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Influence of Light Intensity, Light Quality, Temperature, and Daylength on Uptake and Assimilation of Carbon Dioxide and Sulfate by Lake Plankton

by

Russell L. Cuhel[1] and David R.S. Lean[2]

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[1]Rosenstiel School of Marine and Atmospheric Sciences
Division of Biology & Living Resources
University of Miami
4600 Rickenbacker Cswy.
Miami, Florida 33149, U.S.A.

[2]National Water Research Institute
Canada Centre for Inland Waters
P.O. Box 5050, Burlington, Ontario
L7R 4A6, Canada

MANAGEMENT PERSPECTIVES

The biochemical composition (protein, carbohydrate and lipid) of newly-produced phytoplankton biomass in Lake Ontario was determined under environmentally controlled conditions to determine the variables which control production. The quantity was related to incident light intensity and water temperature but was insensitive to light quality and nutrient additions. The allocation of carbon to carbohydrate polymer storage for night growth was strictly proportional to nightlength. The proportion of carbon contained in protein was strongly correlated with in situ water temperature. Lack of cross-correlation suggests that temperature and daylength exert independent constraints on the biochemical composition of lake microplankton.

PERSPECTIVE-GESTION

La constitution biochimique (protéines, glucides et lipides) de la biomasse de phytoplancton nouvellement formée dans le lac Ontario a été analysée en milieu contrôlé afin de connaître les variables qui règlent la croissance de ces organismes. Bien que l'intensité de la lumière incidente et la température de l'eau se soient traduites par des variations quantitatives, la longueur d'onde de l'illumination et l'ajout de substances nutritives n'ont eu aucun effet. L'affectation du carbone au stockage de glucides polymériques pour la croissance nocturne s'est avérée strictement proportionnelle à la durée de la nuit. La proportion de carbone que renferme les protéines était fortement liéeà la température de l'eau in situ. L'absence de corrélation croisée porte à croire que la température et la durée de la phase diurne exercent des contraintes indépendantes sur la constitution biochimique du microplancton lacustre.

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.

The biochemical composition of newly-produced phytoplankton biomass in Lake Ontario varied systematically with respect to experimentally manipulated incident light intensity and environmentally imposed water temperature and daylength, but was insensitive to light quality. Total uptake of ^{14}C-bicarbonate was light dependent (P_{opt}:dark=60-200) while $^{35}SO_4^{2-}$ uptake was light stimulated (P_{opt}:dark <5). Subcellular allocation of ^{14}C for relative protein, carbohydrate, and lipid polymer synthesis responded sensitively to sub-saturating light. Pathways of ^{35}S assimilation were unaffected by light intensity. Night protein synthesis and attendant respiration of polymeric carbohydrates was a function of prior light history: with daytime illumination at P_{opt}, day and night rates of $^{35}SO_4^{2-}$-S incorporation into protein were often indistinguishable. Using April-November data from P_{opt} only, allocation of carbon to carbohydrate polymer storage for night growth was strictly proportional to nightlength. The proportion of carbon contained in protein was strongly correlated with in situ water temperature. The lack of cross-correlation suggests that temperature and daylength exert independent constraints on the biochemical composition of lake microplankton.

Cuhel, R.L. and D.R.S., Lean. 1987. Influence de l'intensité et de la qualité de la lumière, de la température et de la durée de la phase diurne sur la captation et l'assimilation du bioxyde de carbone et du sulfate par le plancton lacustre. Journal canadien de sciences halieutiques et aquatiques. 44:000-0000.

En faisant varier expérimentalement l'intensité du rayonnement lumineux et en maintenant la température de l'eau ambiante et la durée de la phase diurne, on a constaté des variations systématiques dans la constitution biochimique (protéines, glucides et lipides) de la biomasse de phytoplancton nouvellement formée dans le lac Ontario. Par contre, la longueur d'onde de la lumière n'a eu aucun effet. La captation totale de bicarbonate-^{14}C variait en fonction de la lumière (P_{opt}:obscurité=60-200) tandis que la captation de $^{35}SO_4^{2-}$ était stimulée par la lumière (P_{opt}:obscurité 5). L'affectation infracellulaire de ^{14}C à la synthèse de protéines, de lipides et de glucides polymériques a réagi de façon marquée à la lumière de niveau sous-saturé. L'intensité n'a toutefois eu aucun effet sur les voies d'assimilation du ^{35}S. La synthèse nocturne des protéines et la respiration des glucides polymériques qui l'accompagne était fonction des conditions d'illumination antérieures: de jour, lorsque le niveau de lumière correspondait à P_{opt}, les taux d'assimilation diurnes et nocturnes de $^{35}SO_4^{2-}$-S sous forme de protéines étaient virtuellement identiques. D'après les données de P_{opt} pour la priode d'avril à novembre, l'affectation de carbone au stockage de glucides polymériques pour la croissance de nuit était strictement proportionnelle à la durée de la nuit. La porportion de carbone que renferme les protéines était fortement liée à la température de l'eau in situ. L'absence de corrélation croisée porte à croire que la température et la durée de la phase diurne exercent des contraintes indépendantes sur la constitution biochimique du microplancton lacustre.

Photoautotrophic assimilation of nutrients and generation of energy for biosynthesis often may be limited by the availability of photosynthetically active radiation. Photosynthetic activity responds predictably to variations of instantaneous intensity, total daily flux, daylength, and spectral distribution; each may impose limits upon total daily carbon fixation and/or macromolecular products of photosynthesis by natural populations.

Few freshwater studies have examined what fraction of total carbon fixed goes to reproductive growth (i.e. biosynthesis of the complete suite of macromolecules required to promote cell division) relative to synthesis of storage products. While the literature on quantitative relationships between light intensity and photosynthesis is copious, little attention has been paid to specific end products of photosynthetic carbon fixation in freshwater algae. In freshwater ecosystems this approach has been applied only to the study of Cyanobacteria (cf. Konopka and Schnur 1980). The relative flow of fixed carbon into specific macromolecular end products (e.g. lipid, carbohydrate, protein) may serve as an independent means of assessing the nutritional status of the phytoplankton (Bates 1981; Morris et al. 1974). Biochemical composition studies may additionally provide information on algal nutritional value as food for herbivores (c.f. Scott 1980).

Several hypotheses have been drawn from culture and field

work on light intensity modulation of photosynthetic products in marine phytoplankton which may also be applicable to freshwater studies. Morris et al. (1974) have shown that the proportion of ^{14}C-bicarbonate incorporated into protein is often inversely proportional to light intensity. This suggests that subsaturating light favors protein synthesis rather than storage of carbohydrates. They have used these results to explain observations of an increase in the proportion of protein-^{14}C with depth of incubation in the euphotic zone of coastal and open ocean environments.

Unfortunately, it cannot be assumed that the amount of $^{14}CO_2$-C entering specific biochemical compartments is always quantitatively related to their net biosynthesis. Although the pools of low molecular weight (LMW) metabolites reach isotopic equilibrium with the added tracer rapidly enough in light incubations of growing populations (Cuhel and Waterbury 1984; Lancelot and Mathot 1985; but see also Di Tullio and Laws 1983), turnover of macromolecules or use of polymeric carbon reserves during night metabolism can alter the specific activity of the cellular ^{14}C incorporated into new products. The magnitude of potential interference is not readily detectable with $^{14}CO_2$ assimilation studies alone.

Sulfate is an essential nutrient for microbial growth, being required for protein synthesis and photosynthetic electron transport systems, yet measurement of its assimilation into

microbial biomass is not hampered by several of the problems common to phosphorus and nitrogen uptake studies. Sulfate is so abundant in aquatic systems that substrate depletion during incubation never occurs (typical uptake rates of added ^{35}S-sulfate are <Ø.ØØ1% h^{-1}). As a result, there is no need for microorganisms to store sulfur in excess of immediate growth requirements. Incorporation of sulfate into protein therefore provides a rate measurement of protein synthesis which is useful in comparison with subcellular redistribution of ^{14}C from bicarbonate for investigation of night protein synthesis (Cuhel et al. 1984).

The influences of light intensity and incubation time are important factors controlling the total amount and biochemical incorporation patterns of CO_2-C and SO_4-S (Cuhel and Lean 1987). Superimposed on incubation time is the effect of "local time" relative to the day/night cycle as well as the presence or absence of light itself. Metabolism and growth of algae at night has been frequently overlooked in both laboratory and field studies, despite a developing literature stressing the widespread occurrence of cell division periodicity cued to day/night cycles (c.f. Brand and Guillard 1981; Jorgensen 1966; Weiler and Eppley 1979; reviewed by Chisholm 1981).

The spectral distribution of solar radiation differs markedly from that of light at various depths in the water column due to selective extinction by the water itself and through

absorption by dissolved organic matter and photosynthetic pigments. This difference is one of the reasons used to promote in situ rather than incubator measurements of primary productivity. Surprisingly little is known about the actual influence of light quality on quantitative aspects of phytoplankton photosynthesis and macromolecular synthesis. Major contributions by Wallen and Geen (1971a,b) documented stimulation of protein synthesis by blue and green light in pure cultures of algae. Synthesis of tricarboxylic acid cycle-related amino acids (ASP, GLU) is stimulated by as little as 5 minutes exposure to blue light (Hauschild et al. 1962) and is enhanced 3-fold over white light in only 30 minutes. Field measurements of nearly unialgal populations of colonial chrysophytes often found at metalimnetic chlorophyll maxima also demonstrated green light stimulation of protein synthesis (Pick and Cuhel, in press). Algae in nature grow under variable wavelength-distribution conditions, and their pattern of carbon incorporation into macromolecules may reflect enhanced photosynthesis and action spectrum shifts due to auxilliary pigments. An experiment was therefore conducted to ascertain the magnitude of light quality effects which might lead to differences in results from controlled light and in situ productivity studies.

Our diel studies of $^{14}CO_2$-C and $^{35}SO_4$-S incorporation as a function of light intensity were designed to (1) test the similarity of freshwater and marine phytoplankton light

intensity-dependent macromolecular synthetic patterns, (2) document the influence of light intensity on sulfate assimilation, (3) provide collaborating data for the in situ experiments to assess the proportion of carbon fixation variability attributable to light intensity alone, and (4) establish the dependence of night protein synthesis on prior light intensity history. The experiments spanned diel light cycles from 10L:14D to 16L:8D and temperatures from 1.2-18.7°C; thus we were able to document several seasonal trends for which light intensity effects may have been secondary to other factors.

MATERIALS AND METHODS

The transmission spectra for incident light, near-surface and deeper euphotic zone water, unfiltered white light from the primary incubator used for light intensity experiments, and a second incubator used for the control samples are compared in Fig. 1. For the light quality experiment, incoming light was modified with red, blue, or green color filters (Fig. 1d). The light from the control incubator closely resembles incident radiation and near-surface water, whereas the experimental incubator provides light resembling that found between about 5-15 m. The only serious trough in the spectrum from the experimental incubator (500-520nm) fortuitously occurs at the absorption minimum for chlorophylls a, b, and c and is well below the ca. 550nm absorption maximum of phycobiliproteins (O'Carra and O'h

Eocha 1976). Fucoxanthin, a principal accessory pigment in diatoms, does have maximum absorbance just above 500nm (Jorgensen 1977), but the very broad absorption maximum of this pigment imparts good light harvesting capability at the wavelength of maximum output in the incubator. Thus, selective elimination of photosynthesis by groups of algae containing specific pigments is not likely. Each of the color filters effectively restricted the range of output wavelengths. The spectral distribution of light from the different sources was measured with a LiCor QSM 1-2500 quantum spectrometer; light flux in the photosynthetically active 400-700nm waveband was measured with a LiCor LI-185A quantum meter.

Water samples were collected before dawn in 5-liter Niskin bottles, usually at a depth of 5 m, pooled in a 20 L carboy, and dispensed into 300 mL glass BOD bottles. Replicate bottles, enough to provide for two isotopes at 5-6 light intensities plus zero time blanks, were inoculated with either ^{14}C-bicarbonate (2GBq $mmol^{-1}$; 20DPM ngC^{-1} final specific activity) or ^{35}S-sulfate (carrier free; 150DPM ngS^{-1} final specific activity) and the bottles placed on a rotating wheel in one of two temperature- and light-controlled incubators. The light cycle was coincident with local apparent sunrise and sunset. Filtration methods and subcellular fractionation procedures used to separate low molecular weight (LMW), alcohol-soluble protein, lipids, hot trichloroacetic acid (TCA)-soluble

material, and residue protein are detailed in the accompanying in situ paper (Cuhel and Lean 1987).

Water for the light quality experiment was collected from 7.5 m at 2.5-3 hour intervals at mid-lake station 403 and dispensed into steam-cleaned 300 mL BOD bottles. Replicate bottles were inoculated with ^{14}C-bicarbonate (final specific activity 17 DPM ngC^{-1}) and placed in the principal incubator. Freshly collected water samples were used for each color to minimize dark containment-induced changes in macromolecular synthesis; therefore, duplicate reference samples were also incubated at saturating white light in a second incubator at the same temperature. After 2-3 hours, the bottles were removed and 100 mL subsamples filtered through Whatman GF/F filters. One of the samples was processed by the subcellular fractionation procedure previously described for this study; the remaining samples were counted whole. Overall, both internal and external duplicates agreed well, with average error under 7%.

To provide quantitative comparison of photosynthetic rates with plankton samples collected at different times, we normalized all uptake data to a constant hourly rate for the control samples in the second incubator. The mean rate shown in Table 1 was used to determine the normalization factor for the individual incubations; all factors were <10%. The agreement of subcellular ^{14}C distribution among incubations was sufficient to distinguish differences of about 2% within an incubation and about 5% between

incubations.

RESULTS

Macromolecular Incorporation Patterns of C and S

Several features common to all light gradient experiments are illustrated in Fig. 2 for the inshore station 401 on 20 May. Uptake of ^{14}C-bicarbonate and ^{35}S-sulfate was light saturable, but sulfate uptake approached saturation at an intensity significantly less than that required for maximal carbon dioxide fixation. The characteristic decreasing proportion of carbon incorporated into protein with increasing light intensity (summarized by Morris [1980]) offset by increasing proportional lipid incorporation is clearly distinguishable from the light intensity-independent assimilation pattern of sulfate. At this station early in the season, the dominance of diatoms (Gray 1987) is reflected in the very high proportion of sulfate esters, which account for 70% of the total sulfur fixed in this example. High diatom productivity and associated non-reductive ester-SO_4^- synthesis was marked by very low whole cell ^{14}C:^{35}S uptake ratios (ca. 20) while total ^{14}C:reduced-^{35}S values remained typical of microbial biomass (ca. 70).

Light Quality

While chromatic adaptation is of advantage to populations residing for prolonged periods in subsurface waters, plankton of

the mixed layer may not experience restricted light fields long enough to induce physiological response (Falkowski 1984). Hence the lack of consistent effect of light quality on total carbon dioxide fixation (Fig. 3a) of midsummer epilimnetic phytoplankton was not unexpected. Both blue and red light yielded reduced total photosynthesis at intermediate intensities (ca. 200 uEinst. m^{-2} s^{-1}) but initial slopes and final apparent maximum photosynthetic rates were similar for all colors. At the highest light intensities, deviation from the rate in white light was less than 15% for red light (enhancing) and blue light (inhibiting).

Fig 3

The amount of $^{14}CO_2$-C incorporated into protein, lipid, and hot TCA-soluble material increased similarly with light intensity for the different experiments (Fig. 3b, c, d). The white light control experiment, conducted on a very late afternoon sample, had a lower percentage incorporation of ^{14}C into protein than the standards from the second incubator for the color experiments (Table 1); the dashed line in Fig. 3b denotes the rate corrected for the mean protein synthesis of all the white controls. Even without this consideration, there was but a small enhancement of ^{14}C incorporation into protein in colored light. No distinguishable differences were found for either lipid or carbohydrate (HTCA) incorporation. The absence of adaptation to a specific light field is likely to benefit these populations because the mixed layer encompasses the entire euphotic zone

(mixed layer ca. 10 m, 1% I_0 ca. 11.5 m).

Time Course and Diel Components of Photosynthesis

While absolute uptake and biochemical assimilation of $^{14}CO_2$ and $^{35}SO_4^{2-}$ are influenced by light intensity, both duration of light incubation and time-of-day also may be important in the products of photosynthesis over the 24-hour light/dark cycle. The interaction of these factors will be described using the light gradient experiment of 24 August at inshore station 401 (Fig. 4).

FIG 4

It is first necessary to emphasize the difference between ^{14}C distribution (as percent of total fixed carbon) and the amount of carbon fixed into specific components. In this example, although the proportion of ^{14}C in protein decreases with increasing irradiance (Fig. 4b), the amount of ^{14}C in protein increases and the effect of photoinhibition is less severe than for other fractions (Fig. 4c). Furthermore, incorporation of ^{14}C into protein was light saturated at lower irradiance than total ^{14}C uptake. Consideration of both proportional and absolute subcellular isotope distribution is thus essential for interpretation of isotope incorporation data.

Total CO_2 fixation was light saturated between 336 and 760uEinst. m^{-2} s^{-1} and displayed a photoinhibitory response at 1680uEinst. m^{-2} s^{-1} (Fig. 4a). The extent of photoinhibition was the same for all incubation times, averaging 57% reduction of

photosynthesis relative to P_{opt} (336uEinst. m^{-2} s^{-1}). Dark CO_2 uptake was less than 2% of the light-saturated rate for all time points.

Light intensity strongly influenced the subcellular distribution of newly-fixed carbon, represented in Fig. 4b for the full day (14.8 h) incubation. The most pronounced effect was on the proportion of carbon incorporated into protein. Dark metabolism resulted in over 50% of the total ^{14}C appearing in the protein fraction, whereas only 15% was found in protein at the optimum light intensities. At photoinhibiting light intensity (1680uEinst. m^{-2} s^{-1}) there was also an increase in percent carbon incorporation into protein, while total carbon fixation was decreased by 57% relative to P_{opt}. Conversely, assimilation of carbon into lipids was directly proportional to light intensity, and showed no indication of photoinhibition (as percent of total fixed carbon). Both LMW and hot TCA-soluble (nucleic acid and carbohydrate) carbon increased from dark incorporation levels to a small extent (not shown) reaching a relatively stable plateau near the lower bound of saturating light intensities; however, by far the most significant effects of light intensity were on protein and lipid incorporation.

In all cases during this study, photoinhibition was accompanied by an increase in the percentage of $^{14}CO_2$-C in protein, indicating that protein synthesis was less adversely affected by high light than other biosynthetic reactions. The

proportion of carbon in protein was lowest at optimum light intensities for photosynthesis.

Sulfate uptake was also a positive function of light intensity (Fig. 5a) and incubation time, but there were several important differences between carbon and sulfur uptake. There was a much smaller photoinhibitory response at the highest light intensity, but inhibition increased with incubation time from 14% at 3.9 h to 28% for the full day (14.8 h) incubation. It was also immediately apparent that the ratio of light-saturated to dark sulfate uptake was much lower than for $^{14}CO_2$ fixation, particularly in the short incubation. Light enhancement of sulfate uptake (P_{opt}:dark) increased with exposure time (Table 2) to a greater extent than for carbon fixation but reached only 5% of the maximum P_{opt}:dark ratio for CO_2.

Light intensity exerted a smaller influence on the subcellular distribution of newly-incorporated sulfur (Figure 5b). A minor but very reproducible reduction in the percentage of sulfur in protein occurred with increasing light intensity. No influence whatsoever was seen in the proportion of either sulfur-containing lipids or LMW components, and the hot TCA-soluble sulfur (primarily the ester-SO_4 component) increased only at the highest light intensity. Note the much lower % ester-SO_4^- relative to the early-season experiment (Fig. 2).

Other than the weak but consistent influence of exposure time on the P_{opt}:dark sulfate uptake ratio, the was no difference

in the subcellular distribution of either $^{14}CO_2$-C or $^{35}SO_4^{2-}$-S as a function of incubation time (Table 3). The consistent absence of incubation time effects in this study facilitates comparison of data from experiments of differing incubation time, as long as these are confined to the natural light period.

TAB 3

Night metabolism

Carbon metabolism of algae at night, in the absence of net CO_2 fixation, became apparent only when the subcellular distribution of previously-fixed carbon was examined during the night relative to that of samples harvested at the end of the light period. Due to the nature and extent of the redistribution of ^{14}C in the subcellular fractions (Fig. 6) and the low dark CO_2 fixation during day and night incubations, the contribution of incidental CO_2 fixation by chemolithotrophs and other non-photosynthetic CO_2-fixing microorganisms was discounted. The events shown for the sample previously exposed to optimum photosynthetic light intensity (336uEinst. m^{-2} s^{-1}) conformed to certain generalities regarding night algal metabolism: (1) protein-^{14}C continued to increase during the dark period at a rate not substantially different from the light-driven rate; (2) both HTCA (i.e. carbohydrate-containing) and LMW components lost ^{14}C; (3) lipid-^{14}C remained constant or increased slightly but did not decrease at night; and (4) loss of total cellular ^{14}C occurred, but to a lesser extent than the sum of losses from LMW

FIG. 6

and HTCA compartments. The increase in protein-^{14}C therefore contained label derived from the LMW metabolic intermediates and/or carbohydrate reserves, both of which function as dual carbon and energy sources for night metabolism.

With respect to whole cell elemental composition, night metabolism produced a relatively uniform effect at all but the lowest intensities of prior light exposure (Fig. 7). Small losses of ^{14}C, about 15% of that fixed during the day, were accompanied by much larger increases in total cellular ^{35}S, so the whole cell ratio of ^{14}C:^{35}S dropped by almost 50% overnight (Fig. 7c).

As shown in Fig. 6, carbon from LMW intermediates and storage products was partially redistributed into the protein fraction at P_{opt} during night growth. This phenomenon was not restricted to samples previously exposed to optimum light. Total protein-^{14}C (Fig. 8a) and protein-^{35}S (Fig. 8b) both increased at night across the light gradient, and the overnight protein component retained the general shape of the day's P vs. I curve. As in light-driven photosynthesis and protein synthesis, the photoinhibitory effect of the highest light intensity was more pronounced for ^{14}C incorporation into protein than for ^{35}S. In contrast to whole cell results described above, however, there was little change in the protein ^{14}C:^{35}S ratio after overnight incubation (Fig. 8c). The ^{14}C:^{35}S ratio of the isolated protein for all light intensities compared favorably with the value of 48 derived from sequenced proteins (cf. Jukes et al. 1975),

suggesting that CO_2-fixing microorganisms dominated the assimilation of sulfate into protein and that heterotrophic microorganisms accounted for a relatively small proportion of total net protein synthesis in the upper water column. The upper panel of Fig. 8 shows the night only increase in protein-^{14}C and protein-^{35}S, which further emphasizes the prior light dependence of night protein synthesis. The proportion of protein synthesized at night, based on the ^{35}S data, was about 50% of the light day amount or 30% of the full 24 hour production, in balance with the proportion of light in the 15:9 light/dark cycle. Thus rates of night protein synthesis were nearly equal to daytime rates.

It is necessary to differentiate between incubation time-dependence and time-of-day-dependence when considering temporal changes of biochemical composition measured by radioisotopic methods. Fortunately, in the great majority of cases in this study, assimilation of ^{14}C-bicarbonate during light incubation proceeded with uniform incorporation of carbon into the major end-products of photosynthesis. For the experiment of 24 August, the relative subcellular distribution of both ^{14}C and ^{35}S was constant throughout the light day (Fig. 9). The redistribution of ^{14}C at night was readily discernable as increased % ^{14}C in protein and to a lesser extent in lipid as protein synthesis utilized carbon from LMW and carbohydrate compartments. Combined with a small overall loss of ^{14}C due to respiratory energy production, the proportional distribution reflected the net flows

FIG 9

depicted in Fig. 6. Sulfur requirements for net protein synthesis at night were met with exogenous $^{35}SO_4^{2-}$, and there was no change in subcellular distribution of ^{35}S at any time.

Night protein synthesis and carbohydrate degradation were dependent on prior light exposure. Ratios of the nutritionally significant carbon-containing bioploymers to one another were also sensitive to prior light history (Fig. 10), and graphically illustrated the potential alteration of cellular composition by night metabolism. In the dark, protein was the major sink for carbon, with little incorporation of ^{14}C-bicarbonate into lipid. The absence of a light/dark cycle prevented change of the resultingly high ratios of protein to other polymers. Subsaturating light (55uEinst. m^{-2} s^{-1}) produced overnight changes amplifying the dominance of protein in cellular composition. Prior light intensities promoting near-maximum but not photoinhibited carbon dioxide fixation (140-756uEinst. m^{-2} s^{-1}) lead to consistent night increases in protein:carbohydrate and protein:lipid, and dawn samples contained nearly equal proportions of ^{14}C in the three macromolecular components (Fig. 10). At photoinhibiting irradiances, the less pronounced inhibition of protein synthesis was reflected in higher ratios of protein to other components after overnight incubation.

Fig 10

Seasonal Effects: Temperature and Daylength

Prior to water column stratification the photosynthetic

characteristics of surface and deep water phytoplankton at the central Lake Ontario station 403 were very similar. On 11 June 1982 water from 1 and 150 m (water column depth 178 m) yielded similar total uptake of $^{14}CO_2$-C or $^{35}SO_4$-S in all day incubations (Fig. 11). The % ^{14}C incorporated into protein declined from a dark maximum of 25-35% to 4-6% at light saturation. The lowest light level, 8uEinst. m^{-2} s^{-1}, was well resolved from the dark control and light saturation was reached at lower light intensity than for inshore, stratified populations. There was no light intensity influence on the % ^{35}S in protein, but a small increase did occur at all light intensities relative to the dark control. Ester-SO_4^- contained about 20% of the total assimilated sulfur.

During the season a total of 14 light intensity experiments were performed at the two major stations, encompassing a wide range of physical, chemical, and biological conditions. Comparison of potential photosynthesis and biochemical composition at P_{opt} with light intensity-dependent trends in the pathways of $^{14}CO_2$ and $^{35}SO_4^{2-}$ assimilation (summarized in Table 4) permitted several generalities to be drawn from the study.

Sulfate uptake and incorporation of both carbon and sulfur into protein attained maximum light-stimulated rates at lower light intensities than total $^{14}CO_2$ fixation: the remaining photosynthetic potential was channeled largely into polymeric carbohydrate production and enhanced LMW metabolite pools.

Consequently the proportion of ^{14}C incorporated into protein was always inversely related to light intensity. Relative synthesis of lipids generally increased with light intensity, but instances of no light effect were found, particularly during the short days of spring and fall. The relative incorporation of carbon into polysaccharides + nucleic acids (HTCA) was surprisingly often inversely proportional to light intensity. Only weak trends, if any, were observed for protein-^{35}S as a % of the total label taken up.

We were most intrigued by seasonal trends of $^{14}CO_2$-C assimilation into macromolecular end products at P_{opt}. Among the many physical and nutritional influeneces experienced by the plankton during the April-November sampling period, water temperature and daylength emerged as the most important environmental factors controlling plankton metabolism and biochemical composition. While warming of surface waters resulted in only mildly increased assimilation number at P_{opt} ($Q_{10} = 1.1$; $r^2 = 0.529$; not shown), incorporation of ^{14}C into protein was tightly coupled to temperature ($r^2 = 0.812$; Fig. 12d).

FIG 12

Carbohydrate synthesis (i.e. $^{14}CO_2$-C incorporation into the HTCA fraction) provides reserve energy for night metabolism and therefore may be expected to vary as a direct function of nightlength (Hitchcock 1980). At P_{opt} (data from Table 4) Lake Ontario phytoplankton accurately tracked the changing daylength with storage of energy reserve polymers (Fig. 12a): the longest

day (i.e shortest night), 21 June, is the vertex of a parabola describing the percentage of newly-fixed $^{14}CO_2$-C incorporated into the HTCA-soluble fraction. The proportion of HTCA-^{14}C was quantitatively related to daylength (r^2 = -Ø.933) as demonstrated in Fig. 13.

The independent nature of temperature and daylength regulation of phytoplankton metabolism is demonstrated in part by the absence of correlation between % C in HTCA and temperature (Fig. 12b) and the weak relationship between daylength and % C in protein (Fig. 12c). The validity of daylength as a forcing function for carbohydrate storage is strengthened by experiments performed early in the season (paired sets including Julian Days [138, 140], [160, 162], and [181, 183]). The very late development of thermal stratification at the central lake station fortuitously afforded an opportunity to compare carbon incorporation patterns of inshore, warm-water populations with plankton from cold water under identical day/night cycles. In the absence of daylength differences, phytoplankton at both stations produced identical proportions of HTCA material but had substantially different proportions of protein-C, with the offshore station yielding the lower percentage of protein-C. Therefore, physical factors such as temperature and daylength may, in some cases, prevent changes in cellular composition in response to other regulators such as nutrient deficiency (Myklestad and Haug 1972).

It is noteworthy that no temperature influence was recorded for the proportion of ^{35}S incorporated into protein. In fact, the % S in protein was relatively constant throughout the summer (Table 4), but during the spring incorporation of ^{35}S-sulfate into the ester-SO_4^- fraction was very high (>50% of the total sulfur assimilated). From June on the importance of this fraction declined steadily to minimum values (ca. 15% of the total sulfur assimilated) in late August. No late season increase was observed.

DISCUSSION

The extent to which light stimulated ^{14}C-bicarbonate uptake and controlled the metabolic fate of newly-fixed carbon contrasted sharply with its mild enhancement of $^{35}SO_4^{2-}$ uptake and virtual impotence over the pathways of sulfate assimilation. The differences highlighted the manner in which photosynthetic microorganisms have adapted to maintain temporal continuity of growth in the energetically discontinuous environment imposed by the day/night cycle. Central metabolic processes imperative to growth (e.g. protein synthesis, nucleic acid synthesis and repair) were uncoupled from the external source of energy and activated through an intermediate and continuously available internal supply. Reduced carbon acted as a storehouse of potential energy (as reducing power) as well as a precursor in biosynthesis. In addition to the primary value of making the

night available for biosynthetic purposes, substantial shorter-term benefits were obtained: the biochemically facile dehydration/polymerization and hydrolytic mobilization of carbohydrate polymers involved monomers which were direct metabolites of the primary photosynthate and immediate precursors to the tricarboxylic acid cycle of structural transformation and rapid energy generation. Consequently, excess production of photosynthate could be rapidly channelled into storage material and just as readily recovered to supplement or replace insufficient photosynthetic production. The energetic buffering capacity and quick response potential of polymeric carbohydrate reserves could thus ameliorate the unpredictable, short-term disturbances caused by shading or mixing through the water column as well as power the anticipated biosynthetic activities of impending night.

Photosynthesis by definition is obligately dependent on light, and it is the products of CO_2 fixation that yield information on the relative physiological status of the phytoplankton. The occurrence of carbon in nearly all cellular constituents provided an opportunity for detailed observation of metabolic activities using $^{14}CO_2$ but it was correspondingly more difficult to interpret $^{14}CO_2$ incorporation data quantitatively, particularly at night. Because the process of protein synthesis itself was not directly light dependent, $^{35}SO_4^{2-}$ assimilation measurements reflected growth which integrated the recent light

history of the population. Reduced sulfur was much more restricted in subcellular processes, and by virtue of its ready availability (as sulfate) luxury uptake or mass storage mechanisms have not been necessary. Uptake and reduction occurred as needed for biosynthesis and provided a direct, quantitative means for measuring protein synthesis. $^{14}CO_2$ enabled sensitive analysis of de novo photosynthetic production as a function of imposed variables and with respect to diverse metabolic pathways. The understanding gained from assimilation patterns of $^{14}CO_2$ was enhanced substantially through concurrent study of $^{35}SO_4^{2-}$ incorporation, which distinguished between net production and turnover (both of which incorporate CO_2 into biopolymers in the light) and was an effective tracer of night production in quantitative terms.

In the majority of our experiments, by far the greatest light intensity-induced changes of carbon distribution occurred in the protein and lipid fractions. Frequently no discernable effect was found in either carbohydrate + nucleic acids (HTCA) or LMW components (Table 4). Similar experiments with pure cultures and natural populations of Cyanobacteria (Konopka and Schnur 1980) displayed much greater changes in hot TCA-soluble and LMW components. These differences may be useful in identifying principal contributors to total photosynthesis in mixed populations, but better understanding of phylogenetic characteristics of macromolecular synthesis is required.

For open lake phytoplankton, the percentage of ^{14}C-bicarbonate incorporated into protein in white light decreased with increasing light intensity, reaching a minimum at light saturation. This intensity-dependent relationship was also valid for red, blue, and green light exposure. During in situ incubations the percent ^{14}C in protein increases with depth below a minimum which is usually coincident with the depth of maximum photosynthesis and chlorophyll-specific assimilation efficiency (Cuhel and Lean 1987). Deeper penetration of blue-green light combined with decreasing light intensity complicate interpretation of in situ macromolecular synthesis patterns when the phytoplankton display chromatic adaptation, but for mixed-layer phytoplankton light quality effects on total photosynthesis and its macromolecular end products were negligible. Thus for Lake Ontario plankton the depth of minimum $\%^{14}C$ in protein may be used as a bioassay for P_{opt}, especially when community structure and/or biomass varies markedly with depth. In such cases, the depth of maximum productivity may not necessarily be the same as the depth of biomass-specific maximum photosynthesis.

Light provided much less stimulation for sulfate uptake than for CO_2 and the pathways of its assimilation were only weakly influenced by light intensity. Even though bacteria accounted for 50% of the biomass of lake plankton <20um in cross-section (Caron et al. 1985) a surprising number of indices such as the proportion of sulfur incorporated into protein (Roberts et

al. 1963; Kelley et al. 1976), presence of sulfolipids (Benson 1963; Sinensky 1977), occurrence of carbohydrate polymer-bound inorganic sulfate (ester-SO_4^-: Ramus 1974; Percival et al. 1980), and parallel patterns of $^{14}CO_2$ and $^{35}SO_4^{2-}$ metabolism at night implicate photoautotrophs as the dominant producers of particulate protein in the surface microplankton community. Bacterial activity was however indicated by $^{35}SO_4^{2-}$ incorporation patterns in samples from the base of the euphotic zone/mixed layer in in situ incubations (Cuhel and Lean 1987).

We have found low P_{opt}:dark ratios for sulfate to be quite general for lake plankton throughout the year, whereas marine plankton display more pronounced light enhancement of sulfate uptake (Cuhel et al. 1984). Low light:dark uptake ratios were also obtained by Monheimer (1981) at depths of >25% P_{max} in Lake St. George. Possible explanations include a large, light-independent component resulting from microheterotrophic activity or lack of direct coupling between incident light intensity and essential biosynthetic processes (e.g. protein and nucleic acid synthesis) in freshwater algae. As discussed above, we favor the latter for several reasons. In pure cultures of freshwater algae, Monheimer (1981) demonstrated that dark sulfate uptake often exceeded rates under low light, and the P_{opt}:dark ratios were low overall in short-term incubations. In both our light gradient and in situ studies, night rates of sulfate assimilation from optimal prior light exposures exceeded light rates from

lower intensities or depths.

As an index of nutritional status of microplankton, the C:S ratio of whole cells is complementary to the C:N ratio. Like nitrogen, sulfur is primarily associated with essential growth factors (e.g. protein and nucleic acids) whereas C represents both growth-related and energy generation components. Lower C:S and C:N ratios imply more protein-rich composition, and provide a gross indication of substantial change in plankton nutritional value resulting from night metabolism.

Continuation of protein synthesis at night, drawing on LMW and carbohydrate components of the plankton for both energy and amino acid skeletons, will result in increased protein:carbohydrate and protein:lipid ratios as predicted by the overnight decrease in whole cell $^{14}C:^{35}S$. The relative abundance of protein, carbohydrate, and lipid in microplankton has been shown to influence the growth rate and assimilation efficiency of herbivores (Scott 1980). Pure cultures of Dunaliella tertiolecta have been used to provide examples of the possible range of cellular composition (with respect to C, S, N, protein and carbohydrate) during phases of the day/night cycle (Cuhel et al. 1984). Night growth may be a factor resulting in diel cycles of microplankton nutritional value to herbivores. Our studies on Lake Ontario at first suggested that diel cycles in the biochemical composition of plankton occur and may be of significance to the trophodynamics of lake plankton.

Because of the higher proportion of protein-^{14}C at the beginning of the night for sub- and supersaturated light regimes, it was only during prior light exposures in the range of optimum photosynthesis that overnight metabolism produced plankton with uniform ratios of carbon-containing macromolecules, based on ^{14}C distribution. In all likelihood, protein carbon was even more abundant after night metabolism than suggested, due to the differing specific activity of carbon during night redistribution processes (see above).

The implications of Scott's work with a marine rotifer (Scott 1980) relate to the preferred food composition of a zooplankter obeying indeterminant growth. He found that the best growth and highest assimilation efficiencies were obtained when the food source contained an equal ration of protein (for biomass production), carbohydrate (for short-term energy generation), and lipid (for membrane synthesis and long-term maintenance energy during food inavailability). Based on the ^{14}C data presented above, zooplankton with these requirements would be best adapted to feeding in the middle euphotic zone late at night and near dawn, when night metabolism had altered the chemical composition of the food source to meet these criteria.

Other zooplankton may benefit from different food composition. For example, those with determinant growth will prefer a balanced food source during the growing stages (e.g. naupliar and copepodite stages) but may need more carbohydrate-

rich food during mature stages when the energy-expensive process of reproduction is the dominant metabolic event. In addition, eggs contain a high proportion of energy reserves as either carbohydrate or lipid (cf. Taylor et al. 1987). Egg production by Acartia clausi, for example, was concentrated in the late night following major episodic feeding during the afternoon and early evening (Saint-Jean and Pagano 1983) when ingested energy reserves would be greatest. Thus, the varied chemical composition of algae at different depths in the water column and at different times in the day/night cycle may provide a useful range of foods from which zooplankton may select the optimum type required for their particular metabolic needs.

In addition to alterations of nutritional quality of plankton, night protein synthesis is a major component of energy metabolism resulting in net losses of fixed carbon from the plankton. Uptake of sulfate and its reductive assimilation into the sulfur-containing protein amino acids cysteine and methionine is an energy-expensive process on a molecular basis. Taking into account transport, activation, and reduction to the level of sulfide, incorporation of one molecule of sulfate into protein requires approximately 21 ATP or high energy equivalents. Since sulfur-containing amino acids account for about 3 moles percent of total protein amino acids (Jukes et al. 1975), 33 peptide bonds (each requiring 5 ATP equivalents) are formed for each SO_4-S incorporated. Altogether the incorporation of one mole of SO_4-S

into protein requires about 190 moles of ATP, excluding biosynthetic requirements for generation of other amino acids and the carbon skeleton of the S-amino acids. Assuming that fixed carbon used for night respiration is reduced to the level of carbohydrate and allowing 38 moles of ATP generation for complete oxidation of each mole of glucose [$(CH_2O)_6$], we can estimate that 11ugC must be oxidized for every ugS (or 48 ugC) incorporated into protein. For the August 24 example described above (Fig. 6), night protein synthesis in the sample at P_{opt} incorporated Ø.126ugS, corresponding to oxidation of 1.4ugCH_2O-C for energy. Total loss of ^{14}C at P_{OPT} amounted to 4.5ugC; that is, 31% of the apparent night respiration can be accounted for by protein synthesis. The proportion of night ^{14}C respiration thus accounted for varied up to two-fold within an experiment, but there was no systematic relationship to light intensity. Given that this calculation is the ratio of differences containing error, we have considered the average value for all non-dark incubations within an experiment to be indicative of the energetic demands of night protein synthesis.

At this point it is necessary to reconsider terminology as it relates to ^{14}C vs. CO_2-C. During daylight incubations of even slowly-growing plankton populations, correction of ^{14}C-bicarbonate uptake for ambient CO_2 concentration and isotope discrimination enables accurate measurement of total carbon fixation, since active increases in all LMW and macromolecular

components occur. At night however, this assumption may no longer be valid. There is little information available as to the compartmentalization of recently-synthesized vs. extant LMW and/or carbohydrate pools. We will assume that all carbohydrate polymers are equally available for degradation and metabolism at night. The carbon flowing from these compounds into either protein synthesis or respiration will have a lower specific activity than that of the ^{14}C-bicarbonate added at the start of the experiment, by a factor of approximately $(^{14}CH_2O)_n$ / $[(^{14}CH_2O)_n + (^{12}CH_2O)_n]$, where n indicates polymeric material and the ^{12}C term is the initial phytoplankton content of carbohydrate. If the daytime production of $(CH_2O)_n$ equals the cell contents at dawn, night processes may be underestimated by up to 50% due to isotopic dilution from carbohydrate catabolism. Night respiratory loss of ^{14}C from daylight incubations therefore can only be considered as a minimum unless >3 doublings of phytoplankton biomass occur during the light period of isotope labeling (Taylor 1979).

In contrast, night metabolism and protein synthesis utilizes exogenous sulfur (as sulfate) rather than internal pools (Cuhel et al. 1984). Thus net increases in total and protein sulfur occur, with the same relative distribution of the newly-fixed sulfate among metabolic products. In this case, the specific activity of the sulfur incorporated at night remains the same as that of the added tracer, and quantitative measurements

are acheived. As a result, the incorporation ratios of $^{14}C:^{35}S$ will be less than the true weight ratio by the factor described above. In all cases, however, the amount of C flux will be underestimated by ^{14}C.

We now recognize that night protein synthesis is an important component in overall primary production. In most cases the proportion of protein synthesized at night was in accordance with the relative length of the night period, resulting in nearly continuous protein synthesis throughout the day/night cycle.

Night protein synthesis has implications for several aspects of lake dynamics. Obviously, the increase of protein to the food web is a primary consideration. Since few studies have been directed specifically towards estimation of protein production, no revision of current dogma is necessary. However, future studies in this area should take night growth into account. An indirect effect on nutrient cycling results from the need for plankton to assimilate nitrogen, as either nitrate or ammonium, from the water column to support amino acid synthesis. Accumulation of excess nitrate-N during the day or continuous utilization of NH_4^+-N may contribute significantly to total nitrogen dynamics.

When the mixed layer is deep and reaches below the depth of 1% $I_\emptyset$, as in many oceanic environments, circulation of cells throughout the mixed layer may prevent sufficient light exposure to enable night metabolism. Lake Ontario has a summer mixed layer

of about 10 m, which corresponds to 1-5% of surface light intensity. However, the depth of maximum assimilation number for in situ incubated samples was usually 5 m at mid-day, above which apparent photoinhibition occurred (Cuhel and Lean 1987). The results of concurrent studies of DCMU-enhanced fluorescence in incubator, in situ, and freshly-collected water samples demonstrated that the apparent photoinhibition found for static incubations was not realized by natural populations due to advective removal from high surface light intensities (Putt et al. 1987). The arguement holds equally well for limited residence time in the very light-limited bottom of the mixed layer. It is likely that vertical profiles of night protein synthesis in situ would be uniformly high throughout the upper water column. Maintenance of sub-surface maxima of night protein synthesis (Cuhel and Lean 1987) is probably as rare as the associated daytime primary productivity profile resulting from photoinhibition of samples held at fixed depth.

Prolonged exposure below the mixed layer (e.g. at 15 m depth) can also have a significant effect on biochemical composition. Although both production and night synthesis are low, due to light intensity effects on the immediate incorporation patterns of CO_2-C the net effect is production of relatively protein-rich food. This physiological stratification may also influence vertical distributions of zooplankton.

We have discussed the potential changes in biochemical

composition of microplankton resulting from night increases in protein at the expense of carbohydrate reserves, based upon changes in the subcellular distribution of newly-incorporated $^{14}CO_2$-C during night metabolism. In order for the observed alterations to actually influence the entire standing crop of plankton, it is necessary that the growth rate of the microplankton be sufficiently fast that one night's metabolism can significantly increase protein relative to carbohydrate and/or lipid. For this condition to be met, population doubling times must be on the order of one day or less. In Lake Ontario, however, such growth rates are rarely encountered. Estimates of doubling times for total POC and protein, based on $^{14}CO_2$-C uptake and $^{14}CO_2$-C or ^{35}S-sulfate assimilation into protein, are given in Table 5 for light intensity experiments and in Cuhel and Lean (1987) for in situ work. A significant discrepancy between POC and protein doubling times exists, although protein doubling times estimated from ^{14}C and ^{35}S incorporation into protein generally agree well. In any case, the growth rates are at best slow, and not sufficient for night growth to affect changes in total plankton crop composition. Perhaps this contributes to the lack of significant vertical migration of zooplankton in Lake Ontario (Taylor et al. 1987).

Environmentally imposed physical factors appear to have a large influence on phytoplankton growth rate and biochemical composition. Water temperature places an upper limit on cell

division frequency (Eppley 1972) and may exert equally strong regulation of cell composition. Relative incorporation of carbon into protein at optimum light was shown to be directly proportional to water temperature in Lake Ontario, consistent with pure culture (Morris and Farrell 1971) and field data from marine environments (Smith and Morris 1980; Morris et al. 1981; Cuhel et al. 1984). Water temperature sets the maximum potential growth rate of phytoplankton (Eppley 1972), and it may influence the biochemical composition (and hence nutritional value) of the phytoplankton as well.

A second significant factor, daylength, was well correlated with relative production of carbohydrate, the principal energy reserve for dark (night) metabolism of algae. This relationship has been demonstated in pure culture as well (Hitchcock 1980). Comparison of temperature and daylength effects on the two processes indicate that they act independently on phytoplankton metabolism. These long-term controls may define limits of cellular composition within which superimposed short-term variables such as nutrient availability must operate.

The direct relationship between nightlength and carbohydrate synthesis suggests a relatively sophisticated seasonal timing mechanism in phytoplankton. Time-course measurements demonstrated the independence of relative carbon incorporation into carbohydrate from incubation time during the day, yet carbohydrate production was well related to the duration

of the impending night. Moreover, the amount of storage far exceeds that required for "basal metabolism", suggesting that significant growth-related processes have been anticipated.

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Table 1. Hourly ^{14}C–bicarbonate fixation rates and percentage of total fixed carbon in protein and lipid for white light controls. The normalization factor for each incubation was the ratio of the mean for all incubations to the average of duplicate bottles for each incubation.

Experiment		$\mu g\ C \cdot L^{-1} \cdot h^{-1}$	Correction Factor	% Total ^{14}C In: Lipid	Protein
RED	A	9.183		23.9	19.9
	B	8.838		23.9	18.4
Average		9.011	0.912	23.9	19.2
BLUE	A	8.359		29.7	24.2
	B	8.713		28.6	24.7
Average		8.536	0.963	29.2	24.5
GREEN	A	7.733		27.5	20.8
	B	7.749		26.3	20.1
Average		7.741	1.068	26.9	20.5
WHITE	A	7.534		25.7	16.8
	B	7.641		27.4	15.8
Average		7.588	1.083	26.6	16.3
Mean		8.219		26.6	20.1
SD		0.637		2.1	3.2
C. V. (%)		7.8		7.9	15.9

Table 2. Effect of incubation time on P_{opt}:dark uptake ratios for $^{35}SO_4^{2-}$-S and $^{14}CO_2$-C at Station 401 on 24 August 1982. P_{opt} data is from the 336 uEinst. m^{-2} s^{-1} light level.

	Carbon			Sulfur		
	ug C L^{-1}			ng S L^{-1}		
Hours of Incubation	P_{OPT}	Dark	P_{OPT}:Dark	P_{OPT}	Dark	P_{OPT}:Dark
3.9	30.62	0.53	57.8	270.6[a]	136.7	2.0
8.0	59.38	0.74	80.2	510.9	199.2	2.6
14.8	92.57	1.14	81.2	757.2	215.2	3.5

[a] P_{opt} value from 756 uEinst. m^{-2} s^{-1} light level due to loss.

Table 3. Percent distribution of $^{14}CO_2$-C and $^{35}SO_4^{2-}$-S as a function of incubation time at station 401 on 24 August 1982. Data are given for two light levels; 140 and 336 uEinst. m^{-2} s^{-1}.

	Fraction % of Total Radioactivity											
	Carbon						Sulfur					
							LMW		Ester			
	Lipid		HTCA		Protein		Organic-S		Sulfate		Protein	
Hours of Incubation	140	336	140	336	140	336	140	336	140	336	140	336
3.9	22.8	22.5	26.1	30.5	19.2	15.2	17.0	LOST	28.7	LOST	31.3	LOST
8.0	21.8	23.1	28.2	30.3	18.2	15.1	17.7	17.4	27.5	28.6	30.5	28.0
14.8	20.7	22.8	25.8	30.2	17.3	15.4	17.2	19.2	25.1	24.8	31.8	30.2

Table 4. Seasonal summary of photosynthetic parameters during light intensity experiments for the Lake Ontario Nutrient Assessment Study, 1982. Data are for 5 m samples unless indicated otherwise. Light intensity– dependent changes in subcellular distribution are described as: NC, no change; POS, positive; NEG, negative; values in parentheses indicate weak trends. N/A, not available.

					FRACTION % TOTAL LABEL @ P_{opt}				CHANGE IN FRACTION % TOTAL LABEL WITH INCREASING LIGHT INTENSITY				WEIGHT RATIO	
					CARBON			SULFUR	CARBON			SULFUR		
DATE	STN.	TEMP. (°C)	CHL a (ug l^{-1})	ASSIM. #[a]	LIPID	HOT TCA	PROTEIN	PROTEIN	LIPID	HOT TCA	PROTEIN	PROTEIN	TOT.C: RED.-S	PROT. C:S
6 APRIL	401	1.2	1.6	0.4	12.3	38.6	3.9	18.0	NC	NC	NEG	NC	109	9.7
18 MAY	403	2.4	1.7	1.0	15.5	21.4	6.5	9.3	POS	NC	NEG	NC	263	35.9
20 MAY	401	8.9	4.1	1.4	35.9	22.1	12.1	9.5	POS	NC	NEG	NC	70	25.7
9 JUNE	401	10.4	2.9	1.3	34.9	17.7	13.6	26.7	POS	NEG	NEG	(NEG)	133	38.3
11 JUNE	403[b]	3.2	1.9	1.7	18.8	18.5	4.7	28.2	(NEG)	NEG	NEG	NC	447	65.9
30 JUNE	401	10.0	5.1	2.4	39.5	19.4	10.9	27.6	POS	NEG	NEG	(NEG)	135	32.7
2 JULY	403[c]	3.8	3.8	1.0	22.8	19.0	6.6	27.5	POS	(NEG)	NEG	NC	474	83.3
13 JULY	403	15.7	6.7	1.3	31.2	22.3	20.0	28.9	POS	NEG	NEG	NC	82	32.7
12 AUG	403	18.7	2.5	3.7	21.3	26.2	20.3	N/A	POS	NEG	(NEG)	N/A	N/A	N/A
24 AUG	401	14.5	4.4	1.4	22.8	30.2	15.4	30.2	POS	NC	NEG	(NEG)	176	62.5
25 AUG	403	15.0	2.7	2.6	19.6	34.8	11.6	34.9	POS	NC	NEG	(NEG)	220	52.3
23 SEPT	403	16.0	3.5	2.9	17.0	36.3	12.4	7.0	NC	NC	NEG	NEG	58	88.5
20 OCT	403	12.3	5.7	1.7	18.6	44.3	11.2	19.8	NC	NC	NEG	(NEG)	493	189.0
22 NOV	403[d]	6.1	1.1	1.5	21.1	36.6	6.7	N/A	NC	NC	(NEG)	N/A	N/A	N/A

[a] Assimilation # in g C g Chl a^{-1} h^{-1}.

[b] Sample from 1m.

[c] Sample from 20m due to developing stratification.

[d] Incubation light/dark cycle 15 h out of phase.

Table 5. Standing crops of POC and protein at 5 m, with 24 h production of POC and protein by $^{14}CO_2$-C and protein by $^{35}SO_4^{2-}$-S assimilation techniques. All values are ug L^{-1}. Protein production based on CO_2-C or SO_4^{2-}-S are presented as protein based on 52% C, 1% S in protein by weight.

		Standing Crop		24 h Production			Days To Double		
				POC	Protein		POC	Protein	
Date	Stn.	POC	Protein	(^{14}C)	(^{14}C)	(^{35}S)	(^{14}C)	(^{14}C)	(^{35}S)
9 June	401	328	326	40.1	17.5	31.4	8.2	18.6	10.4
11 June	403	194	123	47.0	5.8	6.3	4.1	21.1	19.4
30 June	401	434	394	153.5	60.4	99.5	2.8	6.5	4.0
2 July[a]	403	516	218	50.0	8.1	6.9	10.3	26.9	31.6
24 Aug	401	457	323	88.1	38.8	35.4	5.2	8.3	9.1
25 Aug	403	314	353	80.7	34.0	33.1	3.9	10.4	10.7

[a] 20 m sample.

FIGURE LEGENDS

Fig. 1. Wavelength distribution of natural and artificial light relevant to the light quality experiment. Panel A: incident sunlight (solid line), solar radiation at 2 m (dotted line) and 10 m (dashed line). Panel B: Artificial light in the incubator used for controls. Panel C: Artificial light in the experimental incubator without filters. Panel D: As Panel C but with blue (dotted line), green (solid line), or red (dashed line) filters.

Fig. 2. Light intensity stimulation of short-term (3.2 h) $^{14}CO_2$-C and $^{35}SO_4^{2-}$-S uptake (lower panel) and proportional incorporation into selected macromolecular components (upper panel) for station 401 on 20 May.

Fig. 3. Total uptake (panel A) and incorporation of $^{14}CO_2$-C into protein (panel B), lipid (panel C), and carbohydrate (panel D) components of Lake Ontario plankton under red, blue, green, and white light.

Fig. 4. $^{14}CO_2$-C uptake and metabolism as a function of light intensity for station 401 on 24 August. Panel A: time course of total ^{14}C fixation. Panel B: proportion of total fixed ^{14}C in protein and lipid after 14.8 h. Panel C: amount of ^{14}C fixed into protein, lipid, and hot TCA-soluble material after 14.8 h.

Fig. 5. $^{35}SO_4^{2-}$-S uptake and metabolism as a function of light intensity for station 401 on 24 August. Panel A: time course of

total ^{35}S assimilation. Panel B: proportional distribution of fixed ^{35}S after 14.8 h.

Fig. 6. 24 h time course of $^{14}CO_2$-C incorporation into macromolecular products at P_{opt} (336uEinst. m^{-2} s^{-1}).

Fig. 7. Whole cell uptake of $^{14}CO_2$-C (panel A), $^{35}SO_4^{2-}$-S (panel B), and the whole cell-^{14}C:reduced-^{35}S ratio (wt.:wt.) as a function of light intensity for station 401 on 24 August. All day and overnight incubations are shown, with the net gain (panel B) or loss (panels A,C) of radioactivity shaded.

Fig. 8. Protein synthesis as a function of light intensity for station 401 on 24 August. All day and overnight incorporation of $^{14}CO_2$-C (panel A) and $^{35}SO_4^{2-}$-S (panel B) into residue protein, with protein ^{14}C:^{35}S weight ratio (panel C) and night only increase in protein ^{14}C and ^{35}S. Net gain (panels A, B) or loss (panel c) is shaded.

Fig. 9. Proportional distribution of $^{14}CO_2$-C (lower) and $^{35}SO_4^{2-}$-S (upper) in LMW-metabolites and macromolecular products during incubation at 336uEinst. m^{-2} s^{-1}.

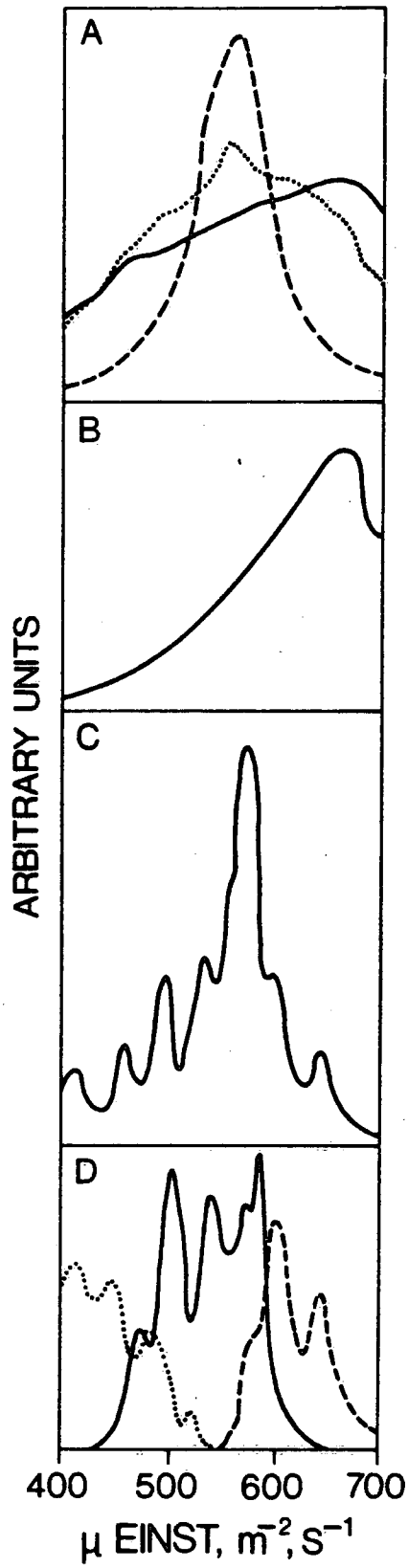
Fig. 10. Ratios of ^{14}C in newly synthesized major biopolymers throughout a day/night cycle as influenced by daytime light intensity exposure. Note scale change for dark control (Panel A).

Fig. 11. Comparison of photosynthetic character between surface

(1 m) and bottom water (150 m; water column depth 178 m) of Lake Ontario on 11 June, prior to seasonal stratification. Both panels: circles, 1 m samples; triangles, 150 m. Lower panel: Total uptake of $^{14}CO_2$ (open symbols, dashed line) and $^{35}SO_4^{2-}$ (closed symbols, solid line). Upper panel: Proportional incorporation of $^{14}CO_2$-C into protein (open symbols, dashed line) and LMW metabolites (closed symbols, solid line). All data are plotted.

Fig. 12. Relative incorporation of $^{14}CO_2$-C into protein and carbohydrate-containing (HTCA) fractions of Lake Ontario phytoplankton as a function of water temperature and daylength (in chronological Julian Days). Upper panels: incorporation into HTCA. Lower panels: incorporation into protein. Right-hand panels: water temperature at 5 m, C. Left-hand panels: Julian Days. Note: Light/dark cycle was 15 h out of phase for the last experiment (J.D. 326; water temperature 6.1 C; data circled).

Fig. 13. Incorporation of $^{14}CO_2$-C into HTCA as a direct function of daylength for data from Fig. 12. The value from November (circled in Fig. 12) was excluded because of the improper light/dark cycle.



L1 FIG 1 CONT

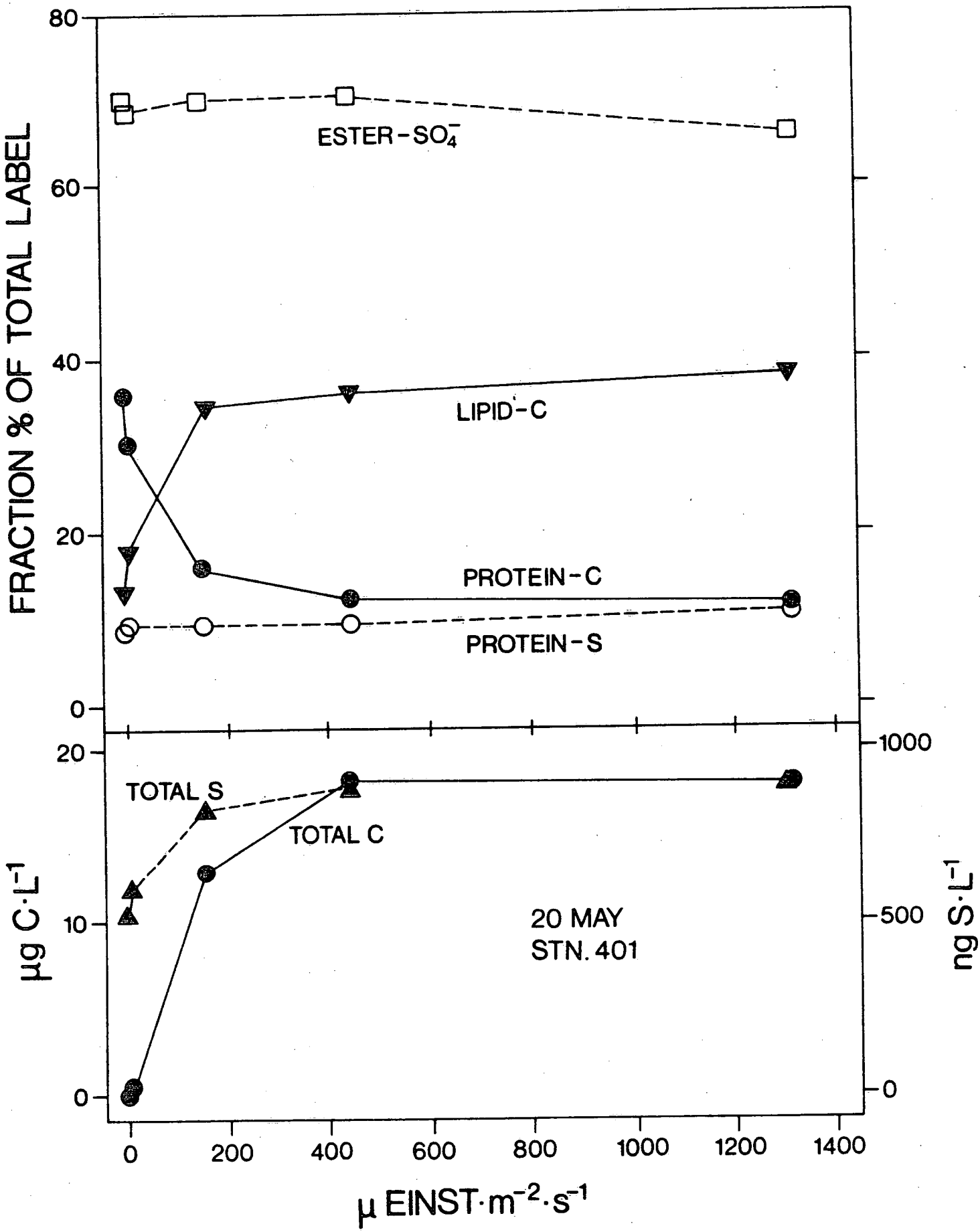
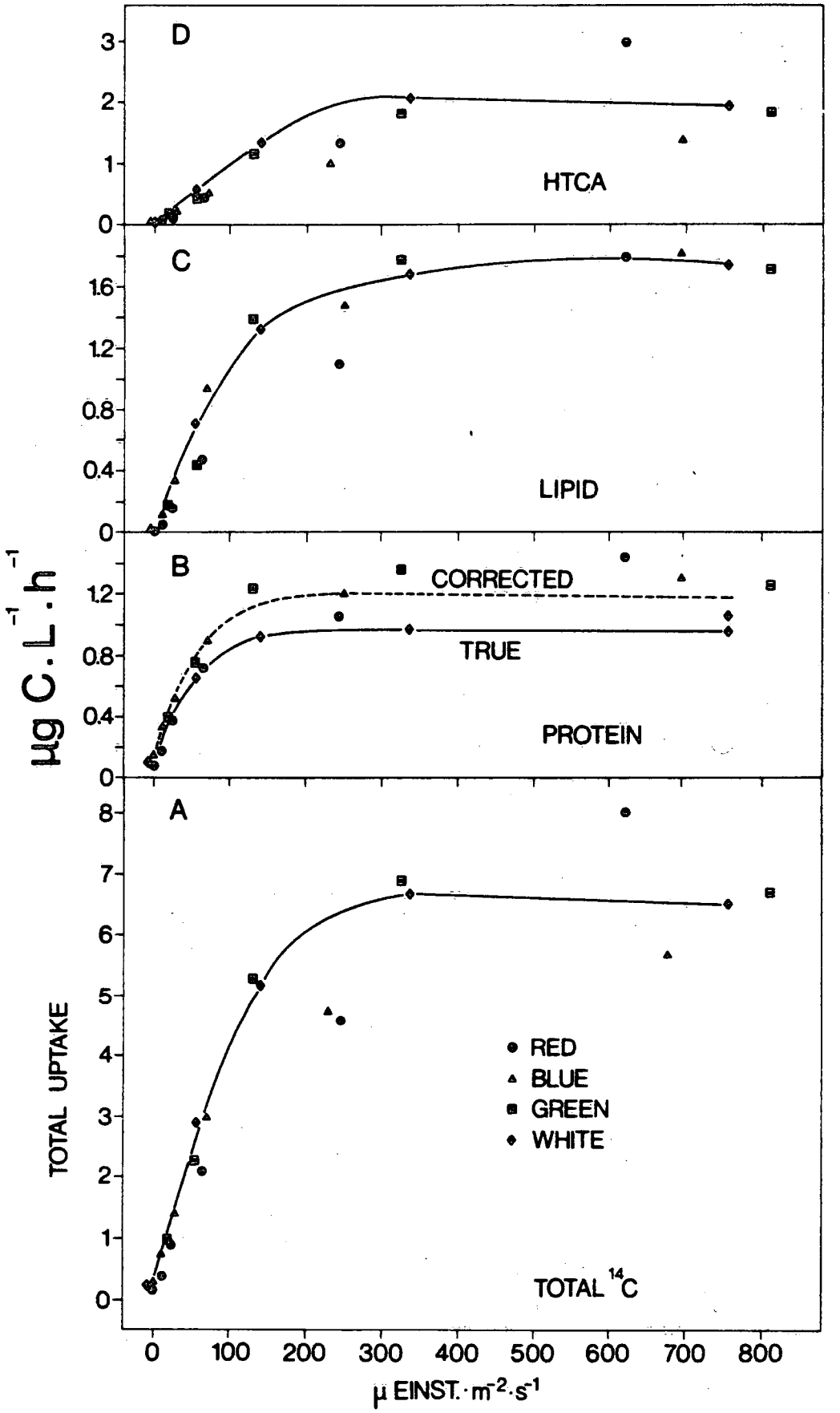

FRACTION % OF TOTAL LABEL
80
60
40
20
0
ESTER-SO_4^-
LIPID-C
PROTEIN-C
PROTEIN-S
20
10
0
TOTAL S
TOTAL C
20 MAY
STN. 401
μg C·L⁻¹
1000
500
0
ng S·L⁻¹
0
200
400
600
800
1000
1200
1400
μ EINST·m⁻²·s⁻¹

Fig 2. LI col.


D
HTCA
C
LIPID
B
CORRECTED
TRUE
PROTEIN
A
TOTAL ^{14}C
RED
BLUE
GREEN
WHITE
μg C.L^{-1}.h^{-1}
TOTAL UPTAKE
μ EINST.·m^{-2}·s^{-1}
0
100
200
300
400
500
600
700
800

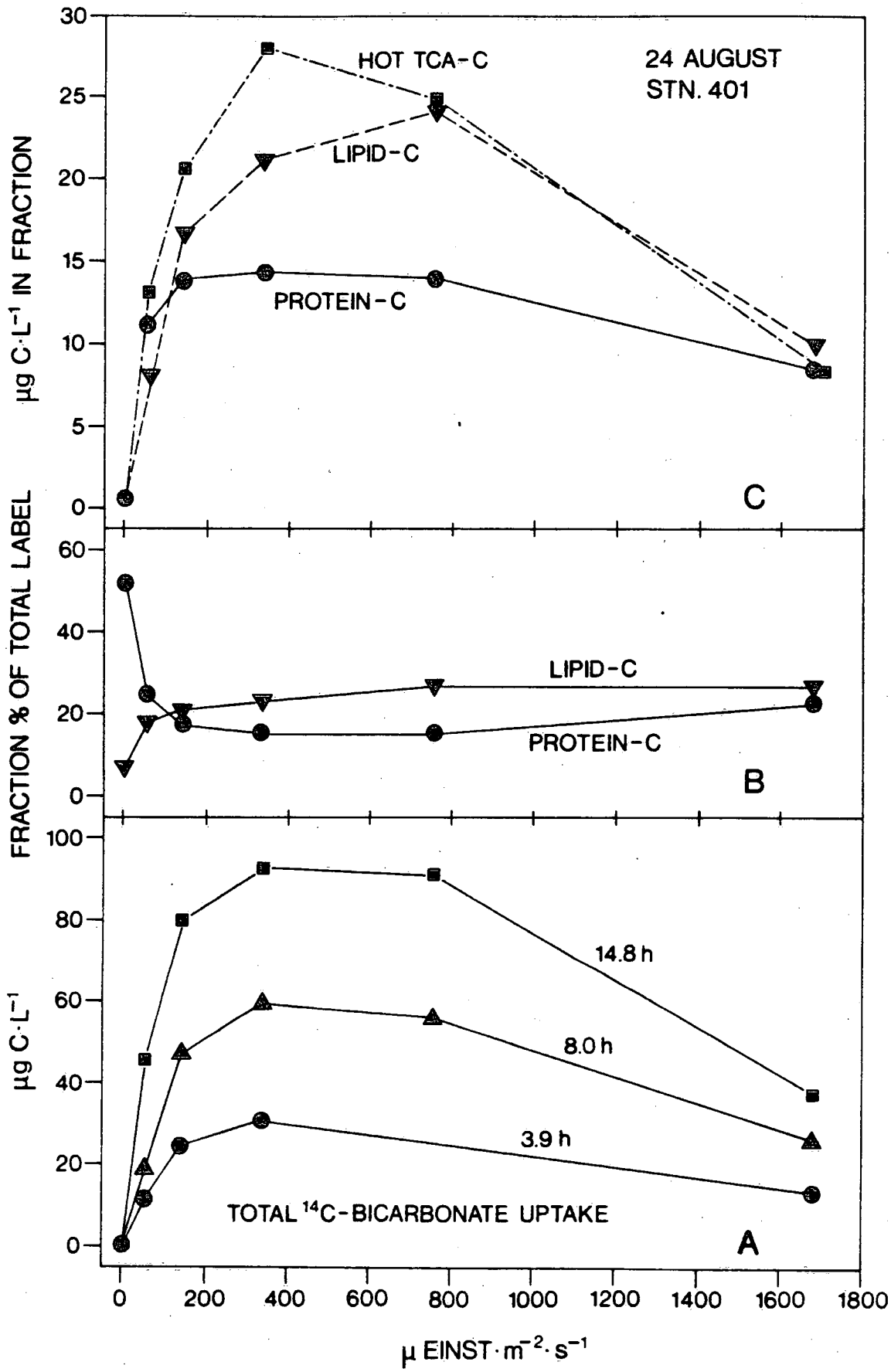

HOT TCA-C
24 AUGUST
STN. 401
LIPID-C
PROTEIN-C
C
μg C·L⁻¹ IN FRACTION
FRACTION % OF TOTAL LABEL
LIPID-C
PROTEIN-C
B
14.8 h
8.0 h
3.9 h
TOTAL ¹⁴C-BICARBONATE UPTAKE
A
μg C·L⁻¹
μ EINST·m⁻²·s⁻¹

FIG. 4 LI C & L

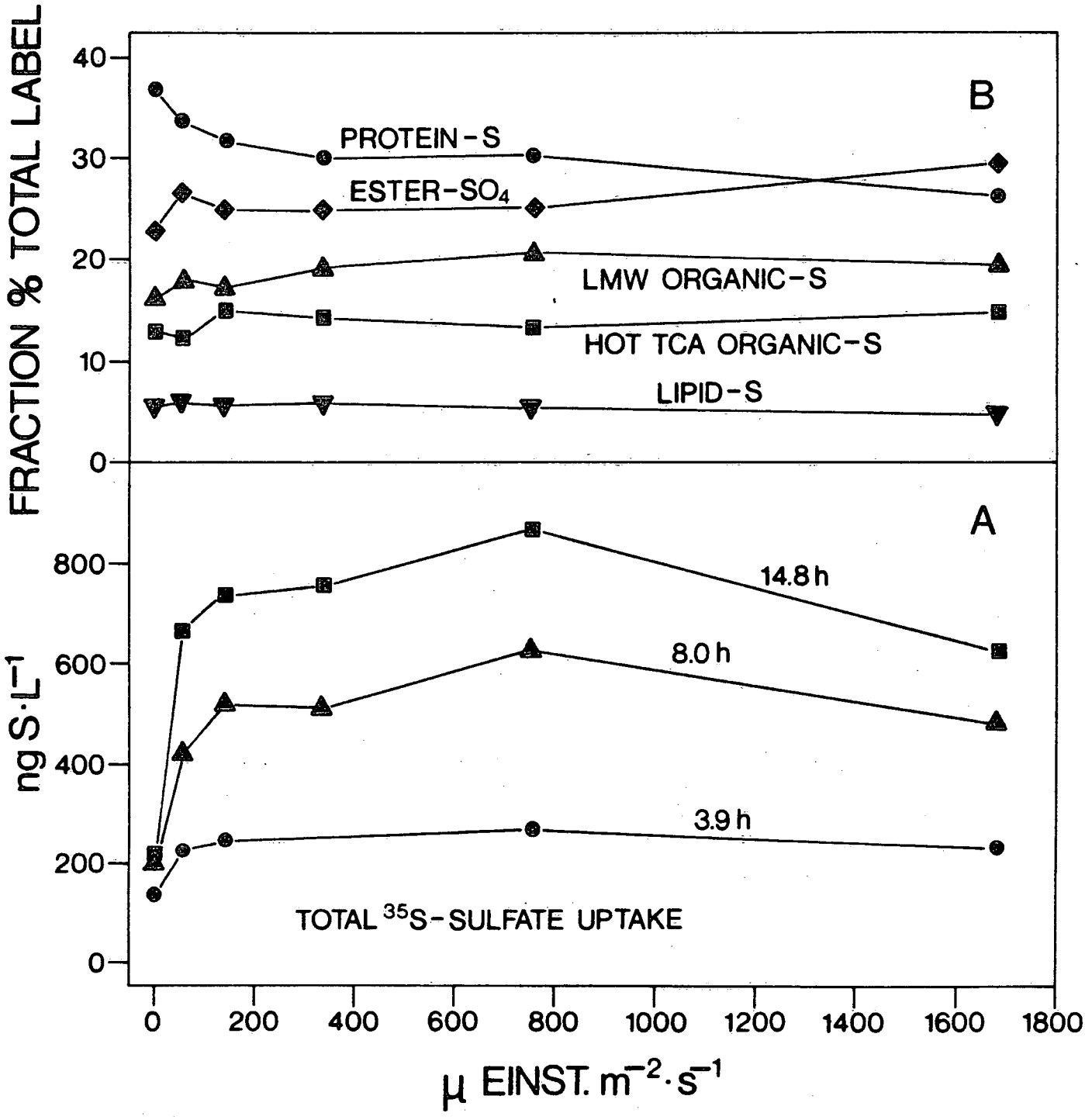

FRACTION % TOTAL LABEL
40
30
20
10
0
B
PROTEIN-S
ESTER-SO4
LMW ORGANIC-S
HOT TCA ORGANIC-S
LIPID-S
A
ng S·L-1
800
600
400
200
0
14.8 h
8.0 h
3.9 h
TOTAL 35S-SULFATE UPTAKE
0
200
400
600
800
1000
1200
1400
1600
1800
μ EINST. m-2·s-1

FIG. 5 W C&C

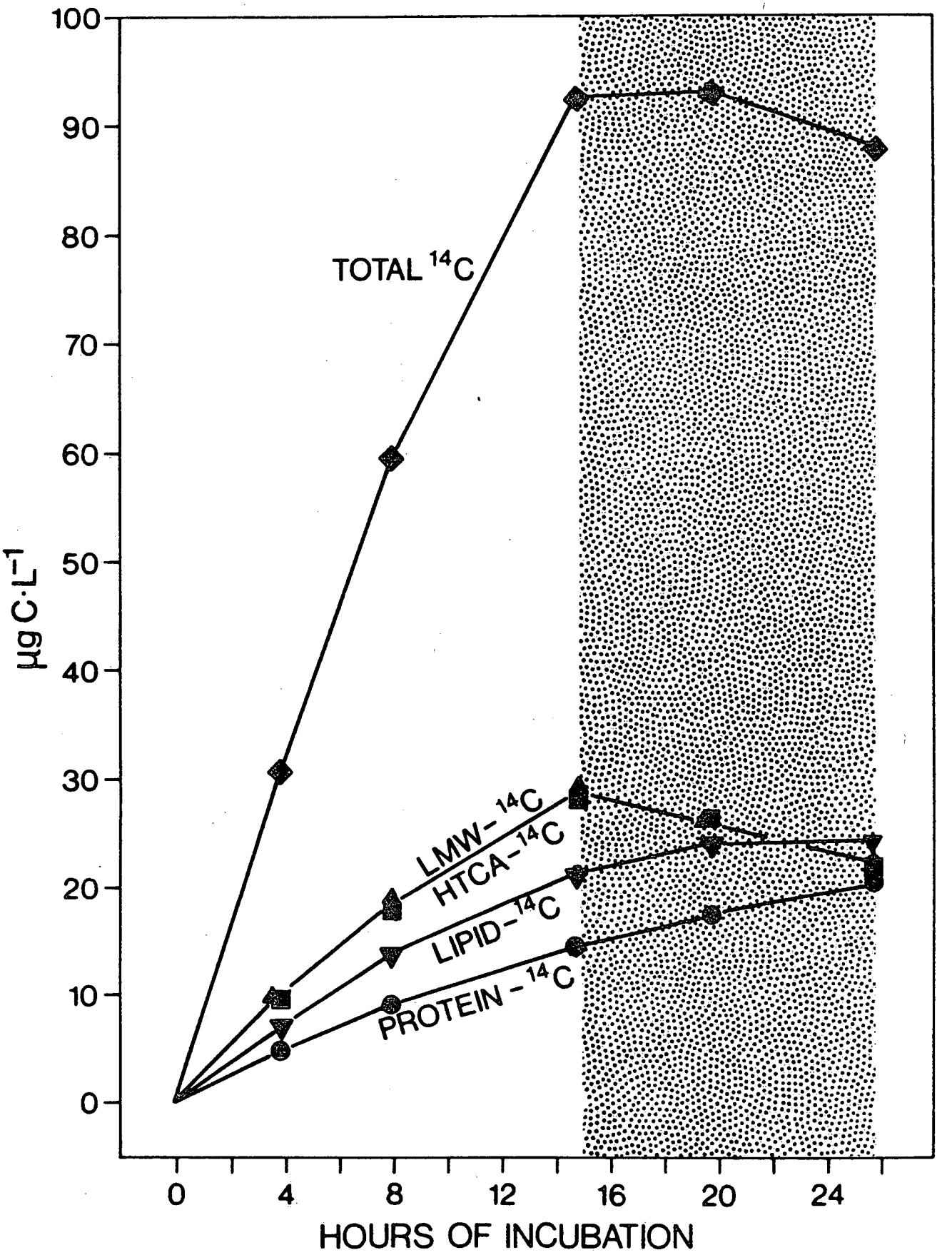

100
90
80
70
60
50
40
30
20
10
0
µg C·L⁻¹
TOTAL ¹⁴C
LMW-¹⁴C
HTCA-¹⁴C
LIPID-¹⁴C
PROTEIN-¹⁴C
0
4
8
12
16
20
24
HOURS OF INCUBATION

FIG 6 LI C& L

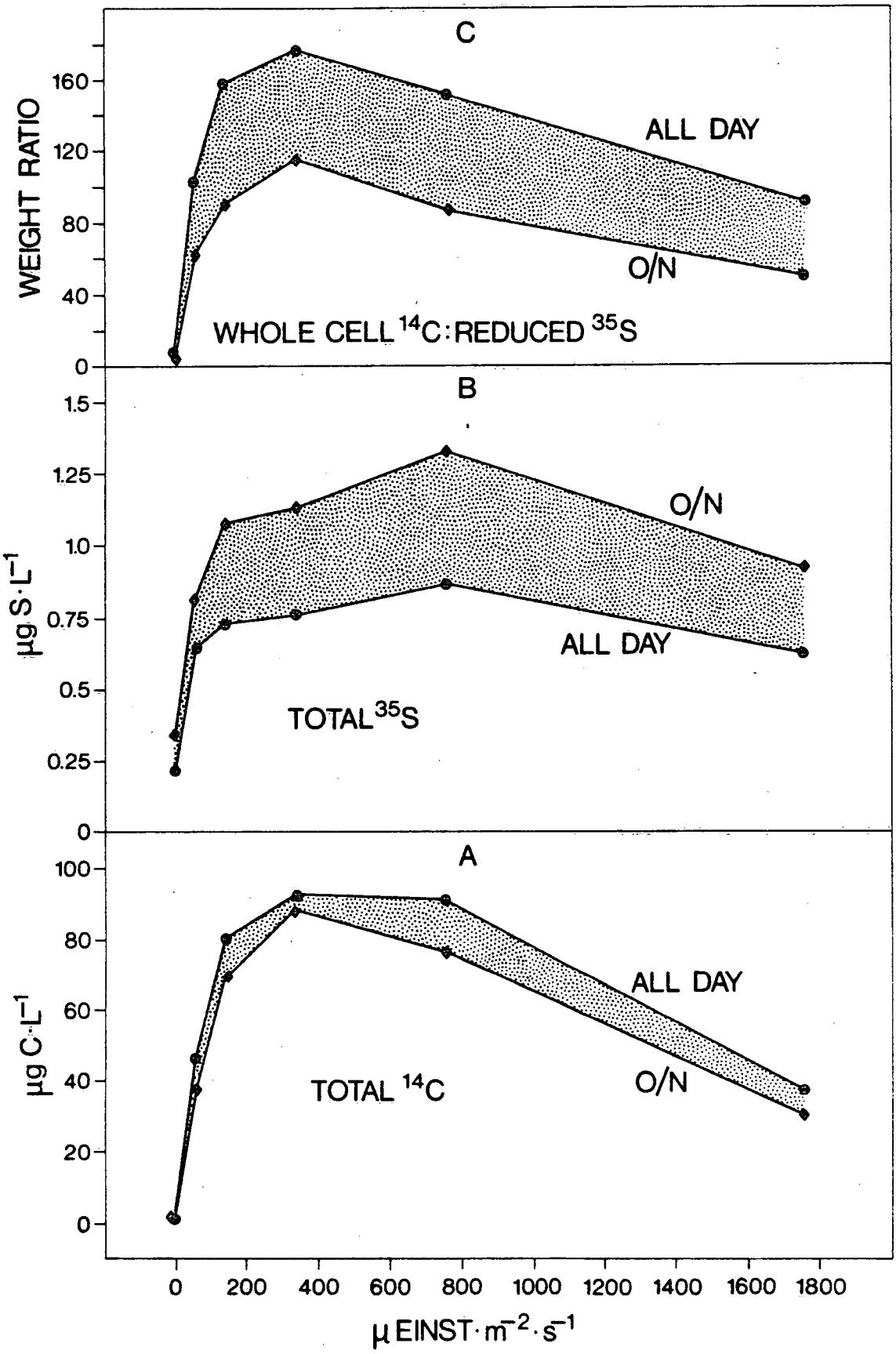

C
WEIGHT RATIO
160
120
80
40
0
ALL DAY
O/N
WHOLE CELL ^{14}C:REDUCED ^{35}S
B
1.5
1.25
1.0
0.75
0.5
0.25
0
$\mu g\ S \cdot L^{-1}$
O/N
ALL DAY
TOTAL ^{35}S
A
100
80
60
40
20
0
$\mu g\ C \cdot L^{-1}$
ALL DAY
TOTAL ^{14}C
O/N
0
200
400
600
800
1000
1200
1400
1600
1800
μ EINST $\cdot m^{-2} \cdot s^{-1}$

FIG 7 LI C & L

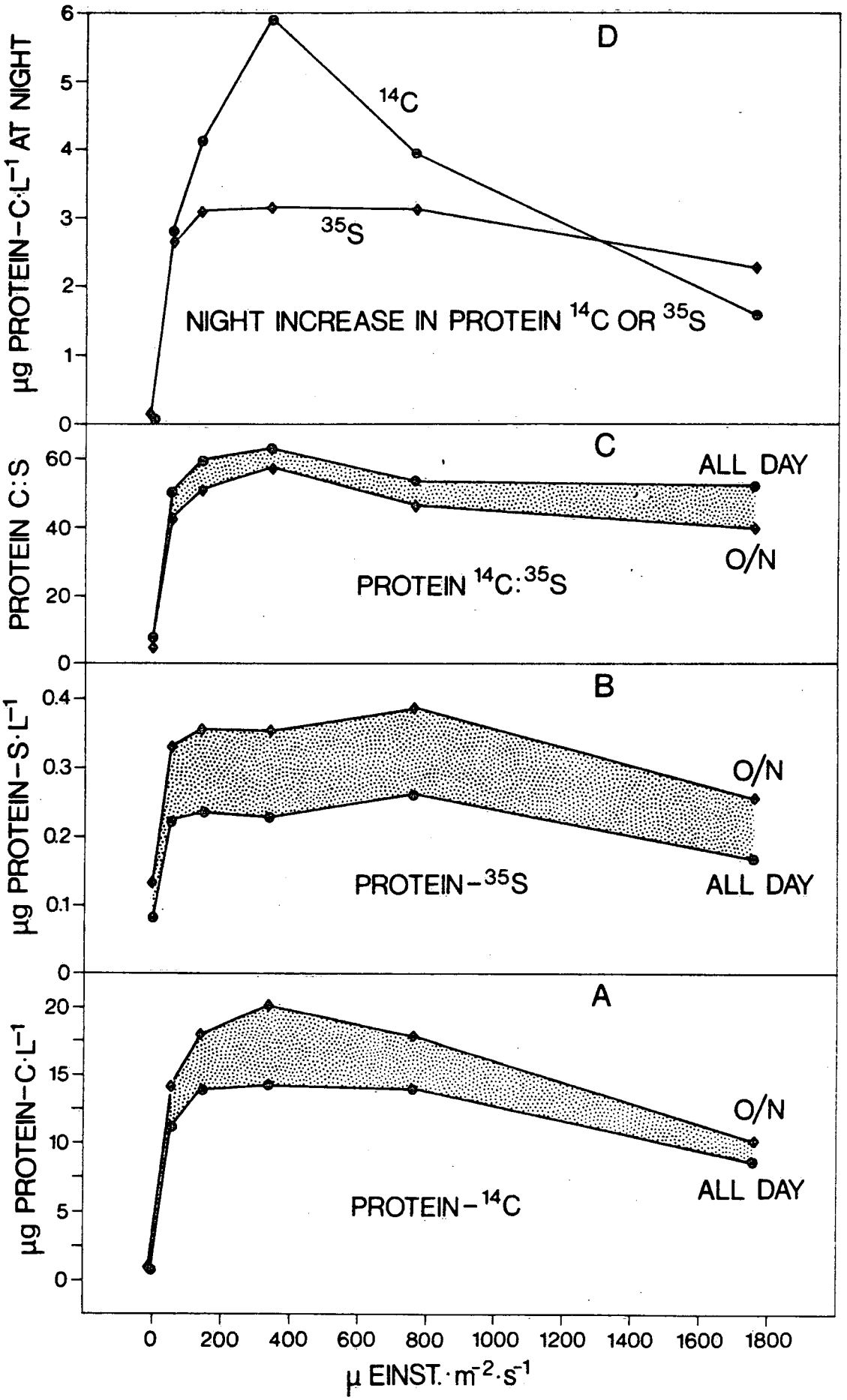

D
^{14}C
^{35}S
NIGHT INCREASE IN PROTEIN ^{14}C OR ^{35}S
µg PROTEIN-C·L^{-1} AT NIGHT
C
ALL DAY
O/N
PROTEIN ^{14}C:^{35}S
PROTEIN C:S
B
O/N
ALL DAY
PROTEIN-^{35}S
µg PROTEIN-S·L^{-1}
A
O/N
ALL DAY
PROTEIN-^{14}C
µg PROTEIN-C·L^{-1}
µ EINST.·m^{-2}·s^{-1}

FIG 8 LI C&C

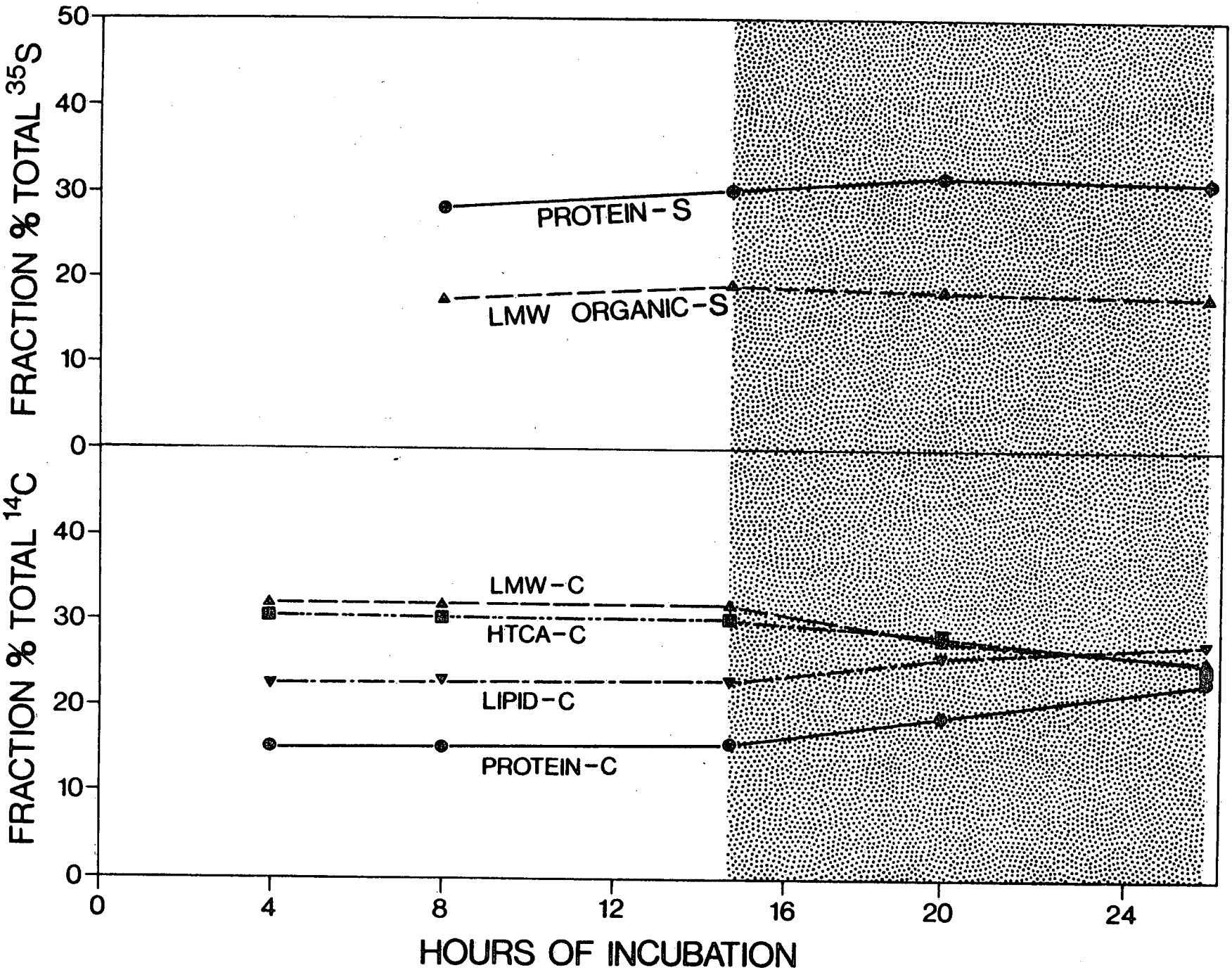

FRACTION % TOTAL ^{35}S
FRACTION % TOTAL ^{14}C
50
40
30
20
10
0
PROTEIN-S
LMW ORGANIC-S
LMW-C
HTCA-C
LIPID-C
PROTEIN-C
0
4
8
12
16
20
24
HOURS OF INCUBATION

Fig 9 11 c & c

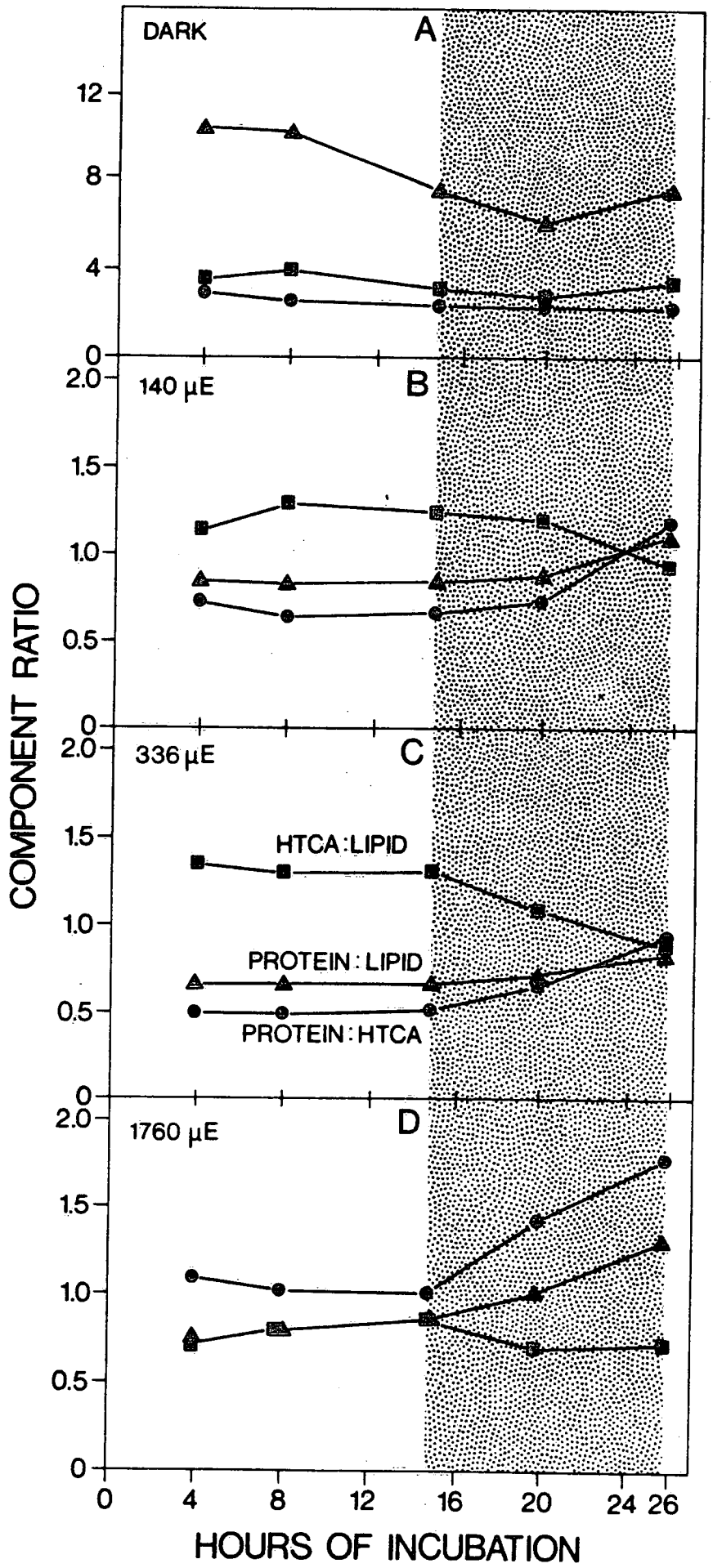

DARK
A
12
8
4
0
140 µE
B
2.0
1.5
1.0
0.5
0
336 µE
C
HTCA:LIPID
PROTEIN:LIPID
PROTEIN:HTCA
1760 µE
D
COMPONENT RATIO
0
4
8
12
16
20
24
26
HOURS OF INCUBATION

FIG 10 LI C & L

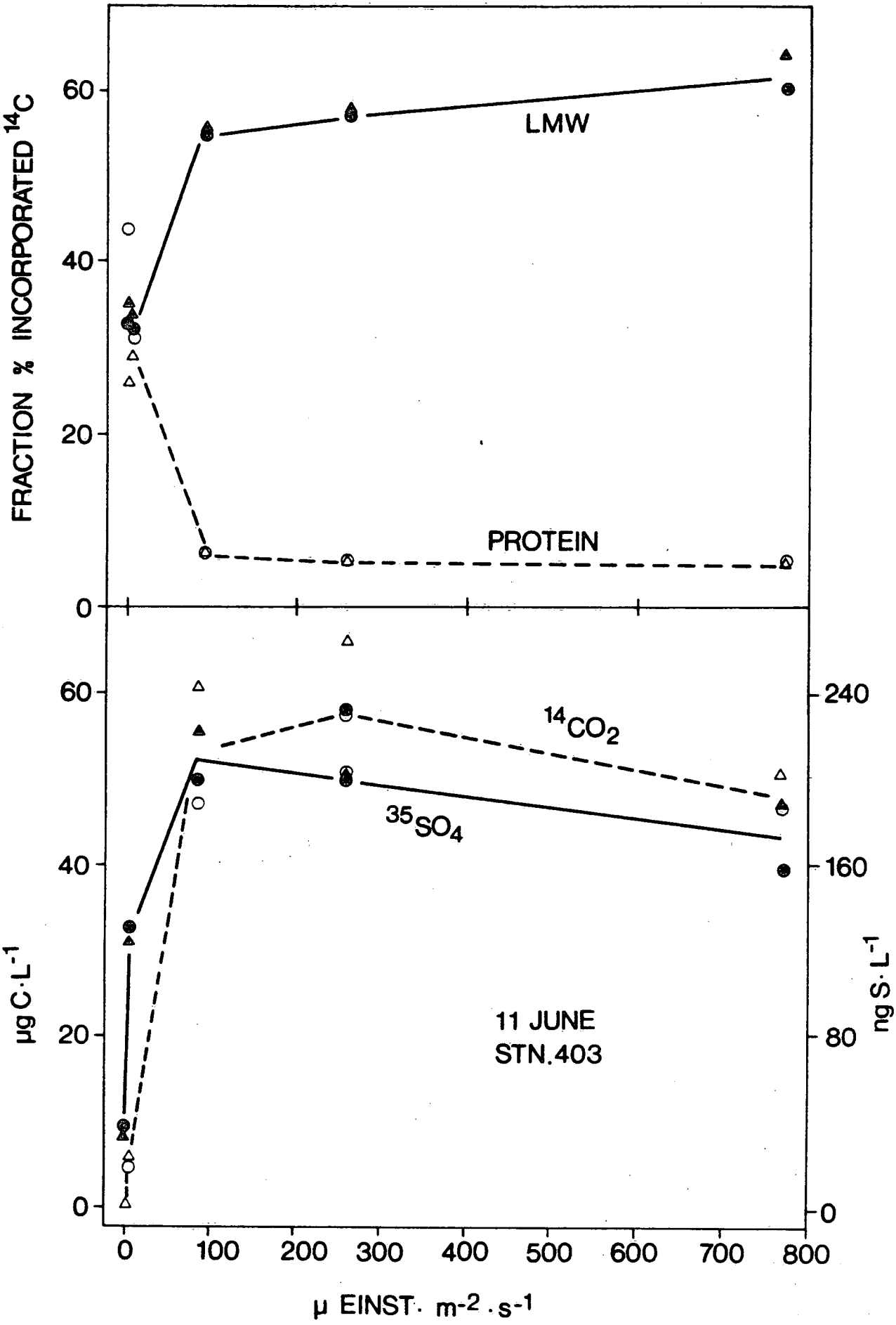

FRACTION % INCORPORATED ^{14}C
LMW
PROTEIN
$^{14}CO_2$
$^{35}SO_4$
11 JUNE
STN.403
µg C·L^{-1}
ng S·L^{-1}
µ EINST· m^{-2} .s^{-1}
0
20
40
60
80
100
160
200
240
300
400
500
600
700
800

FIG 11 LI et L

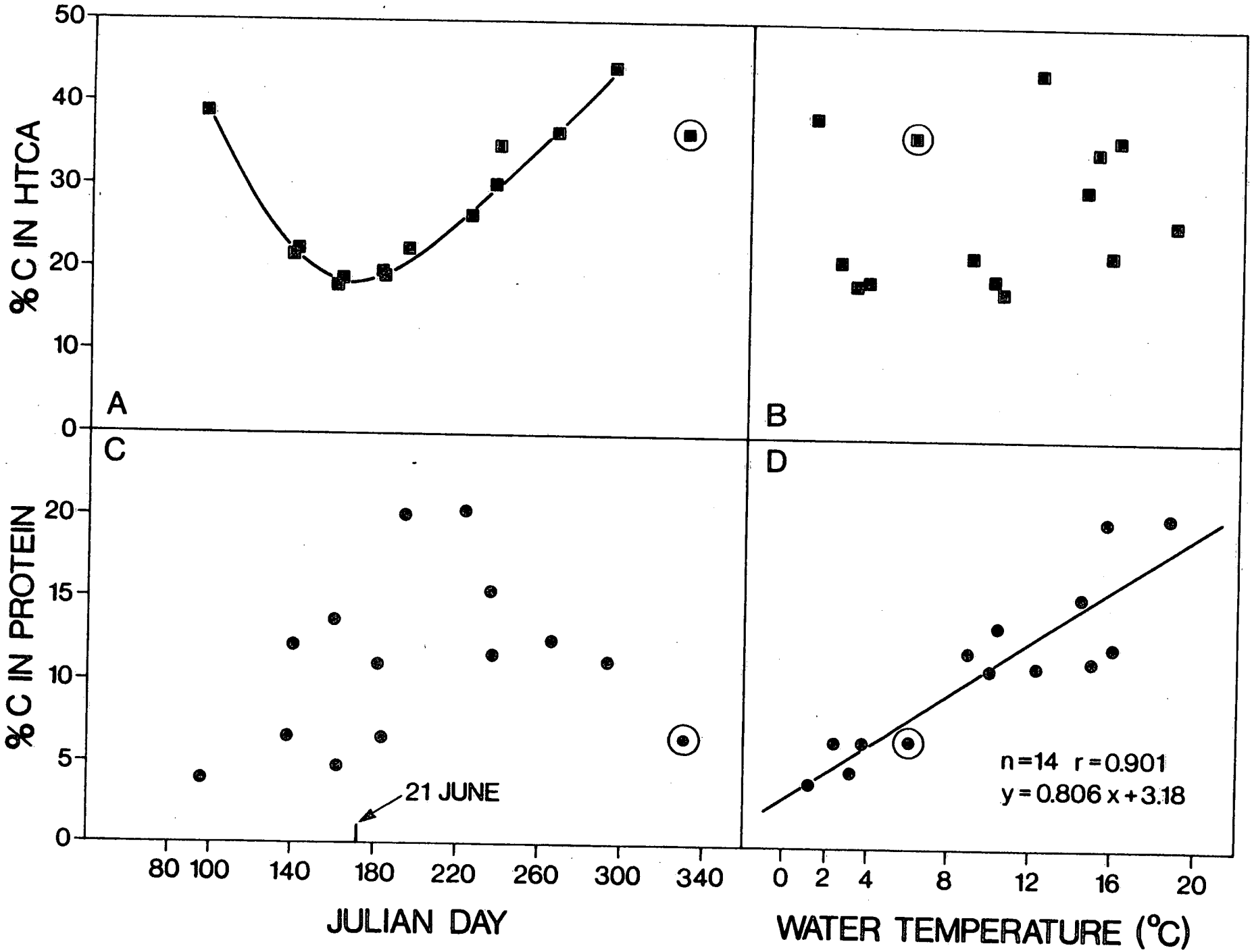

% C IN HTCA
% C IN PROTEIN
A
B
C
D
21 JUNE
n=14 r=0.901
y=0.806 x+3.18
JULIAN DAY
WATER TEMPERATURE (°C)

FIG 2

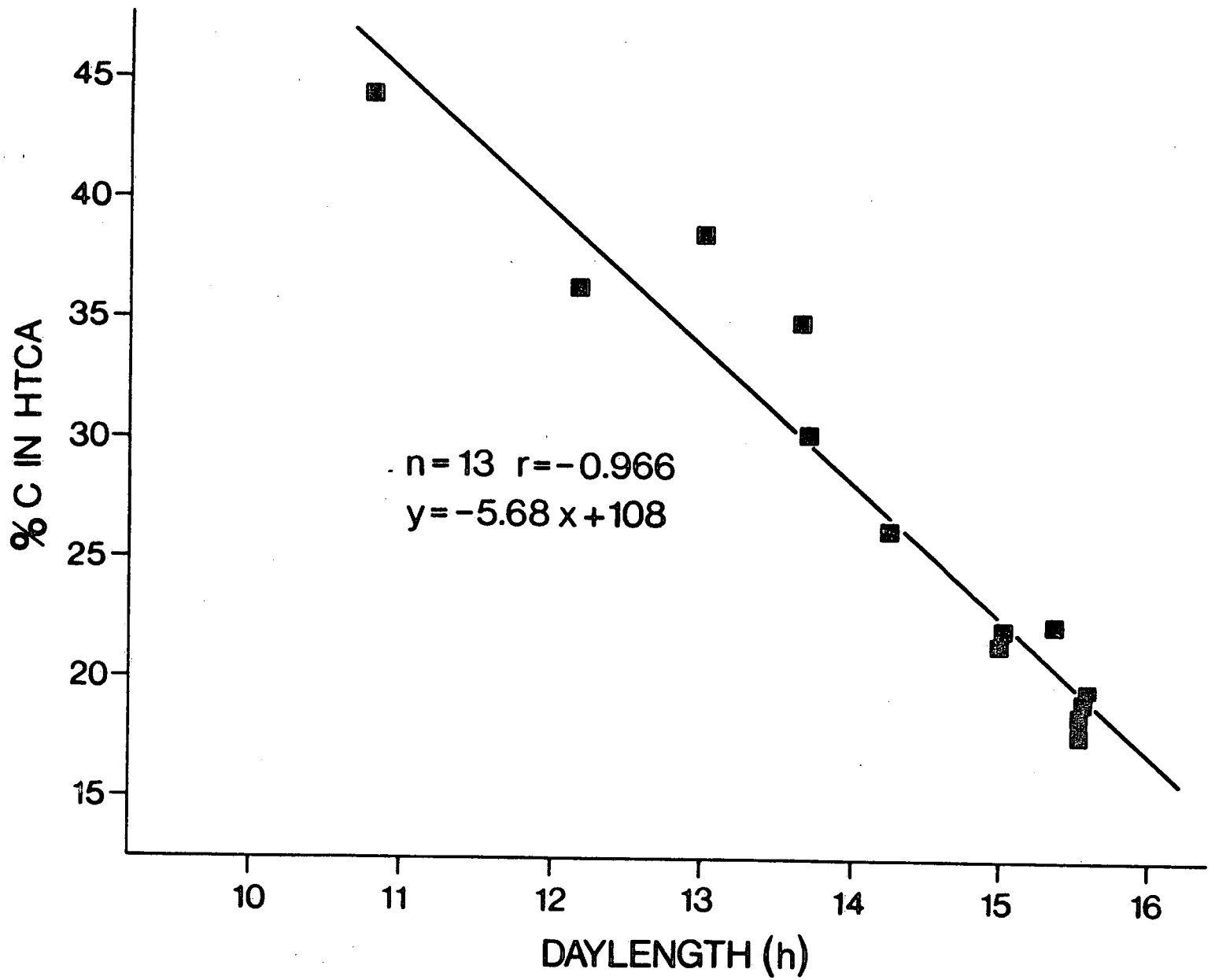

45
40
35
30
25
20
15
% C IN HTCA
n = 13 r = −0.966
y = −5.68 x + 108
10
11
12
13
14
15
16
DAYLENGTH (h)

FIG 13