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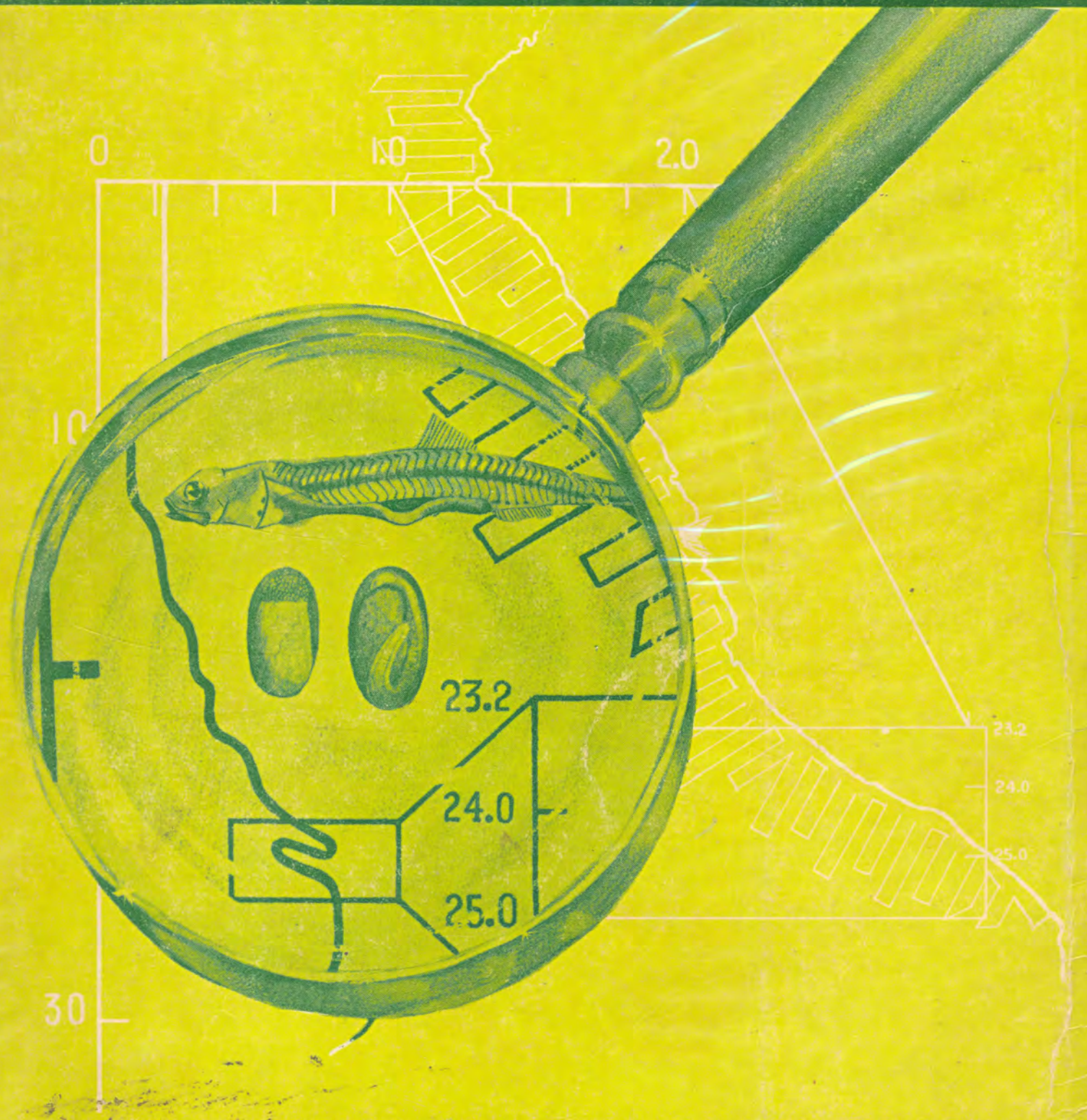

INVESTIGACION COOPERATIVA DE LA ANCHOVETA Y SU ECOSISTEMA - ICANE - ENTRE PERU Y CANADA CALLAO 1981 PERU

PHYSIOLOGICAL STATE AND RATE OF PROTEIN SYNTHESIS OF PHYTOPLANKTON OFF THE COAST OF PERU AS MEASURED BY SULFUR-35 INCORPORATION

by

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ABSTRACT

The physiological state of phytoplankton from coastal waters in the vicinity of Peru was assessed by measuring the rate of ^{35}S incorporation (S inc) into the cellular protein fraction. S inc increased during a north to south transect parallel to the coast and was maximum at station 71 located 6.7 km from the Peruvian coast. When normalized per unit chlorophyll a, S inc was generally similar at all stations, indicating actively growing phytoplankton populations. Maxima in S inc generally occurred at 11-25o/o of the surface light intensity, and were associated with maxima in phytoplankton biomass. However, elevated rates of S inc observed at lower light depths suggests that not all of the synthesis was due to phytoplankton. Protein synthesis, calculated form S inc, was high in upwelling areas. A positive relationship was found between protein synthesis calculated from S inc and $^{15}NO_3^-$ plus $^{15}NH_4^+$ uptake. S inc gave estimates of protein synthesis about three times greater than did ^{15}N.

SUMARIO

El estado fisiológico del fitoplancton de aguas costeras en las inmediaciones del Perú fue determinado midiendo la tasa de incorporación del ^{35}S (S inc) a la fracción proteica celular. El S inc aumentó de norte a sur en una línea paralela a la costa alcanzando el máximo en la estación 71 á 6.7 km de la costa. Normalizado por unidad de clorofila a, el S inc resultó en general similar en todas las estaciones, indicando un activo crecimiento de las poblaciones de fitoplancton. Los máximos de S inc ocurrieron generalmente a intesidades luminosas entre 11 y 25o/o de la de superficie y estuvieron asociados con los máximos de biomasa del fitoplancton. Sin embargo, valores elevados de S inc observados a niveles luminosos más bajos sugieren que no toda la síntesis se debió al fitoplancton. La síntesis proteica, calculada mediante la S inc, fue alta en las áreas de afloramiento. Se encontró una relación positiva entre la síntesis de proteína calculada por la S inc y la captación de $^{15}NO_3^-$ más $^{15}NH_4^+$. Los estimados de síntesis proteica por S inc resultaron cerca de tres veces mayores que los estimados por ^{15}N.

INTRODUCTION

The physiological state of marine phytoplankton can be assessed by measurement of chemical composition (Sakshaug and Holm-Hansen, 1977; Slawyk **et al.**, 1978) or by the determination of rates of selected metabolic processes (Healey, 1973; Glover and Morris, 1979). An alternative to the use of chemical composition, ^{14}C or ^{15}N to assess the physiological state has recently been developed (Bates, 1979). The method measures the rate of incorporation of ^{35}S, provided as radiosulfate, into the cellular protein fraction.

The use of radiosulfate has several advantages: addition of radiosulfate to the incubation bottle should not perturb the nutritional state of the system because sulfate, at about 2.7 g L^{-1} in seawater, is not likely to be a growth-limiting nutrient. Phytoplankton should therefore utilize sulfate in response to physiological needs rather than on the availability of the anion. Sulfate is not metabolized by animals. Sulfur-35 is a weak beta-emitting isotope of energy (0.167 MeV) similar to that of ^{14}C (0.156 MeV), and has an 87.2 d half-life.

The Peruvian upwelling ecosystem is patchy in both spatial and temporal scales (Beers et al., 1971; Sorokin and Mikheev, 1979). Knowing the physiological state of the phytoplankton within and adjacent to upwelling areas may help to predict the growth potential of phytoplankton assemblages and hence their availability to the anchovy in terms of food quantity and quality.

MATERIALS AND METHODS

Radiosulfate, as carrier-free $H_2{}^{35}SO_4$, was obtained from New England Nuclear Corp. The solution was dialyzed (1 cm diameter tubing, 12,000 mw. cutoff) into sterile, filtered distilled water for 24 h in the dark and at 20°C to eliminate possible particulate radioactive contaminants (Bates, 1979). The solution (600 μCi $^{35}SO_4^{2}$ ml^{-1} was then sealed and autoclaved in 10 ml glass ampoules. Immediately prior to an experiment, the ^{35}S stock solution was passed through a 12 mm diameter, 0.22 μm Millipore filter mounted in a Swinnex filter holder.

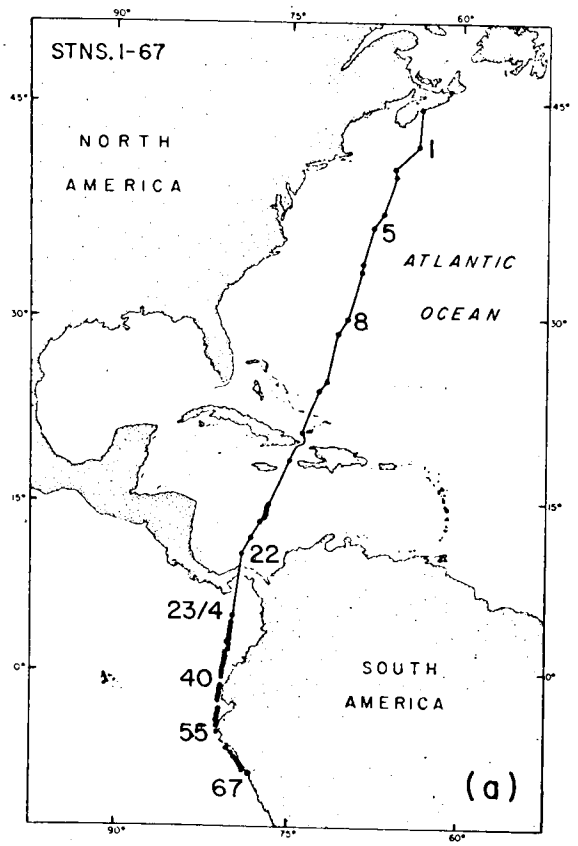
Experiments were carried out during Legs 1 and 2 at stations 23, 40, 48, 56, 71 and 75 (Fig. 1). Samples were collected at depths corresponding to 100, 50, 25, 10 and 1% light levels, and were screened through a 202 μm mesh at all stations except 71 and 75. They were then incubated with 390 μCi of radiosulfate in 150 ml bottles placed in deck incubators cooled by surface water and screened to simulate in situ light intensities. After 24 h, the labelled phytoplankton cells were collected on a 25 mm diameter, 1.0 μm Nuclepore filter. The filter was washed with 100 ml of filtered seawater, the filter chimney was removed and the filter edge was rinsed with about 20 ml of seawater from a sqeueeze bottle.

Sulfur-35 incorporation into the protein fraction was determined by extracting the labelled cells retained on the washed filter with 10 ml of 20°C 10% w/v) trichloroacetic acid for 1 min followed by 10 ml of 20°C Methanol: ether (1:1, v/v) for 1 min while the filter remained on the filter assembly. A "time-zero" filter blank was determined on a sample withdrawn immediately after addition of radiosulfate and treated identically to the experimental sample.

Rates of sulfur incorporation were claculated from:

$$U = \frac{(R_s - R_b)\,S}{R\,T}$$

where U = the rate of sulfur incorporation (mg S m^{-3} d^{-1}), R_s = the radioactivity of the filtered sample (d.p.m. ml^{-1}), R_b = the radioactivity of the time-zero filter blank (d.p.m. ml^{-1}), S = the sulfate sulfur concentration of seawater (2.7 x 10^6 mg m^{-3}), R = the radioactivity of the incubation medium (d.p.m. ml^{-1}), and T = the incubation time (d).



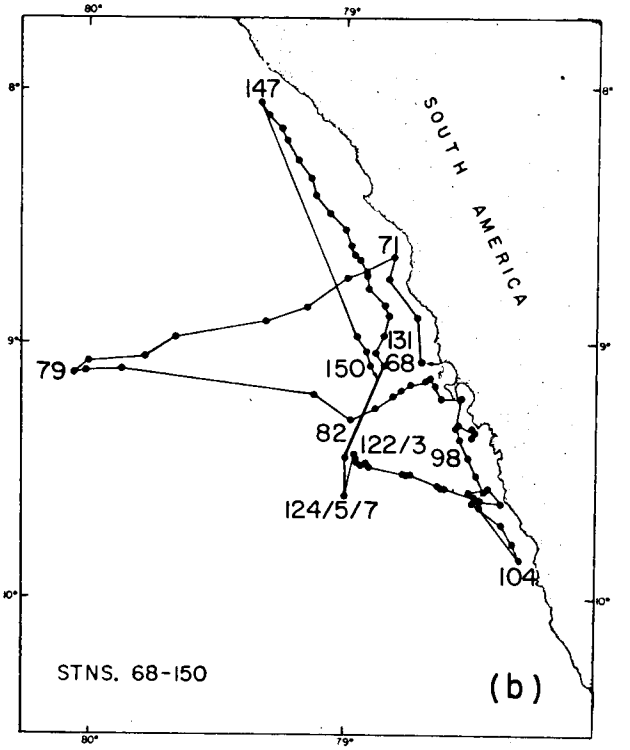


Fig. 1. Cruise track of the CSS Baffin. (a) Stations 1-67 and (b) stations 68-150.

Fig. 2. Nutrient fluxes and biomass from stations off the north western coast of South America. Sulfur incorporated (●), photosynthetic carbon uptake (○), chlorophyll a concentration (■), and particulate organic carbon concentration (▲).

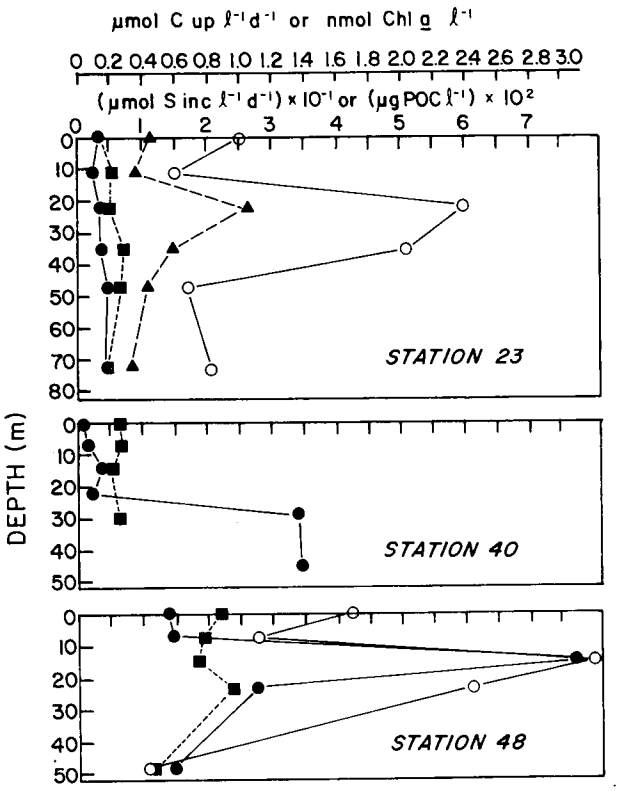
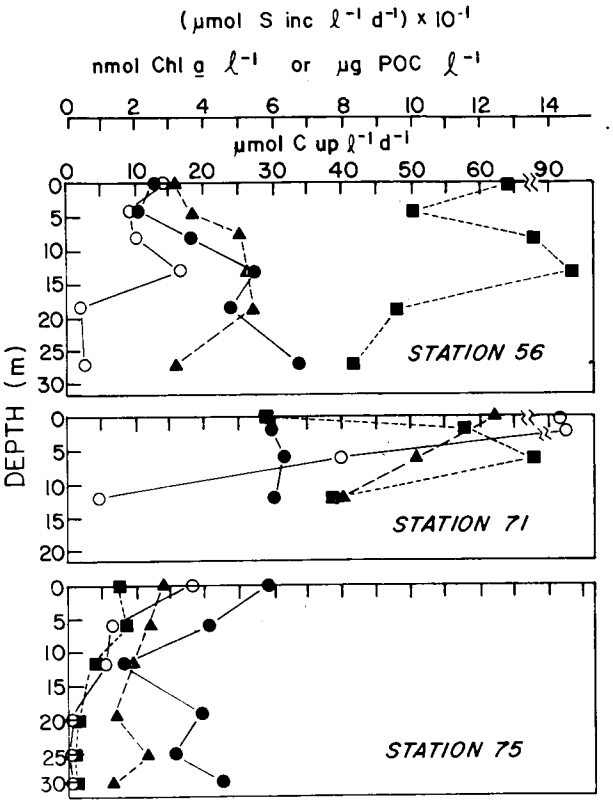


Fig. 3. Nutrient fluxes and biomass from stations off the coast of Peru. Symbols as in Fig. 2.



Radioactivity was measured with a Searle Mark II Model 6847 scintillation spectrometer, using fluor containig Spectrafluor, Triton X – 100 and toluene (0.12:1.00:1.88 v/v). Counts were corrected for quenching and for decay of the isotope.

Rates of protein synthesis were calculated from rates of ^{35}S incorporation using a protein: sulfur weight ratio of 36:1 (Bates, 1979), and from rates of $^{15}NO_3^-$ plus $^{15}NH_4^+$ uptake (W.G. Harrison, unpublished data) using a protein: nitrogen weight ratio of 6.25:1. Values for chlorophyll a (Chl a), particulate organic carbon (POC) and photosynthetic carbon uptake were obtained from Doe (1978).

RESULTS AND DISCUSSION

Rates of ^{35}S incorporation (S inc) increased along a north to south transect parallel to the coast (Fig. 2). This reflects a gradient in phytoplankton biomass from low at station 23 to high at station 71 (Figs. 2 and 3). A gradient in biomass concentration was likewise seen during the transect run perpendicular to the coast, but S inc remained about equal at the inshore and offshore stations (Fig. 3). S inc at stations 71 and 75 may have been high in part because samples were not pre-screened through a 202 µm mesh prior to incubation.

When normalized per unit Chl a, rates of S inc by phytoplankton from the northern stations (23, 40 and 48) were generally similar to those from coastal waters of Peru (Table 1). Phytoplankton from station 75 offshore exhibited higher S inc to Chl a ratios than those from station 71 inshore (Table 1). Areas of upwelling can support a higher phytoplankton biomass than non-upwelling areas due to elevated nutrient inputs. However, the physiological activity of the phytoplankton per unit biomass in the two areas may remain equally high. Active growth in non-upwelling areas may be sustained by localized nutrient regeneration on the scale of the phytoplankton cell (Sheldon and Sutcliffe, 1978; McCarthy and Goldman, 1979).

A maximum in S inc was generally found at depths coresponding to 11-25% of the surface light intensity. This maximum was roughly associated with maxima for Chl. a POC and ^{14}C uptake (Figs. 2 and 3), suggesting that phytoplankton were primarily reponsible for the ^{35}S incorporated at those depths. S inc became elevated where phytopalankton biomass decreased at depths corresponding to 1% of the incident light intensity at stations 56 and 75. Because bacteria also take up sul-

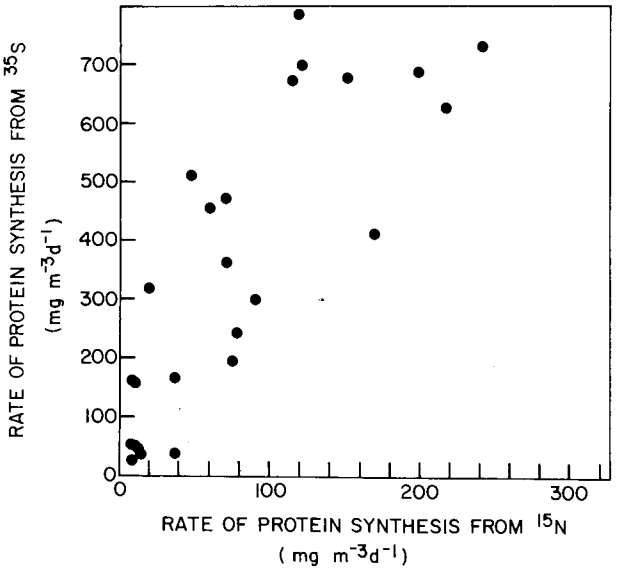
Table 1. Rates of sulfur incorporation (S inc), molar ratios of sulfur incorporation to chlorophyll a concentration, and rates of protein synthesis calculated from ^{15}N and ^{35}S data for microplankton from the west coast of South America.

Station No. and date (d-mo)	Depth (m)	S inc (mg m^{-3} d^{-1})	S inc / Chl a (mol mol^{-1} h^{-1})	Protein synthesis (mg m^{-3} d^{-1}) ^{15}N*	^{35}S**
23	0	1.0 ± 0.2	10.1	14.8	36.4
	11	0.8 0.2	4.5	8.7	27.4
(31-10)	22	1.1 0.1	7.1	37.2	38.9
	35	1.2 0.1	5.6	12.9	43.2
	47	1.5 0.2	7.6	8.3	54.4
	72	1.4 0.2	11.0	11.3	51.8
48	0	4.4 ± 0.7	6.5	10.2	157.7
	7	4.5 0.2	6.7	9.6	160.9
(02-11)	15	24.8 0.9	43.6	15.7	(893.3)
	23	8.9 1.4	12.1	19.5	319.0
	48	4.7 0.6	13.5	36.5	168.5
56	0	8.3 ± 1.3	0.8	90.8	299.9
	4	6.8 0.6	0.9	78.0	243.4
(03-11)	8	11.4 0.5	1.1	169.7	410.8
	13	17.4 7.6	1.6	218.4	625.3
	18	15.3 1.1	2.1	209.1	550.8
	27	21.8 0.2	3.4	118.9	785.9
71	0	18.7 0.4	4.2	114.0	673.2
	2	19.1 1.8	2.1	198.3	686.5
(05-11)	6	20.3 5.5	2.0	241.5	731.9
	12	19.4 2.4	3.3	121.9	699.1
75	0	18.8 ± 1.5	16.3	151.0	675.7
	6	13.2 5.2	9.6	69.9	474.1
(06-11)	12	5.4 0.7	7.8	75.4	193.0
	19	12.7 0.0	54.9	60.0	466.1
	25	10.1 6.8	109.3	71.3	362.9
	29	14.2 6.1	63.6	47.2	510.1

*(mg-at $NO_3^- + NH_4^+$ m^{-3} d^{-1}) (14) (6.25)

**(mg S m^{-3} d^{-1}) (36.0)

Fig. 4. Regression of the rate of protein synthesis calculated from the rate of ^{35}S incorporation on the plus $^{15}NH_4^+$ uptake. Data were obtained from Table 1.



fate (Schiff and Hodson, 1973), the elevated rates of S inc at those depths may be due to heterotrophic bacteria. Sorokin and Mikheev (1979) found significant bacterial production in the Peruvian upwelling ecosystem.

It is possible to calculate rates of protein synthesis from ^{35}S data because ^{35}S is incorporated into the protein fraction. Rates of protein synthesis can also be calculated independently from ^{15}N data. There is little correspondence between rates of protein synthesis calculated from ^{35}S and ^{15}N data when compared on an individual datum basis (Table 1). However, when all of the data were pooled, a positive but non-linear relationship was found between the two methods (Fig. 4). A linear regression gives $y = 3.0x + 116.3$ ($n = 25$; $r = 0.66$). Estimates of rates of protein synthesis were about three times greater using ^{35}S compared to ^{15}N. This may indicate that the ^{15}N-labelled nitrate or ammonia added to the incubation bottles did not represent all forms of nitrogenous nutrients available to the phytoplankton in the water column. In addition, protein synthesis would be underestimated if unlabelled nitrogen compounds were preferentially incorporated into the cellular protein fraction.

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