models for estimating production.]{? }*

Cloern, J. E, C. Grenz and L. Videgar-Lucas. 1995. An empirical model of the phytoplankton chlorophyll:cabon ratio - the conversion factor between productivity and growth rate. Limnol. Oceanogr. *40*: 1313-1321.

Collos, Y. and G. Slawyk. 1985. On the compatibility of carbon uptake rates calculated from stable and radioactive isotope data: implications for the design of experimental protocols in aquatic primary productivity. J. Plankton Res. *7*: 595-605. *[^{13}C vs. ^{14}C]*

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. *[DCMU-induced fluorescence remained unchanged after sampling indicating that phytoplankton cells were not killed or physiologically harmed by metal contamination.]*

Dandonnneau, Y. and A. Le Bouteiller. 1992. A simple and rapid device for measuring planktonic primary production by *in situ* sampling, and ^{14}C injection and incubation. Deep-Sea Res. *39*: 795-803. *[The ^{14}C is injected at depth instead of on the surface]*

Dortch, Q., T. L. Roberts, J. R. Clayton, and S. I. Ahmed. 1983. RNA/DNA ratios and DNA concentrations as indicators of growth rate and biomass in planktonic marine organisms. Marine Ecology Progress Series *13*: 61-71. *[Estimates of μ]*

Furnas, M. 1990. *In situ* growth rates of marine phytoplankton: approaches to measurement, community and species growth rates. J. Plankton Res. *12*: 1117-1151. *[A comprehensive listing of estimates of μ and listing of methods]*

Furnas, M. 1991. Net in situ growth rates of phytoplankton in an oligotrophic tropical shelf ecosystem. Limnol. Oceanogr. *36*: 13-29. *[Grazers are reduced in diffusion incubators and the change in the abundance of major phytoplankton species are recorded to obtain estimates of μ for major phytoplankton species and groups.]*

Gallegos, C. L. and T. Platt. 1985. Vertical advection of phytoplankton and productivity estimates: a dimensional analysis. Mar. Ecol. Prog. Ser. *26*: 125-134. *[Standard incubations may underestimate primary production by using fixed depth and light levels.]*

Geider, R. J. 1993. Quantitative phytoplankton physiology: implications for primary production and phytoplankton growth. ICES mar Sci. Symp. *197*: 52-62.

Geider, R. J., T. Platt, and J. A. Raven. 1986. Size-dependence of growth and respiration in diatoms: a synthesis. Mar. Ecol. Prog. Ser. *30*: 93-104. *[There is no physiological basis for competitive dominance by large phytoplankton cells. Small cells, in the absence of grazing should dominate. Grazing by microzooplankton is invoked as the major reason for dominance by large diatoms]*

Geider, R. J. 1988. Estimating the growth and loss rates of phytoplankton from time series observations of ^{14}C-bicarbonate uptake. Mar. Ecol. Prog. Ser. *43*: 125-138.

Gieskes, W. E. C., G. W. Kraay, and M. A. Baars. 1979. Current ^{14}C methods for measuring primary production: gross underestimates in oceanic waters. Netherlands Journal of Sea Research *13*: 58-78. *[The underestimates may not be as severe as noted here. Historically, this paper was very influential in the great debate]*

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 Press, New York. *[To be discussed and outlined in the class on Gyre productivity]*

Goldman, J. C. 1980. Physiological processes, nutrient availability, and the concept of relative growth rate in marine phytoplankton ecology. Pp. 179-194 *in* P. G. Falkowski, *ed*., Primary productivity in the sea. Plenum Press, New York.

Goldman, J. 1986. On phytoplankton growth rates and particulate C:N:P ratios at low light. Limnol. Oceanogr. *31*: 1358-1363. *[C:N:P ratios=f(μ), but are not greatly affected by light]*

Goldman, J. C., J. J. McCarthy, and D. G. Peavey. 1979. Growth rate influence on the chemical composition of phytoplankton in oceanic waters. Nature *279*: 210-215. *[A very important paper! The C:N and C:P ratios from chemostat studies are reviewed and it is shown that phytoplankton only grow with Redfield ratios at high relative growth rates $0.9 < \mu/\mu_{max} < 1$. They introduce*

the controversial hypothesis that patches of nutrients from zooplankton excretion explain high relative growth rates in the field]

Harrison, W. G., T. Platt, and M. K. Lewis. 1985. The utility of light-saturation models for estimating marine primary productivity in the field: a comparison with conventional *in situ* methods. Can. J. Fish. Aquat. Sci. *42*: 864-872. *[Given the P vs. I parameters determined for one or a few samples, Chl a & light distributions, primary production can be estimated accurately]*

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 ^{14}C method produces *A higher than total algal C, too high an estimate of μ]*

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]*

Laws, E. A. and T. T. Bannister. 1980. Nutrient and light-limited growth of *Thalassiosira fluviatilis* in continuous culture, with implications for phytoplankton growth in the ocean. Limnol. Oceanogr. *25*: 457-473. *[The specific growth rate can be predicted from the C:Chl ratio. A C:Chl ratio of 30 corresponds to about an 8-h doubling time]*

Laws, E. A., D. J. Redlaje, 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. *[Documents high production and μ]*

Laws, E. A., G. R. DiTullio, and D. J. Redlaje. 1987. High phytoplankton growth and production rates in the North Pacific subtropical gyre. Limnol. Oceanogr. *34*: 905-918.*[Documents high production and μ]*

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Li, W. K. W., D. V. Subba Rao, W. G. Harrison, J. J. Cullen, B. Irwin, and T. Platt. 1983. Autotrophic picoplankton in the tropical ocean. Science *219*: 292-295. *[Most of the phytoplankton standing stock and production is in the picoplankton size category.]*

Lohrenz, S. E. and C. D. Taylor. 1987. Primary production of protein: I. Comparison of net cellular carbon and photosynthesis with ^{14}C-derived rate estimates in steady-state cultures of marine phytoplankton. Mar. Ecol. Prog. Ser. *35*: 277-292.

Mitchell, B. G. and D. A. Kiefer. 1988. Chlorophyll a specific absorption and fluorescence excitation spctra for light-limited phytoplankton. Deep-Sea Res. 35: 639-663. *[Kiefer & Mitchell propose using natural fluorescence to measure primary production]*

Mitchell, B. G. and D. A. Kiefer. 1988. Variability in pigment specific particulate fluorescence and absorption spectra in the northeastern Pacific Ocean. Deep-Sea Res. *35*: 665-689.

Orellana, M. V. and M. J. Perry. 1992. An immunoprobe to measure Rubisco concentrations and maximal photosynthetic rates of individual phytoplankton cells. Limnol. Oceanogr. *37*: 478-490.

Platt, T., M. R. Lewis, and R. Geider. 1984. Thermodynamics of the pelagic ecosystem: elementary closure conditions for biological production in the open ocean. Pp. 49-84 *in* M. J. Fasham, *ed*. Flows of Energy and Materials in Marine Ecosystems. Plenum Press, New York.

Platt, T., W. G. Harrison, E. P. W. Horne and B. Irwin. 1987. Carbon fixation and oxygen evolution by phytoplankton in the Canadian high arctic. Polar Biology *8*: 103-113.

Platt, T., W. G. Harrison, M. R. Lewis., W. K. W. Li, S. Sathyendranath, R. E. Smith, and A. F. Vezina. 1989. Biological production of the oceans: the case for a consensus. Mar. Ecol. Prog. Ser. *52*: 77-88. *[Many of the primary production estimates in gyres produce assimilation numbers exceeding the energetic maxima for photosynthesis]*

Pregnall, A. M. 1983. Release of dissolved organic carbon from the estuarine intertidal macroalga, *Enteromorpha periphery*. Marine Biology *73*: 37-42.

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]*

Prezelin, B. B. and A. Alldredge. 1983. Primary production of marine snow during and after an upwelling event. Limnol. Oceanogr. *28*: 1156-1167. *[Mats of Thalassiosira observed at 100 m depth]*

Prezelin, B. B., R. R. Bidigure, H. A. Matlick, M. Putt. and B. Ver Hoven. 1987. Diurnal patterns of size-fractionated primary productivity across a coastal front. Marine Biology *96*: 563-574.

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Spitzer, W. S. and W. J. Jenkins. 1989. Rates of vertical mixing, gas exchange and new production: estimates from seasonal gas cycles in the upper ocean near Bermuda. J. Mar. Res. *47*: 169-196.

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Topliss, B. J. and T. Platt. 1986. Passive fluorescence and photosynthesis in the ocean: implications for remote sensing. Deep-Sea Res. 33: 849-864. *[Passive fluorescence used to estimate primary production]*

Weger, H. G., R. Herzig, P. G. Falkowski and D. H. Turpin. 1989. Respiratory losses in the light in a marine diatom: measurement by short-term mass spectrometry. Limnol. Oceanogr. *34*: 1153-1161. *[Mass spectrometry of $^{18}O_2$ used to estimate mitochondrial respiration and photorespiration. There is virtually no photorespiration in Thallassiosira weisflogii]*

Zlotnick, I. and Z. Dubinsky. 1989. The effect of light and temperature on DOC excretion by phytoplankton. Limnol. Oceanogr. *34*: 831-839.

REDALJE-LAWS CHL -SPECIFIC LABELING

Calliau, C., H. Claustre, F. Vidussi, D. Marie, and D. Vaulot. 1996. Carbon biomass, and gross growth rates as estimated from ^{14}C pigment labeling, during photoacclimation in *Prochlorococcus* CCMP 1378. Mar. Ecol. Prog. Ser. *145*: 209-221. *[Lab studies indicate that ^{14}C incorporation into zeaxzanthin better than into Dv-chl a to estimate prochlorophyte μ]*

DiTullio, G. R. and E. A. Laws. 1986. Diel periodicity of nitrogen and carbon assimilation in five species of marine phytoplankton: accuracy of methodology for predicting N-assimilation rates and N/C composition ratios. Mar. Ecol. Prog. Ser. *32*: 123-132. *[Estimates of the percentage of ^{14}C incorporated into protein after 24 h (12L:12D) can provide estimates of the relative rates of C and N assimilation and hence relative growth rates] [**14**]*

DiTullio, G. R., D. A. Hutchins, and K. W. Bruland. 1993. Interaction of iron and major nutrients controls phytoplankton growth and species composition in the tropical North Pacific Ocean. Limnol. Oceanogr. 38: 495-508. *[Chl-a specific labeling used to estimate the specific growth rate of pico-, nano- and microplankton at 9° N in the Pacific. Large diatoms are limited by Fe; prochlorophyte specific growth {measured by specific activity of divinyl Chl a labeling} limited by macronutrients. Picoplankton appeared to be controlled by grazers, but diatoms were controlled by Fe limitation]*

Eppley, R. W., E. Swift, D. G. Redalje M. R. Landry and L. W. Hass. 1988. Subsurface chlorophyll maximum in August-September 1985 in the CLIMAX area of the North Pacific. Mar. Ecol. Prog. Ser. *42*: 289-301.

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 Press, New York. *[To be discussed and outlined in the class on Gyre productivity]*

Gieskes, W. W. and G. W. Kraay. 1986. Floristic and physiological differences between the shallow and the deep nanophytoplankton community in the euphotic zone of the open tropical Atlantic revealed by HPLC analysis of pigments. Marine Biology *91*: 567-576. *[Primary production incubations done on deck with neutral density filters. Reverse-phase HPLC used to analyze the floral composition of the surface and deep communities off Africa. Diatoms have high concentrations of fucoxanthin, cyanobacteria have high concentrations of zeaxanthin, and Prymnesiophyscea (Coccolithophorids) have 19'- hexanolyloxyfucoxanthis). The **Redalje-Laws (1981)** technique is also applied to estimate phytoplankton carbon concentration and specific growth rates. High assimilation numbers were observed (15), despite having relatively low dark-bottle ^{14}C incubations.]*

Gieskes, W. W. and G. W. Kraay. 1989. Estimating the carbon-specific growth rate of the major algal species in eastern Indonesian waters by ^{14}C labeling of taxon-specific carotenoids. Deep-Sea Res. *36*: 1127-1139. *[The **Redalje-Laws (1981)** technique is expanded to analyze the specific growth rate of different floral groups based on the specific activity in Chl a and taxon-specific accessory pigments]* {?}

Goericke, R. 1998. Response of phytoplankton community structure and taxon-specific growth rates to seasonally varying physical forcing of the Sargasso Sea off Bermuda. Limnol. Oceanogr *43*: 921-935.

Goericke, R. 1998. Response of Sargasso Sea phytoplankton biomass, growth rates and primary production to seasonally varying physical forcing., J Plankton Res *20*: 2223-2249.

Goericke, R. and N. A. Welschmeyer. 1993a. The chlorophyll-labeling method: measuring specific rates of chlorophyll *a* synthesis in cultures and in the open ocean. Limnol. Oceanogr. *38*: 80-95.

Goericke, R. and N. A. Welschmeyer. 1993b. The carotenoid-labeling method: measuring specific rates of carotenoid synthesis in natural phytoplankton communities. Mar. Ecol. Prog. Ser. *98*: 157-171. {?}

Gould, D. M. 1989. Epipelic diatoms of Savin Hill Cove: an analysis of species composition, biomass, specific growth rates and primary production. Ph.D. dissertation, Environmental Sciences Program, UMASS/Boston.

Gould, D. G. and E. D. Gallagher. 1990. Field measurement of specific growth rate, biomass and primary production of benthic diatoms of Savin Hill Cove, Boston. Limnol. Oceanogr. *35*: 1757-1770. *[The **Redalje-Laws (1981)** technique adapted for benthic diatoms: high biomass and production, low μ (6-8 day doubling times)]*

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]*

Laws, E. A. 1984. Improved estimates of phytoplankton carbon based on ^{14}C incorporation into chlorophyll *a*. J. Theor. biol. *110*: 425-434.{**11**, **13**}

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.

Laws, E. A., G. R. DiTullio, and D. J. Redalje. 1987. High phytoplankton growth and production rates in the North Pacific subtropical gyre. Limnol. Oceanogr. *34*: 905-918.

Redalje, D. G. 1983. Phytoplankton carbon biomass and specific growth rates determined with the labeled chlorophyll a technique. Marine Ecology Progress Series *11:* 217-225.{?}

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]* {**11**, **28**}

Strom, S. L. and N. A. Welschmeyer. 1991. Pigment-specific rates of phytoplankton growth and microzooplankton grazing in the open subarctic Pacific Ocean. Limnol. Oceanogr. *36*: 50-63. *[48 h, clean bottle, incubations with dilution grazing experiments (**Landry & Hassett 1982**) and pigment-specific μ determinations (by HPLC)]* {?}

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]*

Waterhouse, T.Y. and N.A. Welschmeyer. 1995. Taxon-specific analysis of microzooplankton grazing rates and phytoplankton growth rates. Limnol. Oceanog. *40*: 827-834

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.{**13**, **14**}

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

ESTIMATING μ WITH FREQUENCY OF DIVIDING CELLS

Chang, J. and E. J. Carpenter. 1990. Species-specific phytoplankton growth rates via diel DNA synthesis cycles. IV. Evaluation of the magnitude of error with computer-simulated cell populations. Mar. Ecol. Prog. Ser. *65*: 293-304.

McDuff, R. E. and S. W. Chisholm. 1982. The calculation of *in situ* growth rates of phytoplankton populations from fractions of cells undergoing mitosis: a clarification. Limnol. Oceanogr. *27*: 783-788.

Rivkin, R. B. 1986. Radioisotopic method for measuring cell division rates of individual species of diatoms from natural populations. Appl. Environ. Micro. *51*: 769-775.

Rivkin, R. B. and H. H. Seliger. 1981. Liquid scintillation counting for ^{14}C uptake of single algal cells isolated from natural samples. Limnol. Oceanogr. *26*: 780-785. *[Individual cells isolated by micropipette and transferred serially through 2 washes of unlabeled water; 10-20 min counting times & 2 h counting times for field samples. Can't isolate cells < 8-10μm]*

Rivkin, R. B. and M. A. Voytek. 1986. Cell division rates of eucaryotic algae measured by tritiated thymidine incorporation into DNA: coincident measurements of photosynthesis and cell division of individual species of phytoplankton isolated from natural populations. J. Phycol. *22*: 199-205.

Wailer, C. S. and R. W. Eppley. 1979. Temporal pattern of division in the genus *Ceratium* and its application to the determination of growth rate. J. exp. Mar. Biol. Ecol. *39*: 1-24.

THE DILUTION METHOD

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 dilution method is used with ^{14}C incubations to estimate C:Chl a ratios.]*

Landry, M. R. & R. P. Hassett. 1982. Estimating the grazing impact of marine microzooplankton. Marine Biology *67*: 283-288. *[The dilution method introduced]* [?]

Landry, M. R. J. Constantinous, & J. Kirshtein. 1995a. Microzooplankton grazing in the equatorial Pacific during February & August 1992. Deep-Sea Res. *42*: 657-672.

Landry, M. R., J Kirshtein & J. Constantinou. 1995b. A refined dilution technique for measuring the community grazing impact of microzooplankton, with experimental tests in the central equatorial

Pacific. Mar. Ecol. Prog. Ser. *120*: 53-63.

Strom, S. L. and N. A. Welschmeyer. 1991. Pigment-specific rates of phytoplankton growth and microzooplankton grazing in the open subarctic Pacific Ocean. Limnol. Oceanogr. *36*: 50-63. *[48 h, clean bottle, incubations with dilution grazing experiments and pigment-specific μ determinations (by HPLC)]*

ESTIMATING CHL *a* CONCENTRATIONS IN THE SEA

(the measurement of Chl *a* in sediments will be covered in a later handout on microphytobenthic production):

General description

Parsons, T. R., Y. Maita, and C. M. Lalli. 1985. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford. *[Lorenzen's 1966 in vivo method is discussed on pp. 111-112.]*

Trichromatic method (i.e., spectrophotometric method)

Jeffrey, S. W. and G. F. Humphrey. 1975. New spectrophotometric equations for determining chlorophylls a, b, c and c_2 in higher plants, algae and phytoplankton. Biochem. Physiol. Pflanz. *167*: 191-194.

Fluorometric method

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.]*

Lorenzen, C. J. 1967. Determination of chlorophyll and phaeopigments: spectrophotometric equations. Limnol. Oceanogr. *12*: 343-346.

Lorenzen, C. J. and J. N. Downs. 1986. The specific absorption coefficients of chlorphyllide a and pheophorbide a in 90% acetone, and comments on the fluorometric determination of chlorophyll and pheopigments. Limnol. Oceanogr. *31*: 449-452.

Strickland, J. D. and T. R. Parsons. 1972. Fluorometric determination of chlorophylls. Ppl 201-206 *in* A Practical Handbook of Seawater Analysis (2nd. Ed.) Bull Fish. Res. Bd. Can. *167*.

Sweet, S. T. and N. L. Guinasso. 1984. Effects of flow rate on fluorescence *in vivo* during continuous measurements on Gulf of Mexico surface water. Limnol. Oceanogr. *29*: 397-401.

Trees, C. C., M. C. Kennicutt, and J. M. Brooks. 1985. Errors associated with the standard fluorometric determination of chlorophylls and phaeopigments. Mar. Chem. *17*: 1-12. *[The fluorometric method may underestimate by nearly 40%]*

Venrick, E. L. 1987. On fluorometric determination of filter-retained pigments. Limnol. Oceanogr. *32*: 492-493.

HPLC

Abayachi, J. K. and J. P. Riley. 1979. The determination of phytoplankton pigments by High Performance Liquid Chromatography. Analytica chim. Acta. *107*: 1-11.

Bidigare, R. R., M. C. Kennicutt, and J. M. Brooks. 1985. Rapid determination of chlorophylls and their degradation productions by high-performance liquid chromatography. Limnol. Oceanogr. *30*: 432-435.

Gieskes, W. W. C and G. W. Kraay. 1983a. Dominance of cryptophyceae during the phytoplankton spring bloom in the central North Sea detected by HPLC analysis of pigments. Marine Biology *75*: 179-185.

Gieskes, W. W. C. and G. N. Kraay. 1983b. Unknown chlorophyll *a* derivatives in the North Sea and the tropical Atlantic Ocean revealed by HPLC analysis. Limnol. Oceanogr. *28*: 757-766.

Gieskes, W. W. C. and G. N. Kraay. 1986a. Analysis of phytoplankton pigments by HPLC before, during and after mass occurrence of the microflagellate *Cyrymbellus aureus* during the spring bloom in the open norther North Sea in 1983. Marine Biology *92*: 45-52.

Gieskes, W. W. and G. W. Kraay. 1986b. Floristic and physiological differences between the shallow and the deep nanophytoplankton community in the euphotic zone of the open tropical Atlantic revealed by HPLC analysis of pigments. Marine Biology *91*: 567-576. *[Reverse-phase HPLC analysis of the floral composition of the surface and deep communities off Africa. Diatoms have high concentrations of fucoxanthin, cyanobacteria have high concentrations of zeaxanthin, and Prymnesiophyscea (Coccolithophorids) have 19'-hexanolyloxyfucoxanthis. High concentrations of Chl* b *were found in the subsurface Chl* a *maximum. The Redalje-Laws technique is also applied to estimate phytoplankton carbon concentration and specific growth rates. High assimilation numbers were observed (15), despite having relatively low dark-bottle ^{14}C controls.]*

Gieskes, W. W. and G. W. Kraay. 1989. Estimating the carbon-specific growth rate of the major algal species in eastern Indonesian waters by ^{14}C labelling of taxon-specific carotenoids. Deep-Sea Res. *36*: 1127-1139. *[Reverse-phase HPLC used to separate photosynthetic pigments. The Redalje-Laws technique is expanded to analyze the specific growth rate of different floral groups based on the specific activity in Chl a and taxon-specific accessory pigments]*

Jacobsen, T. 1978. A quantitative method for the separation of chlorophyll a and b from phytoplankton pigments by High Pressure Liquid Chromatography. Mar. Sci. Comm. *4*: 33-47. *[He asserts that the trichromatic spectrophotometric methods can produce factor of 10 overestimates of chl a concentration. Others have not found this to be the case.]*

Jacobsen, T. 1982. Comparisons of chlorophyll a measurements by fluorometric, spectrophotometric and high pressure liquid chromatographic methods in aquatic environments. Arch. Hydrobiol. *16*: 35-45.

Jeffrey, S. W. 1980. Algal pigment systems. PP. 33-58 *in* P. G. Falkowski, ed. Primary productivity in the sea. Plenum Press, New York. *[An excellent review]*

Lorenzen, C. J. and J. N. Downs. 1986. The specific absorption coefficients of chlorphyllide *a* and pheophorbide *a* in 90% acetone, and comments on the fluorometric determination of chlorophyll and pheopigments. Limnol. Oceanogr. *31*: 449-452. *[Carl Lorenzen's last paper]*

Mantoura, R. F. C. and C. A. Llewellyn. 1983. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase High Performance Liquid Chromatography. Analytica chim Acta *151*: 297-314. *[The classic method]*

Murray, A. P., C. F. Gibbs, and A. R. Longmore. 1986. Determination of chlorophyll in marine waters: intercomparison of a rapid HPLC method with full HPLC, spectrophotometric and fluorometric methods. Marine Chemistry *19*: 211-227. *[Introduces a method requiring 10 min/sample.]*

Strom, S. L. and N. A. Welschmeyer. 1991. Pigment-specific rates of phytoplankton growth and microzooplankton grazing in the open subarctic Pacific Ocean. Limnol. Oceanogr. *36*: 50-63. *[48 h, clean bottle, incubations with dilution grazing experiments and pigment-specific μ determinations (by reverse-phase HPLC)]*

Vernet, M. and C. J. Lorenzen. 1987. The presence of chlorophyll b and the estimation of phaeopigments in marine phytoplankton. J. Plankton Res. *9*: 255-265. *[The ratio of chl b to chl a >0.3 at the chl max but only 0.05 in tropical waters. The presence of chl b could account for a 38% increase in the fluorometric estimate of phaeopigments in the Central N. Pacific.]*

Welschmeyer, N., R. Goericke, S. Strom and W. Peterson. 1991. Phytoplankton growth and herbivory in the subarctic Pacific: a chemotaxonomic analysis. Limnol. Oceanogr. *36*: 1631-1649. *[^{14}C-specific pigment labeling, with pigments separated by HPLC (reverse-phase C-18 column)]*

Wright, S. W. and J. D. Shearer. 1984. Rapid extraction and high-performance liquid chromatography of chlorophylls and carotenoids from marine phytoplankton. J. Chromatography *294*: 281-295.

Wright, S. and S. W. Jeffrey. 1987. Fucoxanthin pigment markers of marine phytoplankton analyzed by HPLC and HPTLC. Mar. Ecol. Prog. Ser. *38*: 259-266.

Yacobi, Y. Z., W. Eckert, H. G. Truper and T. Berman. 1990. High performance liquid chromatography detection of phototrophic bacterial pigments in aquatic environments. Microb. Ecology *19*: 127-136.

PIGMENTS & C:CHL A RATIOS

Alberte, R. S., A. M. Wood., T. A. Kursar, and R. L. Guillard. 1984. Novel phycoerythrins in marine *Synechococcus* spp. Characterization and evolutionary and ecological implications. Plant Physiol. *75*: 732-739. *[Phycobiliproteins are the principal light harvesting pigment proteins of the red algae, Cryptomonads and cyanobacteria. Two novel and spectrally distinct PE's are isolated]*

Banse, K. 1977. Determining the carbon-to-chlorophyll ratio of natural phytoplankton. Marine Biology *41*: 199-212.

Caraco, N. and A. H. Puscoon. 1986. The measurement of bacterial chlorophyll and algal chlorophyll a in natural waters. Limnol. Oceanogr. *31*: 887-893.

Chisholm, S. W., R. J. Olson, E. R. Zettler, R. Goericke, J. B. Waterbury and N. A. Welschmeyer 1988. A novel free-living prochlorophyte abundant in the oceanic photic zone. Nature 334: 340-343 .

Chisholm, S. W., S. L. Frankel, R. Goericke, R. J. Olson, B. Palenik, B. B. Waterbury, L. West-Hohnsrud, and E. R. Zettler 1992. Prochlorcoccus marinus nov. gen. nov. sp.: a oxyphototrophic marine prokaryote containing divinyl chlorophyll a and b. Arch. Microbiol. 157: 297-300 .

Demers, S., J-C Therriault, L. Legendre & J. Neveux. 1985. An *in vivo* fluorescence method for continuous *in situ* estimation of phytoplankton photosynthetic characteristics. Mar. Ecol. Prog. Ser. *27*: 21-27.

Falkowski, P. and D. A. Kiefer. 1985. Chlorophyll *a* fluorescence in phytoplankton: relationship to photosynthesis and biomass. J. Plankton Res. *7*: 715-731.

Falkowski, P. G., R. M. Greene and R. J. Geider. 1992. Physiological limitations on phytoplankton productivity in the ocean. Oceanography *5*: 84-91.

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. *[C:Chl a ratio difficult to determine (see **Redalje & Laws 1981**). During balanced growth, C and Chl a are produced proportionate to C:Chl a. Incubations performed using the dilution method, to reduce grazing effects (and grazer biomass). Grazers lead to overestimates of C:Chl a]*

Jacobsen, T. 1978. A quantitative method for the separation of chlorophyll *a* and *b* from photosynthetic pigments by High Pressure Liquid Chromatography. Mar. Sci. Comm. *4*: 33-47. *[Trichromatic spectrophotometric method produces factor of 10 overestimates of Chl a. Later studies disagree with this estimate]*

Jeffrey, S. W. 1980. Algal pigment systems. Pp. 33-58 *in* P. G. Falkowski, *ed*. Primary Productivity in the Sea. Plenum Press, New York.

Jeffrey, S. W. and G. M. Hallegraeff. 1987. Chlorophyllase distribution in ten classes of phytoplankton: a problem for chlorophyll analysis. Mar. Ecol. Prog. Ser. *35*: 293-304. *[HPLC techniques can underestimate true Chl* a *concentrations if the Chl* a *is enzymatically degraded to chlorphyllide a. Use 100% acetone to inactivate the chlorophyllase enzyme that degrades chl a to chlorphyllide a]*

Kiefer, D. A. 1973. Fluorescence properties of natural phytoplankton populations. Marine Biology *22*: 263-269. *[An early paper showing the natural variability in the flu/Chl a signal from Lorenzen's in situ fluorescence method. Photoinhibition clearly reduces the flu/Chl a ratio, but other environmental effects aren't clear]*

Lorenzen, C. J. and J. N. Downs. 1986. The specific absorption coefficients of chlorphyllide a and pheophorbide a in 90% acetone, and comments on the fluorometric determination of chlorophyll and pheopigments. Limnol. Oceanogr. *31*: 449-452.

Oldham, P. B., E. J. Zilloux and I. M. Warner. 1985. Spectral fingerprinting of phytoplankton populations by two-dimensional fluorescence and Fourier-transform-based pattern recognition. J. Mar. Res. *43*: 893-906.

Olson, R. J., S. L. Frankel, S. W. Chisholm, and H. M. Shapiro. 1983. An inexpensive flow cytometer for the analysis of fluorescence signals in phytoplankton: chlorophyll and DNA distributions. J. exp. Mar. Biol. Ecol. *68*: 129-144.

Trees, C. C., M. C. Kennicutt, and J. M. Brooks. 1985. Errors associated with the standard fluorometric determination of chlorophylls and phaeopigments. Marine Chemistry *17*: 1-12. *[The fluorometric technique may underestimate chl by about 40%]*

Vernet, M. and C. J. Lorenzen. 1987a. The relative abundance of pheophorbide a: and pheophytin a in temperate marine waters. Limnol. Oceanogr. *32*: 352-358.

Vernet, M. and C. J. Lorenzen. 1987b. The presence of chlorophyll b: and the estimation of phaeopigments in marine phytoplankton. J. Plankton Res. *9*: 255-265.

Whitney, D. E. and W. M. Darley. 1979. A method for the determination of chlorophyll a in samples containing degradation products. Limnol. Oceanogr. *24*: 183-186. *[Hexane extraction used to separate and estimate chlorphyllide & chlorophyll a concentrations]*

Yentsch, C. M. (& 13 co-authors). 1983. Flow cytometry and cell sorting: a technique for the analysis and sorting of aquatic particles. Limnol. Oceanogr. *28*: 1275-1280.

Yentsch, C. M. 1990. Flow cytometry: near real-time information about ocean biology. Oceanography *3*: 47-50.

Yentsch, C. S. and D. A. Phinney. 1985. Spectral fluorescence: an ataxonomic tool for studying the structure of phytoplankton populations. J. Plankton Res. *7*: 617-632. *[The Chlorophyll accessory pigment ratio =* ***CAP ratio*** *(=F(530,685)/F(450,685)) is proposed and applied as an indicator of phytoplankton species composition.]*

BIOCHEMISTRY OF CARBON FIXATION

Adams, W. W., C. B. Osmand, and T. D. Sharkey. 1987. Responses of two CAM species to different irradiances during growth and susceptibility to photoinhibition by high light. Plant Physiol. *83*: 213-218.

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.]*

Arp, A. J., J. J. Childress and C. R. Fisher. 1984. Metabolic and blood gas transport characteristics of the hydrothermal vent bivalve Calyptogena magnifica. Physiol. Zool. *57*: 648-662.

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]*

Bazzazz, F. A. and W. E. Williams. 1991. Atmospheric CO_2 concentrations within a mixed forest: implications for seedling growth. Ecology *72*: 12-16.

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 $K_m \approx 30$ μM, Cyanobacterial RuBPCO $K_m \approx 100$ 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.

Bedu, S., G. Peltier, F. Surrey, and F. Joset. 1990. Properties of a mutant from *Synechosystis* PCC6803 resistant to acetazolamide, an inhibitor of carbonic anhydrase. Plant Physiol. *93*: 1312-1315.

Beer, S. and A. Eshel. 1983. Photosynthesis of *Ulva* sp. II. Utilization of CO_2 and HCO_3^- when submerged. J. Exp. Mar. Biol. Ecol. *70*: 99-106. *[A bicarbonate-free seawater is used to study uptake kinetics]*

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]*

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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]*

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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]*

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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]*

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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.]*

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Williams, P. J. LeB., K. R. Heinemann, J. Marra, and D. A. Purdie. 1983. Comparison of ^{14}C and O_2 measurements of phytoplankton production in oligotrophic waters. Nature *305*: 49-50.

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