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Metabolism of photosynthate in the chloroplast-retaining ciliate *Laboea strobila*

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ABSTRACT: Assimilation of photosynthate into 4 chemical classes of compounds was compared in the chloroplast-retaining ciliate *Laboea strobila* and the algae from which its chloroplasts were derived (*Isochrysis galbana* and *Pyrenomonas salina*). In laboratory cultures, the ratio of allocation of radiolabel into polysaccharide and protein was higher in *L. strobila* compared to microalgae following a 6 h incubation at irradiances which were saturating or limiting to photosynthesis. In field populations collected in Vineyard Sound, Massachusetts, USA, and in laboratory cultures of *L. strobila*, maximum rates of polysaccharide production from photosynthate were equivalent to ca 7 to 10 % h^{-1} of the pool of polysaccharide carbon. In contrast, maximum lipid and protein production from photosynthate were usually ≤ 1 % h^{-1} of lipid and protein carbon pools, respectively. About half the radiolabel which accumulated during a 3 h incubation in light was respired or excreted when ciliates were placed in light in the presence of the photosynthetic inhibitor dichlorophenyldimethylurea (DCMU), or in the dark for 18 h. Net loss of radiolabel occurred largely from polysaccharide. Mixotrophic ciliates may have higher trophic efficiencies than heterotrophic ciliates because respiratory and excretory needs are supplemented by photosynthesis.

INTRODUCTION

A number of marine oligotrichous ciliates retain functional chloroplasts (Blackbourn et al. 1973, Taylor 1982, Laval-Peuto & LeFebvre 1986, Jonsson 1987, Laval-Peuto & Rassoulzadegan 1988, Stoecker et al. 1988, 1989a). At least several chloroplast-retaining ciliates are functional mixotrophs deriving nutrition from both photosynthesis and ingested food (Jonsson 1987, Stoecker et al. 1988, 1989a). Chloroplast-retaining ciliates occur in neritic and oceanic waters in temperate, subtropical and polar regions and comprise as much as 90 % of the ciliate community (Stoecker et al. 1987a, 1989, Stoecker 1989b, Putt 1990).

The contribution of ciliates (relative to microalgae) to total chlorophyll and primary production appears highly variable. In the Nordic seas during summer 1988, the most common chloroplast retaining ciliate (*Strombidium* sp.) comprised between < 1 and 24 % of total chlorophyll (Putt 1990). Production by a large mixotrophic ciliate, *Laboea strobila* (= *Strombidium strobila*), at 5 stations on Georges Banks (Northwest Atlantic) during early summer was less than 8 % of total production at the same light level (Stoecker et al. 1989b). However, in the same incubations, production by *L. strobila* accounted for up to 90 % of microplanktonic (> 20 µm) production (Stoecker et al. 1989b). Ciliates are within the size range of particles readily consumed by metazoans and, in some cases, are preferred over similarly sized microalgae (Stoecker et al. 1987b, Gifford & Dagg 1988, Wiadynyana & Rassoulzadegan 1989). In contrast, phytoplankton communities are often dominated by nanoplankton (< 20 µm) which are too small to be effectively consumed by metazoans (Sherr et al. 1986). Thus the relative contribution of organic carbon resulting from ciliate and algal photosynthesis to metazoan nutrition may greatly exceed the relative contribution of ciliates and algae to total primary production (Stoecker et al. 1989b).

Understanding the link between photosynthetic carbon assimilation by mixotrophic ciliates and consumption by metazoans requires information about the metabolism of photosynthate by ciliates. Photosynthate fixed by ciliates may enter the food chain directly if ciliates (like microalgae) use photosynthate for the biosynthesis of macromolecules such as protein and lipid. However, in some symbiotic invertebrates and in sacoglossan molluscs which retain chloroplasts, photosynthate is preferentially used for respiration or

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excreted as mucus (Trench et al. 1972, Muscatine et al. 1981). Photosynthate fixed by ciliates would not be directly available to higher trophic levels if ciliates used photosynthate exclusively for respiration. However, the efficiency of production of new cellular material from ingested particles may increase if the respiratory needs of ciliates are partially filled by photosynthate. In this case, the net effect of mixotrophy by ciliates would be increased availability of nutrients derived from nanoplankton to higher trophic levels.

Here the metabolism of photosynthate in field and laboratory populations of the oligotrichous ciliate *Laboea strobila* is examined. *L. strobila* is a common member of the microplankton in coastal waters (Lohmann 1908, McManus & Fuhrman 1986, Dale 1987, Stoecker et al. 1987a, 1989b, Gifford 1988, Laval-Peuto & Rassoulzadegan 1988). It is an obligate mixotroph requiring both food and light for growth (Stoecker et al. 1988). The synthesis of 4 classes of chemical compounds from photosynthate in *L. strobila* and the algae from which its chloroplasts were derived are compared. The hourly contribution of photosynthate to polysaccharide, protein and lipid pools in the ciliate are estimated and temporal patterns of polysaccharide accumulation and utilization described.

MATERIALS AND METHODS

Collection and culture of ciliates and algae. *Laboea strobila* was collected from surface waters of Vineyard Sound (VS) or Perch Pond (PP), Massachusetts, USA, and either used immediately in experiments or cultured. Wild ciliates were collected from VS during May and June 1988 when water temperatures ranged from 15.5 to 20.5°C. Three strains of *L. strobila* were used in laboratory experiments. Strains LABV and LAB7 were isolated from VS; Strain OCTO was isolated from PP

Ciliates were cultured at 15°C at either high (ca 200 to 300 $\mu E\ m^{-2}\ s^{-1}$) or low (15 to 35 $\mu E\ m^{-2}\ s^{-1}$) irradiances on a 14 h light : 10 h dark cycle in polycarbonate bottles containing seawater medium and food algae (Stoecker et al. 1988). Media containing either full strength (32 ‰ S) or 75 % (24 ‰ S) VS seawater enriched with 0.1 $ml\ l^{-1}$ of iron-EDTA (ethylenediaminetetracetic acid) trace metal solution were autoclaved in teflon containers prior to use (Guillard & Ryther 1962). Several strains of *Laboea strobila* were used since individual strains could only be cultured for ca 6 to 9 mo (Stoecker et al. 1988). Late exponential or early stationary phase cultures were used in experiments.

Ciliates were fed a mixture of the prymnesiophyte *Isochrysis galbana* (Strain ISO) and the cryptomonad *Pyrenomonas salina* (Strain 3C) added at initial concentrations of ca 1.0×10^4 and 0.5×10^3 cells ml^{-1}, respectively. Both algae appear to be required for sustained growth of *Laboea strobila* (Stoecker et al. 1988). Microalgae were grown in f/2 medium without added silica at 20°C at an irradiance of ca 50 $\mu E\ m^{-2}\ s^{-1}$ (Guillard & Ryther 1962). Irradiance was measured with a LiCor Model 185B meter equipped with a 4π sensor.

Photosynthesis experiments. All experiments involved separation of ciliates from their food algae, incubation in the presence of $NaH^{14}CO_3$ and subsequent serial solvent extractions.

Separation of ciliates and algae. Ciliates were generally separated from their food algae under a dissecting microscope using a micropipette. On several occasions, natural populations were initially concentrated by centrifuging 250 ml samples into an underlying 5 ml layer of Percoll-sorbitol-seawater (PSSW) (Price et al. 1978). Centrifugation was carried out in an IEC DPR-6000 Centrifuge at 100 to $200 \times g$ for 20 min. Initial studies indicated that centrifugation into Percoll did not alter the photosynthetic rate of *Laboea strobila* relative to cells initially isolated with a micropipette (Putt unpubl.). All ciliates were passed through several washes of sterile medium following the initial concentration step and finally suspended in medium contained in acid-washed polycarbonate incubation containers (50 to 250 ml). These washing procedures were also carried out whenever radiolabelled cells were resuspended in new medium.

Radiolabelling. $NaH^{14}CO_3$ (Amersham) was added to ciliate suspensions (final activity 0.5 to 1.0 $\mu Ci\ ml^{-1}$) which were then incubated in the light (see below) and in the dark (as a control) (Stoecker et al. 1988). Total added activity was determined in 0.1 ml aliquots added to vials containing 0.1 ml phenethylamine. At the end of the incubation, cells were again passed through at least 3 serial washes of sterile medium and a known number of cells (10 to 40) placed in 2 'sets' of scintillation vials. Distilled water (1 ml) was added to 'Set 1' which was stored at −20°C prior to serial solvent extractions. Acetic acid : methanol (1 ml of a 5 % solution) was added immediately to 'Set 2' which was evaporated to dryness at 60°C and used for determination of radioactivity in whole ciliate cells.

Photosynthetic carbon assimilation in algae was determined in an identical incubation procedure except that (1) the final activity of the $NaH^{14}CO_3$ was ca 0.05 $\mu Ci\ ml^{-1}$ and (2) algae were collected on GF/F filters at the completion of the experiment.

Incubation conditions. Two types of incubations were performed. First the assimilation of radiolabelled photosynthate into 4 biochemical pools was investigated in cultured and wild populations of ciliates incubated for a 3 to 6 h period near midday. Cultured

populations were incubated in their growth chambers. Wild populations were incubated outdoors in a flowing seawater incubator where irradiance was attenuated with neutral density screening. Incubation irradiances were determined to be saturating or limiting to photosynthesis based on more extensive photosynthesis-irradiance relationships (Stoecker et al. 1988, Putt unpubl., Stoecker unpubl.).

Second, reallocation of photosynthate was investigated in 'pulse-chase' experiments. Ciliates which had been radiolabelled near midday at an irradiance saturating to photosynthesis for 1 to 3 h were resuspended in the dark in the presence or absence of unlabelled *Isochrysis galbana* (1×10^4 cells ml^{-1}) for periods ranging from 1 to 18 h. On one occasion the radiolabelled ciliates were placed in the light in the presence of food algae and the photosynthetic inhibitor DCMU [1-(3,4-dichlorophenyl)-1,1-dimethylurea]. The final concentration of DCMU was 0.1 % of a saturated aqueous solution. This concentration of DCMU effectively inhibits photosynthesis in *Laboea strobila* with minimal effects on cell motility and survival in an 18 h period (Putt unpubl.).

Identical incubation conditions were used with algae. For short-term midday incubations, algae were prepared by removing ciliates from a mixed culture. For 'pulse-chase' experiments, individual batch cultures of algae in exponential phase were used. For the 'chase' period, algae were placed in the dark in their original medium.

Serial solvent extractions. Set 1 samples were thawed, mixed with 3.5 ml of a chloroform:methanol solution (1:2, v:v) and placed in an ice bath for 20 min (Rivkin 1985). Samples were filtered through a GF/F filter (2.1 mm) using an apparatus equipped with a stainless-steel frit and rinsed sequentially with the chloroform:methanol solution (1.3 ml), chloroform (1.6 ml), and water (1.9 ml). The filtrate was vortexed and stored at 4 °C for at least 3 h to allow phase separation. The filter was incubated for 1 h at 95 to 100 °C in 3.5 ml of a 5 % aqueous TCA (trichloracetic acid) solution, filtered, rinsed twice with cold 5 % TCA (1 ml) and once with distilled water (1 ml). The methanol:water, chloroform and TCA fractions, as well as the filters containing insoluble material, were placed in separate scintillation vials and held at 60 °C until dry. Distilled water (0.5 ml) and scintillation fluor (5 ml of Universol, ICN) were added to all vials. Radioactivity was determined on a Packard Model 1000 liquid scintillation counter with quench correction using an external standard. Reported values were corrected for dark uptake of ^{14}C. The sum of radiolabel in the fractions determined using serial solvent extractions was 98 ± 11 % (mean ± SD, n = 14) of the radiolabel in whole ciliate cells (described above for Set 2).

Serial solvent extraction procedures depend on the relative specificity of organic solvents for different classes of biochemical compounds (Hitchcock 1983, Cuhel & Lean 1987). The procedure used here separates compounds into a methanol:water soluble fraction containing low molecular weight compounds (LMW), a chloroform soluble fraction containing both membrane and storage lipids, a hot TCA soluble fraction containing largely polysaccharide and an insoluble residue containing largely protein (Li et al. 1980, Rivkin 1985).

Cell carbon determinations. Cell volume was estimated from microscopic determination of linear dimensions of samples preserved in 2 % Lugol's iodine (LI). The shape of *Laboea strobila* was assumed to approximate a cone. Cell carbon (C) was determined on the basis of an experimentally determined carbon:volume ratio of 0.19 pg μm^{-3} for cells preserved in LI (Putt & Stoecker 1989).

Chemical composition of uniformly labelled cells. Theoretical and methodological considerations for achieving uniformly labelled ciliates have been previously described (Putt & Stoecker 1989). Briefly, food algae were grown for 4 d using media containing ca 0.005 $\mu Ci\ ml^{-1}$ (final activity) $NaH^{14}CO_3$ in glass Erlenmeyer flasks sealed with silicone stoppers. Ciliates were added and passed through at least 4 divisions prior to sampling. Ciliates were either sampled on 2 different dates during their period of exponential growth or at 6 h intervals through a diel cycle. Sampling and serial solvent extractions were performed as described for photosynthesis experiments, in order to determine relative proportions of each of the 4 chemical pools. For individual experiments, carbon content of specific pools was calculated from knowledge of relative composition and cellular carbon content.

Calculations. Photosynthetic rates were calculated as described in Strickland & Parsons (1972) using dissolved inorganic carbon (DIC) values determined from alkalinity measurements. Hourly contribution of photosynthate to cellular pools was determined from knowledge of the hourly assimilation of photosynthate into each pool and pool size. Statistical tests were performed as described by Sokal & Rohlf (1969).

Feeding experiment. *Isochrysis galbana* grown for 5 d in media containing ca 0.2 $\mu Ci\ ml^{-1}$ ^{14}C-bicarbonate was centrifuged at 2000 rpm in an IEC Model PR-2 centrifuge for 10 min, washed with sterile media, vortexed and recentrifuged, until negligible radioactivity remained in the supernatant, and finally added to a culture of *Laboea strobila* at an initial concentration of 5×10^4 cells ml^{-1}. Following a 3 h incubation, ciliates were isolated, washed and finally resuspended in an identical concentration of unlabelled *I. galbana* and placed in the dark for 6 h. Ciliates were collected for total radiolabel determination and for serial solvent

extractions 3 and 9 h after the initial addition of uniformly labelled *I. galbana*.

RESULTS

Labelling patterns in midday incubations

During incubations at irradiances saturating to photosynthesis, polysaccharide comprised the largest pool of photosynthate (>40 % of total radiolabel) in both *Laboea strobila* and the algae from which its chloroplasts were derived (Fig. 1A). During incubations at irradiances limiting to photosynthesis, polysaccharide comprised the largest pool of photosynthate in *L. strobila* whereas assimilation of photosynthate into lipid, polysaccharide and protein occurred at about the same rate in microalgae (Fig. 1B). Differences between assimilation patterns in *L. strobila* and its food algae are summarized by examining the ratio of photosynthate assimilation into polysaccharide and protein (Table 1). Two-way analysis of variance (ANOVA) indicated that this ratio was significantly higher in cultured ciliates compared to their food algae and at irradiances which were saturating to photosynthesis compared to those limiting to photosynthesis. There was also a significant interaction between light-level and organism indicating that the reduction in the ratio of polysaccharide to protein at irradiances limiting to photosynthesis differed among organisms.

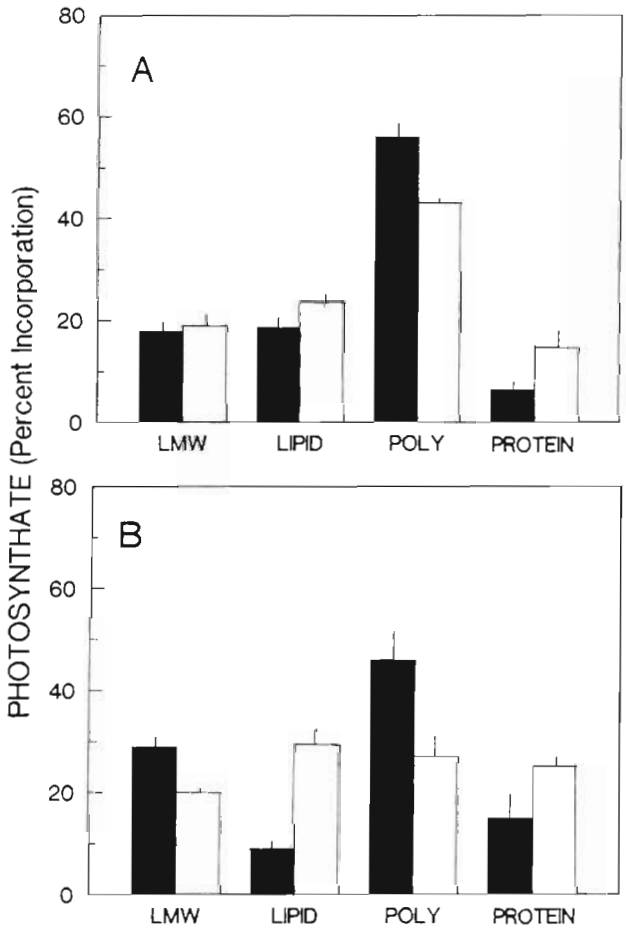

Fig. 1 Relative distribution of photosynthate between low molecular weight (LMW), lipid, polysaccharide (POLY) and protein in batch cultures of *Laboea strobila* (solid bars) and the algae from which its chloroplasts were derived (open bars). Cultures were grown at 15 °C at 15 μE m^{-2} s^{-1} and incubated for 6 h at irradiances which were (A) saturating (250 μE m^{-2} s^{-1}) and (B) limiting (15 μE m^{-2} s^{-1}) to photosynthesis. Photosynthetic rates were 277 ± 11 pg C $cell^{-1}$ h^{-1} at 250 μE m^{-2} s^{-1}. Values are means ± range of duplicate samples

Radiolabelling patterns during midday incubations were also determined for wild populations of *Laboea strobila* collected between 31 May and 24 June 1988 from VS (Table 2). Photosynthetic rates, even at irradiances saturating to photosynthesis, varied by nearly 4-fold. However, ca 40 to 60 % of photosynthate was consistently assimilated into polysaccharide irrespective of incubation conditions. Assimilation into lipid and protein accounted for less than 10 and 25 % of total photosynthate, respectively. Field populations assimilated proportionally more photosynthate into protein than laboratory cultures (Table 3). Both field populations and laboratory cultures assimilated relatively more photosynthate into polysaccharide and less into protein at irradiances saturating to photosynthesis compared to irradiances limiting to photosynthesis.

Serial solvent extractions of uniformly labelled cultures of *Laboea strobila* indicated that lipid, polysac-

Table 1. Ratio of assimilation of photosynthate into polysaccharide and protein in cultures of *Laboea strobila* and algae from which its chloroplasts were derived (a mixture of *Isochrysis galbana* and *Pyrenomonas salina*). Cells were incubated at irradiances which were saturating (250 μE m^{-2} s^{-1}) and limiting (15 μE m^{-2} s^{-1}) to photosynthesis. Values are means with range of duplicate values in parentheses

Organism	Irradiance	
	250	15
Laboea strobila	7.9 (7.7–8.0)	2.7 (1.5–3.9)
Algae	3.0 (2.5–3.5)	1.1 (1.0–1.1)

2-way ANOVA

Source of variation	DF	M_s	F_s	P
Light-level	1	23.5	23.5	< 0.01
Organism	1	22.8	22.8	< 0.01
Interaction	1	5.9	5.9	< 0.05
Error	4	3.0	0.8	

Table 2. Assimilation of photosynthate into lipid, polysaccharide (Poly) and protein in wild populations of *Laboea strobila* incubated at irradiances limiting and saturating to photosynthesis for 3 to 6 h. Incubation irradiance values represent the mean during the incubation period. Means ± SD. NA. not applicable. Units are irradiance: $\mu E\ m^{-2}\ s^{-1}$; Total: ng C $cell^{-1}\ h^{-1}$; Lipid, Poly, Protein: % of total

Date	Incubation irradiance	Photosynthesis			
		Total	Lipid	Poly	Protein
Limiting irradiances					
1 June	90	0.09	11.4	49.2	21.8
15 June	110	0.02	7.7	39.0	21.6
Mean	NA	0.06	9.0	44.1	21.7
Saturating irradiances					
31 May	829	0.27	12.0	48.3	15.8
15 June	460	0.13	15.5	48.9	15.0
22 June	550	0.47	6.7	61.0	15.2
24 June	1245	0.16	4.1	63.0	20.2
Mean ± SD	NA	0.26 ± 0.15	9.6 ± 5.1	55.3 ± 7.8	16.5 ± 2.4

charide and protein pools comprised about 32, 21 and 30 %, respectively, of cell carbon (Table 4). These values were used with cell carbon estimates (Tables 5 and 6) to determine the carbon content of cellular lipid, polysaccharide and protein pools for both field and laboratory populations. The hourly contribution of photosynthesis to the polysaccharide pool was up to 10 % h^{-1} in culture, and up to 14 % h^{-1} in field populations (Tables 5 and 6). In contrast the contribution of photosynthate to lipid and protein pools was typically ≤ 1 % h^{-1}. A notable exception was the contribution of photosynthate to protein in field populations which averaged 1.6 % h^{-1} at irradiances saturating to photosynthesis (Table 6).

Temporal patterns of radiolabel reallocation

In *Laboea strobila,* radiolabel accumulated largely in polysaccharide and LMW pools in an initial 1 h incubation at an irradiance saturating to photosynthesis

Table 3. *Laboea strobila.* Relative proportion (%) of photosynthate assimilated into polysaccharide and protein in ciliates grown in culture or collected from Vineyard Sound and incubated at irradiances which were saturating or limiting to photosynthesis (Fig. 1, Table 2). Values shown are means with range of duplicate values in parentheses. Means ± SD are shown for field populations under light-saturating conditions

Growth conditions	% Polysaccharide		% Protein	
	Saturating	Limiting	Saturating	Limiting
Culture	57.0 (55.2–58.8)	46.0 (43.9–48.2)	6.0 (5.4–7.6)	16.3 (15.0–17.6)
Field	55.3 ± 7.8	44.1 (39.0–49.2)	16.5 ± 2.4	21.7 (21.6–21.8)

2-way ANOVA for differences in Polysaccharide

Source of variation	DF	M_s	F_s	P
Source (culture or field)	1	0	0	NS
Light-level	1	257	257	< 0.05
Interaction	1	8	8	NS
Error	7	250	36	

2-way ANOVA for differences in Protein

Source of variation	DF	M_s	F_s	P
Source (culture or field)	1	113	34	< 0.01
Light-level	1	80	24	< 0.01
Interaction	1	52	16	< 0.05
Error	7	23	3	

Table 4. *Laboea strobila*. Relative proportion (%) of low molecular weight (LMW), lipid, polysaccharide (Poly) and protein carbon in uniformly labelled ciliates cultured at 200 μE m^{-2} s^{-1} Exponentially growing cultures were sampled on 2 successive days; stationary phase cultures were sampled at 6 h intervals over a diel cycle. Values shown for exponential phase cultures are means of samples collected on successive days with range in parentheses. Values shown for stationary phase cultures are means ± SD (n=5). NA: not applicable. Units are Cell C: ng $cell^{-1}$; LMW, Lipid, Poly, Protein: % total cell C

Culture conditions	Cell C	Biochemical fraction			
		LMW	Lipid	Poly	Protein
Exponential Phase	19.3 (18.6–20.0)	17.0 (13.3–21.7)	35.8 (31.8–39.8)	21.5 (20.7–22.3)	26.0 (25.7–26.3)
Early stationary Phase	13.4	17.0 ± 2.0	28.5 ± 4.5	20.2 ± 3.0	34.2 ± 1.4
Mean	NA	17.0	32.2	20.8	30.1

(Fig. 2). In the subsequent 1 h chase period in the dark, about 10 % of the label initially present was respired or excreted. Net changes in protein, low molecular weight and lipid pools were all small.

In a longer (3 h) initial incubation with *Laboea strobila,* the amount of radiolabel which accumulated in polysaccharide was ca 5 times as great as radiolabel in any other pool (Fig. 3). Patterns of radiolabel reallocation in the subsequent 6 to 18 h chase period were similar in all treatments (Table 7). After an 18 h chase period about half the total radiolabel had been respired or excreted. Net loss of radiolabel occurred primarily from the polysaccharide pool. Small increases in radiolabel occurred in protein while net changes in low molecular weight and lipid pools were small.

For *Isochrysis galbana* and *Pyrenomonas salina,* polysaccharide also comprised the largest pool of radiolabel following an initial 3 h incubation (Fig. 4). Patterns of subsequent radiolabel reallocation were similar in the 2 algae (Table 7). After an 18 h chase period, ca 25 % of the radiolabel initially present had been respired or excreted. Net loss of radiolabel occurred out of both lipid and polysaccharide pools for both algae, and out of the low molecular weight pool for *I. galbana.* However, in contrast to *Laboea strobila,* loss of radiolabel out of polysaccharide and lipid, was accompanied by increases in radiolabel of 580 to 960 % in protein by the end of the 18 h chase period.

Feeding experiment

Protein comprised the largest pool of radiolabel following a 3 h incubation in the presence of uniformly labelled *Isochrysis galbana* (Fig. 5). Protein and LMW pools underwent net decreases while negligible changes in lipid and polysaccharide occurred during the subsequent 6 h period in the dark.

DISCUSSION

Most microalgae are capable of growth, i.e. biosynthesis of the complete suite of macromolecules required for cell division, using reduced carbon derived solely from photosynthesis (Morris 1981, Cuhel & Lean 1987). In contrast, the ciliate *Laboea strobila* requires both particulate food and light for growth (Stoecker et al. 1988). The present study indicates that the metabolism of photosynthate in the 2 organisms differs, although they share the same chloroplasts. Serial solvent extraction procedures in various forms have been extensively used to describe variation in the synthesis of compounds related to reproductive growth and storage in algae and cyanobacteria (Morris et al. 1974, Hitchcock 1983, Taguchi & Laws 1985, Pick & Cuhel 1986, Glover & Smith 1988, Smith et al. 1989). Here, these techniques were used to compare patterns of synthesis and

Table 5. *Laboea strobila*. Hourly contribution of photosynthate to lipid , polysaccharide (Poly) and protein pools in ciliates cultured at 200 μE m^{-2} s^{-1} Total cell carbon was estimated from volume determinations (Putt & Stoecker 1989). Carbon pools were estimated from serial solvent extraction of uniformly labelled cells (Table 3). Means with range of duplicate samples in parentheses. Units are irradiance: μE m^{-2} s^{-1}; Total photosynthesis: ng C $cell^{-1}$ h^{-1}; Total, Lipid, Poly, Protein: % of pool

Incubation irradiance	Total photosynthesis	Contribution			
		Total	Lipid	Poly	Protein
200	0.21 (0.18–0.24)	3.2 (2.8–3.6)	0.9 (0.6–1.2)	10.2 (9.8–10.6)	1.1 (0.5–1.7)
25	0.15 (0.13–0.17)	2.0 (1.7–2.3)	1.0 (1.0–1.0)	5.5 (5.1–5.9)	0.6 (0.4–0.8)

Table 6. *Laboea strobila*. Hourly contribution of photosynthate to lipid, polysaccharide (Poly) and protein in wild populations of ciliates at irradiances limiting and saturating to photosynthesis. Incubation irradiance values (μE m^{-2} s^{-1}) represent the mean during the incubation period. Cell carbon was estimated from volume determinations (Putt & Stoecker 1989). Carbon pools were estimated from serial solvent extraction of uniformly labelled cells (Table 4). Means ± SD. NA: not applicable

Date	Incubation irradiance	Contribution			
		Total	Lipid	Poly	Protein
Limiting irradiances					
1 June	90	0.8	0.3	2.1	0.6
15 June	110	0.2	<0.1	0.4	0.1
Mean	NA	0.5	0.2	1.3	0.4
Saturating irradiances					
31 May[a]	829	3.0	1.1	6.9	1.6
15 June	460	1.5	0.8	3.5	0.7
22 June	550	4.7	1.0	14.3	2.9
24 June	1245	1.6	0.2	5.1	1.1
Mean ± SD	NA	2.7 ± 1.4	0.8 ± 0.4	7.4 ± 4.8	1.6 ± 1.0

[a] Cell carbon assumed to be the same as 1 June

utilization of 4 chemical classes of compounds in *Laboea strobila* with the algae from which its chloroplasts were derived.

Algae in this study assimilated a large proportion of photosynthate into polysaccharide when irradiances were saturating to photosynthesis but conserved protein synthesis at the expense of polysaccharide when irradiance limited photosynthesis. Temporal continuity of protein synthesis was achieved by storing photosynthate in polysaccharide and lipid and subsequently using these sources of reduced carbon compounds for protein synthesis during periods of darkness. Similar patterns have been described for microalgae from diverse taxonomic groups in a variety of environmental conditions (Morris et al. 1974, Morris 1981, Cuhel et al. 1984, Lancelot & Mathot 1985, DiTullio & Laws 1986, Cuhel & Lean 1987). Night-time protein synthesis may contribute to the reproductive success of algae in nature where the natural light:dark cycle causes temporal discontinuity of energy supply.

A discussion of the quantitative importance of photosynthesis to ciliate metabolism requires consideration of several sources of error. Two factors may cause underestimates in absolute rates of polymer synthesis based on radiotracer studies. First, as in most aquatic studies which use ^{14}C-bicarbonate to estimate photosynthetic rates, respiration may dilute the specific activity of the carbon pool used in photosynthesis relative to the medium (Taylor 1984, Kuile & Erez 1987). Second, estimates of polymer synthesis based on assimilation of radiolabelled carbon in short-term incubations may underestimate net synthesis because of incomplete equilibration of precursor pools with exogenous ^{14}C (Smith & Platt 1984, Lohrenz & Taylor 1987). Rate estimates in the present study were based on incubations ≥ 3 h in length in an effort to minimize this problem.

Diurnal periodicity of photosynthesis may also contribute to error in daily photosynthetic rates extrapolated from short midday-incubations (e.g. Putt & Prezelin 1985). Initial studies indicate that the ratio of light-saturated photosynthetic rates in *Laboea strobila* at midday, and either early morning or late afternoon, ranges from 1 (no diurnal periodicity of photosynthesis) to 2 (Putt unpubl.). Thus rates derived from midday incubations may overestimate mean daily photosynthetic rates by up to 50 %. Diurnal periodicity of photosynthesis may be the largest source of error in estimating daily photosynthetic rates in the present study. While further quantification of all sources of error discussed here is desirable, overestimates of even 50 % of the contribution of photosynthate to any metabolic pool

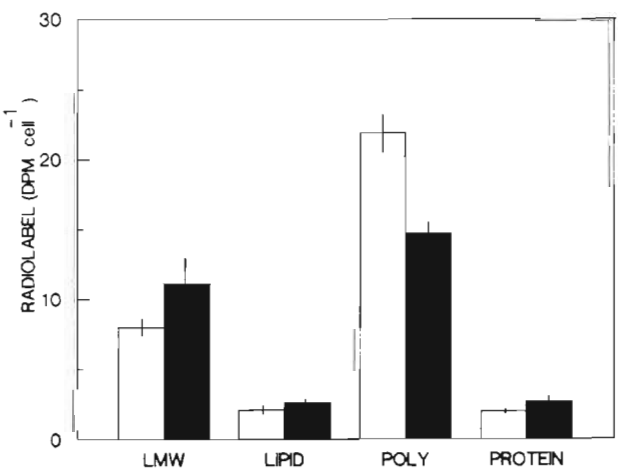

Fig. 2. *Laboea strobila*. Distribution of radiolabel in low molecular weight (LMW), lipid, polysaccharide (POLY) and protein in ciliates incubated at 250 μE m^{-2} s^{-1} for 1 h (open bars) and then placed in the dark for 1 h with food (solid bars). Values are means ± SD (n = 3); DPM: disintegrations min^{-1}

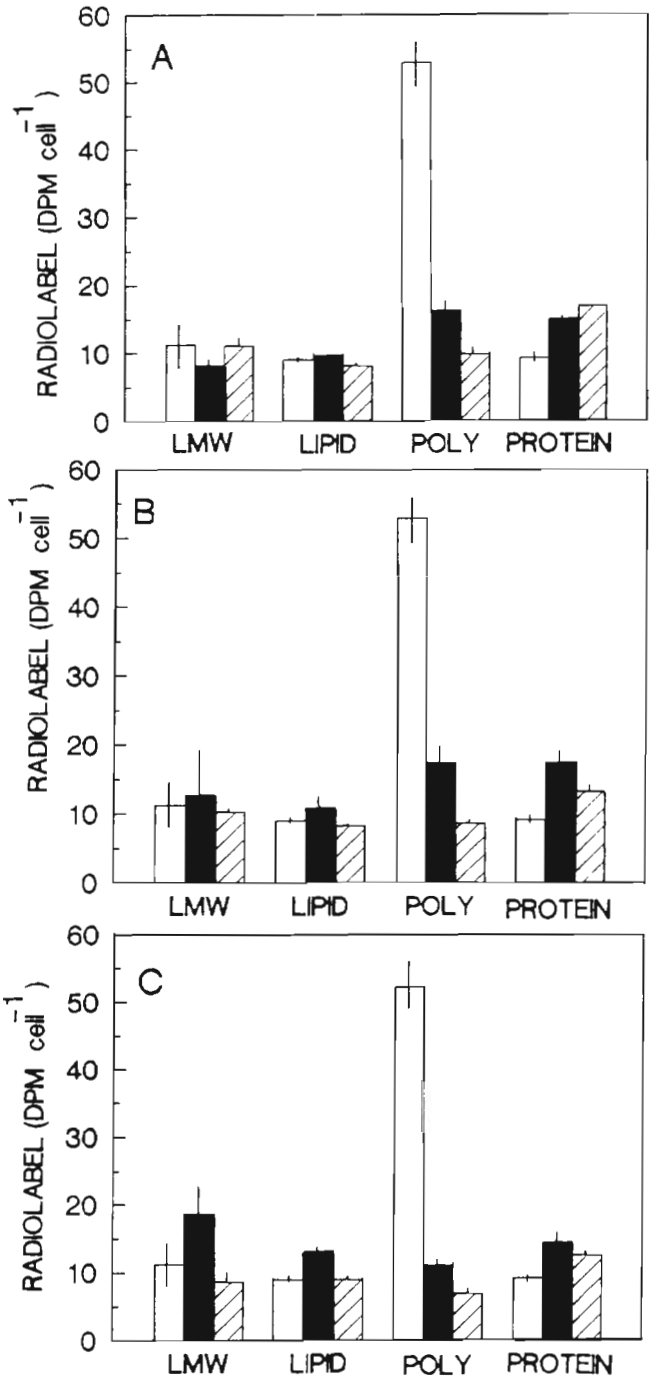

Fig. 3. *Laboea strobila*. Distribution of radiolabel in low molecular weight (LMW), lipid, polysaccharide (POLY) and protein in ciliates incubated at 250 μE m^{-2} s^{-1} for 3 h (open bar) and then placed in (A) darkness with food, (B) darkness without food, and (C) light with food and dichlorophenyldimethylurea (DCMU) for 6 h (solid bar) or 18 h (hatched bar). Initial photosynthetic rate was 229 ± 6 pg C cell^{-1} h^{-1}. Values are means ± SD (n = 3); DPM: disintegration min^{-1}

do not alter the main conclusions of the discussion which follows.

With these caveats in mind, photosynthesis provided no more than ca 16% of the protein pool and 14% of the lipid pool each day in *Laboea strobila* grown in culture. The daily contribution of photosynthate to protein in cultures of *L. strobila* rose to ca 25% if reallocation of photosynthate into protein during the 18 h chase period is assumed to represent events occurring during the natural night. Although *L. strobila* is capable of growth rates up to 1.8 doublings d^{-1}, typical exponential growth rates for *L. strobila* in the present study were about 0.5 to 1.0 doublings d^{-1} (Stoecker et al. 1988, Putt unpubl.). Thus photosynthesis roughly provided 14 to 28% and 25 to 50% of the daily requirements of *L. strobila* for lipid and protein, respectively. The quantitatively small contribution of photosynthate to protein and, particularly to lipid pools may partially explain why *L. strobila* is unable to grow in the absence of particulate food (Stoecker et al. 1988). Sacoglossan molluscs, which retain isolated chloroplasts, also demonstrate relatively small accumulations of photosynthate in protein and lipid (Greene & Muscatine 1972, Trench et al. 1972).

In nature, the actual contribution of photosynthesis to metabolic pools in *Laboea strobila* will depend on in situ irradiances. In the present study, maximum hourly contributions of photosynthate to protein pools were somewhat larger in field populations than in cultures. This finding may reflect genetic differences between wild and cultured strains or a physiological response to spectral quality. Wild populations were incubated in natural sunlight while cultures were grown and incubated under fluorescent lights. Spectral quality is known to influence carbon metabolism in a number of algae (Wallen & Geen 1971, Pick & Cuhel 1986).

In contrast to lipid and protein, daily production of polysaccharide more than doubled the polysaccharide pool in cultures of *Laboea strobila* growing at an irradiance saturating to photosynthesis. In field populations, large variations in photosynthetic rates at saturating and limiting irradiances were accompanied by small variation in the relative assimilation of photosynthate into polysaccharide. The present study gives no information about the site of polysaccharide synthesis in *L. strobila*. However, chromophytic algae such as *Isochrysis galbana* and *Pyrenomonas salina* normally do not store polysaccharide within chloroplasts (Dodge 1979). Not surprisingly, electron micrographs give no evidence for polysaccharide accumulation in the chloroplasts of *I. galbana* and *P. salina* retained by *L. strobila* (Stoecker et al. 1988, Stoecker unpubl.) suggesting that photosynthate is exported from the plastids prior to assimilation into polysaccharide. Photosynthate is generally translocated across chloroplast membranes as small sugars or sugar phosphates (Heldt 1976, Walker 1976). Rapid assimilation of photosynthate into polysaccharide may contribute to maintenance of a concentration gradient of small sugars

Table 7. Change in radiolabel (Change) in polysaccharide (Poly) and protein pools for *Isochrysis galbana*, *Pyrenomonas salina* and *Laboea strobila* incubated initially at a light-saturating irradiance (200 μE m^{-2} s^{-1}) for 3 h and then placed in the dark or light with dichlorophenyl-dimethylurea (DCMU) for 6 or 18 h. Values expressed as percentage of radiolabel present after initial incubation

Organism	Incubation conditions	Change		
		Total	Poly	Protein
I. galbana	6 h dark	−10	−57	+412
P. salina	6 h dark	−14	−43	+572
Mean		−12	−51	+492
L. strobila	6 h dark + food	−38	−74	+63
	6 h dark − food	−20	−67	+91
	6 h light + DCMU + food	−18	−79	+56
Mean		−25 ± 11	−73 ± 6	+70 ± 11
I. galbana	18 h dark	−24	−79	+582
P. salina	18 h dark	−26	−63	+959
Mean		−25	−71	+771
L. strobila	18 h dark + food	−41	−81	+86
	18 h dark − food	−49	−83	+44
	18 h light + DCMU + food	−58	−76	+28
Mean		−49 ± 8	−80 ± 4	+53 ± 30

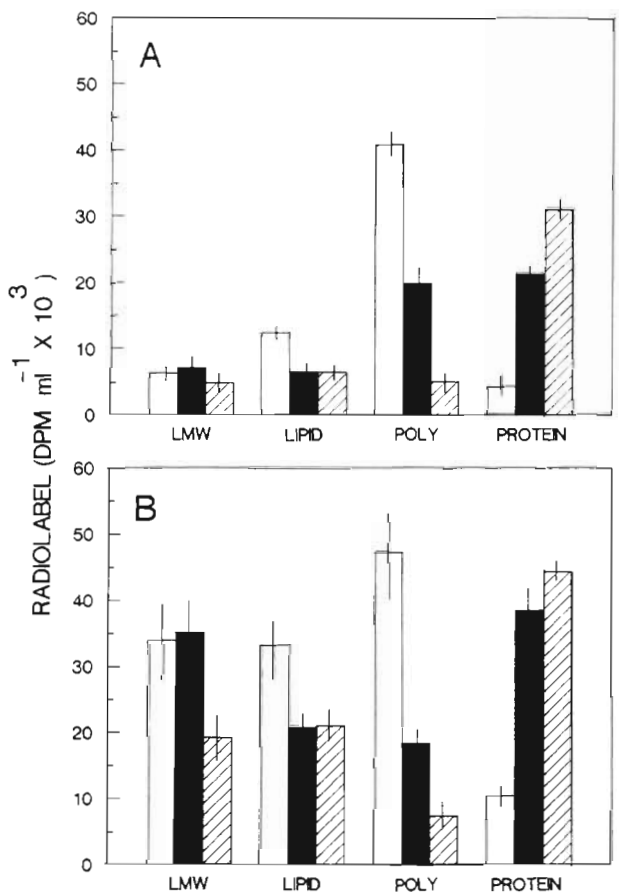

Fig. 4. *Pyrenomonas salina* (A) and *Isochrysis galbana* (B). Distribution of radiolabel in low molecular weight (LMW), lipid, polysaccharide (POLY) and protein in algae incubated at 250 μE m^{-2} s^{-1} for 3 h (open bar) and then placed in the dark for 6 h (solid bar) or 18 h (hatched bar). Values are means ± SD (n = 3); DPM: disintegrations min^{-1}

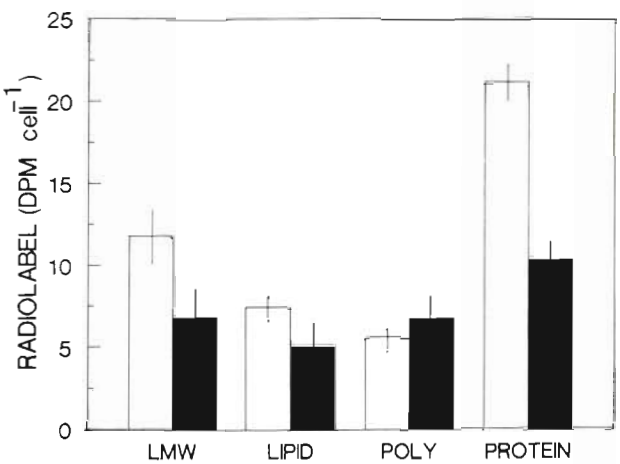

Fig. 5. *Laboea strobila*. Distribution of radiolabel in low molecular weight (LMW), lipid, polysaccharide (POLY) and protein in ciliates fed uniformly labelled *Isochrysis galbana* for 3 h (open bars) and then placed in the dark in the presence of an equal amount of unlabelled *I. galbana* for 6 h (solid bars). Values are means ± SD (n = 3). Total cellular radiolabel decreased from 45.9 ± 5 to 29.2 ± 10.4 disintegrations min^{-1} $cell^{-1}$ during the 6 h dark period

between the chloroplast stroma and the ciliate cytoplasm facilitating net transport of photosynthate across the chloroplast membrane (Hinde 1978). The large accumulation of photosynthate in polysaccharide in incubations as short as 1 h was consistent with this proposed role of photosynthetically derived polysaccharide.

Ciliates contain both storage and structural polysaccharides (Ryley 1967). On the basis of an electron microscopy study, Laval-Peuto & LeFebvre (1986)

speculated that certain structural polysaccharides in plastidic oligotrichs, specifically small 'starch-like' plates located beneath the pellicle, were derived from photosynthate (see also Fauré-Fremiet & Ganier 1970). The present study did not confirm this suggestion. Extensive turnover of photosynthetically-derived TCA-soluble material suggests that metabolic intermediates rather than structural polysaccharides are the major component of this pool. However, some complex polysaccharides such as cellulose are not solubilized by hot TCA (Ryley 1967, Hitchcock 1983). Thus the plates may be derived from photosynthate but may be partitioned into the 'protein' fraction.

Finally, an important finding of this study is that photosynthate contributes to the pool of reduced carbon used by *Laboea strobila* for respiration and excretion. Quantifying respiration based on loss of radiolabel from polysaccharide is not possible because the specific activity of the precursor pool is unknown (Cuhel & Lean 1987). While the specific activity of the total polysaccharide pool can be estimated, it is not possible to estimate the portion of the pool actively used in respiration. However, a rough estimate of respiration of photosynthate was made using the total loss rates observed during pulse-chase experiments. In a typical experiment, the maximum hourly contribution of photosynthate to total cellular carbon in *Laboea strobila* cultures was 3.2 % h^{-1} or 45 % d^{-1}. In pulse-chase experiments, ca 49 % of the radiolabel was respired or excreted within an 18 h period. If similar loss rates occurred during a diel cycle, respiration of photosynthate would account for about 22 % of body carbon d^{-1}. Total respiration and excretion rates of cultured *L. strobila* at a similar growth stage are on the order of 50 % of cell carbon d^{-1} (Stoecker unpubl.). Thus the maximum contribution of photosynthesis to respiratory and excretory requirements of this mixotrophic ciliate was about 44 % d^{-1}. In field populations, maximum hourly contributions of photosynthesis to carbon pools were similar to cultured populations but daily rates would depend on in situ irradiance. Respiration rates in protozoa are highly variable; in particular, some protozoa reduce respiration during periods of starvation and/or slow growth (Ryley 1967, Fenchel & Finlay 1983, Fenchel 1987). In nature, during periods when food is scarce, photosynthesis may be critical in supplementing ciliate respiratory requirements.

Use of photosynthate to supplement respiration in mixotrophic ciliates may also influence oceanic nutrient cycling through an effect on ciliate trophic efficiency. Trophic efficiency reflects the relative amount of ingested food used for reproductive growth compared to respiration, egestion and excretion (Verity 1985, Fenchel 1987). Assuming that the energetic 'costs' of retaining chloroplasts are small, trophic efficiencies may be higher in mixotrophic than in heterotrophic ciliates because photosynthesis supplements ingested material as a source of carbon for fulfilling maintainance costs. Even small increases in the trophic efficiencies of any one component of the food web can increase the amount of biomass supported at higher trophic levels (Fasham 1985, Michaels & Silver 1988). Despite the quantitative significance of oligotrichous ciliates in marine environments there are few direct measurements of trophic efficiencies in heterotrophic oligotrichs (Rassoulzadegan 1982, Scott 1985) and none in mixotrophic forms. More information about whether other mixotrophic ciliates use photosynthate similarly to *Laboea strobila* and the extent to which these patterns are reflected in increased growth efficiencies are needed to understand the impact of mixotrophic ciliates on nutrient cycling through microbial food webs.

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