

ESTIMATING PHYTOPLANKTON PRODUCTION FROM AMMONIUM AND CHLOROPHYLL CONCENTRATIONS IN NUTRIENT-POOR WATER OF THE EASTERN TROPICAL PACIFIC OCEAN^{1,2}

WILLIAM H. THOMAS³ AND ROBERT W. OWEN, JR.⁴

ABSTRACT

Previous work has shown that nitrogen is the limiting nutrient in poor (nitrate-free) water in the eastern tropical Pacific Ocean and has suggested that ammonium is the principal nitrogen source for phytoplankton in this water. Enrichment and uptake experiments with various concentrations of ammonium have provided values for the half-saturation constant, K_s , and the maximum growth rate, μ_{max} , which can be used to calculate actual growth rates with the hyperbolic model relating growth rate to limiting nutrient concentration. At two stations, growth rates calculated from ammonium concentration agreed well with those calculated from chlorophyll and ¹⁴C production, and the hyperbolic equation could be combined with that using production and chlorophyll to calculate production alone. In this paper calculated production rates are compared with those observed from ¹⁴C uptake measurements for a number of EASTROPAC cruises. The regression between calculated production and observed production is highly significant and the slope is close to 1.0, indicating reasonable agreement, particularly when all of the errors in the calculation, especially in K_s , are considered. The results suggest rather close control of phytoplankton production by the limiting nutrient, ammonium, in these near-surface, nutrient-poor waters.

This paper describes how concentrations of a limiting nutrient in sea water and some measure of the standing crop of phytoplankton can be used to estimate phytoplankton production. Estimated production is compared with observed ¹⁴C production, and the two sets of values are shown to agree reasonably well when all the errors in the estimation are considered.

The EASTROPAC Expedition series has delineated particularly well areas that are rich in nutrients and that are nutrient-poor in the eastern tropical Pacific Ocean. Rich areas in-

clude the Peru Current, the Costa Rica Dome, and an area of equatorial upwelling extending across the EASTROPAC area (from the American coast to long 119° W). Poor areas lie to the north and south of the equatorial upwelling zone and to the west of the Peru Current and Costa Rica Dome. Rich and poor near-surface waters were mapped in previous papers (Thomas, 1969, 1970b) and will be shown in detail in the EASTROPAC Atlas (Thomas, unpublished data). Nutrient values for rich and poor water are also given in Table 1 of Thomas (1970a).

Corresponding areal and seasonal changes in the phytoplankton production in this region have been observed and attributed in part to mechanisms of nutrient supply (Owen and Zeitzschel, 1970). No accounting has been possible, however, for the variations observed within the nutrient-poor surface layer of the region.

Near-surface water in poor areas is especially low in nitrate-nitrogen; this nutrient is generally not detectable (<0.1 $\mu\text{g-at./liter}$). Ammonium-N is present in concentrations ranging up to 1 $\mu\text{g-at./liter}$ and organic nitrogen can reach

¹ Contribution from the Scripps Institution of Oceanography.

² This work was part of the research of the STOR (Scripps Tuna Oceanography Research) Program. It is also a result of the EASTROPAC Expedition, a cooperative study of the biological, chemical, and physical oceanography of the eastern tropical Pacific Ocean. The work was supported by National Science Foundation Grant No. GB-8618 to the senior author, and by contracts #14-17-0007-963 and #14-17-0007-989 between the Bureau of Commercial Fisheries (now the National Marine Fisheries Service) and the Institute of Marine Resources.

³ Institute of Marine Resources, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, Calif. 92037.

⁴ National Marine Fisheries Service Fishery-Oceanography Center, La Jolla, Calif. 92037.

concentrations of 17 $\mu\text{g-at./liter}$, but this latter nitrogen source is probably not utilized by phytoplankton (Thomas, Renger, and Dodson, in press).

Prior to EASTROPAC (pre-1967) low nitrate/phosphate ratios in tropical Pacific poor water suggested that nitrogen was a limiting nutrient although ratios were increased when ammonium was included along with nitrate, and it was suggested that this latter nutrient alleviated N deficiency (Thomas, 1966).

Recent EASTROPAC enrichment experiments provided direct evidence for N limitation. Phytoplankton growth occurred in experiments where nutrients were added singly to sea water samples only with N addition, and if N was deleted from an otherwise complete enrichment, little or no growth resulted (Thomas, 1969, 1970b). The fact that photosynthetic assimilation ratios were only slightly (but significantly) decreased in poor water as compared with rich water testified further to the alleviation and control of deficiency by ammonium (Thomas, 1970a).

Having established which nutrient is commonly limiting, one can use a quantitative nutrient requirement in an appropriate mathematical model to estimate growth rates (production) from concentration of the limiting nutrient. Recent work (Caperon, 1967; Dugdale, 1967) indicates that the best model is hyperbolic:

$$\mu = \mu_{\max} \left(\frac{S}{K_s + S} \right) \quad (1)$$

where μ is the phytoplankton specific growth rate, μ_{\max} is the maximum rate which is unlimited by low nutrient concentration, S is a measured limiting nutrient concentration in sea

water, and K_s is the "half-saturation constant"—a nutrient concentration that supports a rate equal to $\mu_{\max}/2$. This equation is equivalent to the Michaelis-Menton formulation for enzyme kinetics and was first applied to bacterial growth rates by Monod (1942). Many biological processes follow the hyperbolic model and since growth is the result of a series of coupled enzymatic reactions, the hyperbolic model is the model of choice.

A previous paper (Thomas, 1970b) provides information on μ_{\max} and K_s (for ammonium) from which μ can be calculated. To obtain these values we enriched samples of nutrient-poor Pacific sea water from a depth of 10 m with a complete mixture of non-nitrogenous nutrients to which various concentrations of ammonium were added. The samples were then incubated in natural light approximating the intensity that would be found at 10 m depth. Growth was estimated by successive daily measurements of *in vivo* chlorophyll (Lorenzen, 1966) in each treatment, and rates integrated over a daily period were calculated from the maximum increases in chlorophyll. These rates were plotted against ammonium concentrations to fit a hyperbolic model and values of K_s and μ_{\max} were obtained from the plot. These values and their 95% confidence limits are given in Table 1 for two such experiments. K_s values can also be determined from uptake experiments since recent work has shown that K_s values for growth and uptake are equivalent (Eppley and Thomas, 1969). Also included in Table 1 are uptake K_s values obtained by MacIsaac and Dugdale (1969) for nutrient-poor tropical Pacific water. Their values for V_{\max} , the maximum uptake rate, are not equivalent to μ_{\max} and thus are not included

TABLE 1.—Rate parameters for growth and uptake on ammonium in nutrient-poor tropical Pacific sea water.

Cruise	Station	K_s (μM)	95 percent limits	μ_{\max} (Doublings/day)	95 percent limits	Reference
EASTROPAC 76	007	1.68	± 3.28	1.12	± 0.83	Thomas (1970b)
EASTROPAC 76	173	1.47	± 0.91	1.22	± 0.27	Thomas (1970b)
Thompson 26	15	0.10	--	--	--	MacIsaac and Dugdale (1969)
Thompson 26	36	0.55	--	--	--	MacIsaac and Dugdale (1969)
Te Vega 13	651-a	0.62	--	--	--	MacIsaac and Dugdale (1969)
Mean values		0.88		1.17		
95 % limits of mean		1.33		0.14		

in Table 1. It will be noted that confidence limits for K_s values in given experiments are large as is the confidence limit for the mean of all five values which is used in subsequent calculations (see Results and Discussion). This can be attributed to lack of precision in measuring either growth or uptake; even in controlled experiments with laboratory cultures, K_s values are imprecise (Eppley, Rogers, and McCarthy, 1969; Eppley and Thomas, 1969).

The integrated daily growth rate, μ , can also be calculated from ^{14}C production estimates and chlorophyll concentrations using the following equation:

$$\mu = \frac{3.32 [\log_{10}(R \cdot \text{chl} + \text{Prod}) - \log_{10}(R \cdot \text{chl})]}{1 \text{ day}} \quad (2)$$

as has been done for laboratory cultures by Thomas (1964) and McAllister, Shah, and Strickland (1964). In this equation R is the carbon/chlorophyll ratio; $R \cdot \text{chl}$ thus is the standing stock of phytoplankton carbon. The constant 3.32 converts logarithms to the base 10 to logarithms to the base 2 and allows μ to be expressed as doublings of phytoplankton carbon per day.

In the previous paper (Thomas, 1970b), μ calculated from ammonium (equation 1) was compared with μ calculated from ^{14}C production and chlorophyll (equation 2) for the two EAS-TROPAC stations where K_s and μ_{max} were determined from enrichment experiments. At station 76.007, μ calculated from ammonium was 0.385 doublings/day while that calculated from ^{14}C uptake and chlorophyll was 0.365 doublings/day. At station 76.173 both values were identical—0.276 doublings/day. For the calculation we used an R value of 98, that found by Eppley (1968) for nitrate-free water off La Jolla.

This excellent agreement suggested that we could set equation (1) equal to equation (2) and solve for production as a function of ammonium and chlorophyll using K_s and μ_{max} as constants. The new equation thus derived is

$$\text{Prod} = \text{chl} \cdot R \left[\text{antilog} \left(\frac{\mu_{\text{max}}}{3.32} \cdot \frac{S}{K_s + S} \right) - 1 \right] \quad (3)$$

This expression allows a direct comparison calculated and measured ^{14}C production (see Results and Discussion).

METHODS

Methods for determining K_s and μ_{max} were given previously (Thomas, 1970b; MacIsaac and Dugdale, 1969)—see also the previous section. Chlorophyll and production samples were taken from the depth of the 50 % light level, which was always in the upper mixed layer and varied from 9 to 16 m. This depth was determined by multiplying the depth at which the Secchi disc disappeared by 0.38. This factor employs the assumption that the Secchi disc disappears at 16 % of surface light intensity (Strickland, 1958).

Chlorophyll was determined in these samples by filtration on glass fiber filters, followed by 90 % acetone extraction of the filters, and measurement of fluorescence of the extract (Yentsch and Menzel, 1963; Holm-Hansen, Lorenzen, Holmes, and Strickland, 1965) using equations developed by Lorenzen (1966).

Simulated *in situ* production was measured by adding 20 μC $\text{Na}_2^{14}\text{CO}_3$ solution to the samples (Steemann Nielsen, 1952) and incubating them in a tubular shipboard incubator space in which natural light intensity was attenuated to 50 % of that incident. Incubation was started at noon and continued until sunset at sea surface temperature. Following incubation the samples were filtered through HA Millipore® filters and their radioactivity assayed ashore by G-M counting of the filters. The ^{14}C solution was standardized by liquid scintillation counting and the efficiency of the G-M counter for these filters was determined by combusting some of these and measuring the evolved $^{14}\text{CO}_2$ with an ionization chamber. Daily uptake was determined by multiplying the activity by 2; we also corrected for the isotope effect by multiplying by 1.05. Darkened samples were incubated with illuminated samples and dark uptake was subtracted from light uptake. No cor-

* The use of trade names is merely to facilitate descriptions: no endorsement is implied.

reactions for respiration by phytoplankton were made.

Ammonium was measured ashore in frozen samples from a depth of 10 m by the method of Richards and Kletsch (1964). Some labile amino-N which is probably available to phytoplankton is measured along with ammonium by this method.

RESULTS AND DISCUSSION

For the comparison of calculated and measured ^{14}C production, we have used samples

from 10 m incubated at light intensities approximating those at 10 m to determine K_s and μ_{\max} , and actual ^{14}C values from the 50% light level. We did this so that light intensities would not be a factor in the comparison—that is, light was presumed to be at saturating intensities but not inhibitory, which would be the case if surface samples had been incubated in the growth experiments and compared with surface production.

Ammonium was not determined at all production stations, and we selected those production values where data were available for

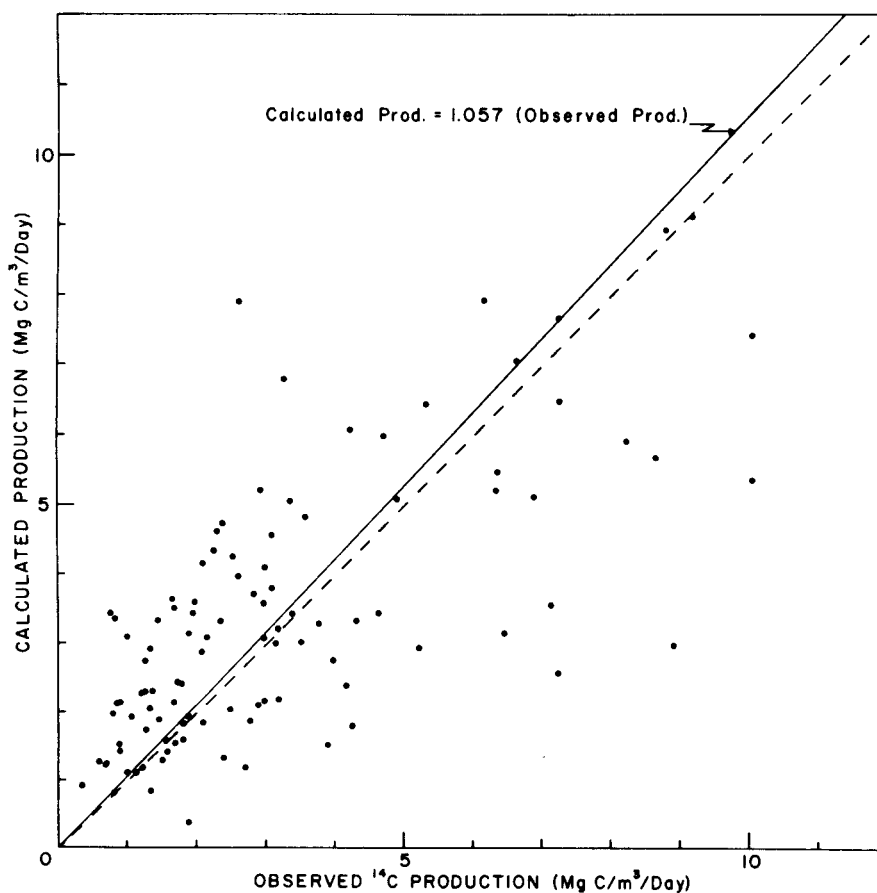


FIGURE 1.—Phytoplankton production calculated from ammonia and chlorophyll concentrations at 10 m compared with simulated *in situ* ^{14}C production at the 50% light level in northerly nutrient-poor water in the eastern tropical Pacific Ocean. The dashed line is the regression that would be expected if agreement between the two sets of production values were perfect.

ammonium and where nitrate was undetectable. One hundred and five such production stations were available from 10 EASTROPAC cruises in this nutrient-poor water.

Production calculated from equation 3 is compared with measured ^{14}C production in Figure 1. There is a highly significant ($P < .01$) relationship between the two sets of values. The slope of the regression line is 1.057, which is very near to the value 1.0 which would be expected if agreement were perfect. Nevertheless, there is a large amount of scatter in the values of Figure 1; that is, the calculation overestimates in some cases and underestimates in others.

TABLE 2.—Errors in the calculation of production.

Parameter	Standard errors	Reference
Chlorophyll	$\pm 12\%$	Holmes, Schaefer, and Shimada (1958)
R	$\pm 17\%$	Eppley (1968)
μ_{max}	$\pm 6\%$	Table 1 (this paper)
S	$\pm 5\%$	Richards and Kletsch (1964)
K_s	$\pm 76\%$	Table 1 (this paper)
Total	$\pm 79\%$	
95% confidence limits	$\pm 152\%$	

Errors in the values used in the calculation are given in Table 2. To figure total error these have been converted to variances and summed. The 95% confidence limit shows that any calculated production value can vary by ± 1.5 fold. Thus, one would expect quite a large scatter in Figure 1.

Most of the error is in K_s . When only the K_s values of Thomas (1970b) are used the calculation generally underestimates the observed ^{14}C production. Use of the mean of the K_s values of MacIsaac and Dugdale (1969) results in an overestimation. Since there is no reason to doubt either set of K_s values, we have used the overall mean K_s from Table 1. In applying this method to any other nutrient-limited waters, it would be well to obtain several values of K_s so that the error due to lack of precision in measuring K_s can be recognized.

Part of the scatter in Figure 1 may also be due to the fact that the parameter K_s is species—and temperature—dependent (Eppley, Rogers, and McCarthy, 1969) and that variations in species composition of the crop or slight var-

iations in temperature may have affected the calculation. The parameters μ_{max} and R are also probably dependent upon the species composition of the crop and on temperature. Because of these factors, which are unknown, it is perhaps surprising that the relationship between calculated and observed production is so good when constant values of K_s , μ_{max} , and R are used.

This evidence supports the hypothesis that phytoplankton production in the upper mixed layer is controlled by the limiting nutrient, ammonium, and shows that the hyperbolic model describes this control very well. In this latter connection it should be noted that if a linear model having a term " S/S_{max} " in equation 3 (where S_{max} is that concentration supporting a maximum growth rate and which has a value near $10.0 \mu\text{M}$ from the data of Thomas, 1970b) is used rather than the term " $S/(K_s + S)$," the calculation very much underestimates the ^{14}C production. The linear model was used previously by Riley (1963) and Steele (1958) but should now be considered obsolete in view of more recent work using the hyperbolic model.

ACKNOWLEDGMENTS

We appreciate the assistance of many persons in gathering these data. Ammonium analyses were performed by Mr. Edward Renger, and Mrs. Anne Dodson aided in the determination of μ_{max} and K_s . Sampling and incubation for production measurements and determination of chlorophyll concentrations were carried out by the following: Messrs. Tapuni Mulitauaopele, Michael Kruse, David Justice, James McCarthy, Lawrence Klapow, David Judkins, Gerald Johnson, Eric Forsbergh, and Jack Metoyer. Dr. Bernt Zeitzschel and Mr. Michael Kruse helped to process and edit the ^{14}C and chlorophyll data. Most of these data were collected aboard the NMFS vessel *David Starr Jordan* and we appreciate the assistance of Capt. C. W. Forster and his crew.

LITERATURE CITED

- CAPERON, JOHN.
1967. Population growth in micro-organisms limited by food supply. *Ecology* 48(5): 715-722.
- DUGDALE, R. C.
1967. Nutrient limitation in the sea: Dynamics, identification, and significance. *Limnol. Oceanogr.* 12(4): 685-695.
- EPPLEY, RICHARD W.
1968. An incubation method for estimating the carbon content of phytoplankton in natural samples. *Limnol. Oceanogr.* 13(4): 574-582.
- EPPLEY, RICHARD W., AND WILLIAM H. THOMAS.
1969. Comparison of half-saturation constants for growth and nitrate uptake of marine phytoplankton. *J. Phycol.* 5(4): 375-379.
- EPPLEY, RICHARD W., JANE N. ROGERS, AND JAMES J. MCCARTHY.
1969. Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. *Limnol. Oceanogr.* 14(6): 912-920.
- HOLMES, R. W., M. B. SCHAEFER, AND B. M. SHIMADA.
1958. SCOPE measurements of productivity, chlorophyll "a", and zooplankton volumes. In Robert W. Holmes, and other members of the Scripps Cooperative Oceanic Productivity Expedition, Physical, chemical, and biological oceanographic observations obtained on Expedition SCOPE in the eastern tropical Pacific, November-December 1956, p. 59-68. U.S. Fish Wildl. Serv., Spec. Sci. Rep. Fish. 279.
- HOLM-HANSEN, OSMUND, CARL J. LORENZEN, ROBERT W. HOLMES, AND JOHN D. H. STRICKLAND.
1965. Fluorometric determination of chlorophyll. *J. Cons. Cons. Perma. Int. Explor. Mer* 30(1): 3-15.
- LORENZEN, CARL J.
1966. A method for the continuous measurement of *in vivo* chlorophyll concentration. *Deep-Sea Res. Oceanogr. Abstr.* 13(2): 223-227.
- MACISAAC, J. J., AND R. C. DUGDALE.
1969. The kinetics of nitrate and ammonia uptake by natural populations of marine phytoplankton. *Deep-Sea Res. Oceanogr. Abstr.* 16(1): 45-57.
- MCALLISTER, C. D., N. SHAH, AND J. D. H. STRICKLAND.
1964. Marine phytoplankton photosynthesis as a function of light intensity: A comparison of methods. *J. Fish. Res. Bd. Can.* 21(1): 159-181.
- MONOD, J.
1942. Recherches sur la croissance des cultures bacterienne. Hermann et Cie, Paris.
- OWEN, R. W., AND B. ZEITZSCHEL.
1970. Phytoplankton production: seasonal change in the oceanic eastern tropical Pacific. *Mar. Biol.* 7(1): 32-36.
- RICHARDS, FRANCIS A., AND RICHARD A. KLETSCH.
1964. The spectrophotometric determination of ammonia and labile amino compounds in fresh and sea water by oxidation to nitrite. In Yasuo Miyake and Tadashiro Koyama (editors), Ken Sugawara festival volume; recent researches in the fields of hydrosphere, atmosphere and nuclear geochemistry, p. 65-81. Maruzen Company, Ltd., Tokyo.
- RILEY, G. A.
1963. Theory of food-chain relations in the ocean. In M. N. Hill (editor), *The sea*, Vol. 2, p. 438-463. Interscience Publishers, New York.
- STEELE, J. H.
1958. Plant production in the northern North Sea. *Scot. Home Dep., Mar. Res.* 7, 36 p.
- STEEMANN NIELSEN, E.
1952. The use of radio-active carbon (C¹⁴) for measuring organic production in the sea. *J. Cons. Cons. Perma. Int. Explor. Mer* 18(2): 117-140.
- STRICKLAND, J. D. H.
1958. Solar radiation penetrating the ocean. A review of requirements, data and methods of measurement, with particular reference to photosynthetic productivity. *J. Fish. Res. Bd. Can.* 15(3): 453-493.
- THOMAS, WILLIAM H.
1964. An experimental evaluation of the C¹⁴ method for measuring phytoplankton production, using cultures of *Dunaliella primolecta* Butcher. U.S. Fish Wildl. Serv., *Fish. Bull.* 63(2): 273-292.
1966. Surface nitrogenous nutrients and phytoplankton in the northeastern tropical Pacific Ocean. *Limnol. Oceanogr.* 11(3): 393-400.
1969. Phytoplankton nutrient enrichment experiments off Baja California and in the eastern equatorial Pacific Ocean. *J. Fish. Res. Bd. Can.* 26(5): 1133-1145.
1970a. On nitrogen deficiency in tropical Pacific oceanic phytoplankton: Photosynthetic parameters in poor and rich water. *Limnol. Oceanogr.* 15(3): 380-385.
1970b. Effect of ammonium and nitrate concentration on chlorophyll increases in natural tropical Pacific phytoplankton populations. *Limnol. Oceanogr.* 15(3): 386-394.
- THOMAS, W. H., E. H. RENGER, AND ANNE N. DODSON.
In press. Near-surface organic nitrogen in the eastern tropical Pacific Ocean. *Deep-Sea Res. Oceanogr. Abstr.*
- YENTSCH, CHARLES S., AND D. W. MENZEL.
1963. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. *Deep-Sea Res. Oceanogr. Abstr.* 10(3): 221-231.