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Carbon–nitrogen relations at whole-cell and free-amino-acid levels during batch growth of *Isochrysis galbana* (Prymnesiophyceae) under conditions of alternating light and dark

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Abstract. *Isochrysis galbana* Parke, Strain CCAP 927/1, was grown in ammonium-limited batch culture under a 12 h light:12 h dark illumination cycle. Samples were taken every 12 h over the 26 d period from lag phase through exponential into stationary phase (no net carbon fixation), with more frequent sampling at points of interest. Exponential cell-specific growth rate was 0.3 to 0.4 d^{-1}. Cell division occurred during the dark phase, while cell volume increase, ammonium uptake, and pigment synthesis occurred during the light. Stationary phase cells were small, and the lag phase was long (5 d) even though the C:N ratio had returned from 18 to 6.5 within 2 d, followed by synthesis of chlorophyll *a*. Net chlorophyll synthesis ceased within 4 d of exhaustion of the nitrogen source. The chlorophyll *c*:chlorophyll *a* ratio remained constant during increasing nitrogen deprivation. Biovolume and carotenoids correlated with carbon biomass. Levels of chlorophyll *a* correlated poorly with carbon fixation and carbon biomass once the nitrogen source had been exhausted. Except after the addition of ammonium to nitrogen-deprived cells (refeeding), the content of intracellular glutamine and the glutamine:glutamate ratio were low during the dark phase, rising to a plateau within the first 1 h of illumination. Refeeding of cells which had only just exhausted the extracellular nitrogen source resulted in a much smaller increase in glutamine than refeeding of nitrogen-starved (stationary-phase) cells. Nitrogen biomass correlated with the presence of an unidentified intracellular amine.

Introduction

Isochrysis galbana has been used widely in research into algal physiology (e.g. Herzig and Falkowski 1989), as a "model phytoplankter" in the development of mathematical models of algal growth (e.g. Caperon 1969, Sukenik et al. 1991), and as a food organism in aquaculture (Renaud et al. 1991).

When attempting to develop models of the interaction between a microalga and a microflagellate, it is necessary to simulate the growth of the alga in terms of both cell numbers and biomass parameters. In any simulation of the interaction between organisms in terms of carbon and nitrogen, it is also clear that a diurnal pattern of carbon input to the system should be included if we are to simulate reality. Furthermore, how the alteration in illumination during the day affects the response of the alga to the availability of nitrogen regenerated by the predator (i.e., nitrogen refeeding of the algae) should also be considered. We therefore collected data describing the growth of *Isochrysis galbana* under conditions of continuous illumination (Davidson et al. 1992) and during growth under alternating light and darkness (present study). For comparison we conducted parallel studies on *Nannochloropsis oculata* (Flynn et al. 1993a).

The aims of this work then, were to (a) obtain a complementary data set describing growth of *Isochrysis galbana* under different light–dark conditions; (b) examine changes in the carbon–nitrogen status of the algae at the subcellular level by determining the contents of the intracellular amino acid pool (including for the first time changes in non-protein amines: Flynn and Flynn 1992); (c) consider the effects of refeeding during light and during dark phases of growth; (d) consider estimators of carbon and nitrogen biomass which may be used in mixed populations where filter-fractionation is not possible.

Materials and methods

Axenic cultures of the prymnesiophyte *Isochrysis galbana* Parke, from the "Culture Collection of Algae and Protozoa", Oban, Scotland (Strain CCAP 927/1), were grown in artificial sea water consisting of 1.6% (w/v) Instant Ocean (Aquarium Systems), 100 μM (1.4 $\mu g\ N\ l^{-1}$) ammonium chloride, 18 μM potassium dihydrogen phosphate, 50 $\mu g\ l^{-1}$ thiamine and 1 $\mu g\ l^{-1}$ vitamin B_{12}. The medium was filter-sterilized (0.22 µm Millipore Durapore membrane)

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Table 1. *Isochrysis galbana.* Composition of main culture in terms of culture volume. Data for ammonium and intracellular amino acids (InAA) are given in terms of nitrogen. Extracted chlorophylls were measured by fluorometry (fl) or spectrophotometry (sp). –: data missing due to instrument malfunction or, for spectrophotometric pigment analysis (sp), because the excessive volumes of culture required for first half of the experiment meant that samples were not taken

Time (d)	Cells (10^6 ml^{-1})	Biovolume (nl ml^{-1})	Ammonium (µg N ml^{-1})	Cell C (µg C ml^{-1})	Cell N (µg N ml^{-1})	InAA (ng N ml^{-1})	Chlorophyll (ng nl^{-1})			Carotenoid (mg N nl^{-1})
							a (fl)	*a* (sp)	*c* (sp)	
0.0	0.04	2.0	1.411	0.60	0.03	0.48	4	4	1	13
0.5	0.04	2.2	1.400	0.61	0.08	0.86	3	–	–	–
1.0	0.04	2.1	1.383	0.58	0.08	0.72	3	–	–	–
1.5	0.04	2.1	1.355	–	0.12	1.02	3	–	–	–
2.0	0.04	2.1	1.355	0.68	0.12	1.13	3	–	–	–
2.5	0.05	2.6	1.350	0.66	–	1.25	4	–	–	–
3.0	0.05	–	1.355	0.75	–	1.93	6	–	–	–
3.5	0.05	2.5	1.333	2.29	0.38	2.27	9	–	–	–
4.0	0.04	2.1	1.316	2.91	0.92	2.29	9	–	–	–
4.5	0.06	2.9	1.288	1.75	0.61	2.49	14	–	–	–
5.0	0.05	2.3	1.288	1.49	0.26	2.77	14	–	–	–
5.5	0.05	3.1	1.260	2.14	0.27	3.90	17	–	–	–
6.0	0.10	4.4	1.257	1.58	0.64	4.14	18	–	–	–
6.5	0.10	5.8	1.226	2.07	0.36	5.07	41	–	–	–
7.0	0.15	7.4	1.226	2.04	0.36	5.40	41	–	–	–
7.5	0.15	9.5	1.092	2.66	0.45	7.81	50	53	52	35
8.0	0.23	9.8	1.092	2.25	0.52	8.14	53	67	70	54
8.5	0.22	13.4	0.935	4.12	0.57	11.67	68	78	43	75
9.0	0.28	13.9	0.932	3.93	0.64	11.12	84	–	–	–
9.5	0.29	18.6	0.806	5.03	0.72	15.76	133	148	46	134
10.0	0.45	18.9	0.795	4.42	0.60	16.14	133	152	55	134
10.5	0.46	31.3	0.577	6.06	0.74	24.39	178	205	75	192
11.0	0.66	28.1	0.566	5.69	0.79	23.16	210	227	80	201
11.5	0.66	41.0	0.274	8.42	1.01	32.66	255	283	104	240
12.0	0.89	38.6	0.291	8.46	1.29	34.13	255	290	115	243
12.5	0.89	54.8	0	11.29	1.37	41.36	323	376	129	320
13.0	1.11	51.6	0	11.51	1.56	39.02	336	410	152	369
13.5	1.13	59.8	0	13.83	1.53	32.42	377	434	160	408
14.0	1.33	62.2	0	13.91	1.61	34.73	399	385	148	387
14.5	1.33	67.8	0	15.95	1.52	32.07	377	468	164	486
15.0	1.47	70.1	0	15.06	1.53	33.43	413	493	181	533
15.5	1.50	76.2	0	20.03	1.60	36.44	440	518	206	597
16.0	1.50	76.5	0	16.40	1.43	35.46	395	478	167	557
16.5	1.64	85.2	0	18.74	1.44	39.91	439	515	195	644
17.0	1.82	93.3	0	18.03	1.54	34.12	399	468	136	594
18.0	1.98	87.9	0	–	–	–	–	–	–	–
18.5	2.00	105.2	0	23.20	1.59	34.83	395	481	164	693
19.0	1.93	106.4	0	22.02	1.32	41.05	386	441	151	657
20.0	1.95	–	0	–	–	–	–	–	–	–
20.5	1.99	101.1	0	23.69	1.30	31.72	377	442	146	674
21.0	1.93	93.4	0	23.66	1.31	30.11	368	439	144	693
21.5	1.95	–	0	–	–	–	–	–	–	–
22.0	2.00	90.3	0	25.80	1.31	30.10	368	415	156	717
23.0	2.00	85.8	0	27.49	1.46	34.24	296	476	153	815
23.5	2.00	90.4	0	25.58	1.38	29.62	268	375	119	645
24.0	1.94	84.2	0	25.53	1.38	27.20	341	407	142	753
24.5	1.90	86.7	0	27.05	1.44	30.83	341	407	150	792
25.0	1.99	90.8	0	26.02	1.36	31.36	323	375	126	731

into 5-litre and 3-litre flasks. Cultures were grown at 18 °C, illuminated at 100 µmol m^{-2} s^{-1}, with a 12 h light : 12 h dark cycle, and occasional shaking. No aeration was provided. Samples were withdrawn aseptically through a syphon tube with the inlet near to the bottom of the flask.

The main culture (initially of 5 litres) was inoculated by the aseptic addition of stationary-phase culture grown on the same medium under the same conditions to give an addition of 3% (v : v inoculum : medium). This culture was sampled at least every 12 h (30 min before the end of the light or dark phase), and more frequently following refeeding during the light (Day 0), for a 24 h period during the exponential phase of growth (Day 9), and whilst entering the stationary phase (Day 15). At the end of the stationary phase (Day 24), a portion of the culture was used to inoculate 2 litres of fresh medium, and the response to refeeding a stationary-phase culture in darkness was examined.

An additional 2-litre volume was also inoculated at Day 0, again with a 3% inoculum from the same initial culture. This culture was sampled occasionally until it had just exhausted the nitrogen source, at which time portions were taken and used to inoculate fresh

Table 2. *Isochrysis galbana*. Predictive regressions (Ricker 1973) for determination of carbon and nitrogen biomass, and for carbon : nitrogen (C : N) ratio. Chlorophyll *a* was determined by fluorometry (fl) or spectrophotometry (sp). $A_{480} : A_{665}$ = ratio of absorbance at 480 nm and 665 nm for extracted pigments

Y (unit)	*X* (unit)	Intercept	Slope	*r*	*df*
Cell carbon (μg)	Biovolume (nl)	0	0.251	0.97	42
	Carotenoid (ng)	0	0.034	0.99	29
Cell nitrogen (μg)	Chlorophyll *a* (ng;fl)	0	0.0045	0.88	28
	Chlorophyll *a* (ng; sp)	0	0.0034	0.91	41
	Ammonium N (μg)	1.477	−0.918	0.89	29
C : N	$A_{480} : A_{665}$	−3.410	11.158	0.94	27

medium, either 1 h into the light phase or 1 h into the dark phase. These cultures were examined over the two subsequent light phases to study the effect of refeeding before the cells had been subjected to a significant period of nitrogen deprivation.

Measurement of cell numbers and cell volumes (Coulter Counter ZM with Channelyzer 256), cell carbon and cell nitrogen (Leco 900 CHN analyzer), ammonium and amino acids [high pressure liquid chromatography of o-phthalaldehyde (OPA) derivatives] and pigments (fluorometric and spectrophotometric estimates from extracted pigments) were made as described previously (Davidson et al. 1992). Amines were detected using the method of Flynn (1988). Since this work was conducted it has become apparent that an unidentified amine, termed TT1, coeluted with alanine during these analyses (Flynn and Flynn 1992). Alanine actually comprizes ~10% of the peak; throughout this work, the TT1 plus alanine peak is termed TT1. There is also another significant amine in *Isochrysis galbana*, termed X (Flynn and Flynn 1992), which elutes between glutamine and histidine in the method of Flynn (1988).

Results

The main data for *Isochrysis galbana* are presented in Table 1; predictive regressions are given in Table 2. Estimates for erroneous or missing values for cell carbon and nitrogen were obtained (and are used in Figs. 1, 2, and 3) from regressions between the concentrations of biovolume and cell carbon, and between ammonium and cell nitrogen (as given in Table 2) computed using methods recommended by Ricker (1973).

Cell numbers, volume, biomass and pigments

Following a lag phase of 5 d, the cells went through five generations before exhausting the nitrogen source during Day 12 (Table 1). Numbers increased two-fold during the post-exponential phase before net carbon fixation ceased. The cell-specific growth rate (cell-μ) was ~0.4 d^{-1} during exponential phase (Days 5–12), falling after the exhaustion of the nitrogen source (Fig. 1 a). The nitrogen-specific growth rate (N-μ) differed from that of the carbon-specific growth rate (C-μ) and cell-μ (Fig. 1 a), with an early maximum as the cells assimilated nitrogen and a value of 0.2 d^{-1} during the lag phase prior to cell division. C-μ and cell-μ peaked at Day 6. There was a synchronized first division of cells leaving the lag phase. The cellular N : C ratio correlated with C-μ after the lag phase (Fig. 1 b); the slope of this line is similar to that for *Isochrysis galbana* grown under continuous illumination.

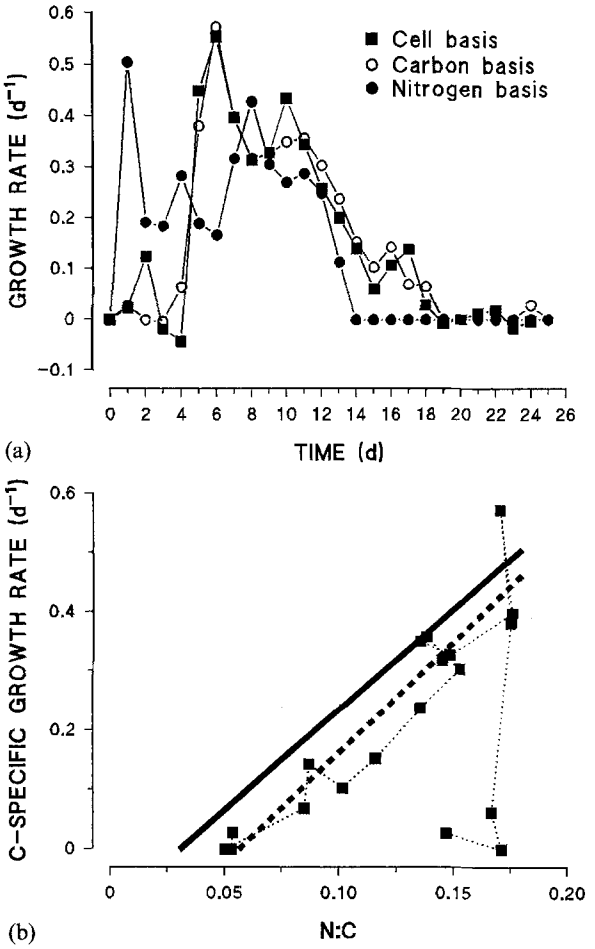

Fig. 1. *Isochrysis galbana*. Changes in growth rate per day on cell, carbon and nitrogen bases (a), and changes in carbon-specific growth rate with cellular N : C ratio (b). Dotted line in (b) links data points in chronological order, revealing increasing growth rate with stable N : C during pre-exponential growth phase, followed by a decline in both growth rate and N : C during post-exponential growth; dashed line in (b) indicates regression line fitted through post-lag phase data (data from end of each light phase was used; carbon-specific growth rate = 3.743 · N : C − 0.212; $r = 0.935$; df 13); continuous line is regression line for data of Davidson et al. (1992) with *I. galbana* grown under continuous illumination (carbon-specific growth rate = 3.383 · N : C − 0.103; $r = 0.865$; df 12)

Cell division occurred during the dark phase, and there was a corresponding cyclic variation in cell volume between the end of the light phase (maximum) and the end of the dark phase. The volume of cells in stationary phase (Day 0 and after Day 19) tended towards the minimum cell volume observed during exponential growth. Biovolume showed a step-wise progression, increasing during the light phase only and correlating (Table 2) with cell carbon. Cell nitrogen also increased only during the

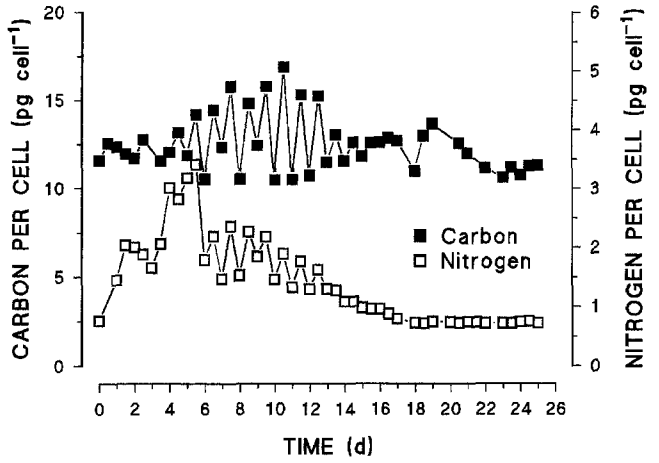

Fig. 2. *Isochrysis galbana.* Changes in cell carbon (computed from biovolume using predictive regression in Table 2) and cell nitrogen from elemental analysis

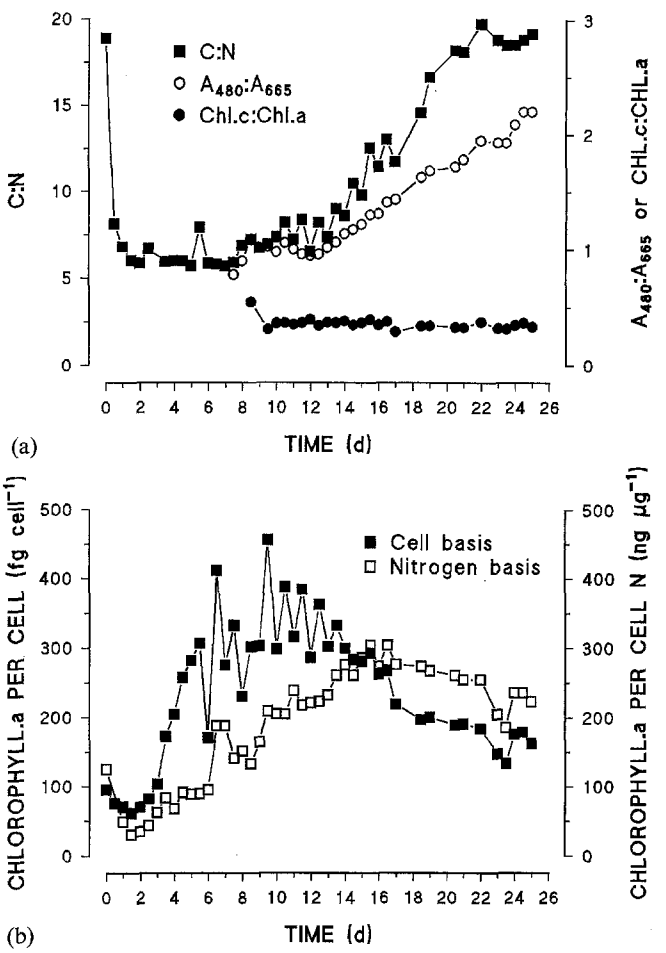

Fig. 3. *Isochrysis galbana.* Changes in C : N, chlorophyll *c* : chlorophyll *a* and in extracted pigment ratio measured at 480 and 665 nm (A_{480} : A_{665}) (a), and in chlorophyll *a* on cell and nitrogen basis (b)

light phase once cell division had commenced at Day 6 (Fig. 2). Average carbon per cell remained relatively constant during the post-exponential phase, while nitrogen per cell declined to 30% of the maximum during exponential growth (Fig. 2). Nitrogen per cell was maximum just before the start of the exponential phase (Day 5). The C : N ratio decreased from an initial value of 18 to ~6.5, and remained at this value during exponential growth, with step-wise increases during the light phase and decreases in darkness after exhaustion of the nitrogen source (Fig. 3a).

Because of the need to minimize the volumes taken for each sample, initially, extracted chlorophyll *a* was determined by fluorometry only. Later, the extracts were also measured spectrophotometrically: values of chlorophyll *a* per ml were ~10% less than values determined by fluorometry. The chlorophyll *c* : chlorophyll *a* ratio was ~0.4 during progression from the exponential to the stationary phase (Fig. 3a). Net chlorophyll *a* synthesis did not commence until Days 2 to 3 (by which time C : N had decreased to its minimum; Fig. 3), and then increased on a cellular basis until the first cell division at Day 6 (Fig. 3b). Chlorophyll contents per ml of culture increased with increasing cell numbers until the nitrogen source was exhausted (Day 12) when net synthesis slowed, ceasing at Day 15. Chlorophyll *a* correlated with cell nitrogen (Table 2). There was a cyclic variation in chlorophyll concentration; this was particularly obvious when considered on a cell basis (Fig. 3b). However, this variation was far less than that observed for the data sets on cell volume and carbon and nitrogen per cell (Fig. 2), suggesting that some chlorophyll synthesis proceeded during darkness.

Carotenoid levels per cell remained relatively constant (computed from Table 1), increasing during the light phase. As with the chlorophylls, the amplitude of the variation was less than that for carbon and nitrogen biomass. Carotenoid per ml followed the same pattern as cell density and correlated with cell carbon (Table 2).

The ratio of absorbance of the extracted pigments at 480 and 665 nm, A_{480} : A_{665} (approximating the ratio of carotenoid to chlorophyll *a*), increased in a linear fashion after exhaustion of the nitrogen source on Day 12 (Fig. 3a), correlating with cell carbon : nitrogen (Table 2).

Pattern of intracellular amino acids

The data are presented as moles of amino acids *not* of amino-N; arginine (Arg) contains 4 *N* per molecule, while the amines X and TT1 are of unknown composition and assumed to react with OPA in the same way as glutamate (see Flynn and Flynn 1992).

The levels of most of the individual intracellular amino acids displayed a diurnal variation (high in the light phase), but a few, notably the unidentified non-protein amines X and TT1 (Fig. 4a), did not vary as obviously. Generally, the levels of intracellular amino acids, and of total amino-nitrogen per ml of culture declined after exhaustion of the nitrogen source on Day 12; this was particularly striking for glutamine (Gln) and asparagine (Asn; Fig. 4b). However, levels of glutamate (Glu; Fig. 4a) and valine (Val; data not shown) per ml continued to increase throughout, and Gln levels also recovered during the stationary phase (Fig. 4b).

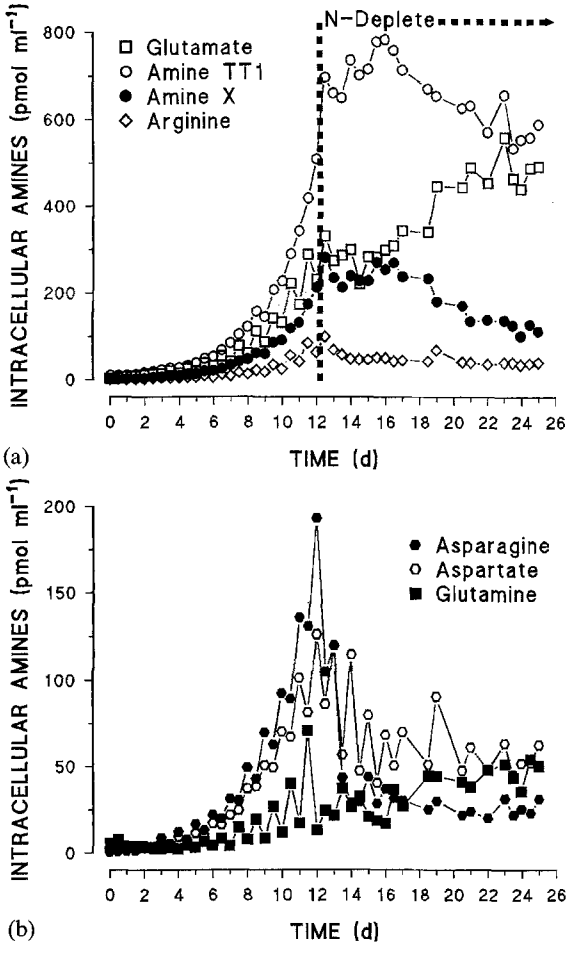

Fig. 4. *Isochrysis galbana.* Changes in intracellular free amines. (a) Glutamate, arginine, and non-protein amines X and TT1; (b) aspartate, asparagine and glutamine

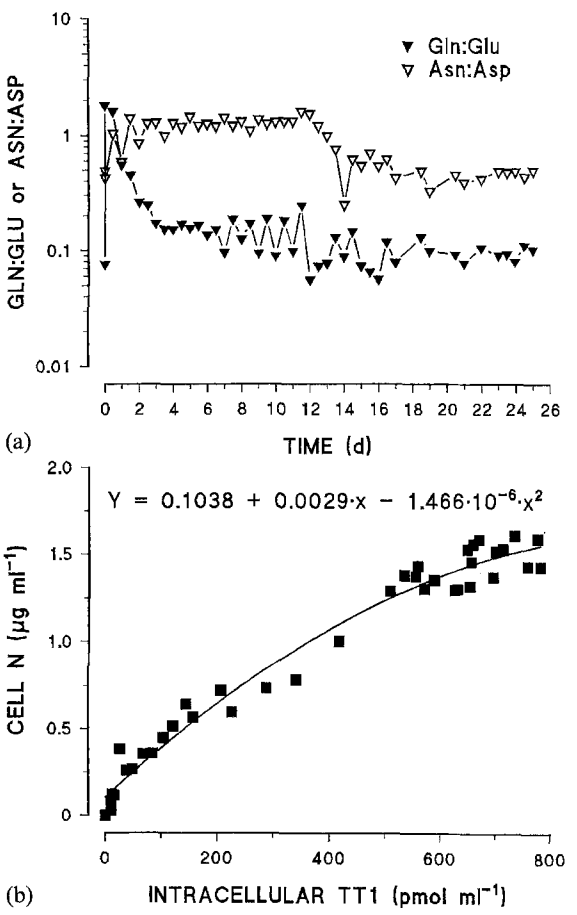

Fig. 5. *Isochrysis galbana.* (a) Changes in ratios of intracellular Gln : Glu and Asn : Asp; (b) relationship between intracellular non-protein amine TT1 and cell nitrogen, together with its quadratic equation

The ratio of Gln : Glu was < 0.1 at Day 0, increasing rapidly to a peak of 2.8 within 4 h of refeeding with ammonium, falling again to ~1.8 by 12 h (as in Fig. 5a) and to 0.2 by Day 2, and subsequently exhibiting diurnal fluctuations once cell division commenced on Day 6 (Fig. 5a). Over the entire growth period, the Gln : Glu ratio exhibited a cyclic pattern of maxima during the light phase (0.2) and minima during the dark (0.1); these values were halved after Day 12, during nitrogen deprivation. The ratio of Asn : Asp climbed from 0.4 to ~1.3 during nitrogen-sufficient growth (with little obvious diurnal fluctuation), before decreasing again to 0.4 after exhaustion of the nitrogen source (Fig. 5a). The proportion of total cell nitrogen, as identified amino nitrogen, increased slowly to a maximum of 2.75% at the point of nitrogen-source exhaustion, and then declined slowly (from Table 1). The unidentified amine TT1 correlated with cell nitrogen in a curvilinear fashion (Fig. 5b).

Diurnal changes in amino acids

Fig. 6 shows changes in the intracellular amines Glu, Gln, Arg, X, and TT1 (expressed per cell) at various points during the growth cycle. On refeeding stationary-phase cells during the light phase (Fig. 6a), Gln increased very rapidly, with a concurrent fall in Glu and TT1. Over the following dark phase, Gln was still high, although it fell on entry into the dark phase. By the end of the second dark phase, levels of Glu, Arg and TT1 were recovering to levels typical of exponentially growing cells (Fig. 6b), while Gln declined.

A diurnal cycle was apparent during exponential growth (Fig. 6b), with an increase in levels of all amines in the light and a decrease in the dark (most notably for Gln). The Gln : Glu ratio fell from ~0.2 in the light to ~0.1 in the dark (Fig. 5a). The diurnal cycle during the post-exponential phase was flat (X and Glu = 0.18, Gln = 0.02, Arg = 0.03, and TT1 = 0.5 fmol cell^{-1}), with patterns of increases during the light phase barely discernible. The Gln : Glu ratio for these cells was −0.12 (light phase) and −0.08 (dark phase).

At the stationary phase, the amount of Glu and TT1 per cell had fallen to around half that in exponentially growing cells. On refeeding these nitrogen-starved cells with ammonium (to give 100 µ*M*) in the dark phase (Fig. 6c), a rapid increase in Gln again occurred and a fall in Glu was apparent, giving a peak Gln : Glu value of

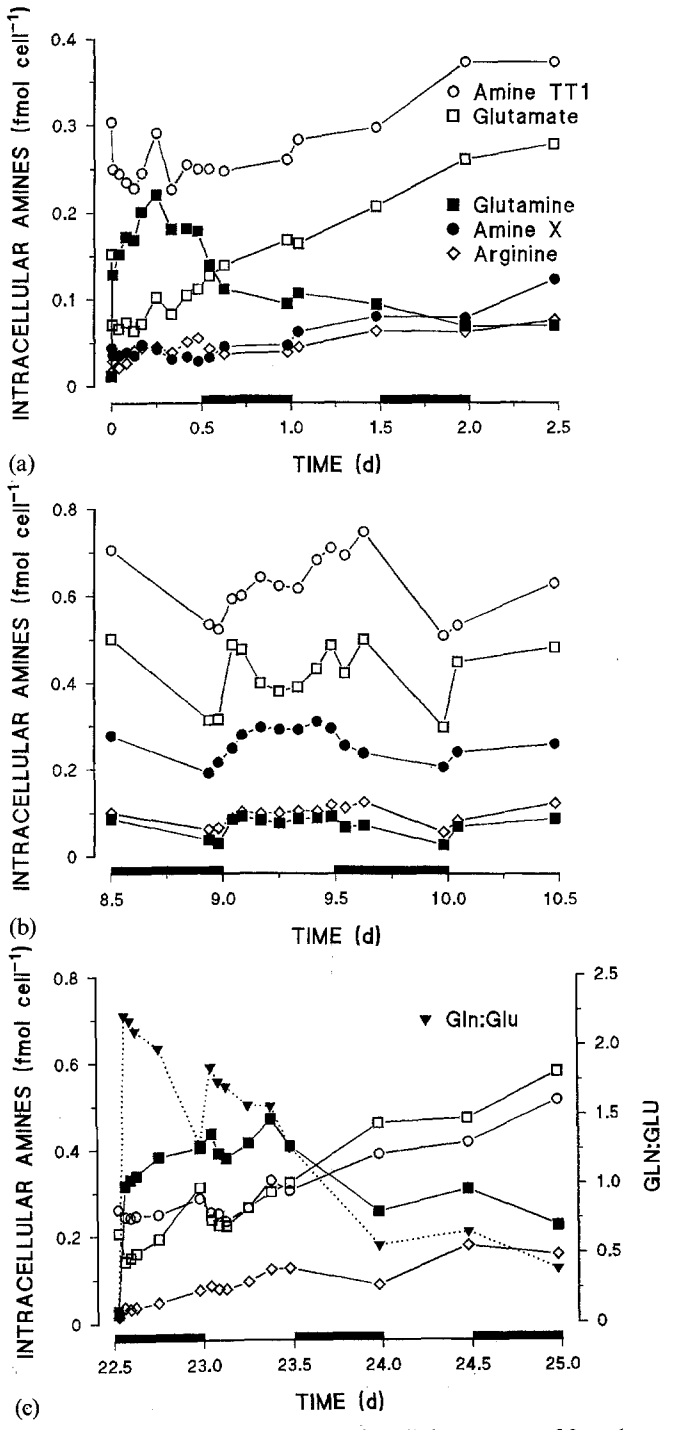

Fig. 6. *Isochrysis galbana.* Changes in cellular content of free glutamate, glutamine, arginine and of non-protein amines X and TT1 during ammonium refeeding in light (a), during exponential growth (b) and during refeeding in darkness (c). In (c), Gln : Glu is shown, but data for amine X have been omitted for clarity

2.2 1 h after refeeding (Fig. 6 c). The C : N ratio decreased rapidly from 17 to 7.7 within 24 h (data not illustrated). However, in contrast to the situation when nitrogen-starved cells were refed in the light (Fig. 6 a), levels of Glu, Arg, and TT1 increased more rapidly after refeeding in the dark (Fig. 6 c), falling slightly when the lights came on.

Refeeding of cells recently deprived of nitrogen

These data came from the separate, parallel culture in which growth was followed until the nitrogen source was exhausted. The culture was then divided into two, with portions refed with ammonium (to give 100 μM) in the light or dark phase. The C : N ratios and pigment contents for these cells were similar to those of exponentially growing cells (data not illustrated); the cells had only just become nitrogen-deprived.

Refeeding with ammonium during the dark phase resulted in no significant changes in the concentration of Glu, Gln, Arg, and TT1 (Fig. 7 b) or of any other amino acid in comparison with the control (Fig. 7 a). During the following light phase there was a restoration of the normal cycle of rising and falling (during the next dark phase) seen during exponential growth (cf. Fig. 6 b). Refeeding in the light phase (Day 15) resulted in a rapid increase in Gln and Arg, but a slower rise following an initial decrease, in Glu and TT1 (Fig. 7 c, cf. Fig. 7 a). The dark refed cells showed no obvious change in Gln : Glu until the following light phase, when there was a recovery to the level observed in nitrogen-sufficient cells. In contrast, the cells refed in the light phase exhibited slightly elevated values of 0.3 during that phase (Fig. 7 d).

Discussion

This study complements that of Davidson et al. (1992) who grew *Isochrysis galbana* under continuous illumination, not in conditions of alternating light and dark. The two studies employed otherwise identical conditions of media, temperature, nutrition; the photon-flux density was the same, but the culture illuminated continuously received twice the total amount of energy per day. The continuously illuminated culture attained a 10% higher cell density, with a 10% higher cell-specific growth rate (cell-μ). Cells grown under light/dark conditions contained 1.6 times more chlorophylls *a* and *c* (but only 1.1 times as much carotenoid) than cells grown under continuous illumination, despite receiving only half the light energy. This was probably a major determinant of the lower rate of growth for the light/dark culture. The loss of nitrogen from the liquid phase, by the adherence of cells to the flask during nitrogen deprivation due to liberation of carbohydrate (Marker 1965), was less marked when the culture was grown under light/dark conditions.

From another study (Flynn et al. 1993 b), we know that there are no significant intermediates of chlorophyll synthesis or breakdown detectable by HPLC of extracted pigments from *Isochrysis galbana* during nitrogen deprivation. Chlorophyll estimates by simple spectrophotometric methods should be accurate. Also, the carotenoid composition changes during nitrogen deprivation, with an increase in echinenone and a decrease in the proportion of fucoxanthin (Flynn et al. 1993 b). During growth in the light/dark cycle, the chlorophyll content per cell did not oscillate to the same degree as cell carbon and cell nitrogen; this presumably reflects a stimulation of pigment synthesis as a response to entry into the dark phase

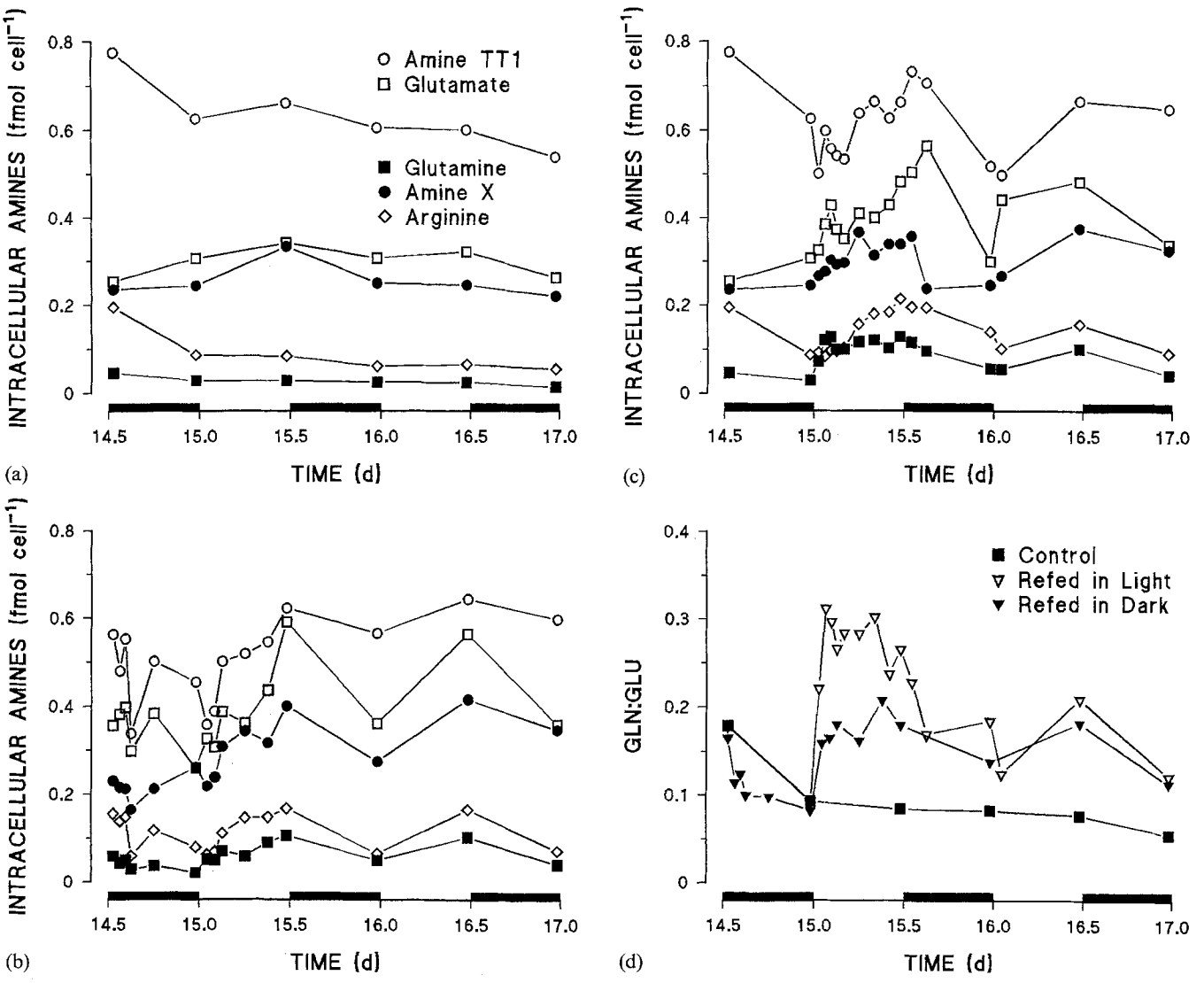

Fig. 7. *Isochrysis galbana.* Changes in cellular content of free glutamate, glutamine, arginine and of non-protein amines X and TT1 in the parallel culture shortly after exhaustion of extracellular ammonium. (a) Control flask with no addition; (b) refed in darkness; (c) refed in the light; (d) changes in Gln : Glu ratio for control, dark-refed and light-refed cells

with the concurrent carbon stress. As noted before (Davidson et al. 1992, Flynn et al. 1993 b), and contrary to the report by Herzig and Falkowski (1989), the chlorophyll *c*: chlorophyll *a* ratio did not alter during nitrogen deprivation in our studies.

For cultures grown under continuous or alternating light/dark illumination, the amount of carotenoid per cell remained relatively constant throughout the growth period, while net chlorophyll synthesis ceased shortly after the nitrogen source had been exhausted. The chlorophyll content per cell varies to such a degree that its usefulness in following algal growth dynamics in terms of cell numbers or carbon content is severely limited. In *Isochrysis galbana*, estimations of carbon biomass by means of biovolume, and of nitrogen biomass by intracellular TT1 content, are more robust than estimations arising from pigment analysis. For both the continuous and light/dark illuminated cultures, the ratio of absorbance at A_{480} : A_{665} gave a good linear correlation with C : N (Table 2 and Davidson et al. 1992). However, the slopes differed by a factor of 1.5 (with continuous light higher). Therefore the use of this absorbance ratio as a means to estimate the C : N ratio (Heath et al. 1990) of a population of unknown light history is not possible.

Cell volumes differed slightly in culture grown under the different light regimes. It is difficult to compare the data directly because of the phasing of cell division under light/dark, but the stationary-phase cell volume was (10 to 20%) smaller for light/dark cells. Carbon and nitrogen per cell, and maximum (~20) and minimum (6.5) C : N ratios, were all similar. The correlation between biovolume and cell carbon was also very good for both cultures, but the light/dark culture contained 0.24 g C ml^{-1} cell volume rather than 0.2 g C ml^{-1} in the continuously illuminated culture.

It was notable that the stationary-phase cells displayed the minimum volume and carbon biomass (Fig. 2 and Davidson et al. 1992), a situation which contrasts with that for the eustigmatophyte *Nannochloropsis oculata* (Flynn et al. 1993 a). *N. oculata* is largest during the

stationary phase; it then undergoes a very short lag phase before recommencing exponential growth. During the stationary phase, *Isochrysis galbana* appears to operate a minimum metabolic configuration, resulting in a long lag phase which is necessary to re-synthesise the biochemical machinery required for growth and division. The process is restricted further when illumination is not continuous. Modelling such lag phases is an important but difficult facet of algal growth simulations (Droop 1975, Davidson et al. 1993).

Laws and Caperon (1976) reported differences in the slope of the correlation between N:C and carbon-specific growth rate (C-μ) in *Pavlova* (*Monochrysis*) *lutheri* (an alga related closely to *Isochrysis galbana*) when grown under continuous or discontinuous illumination with nitrate as the nitrogen source. In our study, using ammonium rather than nitrate, there was no difference. Whether this (as with the differences in chlorophyll *c*:chlorophyll *a* ratios in our study from those reported by Herzig and Falkowski 1989) is a consequence of interspecific differences is not clear. Other differences, such as the utilization of nitrate rather than ammonium (which has profound effects on algal metabolism: see Flynn et al. 1993 b for data on *I. galbana*), and culture types (chemostats vs batch conditions) may well also play a part. It does, however, raise doubts about the universality of conclusions drawn from work done under continuous and batch culture conditions, even with organisms that are closely related.

What is clear, from the amino acid analyses, is that darkness has a profound effect on metabolic processes in *Isochrysis galbana*. During exponential growth, the contribution of amino nitrogen to cell nitrogen in the light/dark culture was less than half that under continuous illumination (Table 1, cf. Davidson et al. 1992). Only Glu concentrations per cell were similar. The diurnal effect of changing illumination was marked, and the response to changes in the light regime rapid, indicating the consequence to the cell of changing the relative supply of carbon and nitrogen. During exponential growth, ammonium uptake occurred only during the light phase. Especially the changes in Gln during refeeding indicate that amino acid synthesis is limited by the supply of carbon skeletons in the dark. The utilization of ammonium in darkness requires the use of carbon accumulated during (especially) periods of nitrogen stress. Dark-incorporation of ammonium was only apparent when ammonium was supplied to nitrogen-deprived cells, when Gln:Glu (Flynn 1990) could exceed that of similar cells fed in the light (Fig. 6c, cf. Figs. 5 and 6a). In darkness, Gln did not rise after refeeding of cells which had just exhausted the extracellular ammonium (Fig. 7b), although there were other changes (increased Glu, decreased TT1 and a modest rise of Gln during the following light phase compared to cells refed in the light; Fig. 7c). This suggests that cellular metabolism in *I. galbana* is affected by even short periods of nitrogen stress and subsequent refeeding.

Changes in the levels of the non-protein amines X and TT1 (Flynn and Flynn 1992) during batch growth and refeeding with ammonium (Figs. 6 and 7) give little clue to the identity of these amines. The concentration of X varied stepwise during the light/dark cycles while that of TT1 did not (Fig. 4a), and X declined after exhaustion of the nitrogen source. This is consistent with TT1 being a secondary metabolite with perhaps a limited role in cell metabolism per se, while X appears to be more closely associated with the synthesis of protein amino acids. The relationship between TT1 and cell nitrogen (Fig. 4b) has subsequently been used in predator–prey studies using *Isochrysis galbana* as food (Flynn et al. in preparation).

As indicated by Vanlerberghe et al. (1990) and Turpin (1991), these results imply that it is important to consider both the carbon and nitrogen status of the cells when measuring either carbon or nitrogen assimilation alone. Although the response of Gln:Glu to changes in the carbon:nitrogen status indicates satisfactorily nitrogen stress in the light, the effect of darkness on, presumably, keto acid synthesis also results in a rapid decrease in Gln:Glu in response to carbon stress in *Isochrysis galbana*. The relatively low levels of Gln:Glu during the light phase of exponential growth (compared with algae from other groups: Flynn 1990) are similar to other observations reported for growth of *I. galbana* (Davidson et al. 1992, Flynn et al. 1993 b). *Dunaliella primolecta* also displays Gln:Glu values of ~ 0.2 to 0.3 when grown under conditions of alternating light and dark (Flynn 1990). Both these flagellates also exhibit a gregarious behaviour in static cultures, with "curtains" of individuals often forming in patterns that are likely to result in some measure of self-shading. The low Gln:Glu values may be indicative of carbon stress incurred from self-shading of flagellates during such behaviour (the ratio fell further, to < 0.1, in darkness when carbon stress would be maximal).

In conclusion, it is clear that there are significant differences between the carbon and nitrogen physiology of cells grown under continuous light, and those grown under conditions of alternating light and dark. Any attempt to relate the physiological responses of laboratory cultures to growth of natural populations should, therefore, be conducted using illumination patterns as near to those found in nature as possible.

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