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Protein Synthesis by Lake Plankton
Measured using in situ Carbon Dioxide
and Sulfate Assimilation

by

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MANAGEMENT PERSPECTIVES

The estimation of "growth" of planktonic communities has been complicated by an inability to separate growth of bacteria which use organic substrates from algae that fix carbon dioxide for growth. Quantification of carbon recycling via algal respiration and excretion also remains elusive. These problems were avoided by measuring protein production. Furthermore, the subcellular composition of other newly formed biopolymers (carbohydrate and lipid) was estimated. These methods provided doubling times for the plankton population of 10 to 20 days instead of the 3-5 days from previous methods. This information makes the modelling of transport rates of nutrients much different. Recyling through zooplankton grazing is in balance with growth. Such food chain studies are a prerequisite for contaminant process studies. As part of the present study, pathways for sulfate metabolism in a lake ecosystem are also described.

PERSPECTIVE-GESTION

Il est difficile d'estimer la croissance des communautés planctoniques dans la mesure où on ne parvient pas à distinguer la croissance des bactéries qui se nourrissent de substrats organiques de celle des algues qui métabolisent le bioxyde de carbone. Les efforts visant à quantifier le recyclage du carbone par suite de la respiration et de l'excrétion des algues n'ont donné que des résultats imprécis. Nous avons contourné ces difficultés en mesurant la production de protéines. Nous avons en outre estimé la constitution infracellulaire d'autres polymères vivants nouvellement formés (glucides et lipides). Nos méthodes ont révélé un temps de doublement de 10 à 20 jours par opposition à celui de 3 à 5 jours avancé antérieurement. Cette information entraîne d'importantes répercussions sur la modélisation des taux de transport des substances nutritives. Le zooplancton phytophage compense la croissance de la fraction végétale et maintient une situation d'équilibre par sa fonction de recyclage. Les études de ce genre sur les chaînes alimentaires sont absolument indispensables pour ouvrir la voie aux études sur le cheminement des contaminants. Dans la cadre de cette même étude, on définit également le cheminement du métabolisme des sulfates dans un écosystème lacustre.

Cuhel, R. L., and D. R. S. Lean. 1987. Protein synthesis by lake plankton measured using in situ carbon dioxide and sulfate assimilation. Can. J. Fish. Aquat. Sci. 44: ØØØ-ØØØ.

Sequential 4-6 h in situ measurements of carbon dioxide and sulfate uptake showed mid-day deepening of the depth of P_{max} and photoinhibition of upper water column samples. Analysis of subcellular fractions accentuated total uptake measurements, with net protein synthesis providing a direct measure of growth. The percentage of carbon assimilated into protein was smallest at the depth of maximum photosynthesis and increased with light limitation. Summed incubations agreed well with all-day deployments for total carbon fixation and protein synthesis. Assimilation numbers were consistently low (<2.5 g C g Chl $\underline{a}^{-1}$ h^{-1}) with integrated (Ø-2Ø m) areal production of 616-1467 mg C m^{-2} and 7.5-32.4 mg S m^{-2} during the light day. Non-reductive sulfate assimilation (predominantly ester-SO_4^-) accounted for up to 4Ø% of the total sulfate uptake when diatoms predominated. Protein synthesis measured with ^{35}S (2ØØ-1ØØØ mg protein m^{-2} during the light day) increased 57-89% overnight. Hourly rates were similar during light and scotophase incubations. Night metabolism substantially altered the biochemical composition (e.g. protein, lipid, and carbohydrate) of the plankton with respect to newly-incorporated carbon. Combined plant-specific $H^{14}CO_3^-$ and general microbial $^{35}SO_4^{2-}$ techniques suggested algal dominance in the mixed layer.

Cuhel, R.L. and D.R.S., Lean. 1987. Mesure de la synthèse de protéines par le plancton à partir de l'assimilation de bioxyde de carbone et de sulfate in situ. Journal canadien de sciences halieutiques et aquatiques. 44:000-0000.

Des mesures séquentielles in situ toutes les 4 à 6 heures de la teneur en bioxyde de carbone et en sulfate ont révélé que le niveau de photosynthèse maximale se situait à une profondeur plus grande au milieu de la journée et que la lumière avait un effet inhibiteur dans les échantillons de la portion supérieure de la colonne d'eau. L'analyse des fractions infracellulaires a permis de mettre en évidence les mesures de l'assimilation totale; nous nous sommes fondés sur la synthèse protéique nette pour mesurer la croissance directement. La proportion de carbone qui est asssimilé pour la synthèse de protéines atteint son point le plus faible à la profondeur correspondant à la photosynthèse maximale et augmente au fur et à mesure que la lumière pénétrante décroit. La sommation des incubations concorde bien avec les les incubations se prolongeant toute la journée pour ce qui est de la fixation totale de carbone et la synthèse de protéines. Les mesures de l'assimilation tendent uniformément à la baisse (2,5g C Chl a^{-1} h^{-1}) tandis que les productions par unité de surface intégrées (de 0 à 20 m) s'échelonnent respectivement de 616 à 1 647mg C m^{-2} et de 7,5 à 32,4mg S m^{-2} au cours de la partie diurne de la journée. L'assimilation non-réductive de sulfate (principalement sous forme d'ester-SO_4^-) représente plus de 40 p. 100 de l'assimilation totale lorsque les diatomées prédominent. La synthèse protéique mesurée à l'aide du ^{35}S (200-1 000mg protéine m^{-2} au cours de la cours de la partie diurne de la journée) augmente de 57 à 89 p. 100 pendant la partie nocturne de la journée. Les taux horaires demeurent semblables pendant les périodes d'incubation illuminées et obscures. Le métabolisme nocturne transforme considérablement la constitution chimique du plancton (c'est-à-dire les protéines, lipides et glucides) pour ce qui est du carbone nouvellement incorporé. L'emploi de techniques de mesure du $H^{14}CO_3^-$ propres au phytoplancton et des techniques microbiennes générales fondées sur le $^{35}SO_4^{2-}$ semble indiquer que les algues dominent dans la couche mixte.

The biochemical composition of newly-formed particulate material resulting from phytoplankton growth is modulated by light, temperature, species composition, and the nutritional state of the plankton. Recently developed methods for measurement of macromolecular synthesis by phytoplankton permit analysis of specific end products of carbon fixation (e.g. protein, carbohydrate, and lipid) and may provide a means of identifying nutrient deficiency in phytoplankton by accumulation of carbon in carbohydrate and lipid storage products beyond daily growth requirements (Antia et al. 1963; Antoine and Tepper 1969; Strickland et al. 1969; Hobson and Pariser 1971; Konopka and Schnur 1981). The relative content of protein, lipid, and carbohydrate in phytoplankton may also affect the value of microplankton as a food source for herbivores (Scott 1980).

Sulfate incorporation into protein provides a quantitative measurement of net protein synthesis by microplankton (Bates 1981; Cuhel et al. 1982). In combination with carbon assimilation studies it enables detection of biomass turnover in circumstances when algal growth is slow or absent (Cuhel et al. 1984). Since sulfate is taken up as needed, the method can be used to demonstrate net protein synthesis by microplankton at night, a phenomenon which is closely linked to diel patterns of carbon metabolism. In addition, total sulfate uptake and the ratio of reductive to non-reductive sulfate

assimilation (see below) gives useful information for construction of geochemical cycling models in aquatic systems.

The combined measurements of sulfate uptake and subcellular incorporation patterns for both carbon and sulfur were applied to several specific problems or questions in lake productivity analysis: (1) investigation of effects of sampling time-of-day and in situ incubation time on productivity estimation; (2) determination of areal rates of reductive sulfate assimilation and protein synthesis, with comparison to ^{14}C-bicarbonate uptake and incorporation patterns; and (3) measurement of night protein synthesis by ^{35}S-sulfate incorporation into protein and related night changes in carbon metabolism following in situ incubation. Because in situ methods take into account small scale temperature gradients, light quality, and differences in phytoplankton species composition, abundance, and light adaptation with depth, they may provide more accurate estimates of production than obtained with artificial light incubators. In most cases, in situ studies were accompanied by time-course light intensity experiments (Cuhel and Lean 1987).

Pathways of Sulfate Assimilation

Sulfate transport is an energy-requiring process which concentrates sulfate against an external-internal concentration gradient (Deane and O'Brien 1975; Jeanjean and Broda 1977; Cuhel

et al. 1981). Once inside the cell, the relatively inert sulfate ion must be activated by ATP (Fig. 1); the activated sulfate (APS or PAPS) is subsequently available for reductive assimilation into cysteine, methionine, and sulfur-containing peptides (e.g. glutathione) and protein (see Tsang and Schiff 1975). Alternatively, several important non-reductive pathways of sulfur assimilation have been described, including synthesis of the plant sulfolipid (Benson 1963) which is an obligate requirement for photosynthetic electron transport and hence a component of all photosynthetic membrane structures (Menke et al. 1976; Sinensky 1977). A second major sulfur-containing product is acidic sulfate esters of polysaccharides, common among diatoms, red, and brown algae (Ramus 1974; Percival et al. 1980). Both sulfolipid and ester sulfate synthesis are carried out by algae to the greatest extent, although some bacterially-derived sulfate esters have been reported (Fitzgerald 1976). Finally, the involvement of S-adenosyl methionine (SAM) in methylation reactions is shown in Figure 1: although the sulfur atom of methionine is not transformed in the methylations, there is a broad range of environmentally important products arising from SAM-catalyzed methylations which may be investigated through studies with radiolabeled methionines.

The geochemical fate of biogenic sulfur in the water column and in sediments depends on the degree to which the sulfur is reduced. Most biomass sulfur is fully reduced to the level of

sulfide (S^{2-}) and can be either incorporated directly by decomposers after protein hydrolysis or released to the environment as H_2S (from amino acid fermentation) or as volatile sulfur compounds (e.g. dimethyl sulfide) to the atmosphere. The latter process accounts for about 30 Tg S per year in the global atmospheric sulfur cycle (Andreae and Raemdonck 1983). The oxidized forms mentioned above are more refractory to degradation and probably become involved in sedimentary processes. By virtue of its hydrolysis-resistant C-S bond, the plant sulfolipid may persist in sediments and provide a marker for phytoplankton sedimentation. More quantitatively significant is the ester sulfate of acidic polysaccharides (Nriagu and Soon 1985): its labile $-C-O-SO_3^-$ linkage is susceptible to arylsulfatase-catalyzed hydrolysis in sediments (Nriagu and Soon 1984); combined with its high proportion of total sulfur fixation in some circumstances can result in substantial inputs of inorganic sulfate required for sedimentary sulfate reduction. We have therefore considered sulfate assimilation in the context of both total and reductive (i.e. excluding sulfate esters and sulfolipids) forms. Whole cell sulfate uptake measurements (cf. Jassby 1975; Campbell and Baker 1978; Monheimer 1978b) do not differentiate between reductive and non-reductive assimilation, but the distinction may be of value in models of biogenic reduced sulfur inputs for acid rain and related considerations.

MATERIALS AND METHODS

Water samples were collected from the CSS LIMNOS in 5-liter Niskin bottles deployed on a rosette sampler with temperature sensor. The samples, collected from fixed depths, were drawn into steam-cleaned 300 mL BOD bottles and spiked with either ^{14}C-bicarbonate (2 GBq $mmol^{-1}$; 20 DPM ngC^{-1} final specific activity) or ^{35}S-sulfate (carrier-free; 125 DPM ngS^{-1} final specific activity). The bottles were returned to the original depths on a mooring several hundred metres from the ship. For back-to-back incubations, three moorings were deployed, with one reserved for an all-day incubation. Short-term experiments were sequentially deployed and removed from the remaining two moorings. Sub-surface light intensity was determined with a submersible LiCor quantum sensor near local noon, and total daily light flux was calculated from pyroheliometer data.

In general, preincubation manipulations were carried out in subdued light before dawn. Unless specified otherwise, "^{14}C" and "^{35}S" refer to filter-retainable radioactivity derived from labelled sodium bicarbonate and sodium sulfate, respectively.

At the end of each incubation, ^{14}C and ^{35}S-labeled samples were filtered through Whatman GF/F filters using the punch funnel described in Cuhel et al. (1981) to reduce isotope adsorption. The filters were then frozen (-20 C) for subsequent laboratory processing. The subcellular fractionation procedure

used to separate low molecular weight (LMW), alcohol-soluble protein, lipids, hot trichloroacetic acid (HTCA)-soluble material, and residue protein is described in Cuhel et al. (1984).

The long turnover time for sulfate (>10 years) requires the use of high radioisotope activities. However, the subcellular fractionation procedure isolates contaminating, unfixed sulfate in the LMW fraction and it is easily removed by barium precipitation. For this reason low blanks in the other fractions can be routinely obtained without the rigorous isotope preparation employed by Bates (1981).

Radioisotopes were obtained from New England Nuclear Corp. and Amersham Searle. Filters for whole cell uptake and aliquots of derived fractions were counted in PCS (Amersham Searle) by liquid scintillation on a Searle Mark III using the external starndards method of quench correction. All data for ^{14}C-bicarbonate uptake were corrected for isotope discrimination using the factor of 1.06 recommended by Steemann Nielsen (1952). Incorporation of ^{35}S-sulfate into biomass occurs without isotope discrimination (McCready et al. 1975).

Synoptic analyses of dissolved nutrients and particulate carbon, nitrogen, phosphorus, and chlorophyll a were provided by the Water Quality Laboratory, CCIW.

Composition of the subcellular fractions

This study relies on a subcellular fractionation procedure

which separates classes of molecules based on their sequential solubilization by a variety of relatively specific solvents under appropriate extraction conditions (Roberts et al. 1963). Because it is critical to understand possible ambiguities arising from the actual composition of particular intracellular fractions, an overview of the types of labelled molecules expected in each fraction is given below:

1) Low Molecular Weight (LMW): The cold trichloroacetic acid (TCA) soluble fraction contains a wide variety of metabolic intermediates and all the inorganic ions. Sugars and sugar phosphates, amino acids and peptides, nucleotides, vitamins and co-enzymes are found in this fraction. Precipitation of inorganic sulfate with barium leaves in solution the "LMW organic-S" component of ^{35}S-labelled samples. The loss of cysteine or methionine is negligible.

2) Alcohol Soluble Protein (EtOH): Samples labelled with ^{35}S can contain a significant amount of label in the alcohol-soluble, ether-insoluble portion of the lipid extract. The aqueous phase of the partitioned extract contains proteins with amino acid compositions similar to that of the residue protein (Roberts et al. 1963) and probably consists of more hydrophobic membrane proteins such as permeases and electron transport system proteins. The proportion of ^{14}C incorporated into this fraction is trivial (<1%) so for ^{14}C it is not separated and is therefore included in the lipid fraction.

3) Lipid: This contains ether-soluble membrane lipids, neutral fats, and sterols. Sulfolipid, an integral part of photosystem II, found only in photosynthetic organisms, is contained in this fraction.

4) Hot TCA-Soluble (HTCA): Mono- and oligosaccharides from the hot acid hydrolysis of polymeric carbohydrates, mono- and oligonucleotides from hydrolysis of ribonucleic acids (RNA) and deoxyribonucleic acids (DNA) are the primary constituents of the HTCA material. Hydrolyzed bacterial cell walls (hexosamines) and a small amount of protein can also appear in this fraction. In ^{35}S-labelled samples the hydrolysis of sulfated polysaccharides releases inorganic sulfate which can be precipitated with barium, subdividing HTCA into "Ester-SO_4^-" and "HTCA Organic-S" components.

5) Protein: The final residue contains the bulk of cellular protein, but cellulose is also resistant to the above treatments and is included in this fraction. Although cellulosic cell walls are not common in algae, they can occur in some species of the Chlorophyceae, Chrysophyceae, Dinophyceae, Prasinophyceae, Rhodophyceae, and Xanthophyceae (Sournia 1981).

While replicate samples generally agree very well (c.f. Cuhel et al. 1984) instances of mismeasurement, filter inefficiency, etc. do occur. We emphasize the fact that within an experimental treatment subcellular distribution, as percent of total fixed label in specific fractions, is independent of the

total amount of isotope retained on the filter. The precision of subcellular distribution is in fact better than that of total uptake, which allows resolution of smaller differences among samples and can help identify aberrant whole cell uptake data. All data reported herein for total uptake are the sum of the derived fractions. Unprocessed filters labelled with ^{14}C were regularly counted to verify recovery. We can assume a similar recovery for ^{35}S in the replicate samples since the fractionation procedure was essentially identical.

RESULTS

Diurnal cycles of photosynthetic capacity (Jorgensen 1966; Prezelin et al. 1977) and incorporation of carbon into macromolecules (Morris and Skea 1978; Cuhel et al. 1984) are well documented. The rhythms may result from mixing in the water column and exposure to variable light fields (Marra 1978a,b); hence, all-day in situ incubations which retain the sample at a fixed depth for prolonged periods may introduce artifacts in total production measurement. Since it is not always practical to make repeated in situ deployments during the day, on several occasions we compared total uptake and subcellular incorporation patterns of ^{14}C-bicarbonate from three sequential in situ incubations to results from samples incubated all day. Physical and standing crop measurements of the stations are summarized in Table 1.

The first example is from inshore station 4Ø1 on 8 June 1982 when the water column was weakly stratified under thermal bar conditions (Simons and Schertzer 1987). Subsurface maxima for total carbon fixation and incorporation into protein and lipid were evident during each incubation period (Fig. 2). Photoinhibition of ^{14}C-bicarbonate uptake was most pronounced in mid-day samples at 1 and 2.5 m. The $\%C_{prot}$, an indicator of photoinhibition in light intensity experiments (Cuhel and Lean 1987), displayed the most marked increase at the surface in the late afternoon incubation. At this time, although the magnitude of the subsurface maximum was less pronounced, the hourly rate of photosynthesis was reduced at all depths to values similar to early morning incubations. Incorporation of CO_2-C into protein was much more uniform with depth and time of day than was total carbon fixation or incorporation into lipids.

The all day incubation yielded lower values for CO_2 uptake and incorporation than the sum of the sequential incubations, though the differences were generally $< 25\%$. Discrepancies were greatest below 5 m, but there was very little difference in the %C among fractions, shown for $\%C_{prot}$ in Figure 2.

Net protein synthesis was measured using ^{35}S-sulfate for the all day incubation. Vertical profiles for total uptake and subcellular distribution of CO_2-C and SO_4-S (Fig. 3) were similar in the depth of the principal subsurface maximum for

total uptake and the shape of the protein production profile. Total light:dark uptake ratios for sulfate increased from the surface to a maximum at the productivity maximum. At 10 m and below the ratio was about half that at P_{max}. Below 45 m microbial growth was essentially undetectable by these techniques.

Ester sulfate dominated the subcellular distribution of sulfur, comprising up to 40% of the total fixed sulfur. Ester-S was far more significant in the upper water column: both $\%S_{ester}$ and total ester decreased rapidly with depth (Table 2). The light:dark ratio of ester sulfate synthesis was also depth dependent. Sulfur incorporation into protein was a relatively constant proportion of total sulfur metabolism in the upper 12.5 m, below which it increased. Samples from deeper in the water column had increasing proportional incorporation of sulfate into protein in the dark (Table 2).

Whole cell and protein C:S ratios were similar, increasing from 50 at 1 m to almost 90 at 10 m (Fig. 3). Below this depth both decreased rapidly. The difference between total-C:total-S (whole cell) and total C:reduced-S emphasized the large contribution of ester sulfate to total sulfur uptake, especially in the upper 10 m. The total-C:reduced-S ratio is more appropriate to physiological interpretation because ester-SO_4^- is generally associated with extracellular polysaccharides, hence total-C:reduced-S will be used for the remainder of this paper.

The in situ experiment decribed above took place during

stratification at the inshore station. The physical instability may have resulted in sampling of different plankton populations at each depth throughout the day. For accurate comparison of sequential and all day incubations it is necessary that similar populations are repetitively sampled at each depth. The following examples were obtained at stations with well defined thermoclines which restricted mixing to the epilimnion.

A three part back-to-back in situ deployment at the south shore station (405) on 1 July 1982 represented conditions of strong stratification and high chlorophyll a concentration (Table 1). Subsurface maxima for total carbon fixation and its assimilation into lipid and protein occurred only during the mid-day incubation (Fig. 4), and there was only slight evidence of photoinhibition based on $\%C_{prot}$, despite nearly 1800 uEinst. m^{-2} s^{-1} of incident solar radiation at noon. Photosynthesis fell off rapidly below the level of P_{max} (depth of 1% light penetration, 10.9 m) accompanied by increased proportional incorporation of carbon into protein indicative of light limitation. In contrast to the previous example, the agreement of summed sequential incubations with the all day deployment was excellent for total photosynthesis and subcellular carbon incorporation patterns.

Depth profiles of total sulfate uptake and incorporation into protein and ester components differed from those for carbon only in the morning incubation (Fig. 5) when the rate of decrease with depth was not as steep and the depth of maximum protein

synthesis was slightly deeper for sulfur. In all other respects the two measurements provided very similar results. Protein-S from the all day deployment agreed well with the sum of the sequential experiments, and the lower all day total sulfur was attributable to lower ester sulfate labelling. As for the previous example, ester sulfate was much less significant deeper in the water column and the proportion of sulfur incorporated into protein increased with depth in all cases (Fig. 5). Both protein C:S and total-C:reduced-S were lower than the June experiment and generally resembled the depth profile for photosynthesis.

Depth profiles of the light:dark sulfate uptake ratio displayed sub-surface maxima at the depth of P_{max} in all cases (Table 3). Light stimulation of sulfate uptake increased markedly with incubation time, such that ratios from all day incubations were generally 2-3 times the average for part day samples. Quantitatively, summed dark sulfate uptake was about 3 times greater than the all day rate, possibly reflecting enhanced algal dark uptake after previous light exposure. The midday dark uptake rates were almost twice those from the early morning sampling which had no prior light exposure. However, hourly dark sulfate uptake decreased to morning values in the late afternoon deployment. Light:dark ratios for $\%S_{ester}$ were >1 and for $\%S_{prot}$ <1 only during the morning incubation (Table 2). These features were completely eliminated in later incubations but were again

apparent in all day samples.

All day incubations may also yield systematically greater daily photosynthesis relative to summed part-day deployments, as shown for station 403 on 26 August. Although temperature was uniform in the upper 10 m (Table 1), there was a surface maximum in chlorophyll a. In turn, subsurface maxima in total carbon dioxide fixation occurred throughout the day, with an extremely steep gradient during the mid-day incubation (Fig. 6). The pronounced mid-day depression of surface photosynthesis was accompanied by the only instance of a subsurface minimum in the $\%C_{prot}$ (Fig. 6).

As in the previous examples (Figs. 2 and 4), the amount of carbon fixed into protein was very similar between all day and sum-of-parts incubations, with even smaller differences than for total carbon uptake (Fig. 6, bottom panel). Only the 1 m all day incubation had lower protein-C as well as much lower total production. The small differences in protein carbon relative to total carbon fixation tended to accentuate a subsurface minimum in $\%C_{prot}$ for the all day incubation. The isothermal composition of the upper 10 m combined with winds increasing to 20 knots on that day may have promoted sufficient mixing to prevent in situ photoinhibition at the surface (Putt et al. 1987 and hence enable greater photosynthesis in the 1 m afternoon sample.

Both total uptake and subcellular distribution of sulfur during the all day incubation were well correlated with those for

carbon (Fig. 7). The extent of surface depression in uptake was less pronounced for sulfur, resulting in a large increase in the cellular C:S ratios between 2.5 and 7.5 m. A slight increase in the light:dark sulfate uptake ratio was also found in this region. A similar, though muted trend was found for protein C:S ratios. The proportion of sulfur incorporated into protein increased below the depths of high productivity to slightly greater than 50% of the total sulfur assimilated in the deepest sample.

The three experiments described above demonstrate a very strong influence of time-of-day on the depth distribution and magnitude of photosynthesis measured by in situ techniques. Protein synthesis, measured by ^{14}C incorporation into protein, was much more uniform with depth than total carbon fixation, and this was supported by sulfur incorporation into protein in parallel incubations at station 405 (1 July 1982; Fig. 5). The smaller decrease in protein synthesis relative to total carbon fixation at photoinhibiting irradiance, documented during light intensity experiments (Cuhel and Lean 1987), was demonstrated for mid-day incubations during all three experiments.

Night metabolism and protein synthesis in situ:

Loss of previously fixed carbon during night respiration of algae is often a significant component of the total daily particulate organic carbon budget. A portion of the energy thus

produced can be used for algal protein synthesis, leading to net assimilation of sulfate-S into protein accompanied by loss of carbon from energy storage reservoirs (e.g. LMW metabolites, polymeric carbohydrates, neutral lipids, etc.). Previously reported measurements from incubations in a light gradient implicated light history as a dominant factor controlling the extent of night metabolism (Morris 1981; Cuhel et al. 1984; Cuhel and Lean 1987). Overnight incubations during in situ productivity measurements provided corroborative data and reaffirmed the potential significance of night metabolism to nutrient flux and phytoplankton chemical composition. The following example, from the 26 August deployment at the central lake station, demonstrates several important processes associated with night algal growth.

All day incorporation vs. depth profiles for $H^{14}CO_3^-$ and $^{35}SO_4^{2-}$ (Fig. 7) included very strong subsurface assimilation maxima for both tracers, and this feature was also evident in the night component of C and S metabolism. At 5 m, the depth of daytime P_{max}, total ^{14}C decreased by nearly 25% (Fig. 8). Respiration and redistribution of ^{14}C-labeled organic material reduced the polymeric carbohydrate (HTCA) and to a lesser extent LMW metabolite pools, but very little change in lipid-^{14}C was noted. Protein was the only fraction to increase in ^{14}C activity overnight. Net synthesis was confirmed by parallel incorporation of ^{35}S-sulfate into protein, although sulfate was assimilated

into cell components in proportions similar to daytime growth. No net loss of sulfur was observed during night incubation, and the sub-surface peak of activity was much less pronounced than for carbon.

Night metabolism of in situ incubated plankton was far from trivial in Lake Ontario experiments. For the three experiments containing overnight components one third to one half of the total daily protein synthesis occurred at night, based on the assimilation of sulfur into the protein fraction (Table 4). The somewhat lower proportional increases of protein carbon reflect the varying specific activity of the newly-incorporated ^{14}C in the different macromolecular fractions contributing to night protein synthesis. In general 1 mg protein-^{14}C was incorporated at night for every 2-4 mg total ^{14}C respired or otherwise lost from the particulate matter (excretion was not measured). We stress the terms mg ^{14}C- since the transformation of carbon among fractions and the environment occurs with a wide range of specific activities and hence need not reflect actual weight values for total particulate carbon. In contrast, ^{35}S is incorporated from exogenous supplies as needed and, with its small precursor pools, provides quantitative rates during both day and night incubations.

DISCUSSION

Though long-term deployments for in situ carbon assimilation

often result in lowered daily estimates of photosynthesis (Vollenweider and Nauwerek 1961; and others as reviewed in Harris 1978), from the results of the three detailed in situ experiments, it can be concluded that in Lake Ontario, all day in situ deployments could be used as quantitative measurements of in situ primary productivity. Each represented a specific situation, i.e. thermal bar development, stratification during maximum phosphorus deficiency, and season's-end destratification. Total uptake and subcellular assimilation patterns of ^{14}C-carbon dioxide were remarkably similar between all day incubations and the sum of sequential deployments: considering the possible differences incurred in repetitive sampling of natural populations the <25% "error" between the methods may be considered negligible. There was also similar agreement for ^{35}S-sulfate in the example provided.

Within the particular experiments, however, there appeared several consistent features bearing on the primary productivity of the water masses themselves. The first of these involves photoinhibition of primary production by surface populations. It has been emphasized that retaining samples in bottles near the surface prevents turbulent mixing through the water column experienced by unrestrained populations (cf. Marra 1978b). While this may well affect many measurements, cases may occur in which the mixed layer is sufficienntly shallow that photoinhibiting irradiance exists throughout the mixed layer.

Such an example may be found in the 8 June experiments (Fig. 2). The apparent depression of photosynthesis in surface samples during midday and afternoon incubations coincides with the only occassion in which an independent measure of photoinhibition, DCMU-enhanced fluorescence, also indicated in situ photoinhibition (Putt et al. 1987). In our other experiments, in situ photoinhibition may not have occurred: to assess the magnitude of error introduced by the in situ incubation we have calculated the integrated daily production with and without photoinhibition (i.e. assuming the chlorophyll a-specific rate at the depth of P_{max} was maintained to the surface, Table 5). In fact the greatest potential error was found on the day when DCMU-enhanced fluorescence suggested the occurrence of in situ photoinhibition. Overall, however, error due to this factor was much less than 10% for full day productivity estimation.

Vertical heterogeneity in the composition of the plankton population was well demonstrated in the 8 June experiment at station 401. Because long-shore current velocities were highly variable (Simons and Schertzer 1987), sequential sampling points at a given depth did not necessarily contain similar populations. Vertical gradients in production characteristics were also encountered and were expressed most clearly in the subcellular distribution of incorporated tracers. While deeper samples were exposed to lower light fluxes, the variability in % ester sulfate (Table 2) with depth was much greater than could be attributed to

light intensity effects alone, based on an incubator experiment at the same station the next day. In fact, very little light effect was demonstrated for the proportion of sulfur entering this product in the homogeneous population of the light intensity experiment, as found for the 1 m in situ sample.

The magnitude of increase in the proportion of protein-S with depth (Table 2) was also much greater than the weak effects of light intensity alone (Cuhel and Lean 1987). The proportion of sulfur in bacterial protein is considerably larger (usually >70%) than for algae (Cuhel et al. 1984): the maximum of 63% at 20 m is reminiscent of typical bacterial sulfur distribution (Cuhel et al. 1982) suggesting increased heterotrophic activity relative to phytoplankton in the deepest samples. Further evidence for bacterial activity was found in comparison of the subcellular distribution of sulfur in light and dark-incubated samples. Algal protein turnover in the absence of net synthesis results in ^{14}C incorporation into protein but not of ^{35}S (Cuhel et al. 1984). Hence the unusually high protein C:S ratios in the upper water column may be indicative of substantial algal protein turnover, but this conclusion remains tentative. Further work is necessary to clarify the meaning of the C:S ratio when applied to mixed microbial populations.

During the 1982 Lake Ontario Nutrient Assessment Study (LONAS), 6 all day in situ measurements of carbon and sulfur metabolism were conducted, three at inshore stations (401 and

405) and three at the central lake station (403). Depth profiles for temperature, chlorophyll *a*, assimilation number, and the % C or S incorporated into protein are shown in Figs. 9 and 10 (inshore and central lake stations, respectively). Maximum assimilation numbers (g C g Chl a^{-1} h^{-1}) were 1.1-1.5 (inshore) and 0.9-2.2 (offshore), significantly lower than the 1.1-4.5 and 1.6-5.7 reported by Glooschenko et al. (1974) and Stadelmann et al. (1974) for similar Lake Ontario stations during the International Field Year of the Great Lakes (IFYGL). In most cases sub-surface maxima for assimilation number coincided with minima in % C incorporated into protein, but no relationship was found between assimilation number and % S in protein. This is consistent with the lack of light intensity effect on patterns of sulfur metabolism in general (Cuhel and Lean 1987).

Even though photosynthetic efficiency and soluble reactive phosphorus (Stevens et al. 1987) were markedly lower in the lake, June-September integrated areal production estimates (0.6-1.5 g C m^{-2} day^{-1}) were in good agreement with those of Stadelmann et al. (1974; 0.3-1.9 g C m^{-2} day^{-1}).

Integrated carbon fixation : sulfate reduction ratios averaged 99 by weight, a value similar to the composition of microorganisms in general (Jordan and Peterson 1978; Cuhel et al. 1984; Cuhel and Waterbury 1984) but not necessarily to short-term uptake (Monheimer 1978a). Non-reductive sulfate assimilation processes, particularly ester sulfate formation, were often more

than 30% of total sulfur assimilation, and can contribute significantly to sediment sulfur cycles as electron sinks for the oxidation of organic matter. In fact our estimates of sulfate ester formation (Table 4), 2-12mg S m^{-2} d^{-1}, are consistent with observed rates of reduction of organic sulfate esters in lake sediments (ca. 8 ng S m^{-2} d^{-1}) reported by Smith and Klug (1981).

Night metabolism of algae can include biosynthesis of protein and nucleic acids at rates equal to daytime growth (cf. Cuhel et al. 1984). For photosynthetic organisms this capability has substantial physiological significance as it represents complete uncoupling between carbon and energy accumulation and utilization. The temptation to consider relative night growth of algae as an index of nutrient deficiency (Lancelot and Mathot 1985) must be yielded to cautiously, however. Since daylength varies considerably in higher latitudes, relative amounts of products must be interpreted in terms of their respective rates, otherwise night metabolism will appear inconsequential when nights are short. More difficult considerations include isotopic equilibration artifacts, discussed below and in Cuhel and Lean (1987), as well as the non-ubiquity of night protein synthesis capability among algae.

Two consequences of night synthesis are a net increase in certain biochemical constituents, e.g. protein, and a continued demand for nutrients. The energy used for night synthesis is derived from carbohydrates, generally requiring degradation of

polymeric carbohydrates stored during daylight hours. Therefore, elemental ratios such as C:N and C:S and cell composition ratios such as protein:carbohydrate and protein:lipid may systematically change throughout the night. Combined ^{14}C-bicarbonate and ^{35}S-sulfate assimilation studies carried out overnight can describe night synthesis unambiguously.

Several of the in situ experiments included an overnight component for which the data of 26 August, Station 403, will serve as an example (Fig. 8). The flow of carbon was primarily into protein from carbohydrates, with all activity mediated by the LMW fraction (through which polymeric carbohydrate degradation products are transformed). The energetic costs of night protein synthesis include at least 5 ATP for each peptide bond formed plus 17-18 ATP for each reductively assimilated SO_4 molecule (one every 30 amino acids) in addition to the transformation of carbon skeletons, transamination reactions, etc. These costs are met through oxidative metabolism of carbohydrates. Thus one carbon unit is incorporated into protein for every 3-5 units removed from the (LMW + polymeric) carbohydrates: the flow of carbon into protein was accompanied by a similar assimilation of exogenous sulfate into protein. One result of these activities is a dramatic alteration in the ratios of ^{14}C-biopolymers to one another (Fig. 11). While incorporation patterns from all day incubation yielded quite variable ratios with dominance of carbohydrates in evidence, overnight incubation

and associated night growth returned the relative abundance of the major biopolymers to near unity in the upper water column (lower panel). Such uniform relative abundance has been suggested to be optimal food quality for some herbivores (cf. Scott 1980).

In order for the observed redistribution of carbon to actually have a noticeable effect on the food quality of plankton requires that the bulk composition of the plankton change in kind overnight. That is, the composition of the total particulate carbon must be altered appreciably. It is not sufficient that the recently-assimilated, hence ^{14}C-labelled component of the particulate material undergo these changes if it accounts for a small fraction of the total particulate carbon. We have therefore considered the approximate doubling time of the plankton population with respect to total particulate carbon and the protein component specifically. Using integrated production values from Table 4 and integrated POC and particulate protein (Pick 1987) we estimated the days required to double either the POC or protein stocks using either light day or 24 hour production measurements (considering each as a "day" for calculation purposes; Table 6). The significance of night metabolism is accentuated in these calculations: since protein synthesis at night is energized by oxidative carbon metabolism, full 24 hour growth measurements indicate faster protein-specific and slower carbon-specific growth rates than the light day estimates. Three stations with 24 hour measurements had average

POC and protein increases of 9.7 and 6.0% day^{-1} using light day data but 8.2 and 10.6% based on 24 hour incubations. In light of these findings, the diel vertical migration of herbivores (Taylor et al. 1987), may be related to food quality but other factors may be involved in Lake Ontario where the growth rate of the particulate material was too slow for a single day's night metabolism to significantly alter the biochemical community structure.

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Andreae, M. O., and H. Raemdonck. 1983. Dimethyl sulphide in the surface ocean and marine atmosphere: a global view. Science 221: 744-747.

Antia, N. J., C. D. McAllister, T. R. Parsons, K. Stephens, and J. D. H. Strickland. 1963. Further measurements of primary production using a large-volume plastic sphere. Limnol. Oceanogr. 8: 166-183.

Antoine, A. D., and B. S. Tepper. 1969. Environmental control of glycogen and lipid content of Mycobacterium phlei. J. Gen. Microbiol. 55: 217-226.

Bates, S. S. 1981. Determination of the physiological state of marine phytoplankton by use of radiosulfate incorporation. J. exp. mar. Biol. Ecol. 51: 219-239.

Benson, A. A. 1963. The plant sulfolipid. Adv. Lipid Res. 1: 387-394.

Campbell, P. G. C., and J. H. Baker. 1978. Estimation of bacterial production in fresh waters by the simultaneous measurement of (^{35}S)-sulphate and D-(^{3}H)glucose uptake in the dark. Can. J. Microbiol. 24: 939-946.

Cuhel, R. L., and D. R. S. Lean. 1987. Influence of light intensity, light quality, temperature, and daylength on uptake and assimilation of carbon dioxide and sulfate by lake plankton. Can. J. Fish. Aquat. Sci. 44: 000-000.

Cuhel, R. L., P. B. Ortner, and D. R. S. Lean. 1984. Night synthesis of protein by algae. Limnol. Oceanogr. 29: 731-744.

Cuhel, R. L., C. D. Taylor, and H. W. Jannasch. 1981. Assimilatory sulfur metabolism in marine microorganisms: characteristics and regulation of the sulfate transport system in Pseudomonas halodurans and Alteromonas luteo-violaceus. J. Bacteriol. 147: 340-349.

Cuhel, R. L., C. D. Taylor, and H. W. Jannasch. 1982. Assimilatory sulfur metabolism in marine microorganisms: considerations for the application of sulfate incorporation into protein as a measurement of natural population protein synthesis. Appl. Environ. Microbiol. 43: 160-168.

Cuhel, R. L., and J. B. Waterbury. 1984. Biochemical composition and short-term nutrient incorporation patterns in a

unicellular marine Cyanobacterium, Synechococcus sp. (WHOI 7801). Limnol. Oceanogr. 29: 370-374.

Deane, E. M., and R. W. O'Brien. 1975. Sulfate uptake and metabolism in the marine chrysomonad, Monochrysis lutheri. Arch. Microbiol. 105: 295-301.

Fitzgerald, J. W. 1976. Sulfate ester formation and hydrolysis: a potentially important yet often ignored aspect of the sulfur cycle of aerobic soils. Bact. Rev. 40: 698-721.

Glooschenko, W. A., J. E. Moore, M. Munawar, and R. A. Vollenweider. 1974. Primary production in Lakes Ontario and Erie: a comparative study. J. Fish. Res. Board Can. 31: 253-263.

Harris, G. P. 1978. Photosynthesis, productivity and growth: The physiological ecology of phytoplankton. Arch. Hydrobiol. Beih. Ergebn. Limnol. 10: 1-171.

Hobson, L. A., and R. J. Pariser. 1971. The effect of inorganic nitrogen on macromolecular synthesis by Thalassiosira fluviatilis Hustedt and Cyclotella nana Hustedt grown in batch culture. J. exp. mar. Biol. Ecol. 6: 71-78

Jassby, A. D. 1975. Dark sulfate uptake and bacterial productivity in a subalpine lake. Ecology 56: 627-636.

Jeanjean, R., and E. Broda. 1977. Dependence of sulfate uptake by Anacystis nidulans on energy, on osmotic shock, and on sulfate starvation. Arch. Microbiol. 114: 19-23.

Jordan, M. J., and B. J. Peterson. 1978. Sulfate uptake as a

measure of bacterial production. Limnol. Oceanogr. 23: 146-150.

Jorgensen, E. G. 1966. Photosynthetic activity during the life cycle of synchronous _Skeletonema_ cells. Physiol. Plant. 19: 789-799.

Jukes T. H., R. Holmquist, and H. Moise. 1975. Amino acid composition of proteins: selection against the genetic code. Science 189: 50-51.

Konopka A., and M. Schnur. 1981. Biochemical composition and photosynthetic carbon metabolism of nutrient limited cultures of _Merismopedia_ _tenuissima_ (Cyanophyceae). J. Phycol. 17: 118-122.

Lancelot, C., and S. Mathot. 1985. Biochemical fractionation of primary production by phytoplankton in Belgian coastal waters during short- and long-term incubations with ^{14}C-bicarbonate. I. Mixed diatom population. Mar. Biol. 86: 219-226.

Marra, J. 1978a. Effect of short-term variations in light intensity on photosynthesis of a marine phytoplankter: a laboratory simulation study. Mar. Biol. 46: 191-202.

Marra, J. 1978b. Phytoplankton photosynthetic response to vertical movement in a mixed layer. Mar. Biol. 46: 203-208.

McCready, R. G. L., E. J. Laishley, and H. R. Krouse. 1975. Stable isotope fractionation by _Closterium_ _pasteurianum_. 1. $^{34}S/^{32}S$: inverse isotope effects during SO_4^{2-} and SO_3^{2-}

reduction. Can. J. Microbiol. 21: 235-244.

Menke, W., A. Radunz, G. H. Schmid, F. Koenig, and R.-D. Hirtz. 1976. Intermolecular interactions of polypeptides and lipids in the thylakoid membrane. Z. Naturforsch. 31c: 436-444.

Monheimer, R. H. 1978a. Difficulties in interpretation of microbial heterotrophy from sulfate uptake data: laboratory studies. Limnol. Oceanogr. 23: 150-154.

Monheimer, R. H. 1978b. Microbial heterotrophic production in two lakes of different productivity. Verh. Internat. Verein. Limnol. 20: 2222-2226.

Morris, I. 1981. Photosynthetic products, physiological state, and phytoplankton growth. p. 83-102. In: T. Platt [ed.] Physiological bases of phytoplankton ecology. Can. J. Fish. Aquat. Sci. 210.

Morris, I., and W. Skea. 1978. Products of photosynthesis in natural populations of marine phytoplankton in the Gulf of Maine. Mar. Biol. 47: 303-312.

Nriagu, J. O., and Y. K. Soon. 1984. Arylsulphatase activity in polluted lake sediments. Environ. Pollut. Ser. B 8: 143-153.

Nriagu, J. O., and Y. K. Soon. 1985. Distribution and isotopic composition of sulfur in lake sediments of Northern Ontario. Geochim. Cosmochim. Acta 49: 823-834.

Percival, E., M. A. Rahman, and H. Weigel. 1980. Chemistry of the polysaccharides of the diatom _Coscinodiscus nobilus_. Phytochem. 19: 809-811.

Pick, F. R. 1987. Protein:carbohydrate ratios as indicators of plankton nutrient deficiency. Lake Ontario 1982. Can. J. Fish. Aquat. Sci. 44:000-000.

Prezelin, B. B., B. W. Meeson, and B. M. Sweeney. 1977. Characterization of photosynthetic rhythms in marine dinoflagellates. I. Pigmentation, photosynthetic capacity, and respiration. Plant Physiol. 60: 384-387.

Putt, M., G. P. Harris, and R. L. Cuhel. 1987. Photoinhibition of DCMU-enhanced fluorescence in Lake Ontario phytoplankton. Can. J. Fish. Aquat. Sci. 44: 000-000.

Ramus, J. 1974. In vivo molybdate inhibition of sulfate transfer to Porphyridium capsular polysaccharide. Plant Physiol. 54: 945-949.

Roberts, R. B., P. H. Abelson, D. B. Cowie, E. T. Bolton, and R. J. Britten. 1963. Studies of biosybthesis in Escherichia coli. Carnegie Inst. Wash. Publ. No. 607: 521 pp.

Scott, J. M. 1980. Effect of growth rate of the food alga on the growth/ingestion efficiency of a marine herbivore. J. mar. biol. Assn. U. K. 60: 681-702.

Simons, T. J. and W. M. Schertzer. 1987. Stratification, currents, and upwelling in Lake Ontario, Summer 1982. Can. J. Fish. Aquat. Sci. 44: 000-000.

Sinensky, M. 1977. Specific deficit in the synthesis of 6-sulfoquinovosyl diglyceride in Chlorella pyrenoidosa. J. Bacteriol. 129: 516-524.

Smith, R. L., and M. J. Klug. 1981. Reduction of sulfur compounds in the sediments of a eutrophic lake basin. Appl. Environ. Microbiol. 41: 1230-1237.

Sournia, A. 1981. Morphological bases of competition and succession. p. 339-346. In: T. Platt [ed.] Physiological bases of phytoplankton ecology. Can. J. Fish. Aquat. Sci. 210.

Stadelmann, P., J. E. Moore, and E. Pickett. 1974. Primary production in relation to temperature structure, biomass concentration, and light conditions at an inshore and offshore station in Lake Ontario. J. Fish. Res. Board Can. 31: 1215-1232.

Steemann-Nielsen, E. 1952. The use of radioactive carbon (^{14}C) for measuring organic production in the sea. J. Cons. perm. int. Explor. Mer 18: 117-140.

Stevens, R. J. J. and M. A. Neilson. 1987. Response of Lake Ontario to reductions in phosphorus load: 1967-1982. Can. J. Fish. Aquat. Sci. 44: 000-000.

Strickland, J. D. H., O. Holm-Hansen, R. W. Eppley, and R. J. Linn. 1969. The use of a deep tank in plankton ecology. I. Studies of the growth and composition of phytoplankton crops at low nutrient levels. Limnol. Oceanogr. 14: 23-34.

Taylor, W. D., H.-J. Fricker, and D. R. S. Lean. 1987. Zooplankton biomass distribution, biomass, and filtering rates. Can. J. Fish. Aquat. Sci. 44: 000-000.

Tsang, M. L. S., and J. A. Schiff. 1975. Studies of sulfate utiliza-tion by algae. 14. Distribution of adenosine-3'-phosphate-5'-phosphosulfate (PAPS) and adenosine-5'-phosphosulfate (APS) sulfotransferases in assimilatory sulfate reducers. Plant Science Letters 4: 301-307.

Vollenweider, R. A., and A. Nauwerek. 1961. Some observations on the ^{14}C method for measuring primary production. Verh. Internat. Verein. Limnol. 14: 134-139.

Table 1. Vertical profiles of temperature, chlorophyll a, particulate carbon and nitrogen, and dissolved nitrate plus nitrite and soluble reactive phosphorus. Data are for near-dawn samples.

		$\mu g \cdot L^{-1}$						
DEPTH m	TEMP. °C	CHL a	POC	PON	CARBO-HYDRATE	PROTEIN	$NO_3 + NO_2$	SRP
8 JUNE 1982 STATION 401								
1	10.46	2.9	352	64	227	394	280	1.0
5	9.84	3.3	358	65	244	428	282	0.8
10	7.56	4.0	394	69	286	362	292	0.9
15	6.15	4.0	325	59	233	362	310	0.9
30	5.43	3.0	276	50	179	286	332	1.5
1 JULY 1982 STATION 405								
1	15.50	7.9	76	134			199	1.4
5	15.50	9.3	762	139	353	583	204	1.6
10	14.40	9.2	629	115			270	1.4
15	9.70	9.2	629	115			270	1.3
29	6.20	5.8	345	55			332	1.0
26 AUGUST 1982 STATION 403								
1	12.53	5.1	349	68			213	0.2
5	12.53	3.4	387	69	205	268	210	0.2
10	12.43	4.0	384	74			206	0.5
15	12.04	3.2	465	95			211	0.2
25	4.82	1.3	230	37			302	0.6

Table 2. Protein and ester sulfate components of light and dark all day in situ incubations at station 401 on 8 June 1982, with incubator data using 5 m water at station 401 on 9 June 1982 for comparison. N/A, not applicable.

	TOTAL S	ESTER SULFATE			PROTEIN S		
DEPTH (m)	L/D	LIGHT	DARK	L/D	LIGHT	DARK	L/D
			% OF TOTAL ^{35}S IN FRACTION				
1	N/A	41.1	43.7	0.94	22.8	21.3	1.07
5	N/A	39.0	24.6	1.59	17.9	24.7	0.72
12.5	N/A	34.0	17.8	1.91	20.3	31.9	0.64
20	N/A	15.9	5.0	3.18	33.8	62.9	0.54
5 m, P_{opt}	N/A	40.5	37.9	1.07	26.7	28.9	0.92
			ng $S \cdot L^{-1} \cdot d^{-1}$ IN FRACTION				
1	2.55	225.5	93.9	2.40	124.9	45.8	2.73
5	4.19	325.9	49.1	6.64	149.3	49.3	3.03
12.5	2.33	142.1	31.9	4.45	84.9	57.1	1.49
20	2.55	14.8	1.8	8.22	31.6	23.1	1.37
5 m, P_{opt}	2.96	298.7	94.5	3.16	197.1	72.0	2.74

Table 3. Protein and ester sulfate components of light and dark all day in situ incubations at station 403 on 26 August 1982, with incubator data using 5 m water at station 403 on 25 August 1982 for comparison. The depth of 1% I_o was 10.8 m and received 7.9 μEinst.$\cdot m^{-2}\cdot s^{-1}$ at midday. N/A, not applicable.

	TOTAL S	ESTER SULFATE			PROTEIN S		
DEPTH (m)	L/D	LIGHT	DARK	L/D	LIGHT	DARK	L/D
% OF TOTAL ^{35}S IN FRACTION							
1	N/A	25.2	31.0	0.81	32.0	39.0	0.82
5	N/A	25.0	31.9	0.78	35.2	39.9	0.88
12.5	N/A	21.2	35.9	0.59	43.5	39.1	1.11
20	N/A	46.8	52.0	0.90	51.6	44.9	1.15
5 m, P_{opt}	N/A	20.5	14.5	1.41	34.9	42.0	0.83
ng $S\cdot L^{-1}\cdot d^{-1}$							
1	1.74	113.0	80.0	1.41	143.7	100.8	1.43
5	2.51	178.6	90.8	1.97	251.7	113.5	2.22
12.5	1.34	51.1	64.9	0.79	104.8	70.6	1.48
20	1.74	38.6	24.7	1.56	42.6	21.3	2.00
5 m, P_{opt}	3.67	125.8	24.2	5.20	214.3	70.4	3.04

Table 4. Light day, night, and 24 hour integrated particulate production for stations with overnight in situ deployments. Night values are the difference between light day and 24 hour measurements.

		PARTICULATE PRODUCTION (mg m^{-2})					
		CARBON		SULFUR			
DATE/STATION	PHOTO-PERIOD	TOTAL	PROT.	TOTAL	REDUCED	OXID.	PROT.
29 June	15.5 L	1080.2	194.6	21.5	12.9	8.6	5.8
STN. 401	8.5 D	-124.1	+96.9	12.7	9.5	3.2	4.9
	24 hour	956.1	291.5	34.2	22.4	11.8	10.7
26 August	13.6 L	713.8	127.9	7.5	5.1	2.4	2.8
STN. 403	10.4 D	-143.7	+54.3	5.2	3.9	1.3	2.5
	24 hour	570.1	182.2	12.7	9.0	3.7	5.3
22 September	12.2 L	890.6	136.8	13.0	11.1	1.9	2.1
STN. 403	11.8 D	-144.2	+30.3	1.0	0.8	0.2	1.1
	24 hour	746.4	167.1	14.0	11.9	2.1	3.2

Table 5. All day in situ production integrated 0-20 m with (+) and without (-) photoinhibition (PI). Integrations without photoinhibition assume uniform chlorophyll a-specific photosynthesis from the depth of P_{max} to the surface. No photoinhibition was apparent on 29 June (Stn. 401) or 26 August (Sta. 403). N/A, not available.

		CARBON		SULFUR		
DATE/STATION	PI	TOTAL	PROTEIN	TOTAL	REDUCED	PROTEIN
8 June	+	616.2	116.3	9.26	5.48	1.99
STN. 401	-	690.3	124.1	10.23	6.08	2.05
	% DIFF	12.0	6.7	10.5	10.9	3.0
10 June	+	552.6	40.4	N/A	N/A	N/A
STN. 403	-	565.4	43.0	N/A	N/A	N/A
	% DIFF	2.3	6.4			
1 July	+	1467.2	299.1	32.38	19.03	9.80
STN. 405	-	1573.0	309.3	33.11	19.50	10.05
	% DIFF	7.2	3.4	2.3	2.5	2.6
22 Sept.	+	890.6	136.8	13.03	11.13	2.13
STN. 403	-	912.9	141.1	13.12	11.18	2.16
	% DIFF	2.5	3.1	0.7	0.4	1.4
AVG. DIFFERENCE		6.0	4.9	4.5	4.6	2.3

Table 6. Euphotic zone doubling times for total particulate organic carbon and protein based on integrated light day or 24 hour in situ measurements and integrated standing stocks of POC and protein. Protein data are from Pick (1986). A protein composition of 52.6% C, 1.1% S is used (Jukes et al. 1975).

DATE/STATION	INCUBATION PERIOD	DAYS TO DOUBLE STANDING STOCK		
		POC(^{14}C)	PROTEIN(^{14}C)	PROTEIN(^{35}S)
29 June	LT DAY	9.2	19.4	13.6
STN. 401	24 hour	10.4	13.0	7.4
26 August	LT DAY	10.8	22.0	21.1
STN. 403	24 hour	13.6	15.5	11.1
22 Sept.	LT DAY	11.0	18.1	24.7
STN. 403	24 hour	13.1	14.9	15.7

FIGURE LEGENDS

Fig. 1. Schematic pathways of sulfate assimilation in microorganisms.

Fig. 2. Total carbon uptake and its incorporation into lipid and protein during back–to–back in situ incubations at station 401 on 8 June. The proportion of carbon incorporated into protein is shown in the right–hand panels. The bottom panels show the sum of the three incubations (closed symbols) compared to all day (0630–2125) in situ incubation (open symbols).

Fig. 3. Details of carbon dioxide and sulfate metabolism during all day in situ incubation at station 401 on 8 June. Panel A: Carbon dioxide. Panel B: Sulfate; right–hand panel shows the light : dark sulfate uptake ratio. Panel C: C:S uptake ratios and proportion of total sulfur incorporated into protein.

Fig. 4. Total carbon uptake and its incorporation into lipid and protein during back–to–back in situ incubations at station 405 on 1 July. The proportion of carbon incorporated into protein is shown in the right–hand panels. The bottom panels show the sum of the three incubations (closed symbols) compared to all day (0600–2105) in situ incubation (open symbols).

Fig. 5. Total uptake and incorporation of sulfur into protein and ester–SO_4 during back–to–back in situ incubations at station 405 on 1 July. The proportion of sulfur incorporated into protein and C:S weight ratios for protein and total–C:reduced–S are shown in the right–hand panels. The bottom panels show the sum of the three incubations (closed symbols) compared to all day (0600–2105) in situ incubation (open symbols).

Fig. 6. Total carbon uptake and its incorporation into lipid and protein during back–to–back in situ incubations at station 403 on 26 August. The proportion of carbon incorporated into protein is shown in the right–hand panels. The bottom panels show the sum of the three incubations (closed symbols) compared to all day (0655–2035) in situ incubation (open symbols).

Fig. 7. Details of carbon dioxide and sulfate metabolism during all day in situ incubation at station 405 on 26 August. Panel A: Carbon dioxide. Panel B: Sulfate; right–hand panel shows the light : dark sulfate uptake ratio. Panel C: C:S uptake ratios and proportion of total sulfur incorporated into protein.

Fig. 8. Night changes in total carbon and sulfur and their subcellular components for station 403 on 26 August. Natural photoperiod 13.5L:10.5D. NOTE: amounts are calculated for the specific activity of the *initial inorganic* ^{14}C and hence underestimate fluxes out of macromolecular components.

Fig. 9. Vertical profiles for temperature, chlorophyll a, assimilation number, and the % of total assimilated C or S in protein for all day in situ incubations at inshore stations during LONAS, 1982. Daily integrated production is given in Tables 4 and 5.

Fig. 10. Vertical profiles for temperature, chlorophyll a, assimilation number, and the % of total assimilated C or S in protein for all day in situ incubations at the central lake station during LONAS, 1982. Both temperature and chlorophyll a were uniform with depth at 3 units on 10 June (upper panels). Daily integrated production is given in Tables 4 and 5.

Fig. 11. Ratios of major ^{14}C–biopolymers following all day and overnight in situ incubation at station 403 on 26 August.

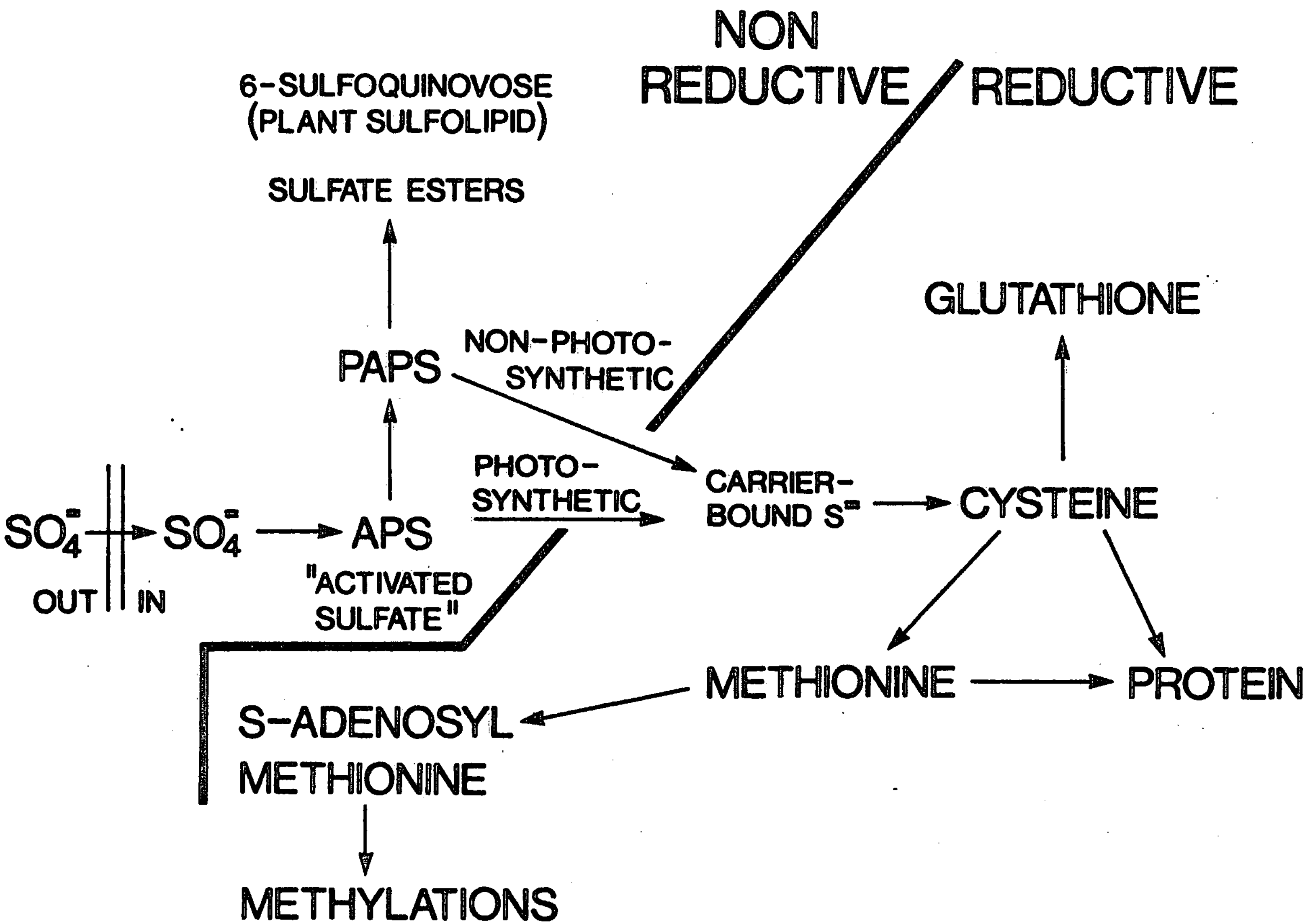

Fig. 1. Protein Synthesis - Cuhel & Lean

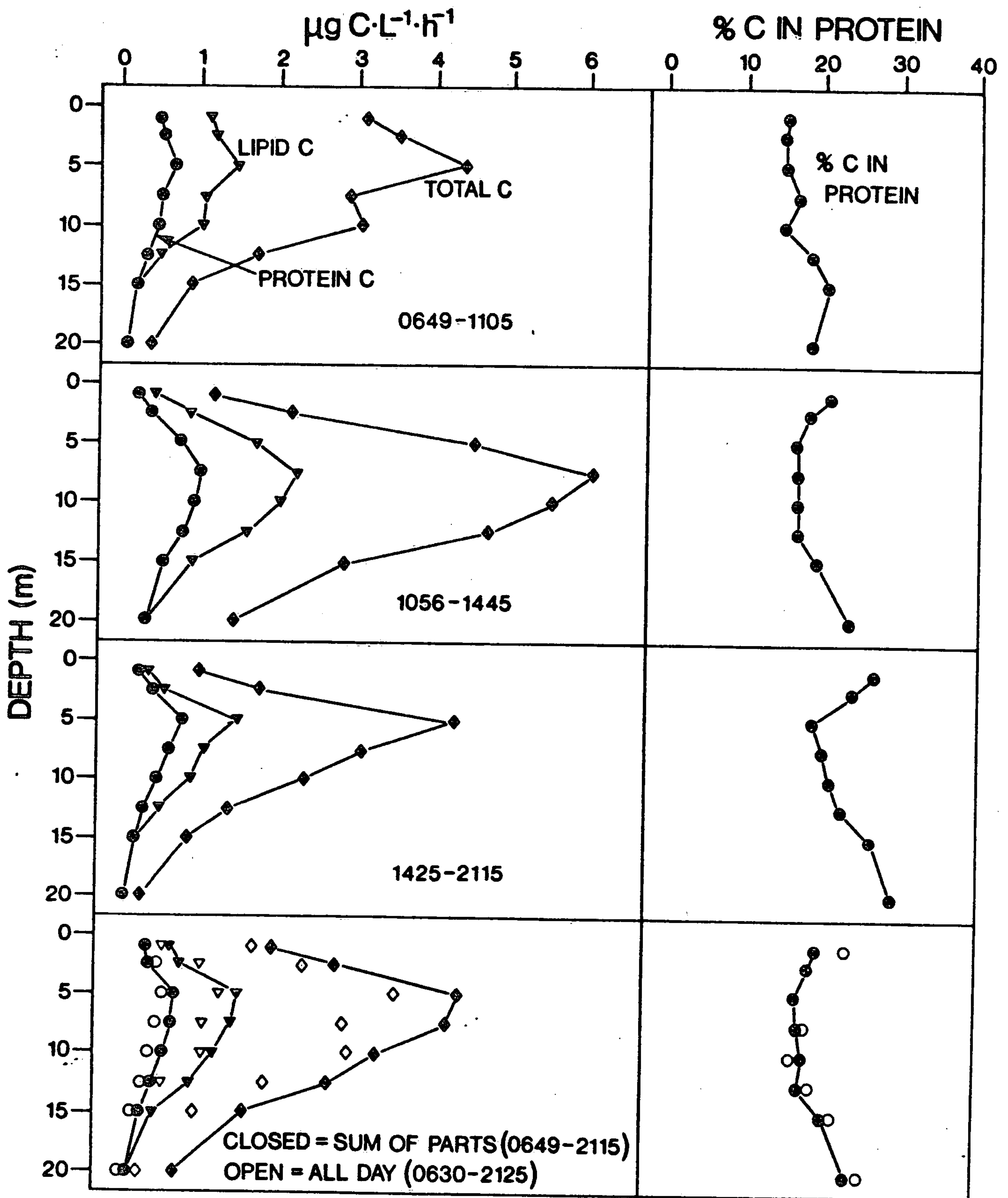

Fig. 2. Protein Synthesis - Cuhel & Lean

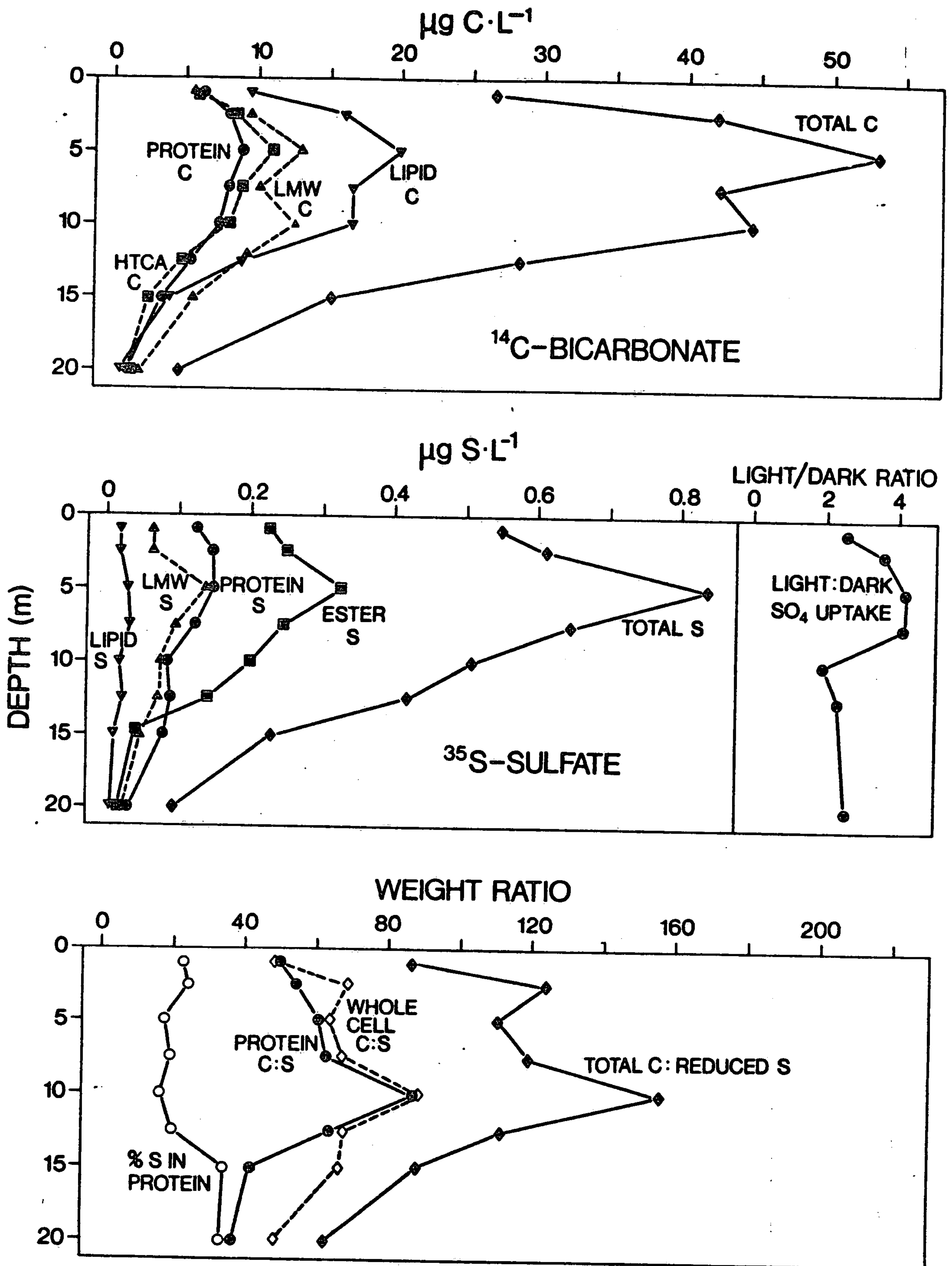

Fig. 3. Protein Synthesis - Cuhel & Lean

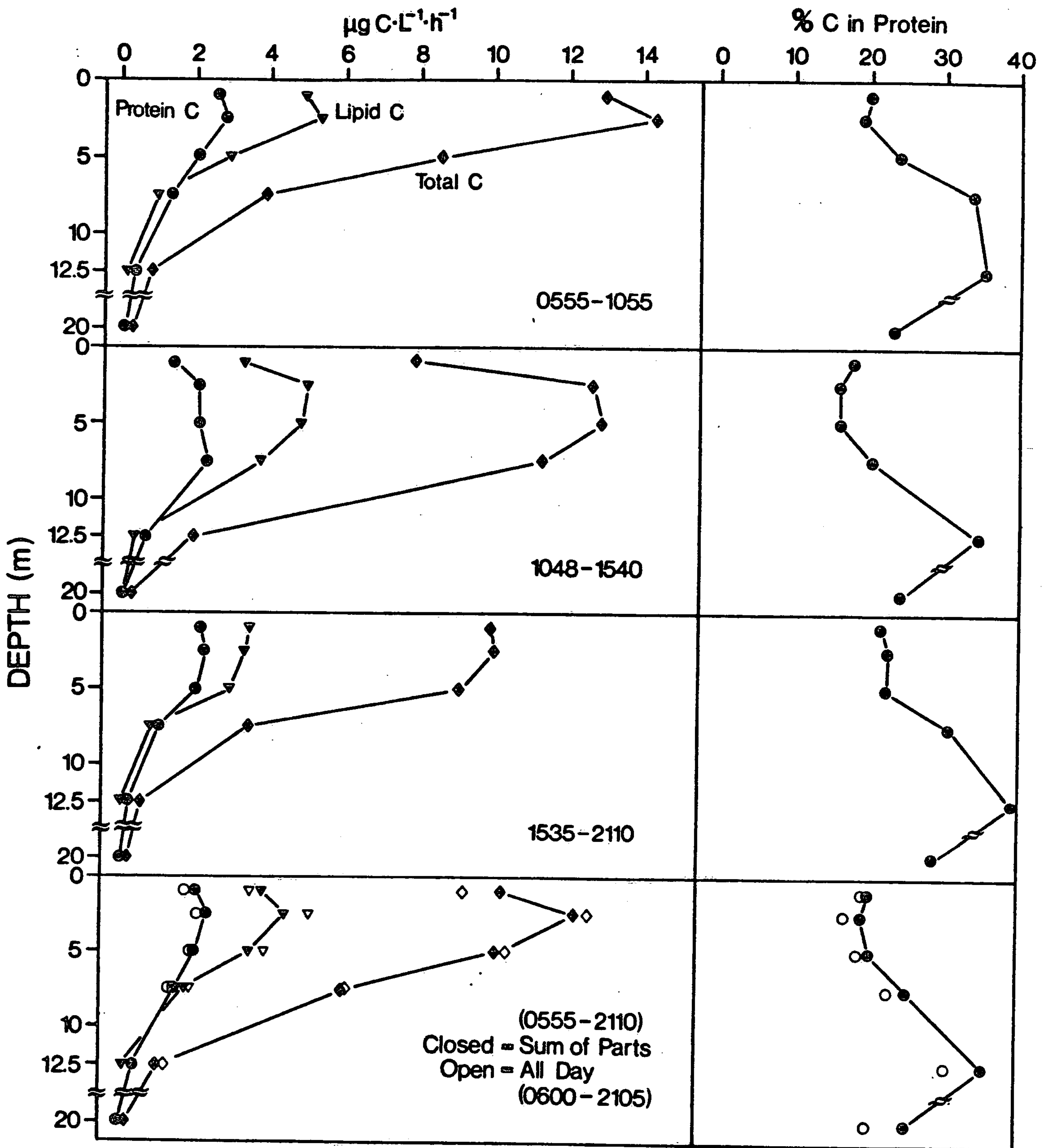

Fig. 4. Protein Synthesis - Cuhel & Lean

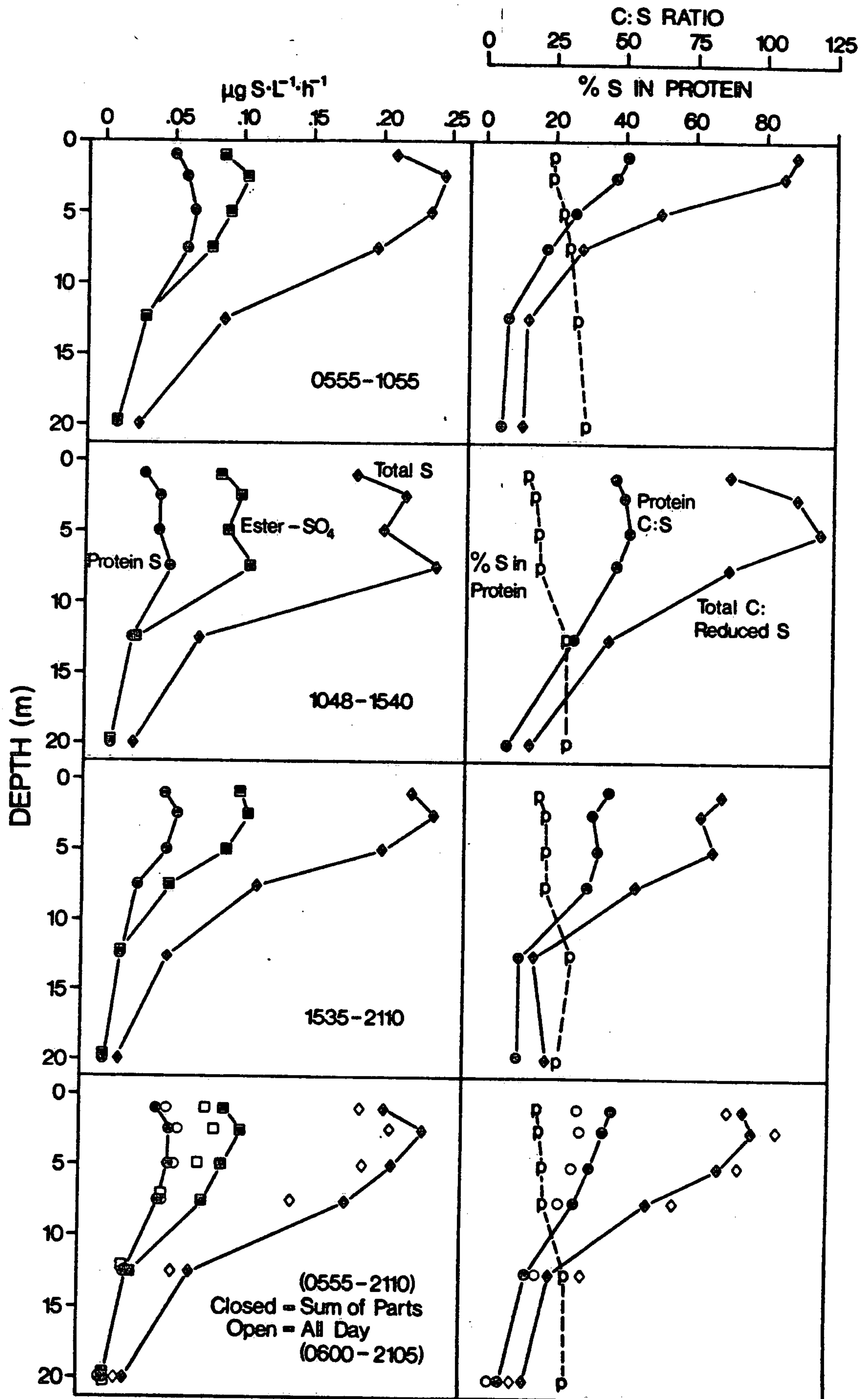

Fig. 5. Protein Synthesis - Cuhel & Lean

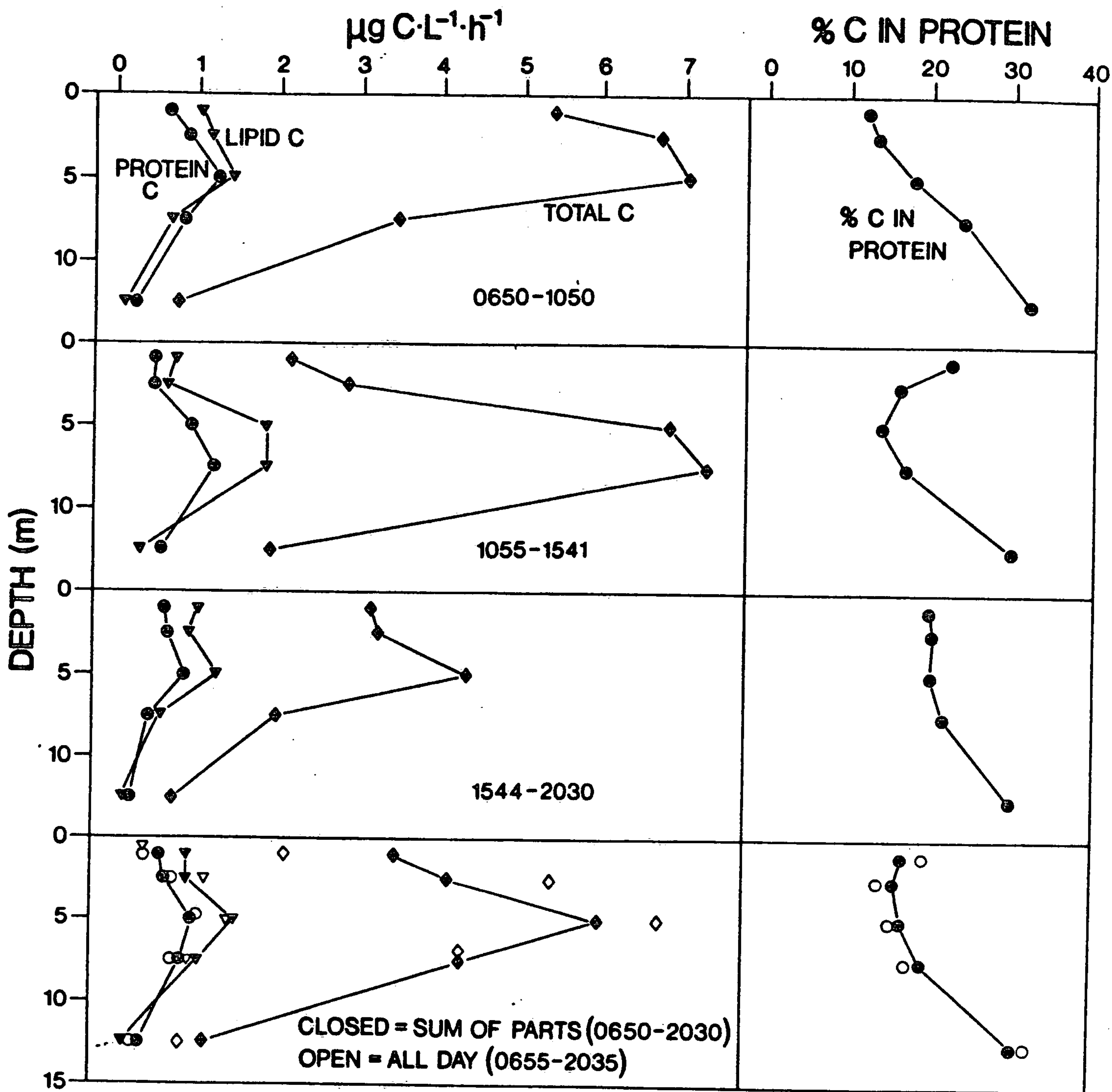

Fig. 6. Protein Synthesis - Cuhel & Lean

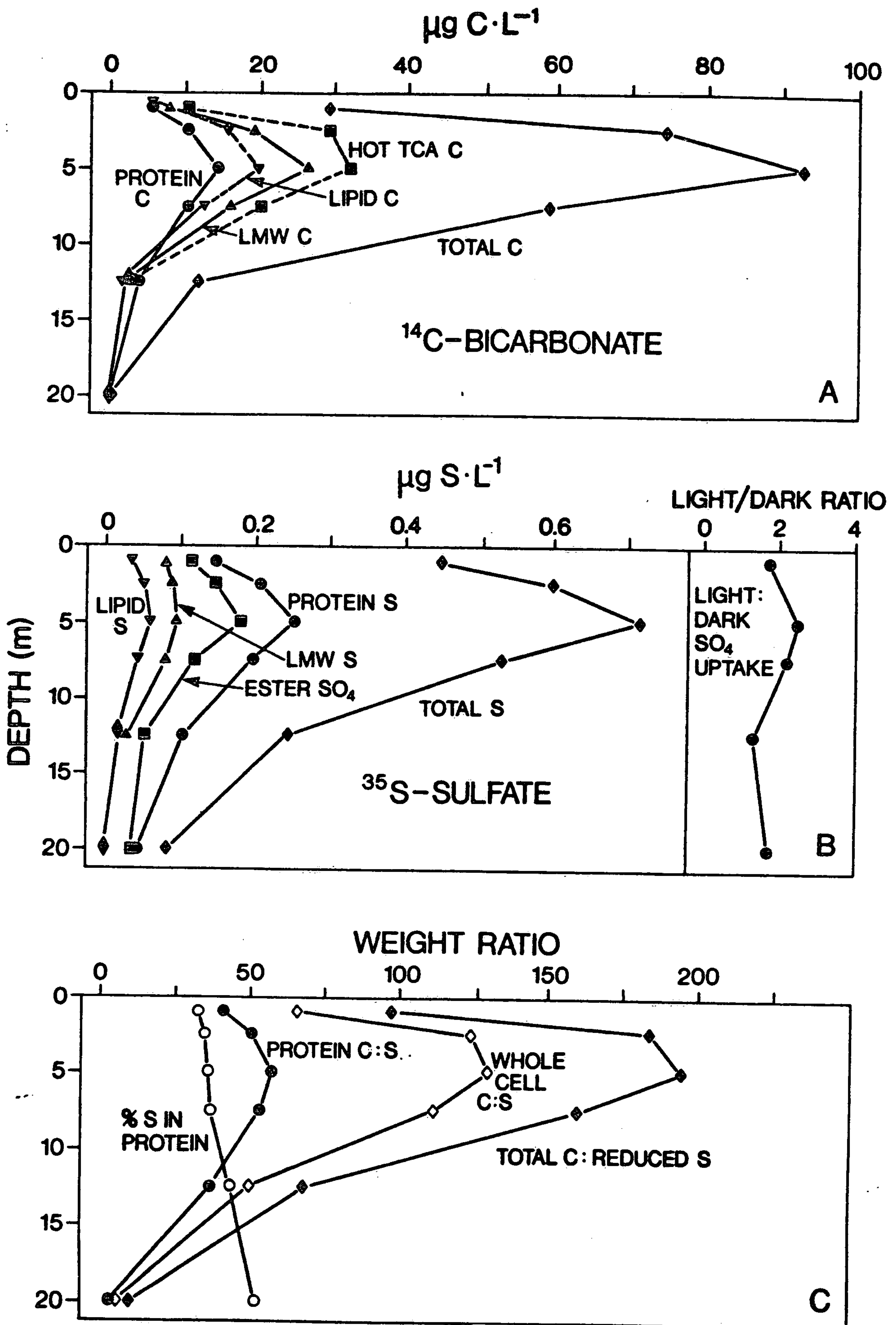

Fig. 7. Protein Synthesis - Cuhel & Lean

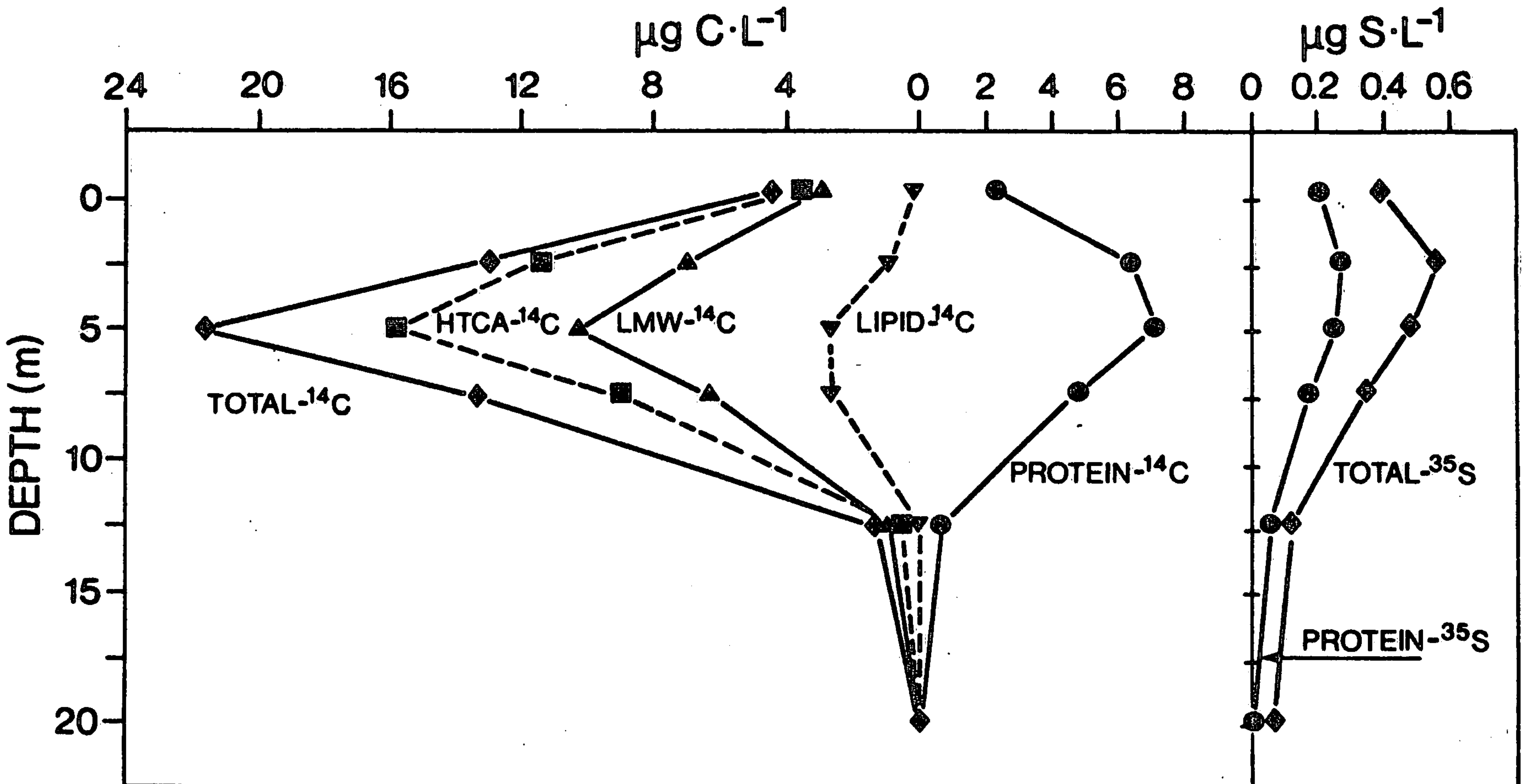

Fig. 8. Protein Synthesis - Cuhel & Lean

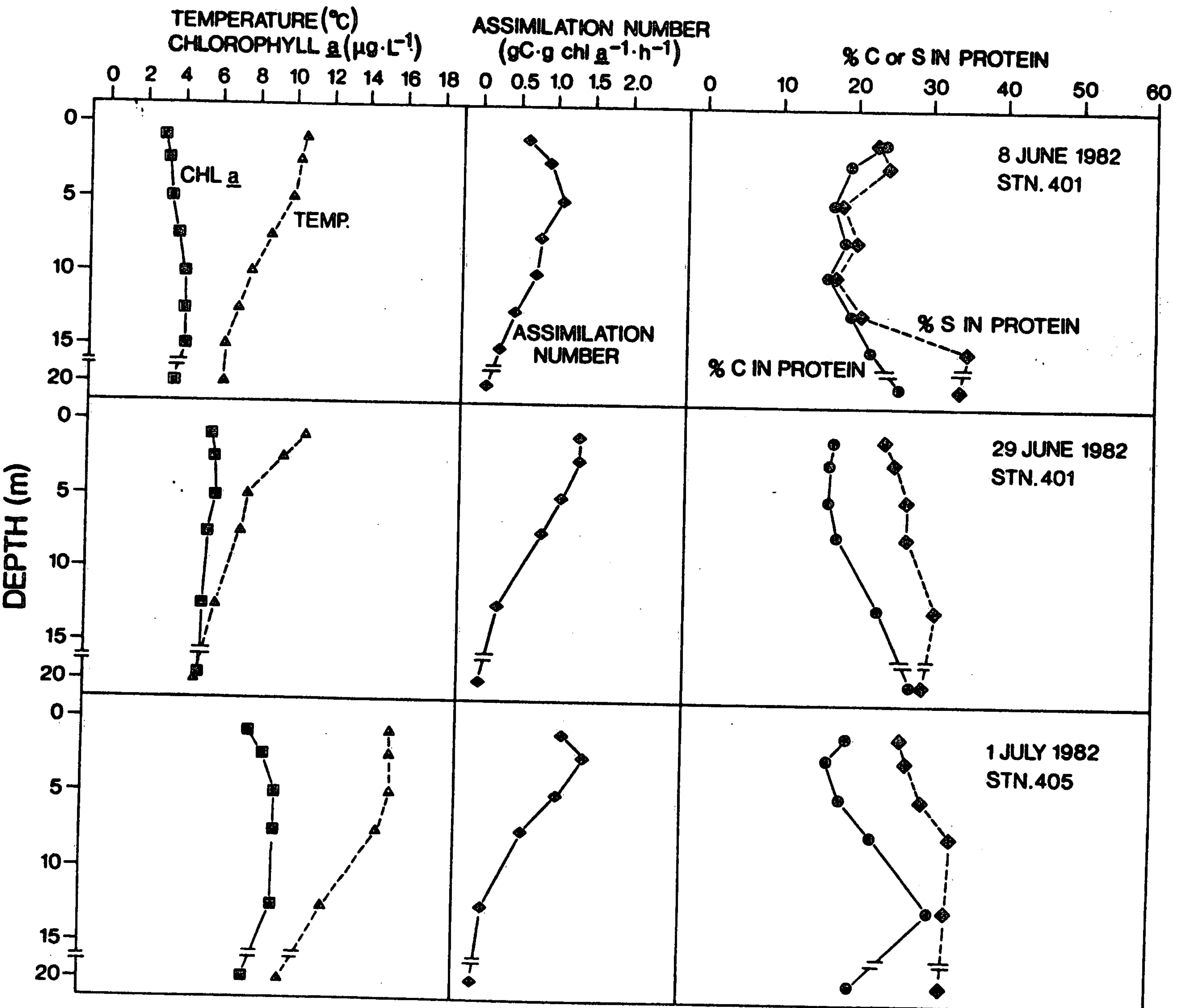

Fig. 9. Protein Synthesis - Cuhel & Lean

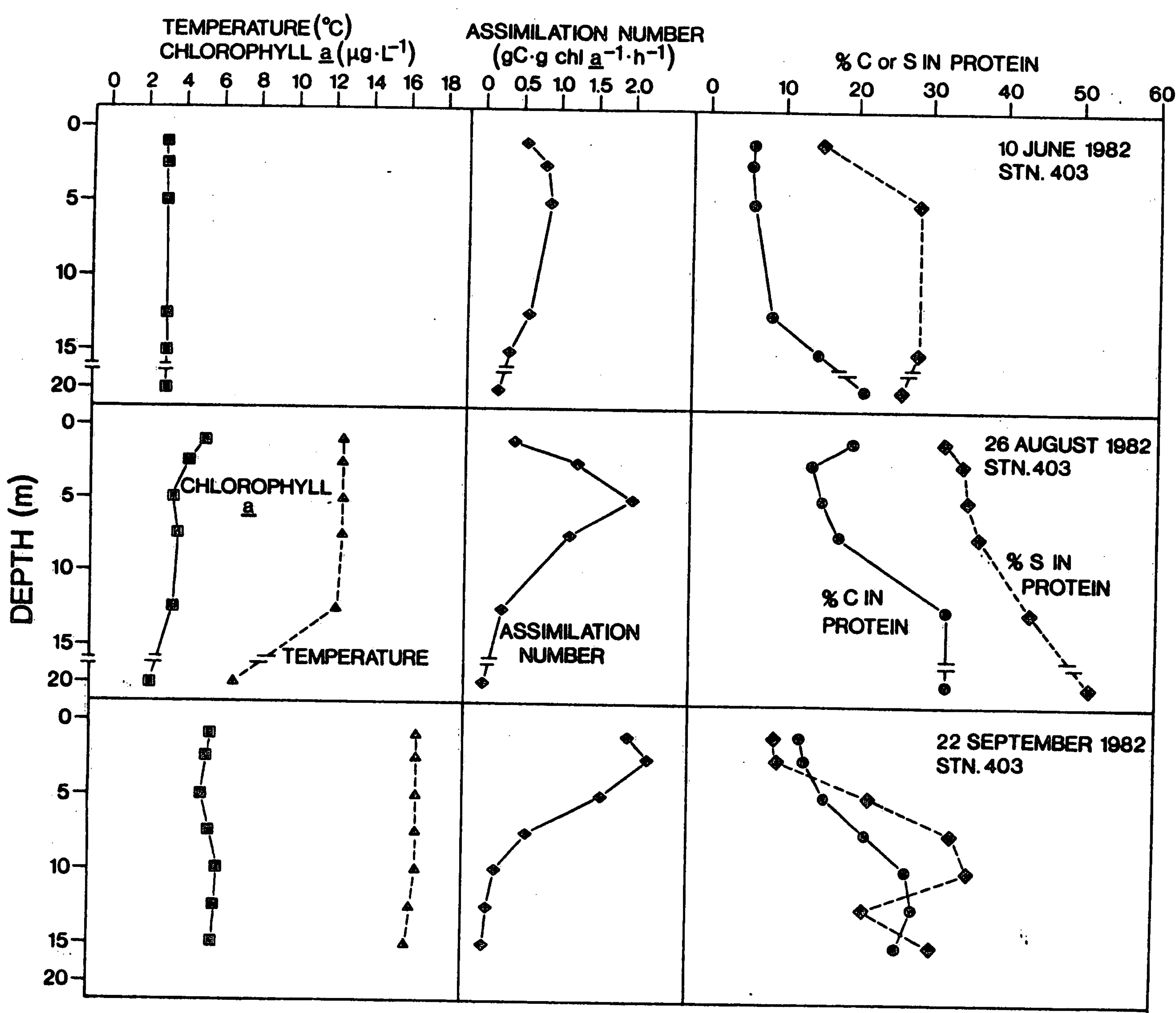

Fig. 10. Protein Synthesis - Cuhel [illegible]ean

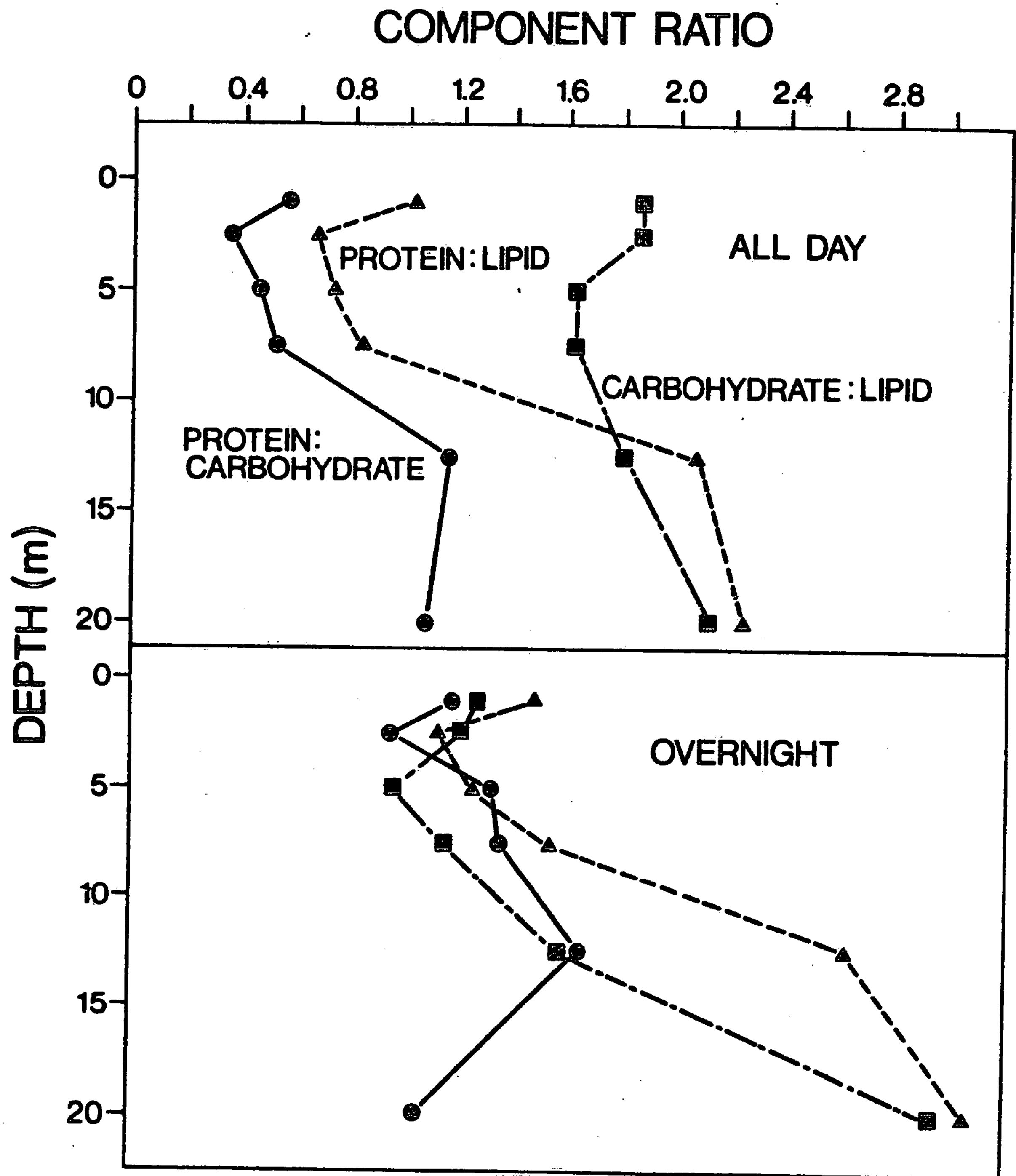

Fig. 11. Protein Synthesis - Cuhel & Lean