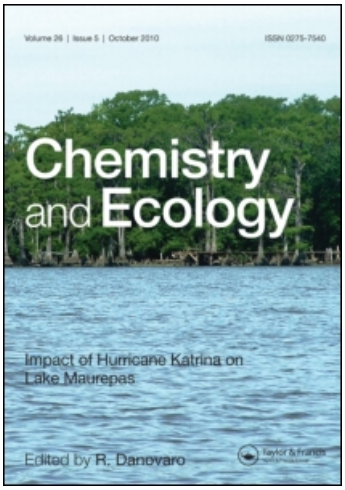

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

Changes in the Biochemical Composition of *Tetraselmis Suecia* and *Isochrysis Galbana* During Growth and Decay

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To cite this Article Pusceddu, Antonio and Fabiano, Mauro(1996) 'Changes in the Biochemical Composition of *Tetraselmis Suecia* and *Isochrysis Galbana* During Growth and Decay', Chemistry and Ecology, 12: 3, 199 — 212
To link to this Article: DOI: 10.1080/02757549608039082
URL: http://dx.doi.org/10.1080/02757549608039082

Chemistry and Ecology, 1996, Vol. 12, pp. 199–212
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CHANGES IN THE BIOCHEMICAL COMPOSITION OF *TETRASELMIS SUECIA* AND *ISOCHRYSIS GALBANA* DURING GROWTH AND DECAY

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(Received 2 January 1996; Revised 22 January 1996)

The variations of the biochemical composition of *Tetraselmis suecica* and *Isochrysis galbana* during growth and decay were determined. The content of chlorophyll *a* (Chl-a) of the cultures, as expected, slowly degraded into phaeopigments during decay, confirming that chlorophyll measurements do not always provide an accurate estimate of phytoplanktonic biomass and, consequently, may fail if used to measure the food availability of particulate matter for consumers. Measurements of total amounts of proteins, carbohydrates and lipids, related to the nutritional value of particles in terms of caloric content, are shown to provide information on the readily available food for consumers, particularly during the blooms. The protein/carbohydrate, C/N and POC/Chl-a ratios were used to evaluate the differences between these two species during the growth and the decomposition processes. A comparison between experimental and field conditions was undertaken to implement our understanding of the growth and degradation processes of particulate organic matter of phytoplanktonic origin in the sea and its role on natural systems, during and after phytoplankton blooms.

KEY WORDS: Phytoplankton, biochemical composition, particulate organic matter, growth, decay, chlorophyll, POC, algae.

INTRODUCTION

In marine ecosystems, the organic fraction of particulate matter is mostly composed of detritus (Velimirov, 1991); only during phytoplankton blooms most of the particulate matter is constituted of living cells. After the blooms, large amounts of dead algae, not grazed by herbivores, become available for microbial degradation. Such phytoplankton detritus is likely to play an important role for pelagic-benthic coupling (Newell, 1983; Posedel and Faganelli, 1991).

Several studies have considered the variation of some biochemical components of the phytoplankton during bloom and decay in natural environments and in micro- or mesocosm experiments (Gabrielson and Hamel, 1985; Fukami *et al.*, 1985a,b; Hansen *et al.*, 1986; Cassiani *et al.*, 1989; Aizaki and Takamura, 1991), in relation to different environmental conditions (Moal *et al.*, 1987; Martin-Jezequel *et al.*, 1988), and to inorganic nutrient loading, uptake and metabolism (Admiraal *et al.*, 1986; Flynn and Butler, 1986). However, few papers have considered the composition of phytoplankton in terms of lipids, proteins and carbohydrates (Moal *et al.*, 1987; Cassiani *et al.*, 1989).

In this paper, we analyzed the variations of the biochemical composition of the living cells of *Tetraselmis suecica* and *Isochrysis galbana* and of the algal detritus originating from the cells after the death. The role and changes in the nutritional value of the living cells and of the phytodetritus are discussed.

MATERIALS AND METHODS

Tetraselmis suecica and *Isochrysis galbana* were grown at a temperature of 25 ± 2 °C in 2 litres of filtered (Whatman GF/F, 0.45 μm nominal pore size) and sterile sea water enriched with the Walne medium (Villani, 1989). The cultures were aerated with prefiltered air (Gelman glassfibre filter, 10 μm pore size) and illuminated (280 ± 20 μ moles of photons $m^{-1} s^{-1}$) with a dark : light period of 14 : 10. The number of cells in the medium was estimated, daily, by counting the cells in 2 ml samples with a reverse microscope from the day following inoculation to the end of the logarithmic growth phase (8 days from inoculation). The cells were then killed with a 48 hours treatment with lethal UV irradiation. After UV irradiation, the cultures were kept in the same medium used for growth and continuously stirred. Subsamples (2 ml) were collected once a day after 2, 3, 5, 7, 9, 12 and 21 days after UV irradiation for *Tetraselmis*, and after 2, 4, 7, 14 and 32 days for *Isochrysis*.

Three ml samples were collected from the cultures and filtered on Nuclepore filters (0.4 μm pore size) for the lipid, carbohydrate and protein analyses. The filters were sonicated for two hours in 1 ml of distilled water. Carbohydrates (CHO) were evaluated according to Dubois *et al.* (1956) using D(+)glucose as a standard. Protein (PRT) determination was carried out according to Hartree (1972), using bovine serum albumin (BSA) as a standard. Lipids (LIP) were extracted by the method of Bligh and Dyer (1959) and measured according to Marsh and Wenstein (1966). Tripalmitine was used as a standard.

Carbon equivalents of carbohydrates, proteins and lipids were calculated using the conversion factors of 0.40, 0.49 and 0.75 g C g^{-1}, respectively, according to the standard utilized. The sum of carbohydrate, protein and lipid carbon (CPOM) was used to estimate the labile fraction of the organic matter (Fichez, 1991). The caloric content of particulate matter was calculated using the equation:

$$\text{Kcal g}^{-1} = 0.041\%\,\text{CHO} + 0.055\%\,\text{PRT} + 0.095\%\,\text{LIP}$$

where CHO, PRT, LIP are respectively the percentage content of carbohydrate, protein and lipid (Winberg, 1971).

For the determination of chlorophyll *a* (Chl-a), phaeopigments (Phae), particulate organic carbon (POC) and nitrogen (PON), 2 ml culture samples were filtered through precombusted (450°, 2h) GF/F Whatman filters (0.45 μm nominal pore size) and stored at -20 °C until analysis. Photosynthetic pigments (Chl-a and Phae) were extracted in 90% acetone. Their concentrations were calculated according to Lorenzen and Jeffrey (1980). POC and PON were analyzed by a Carlo Erba CHN-Analyzer (CHNS-O EA1108 Elemental Analyzer). In this case, filters were treated with hydrochloric acid (5 h) to remove carbonates (Hedges and Stern, 1983).

RESULTS

The results for both species were divided into two groups corresponding to: 1) growth phase (living cells) and 2) decomposition phase (particulate detritus composed of dead algae during the decomposition processes).

Experiments on Tetraselmis suecica

Growth Phase

Chlorophyll *a* concentrations increased from 0.5 to 3.6 μg ml^{-1} between day 1 and 8, while phaeopigments increased from an initial value of 0.28 μg ml^{-1} to 1.36 μg ml^{-1} at day 8 (Fig. 1a). In contrast, chlorophyll *a* per cell decreased with time from an initial

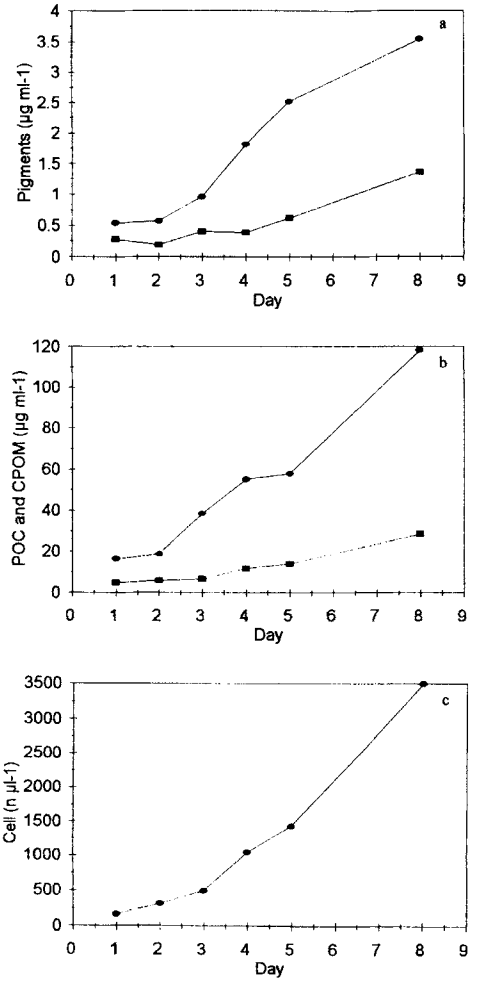

Figure 1 The growth phase of *Tetraselmis suecica*: a) Chlorophyll *a* (●) and Phaeopigments (■); b) particulate organic carbon (POC, ●) and particulate organic matter expressed in carbon equivalents (CPOM; ■); c) number of cells.

value of 3.3 pg cell^{-1} to a value at day 8 of 1.0 pg cell^{-1}. Phaeopigment content of the cells decreased (from 1.7 to 0.4 pg cell^{-1}). CPOM and POC (Fig. 1b) increased with cell concentration (Fig. 1c), reaching their maximum values, 118.5 μg ml^{-1} POC and 28.6 μg C ml^{-1} CPOM on day 8, corresponding to 3.5 10^3 cell μl^{-1}. Except on day 1 the amount of carbohydrates per cell (Fig. 2a) decreased until day 4 (2.1 pgC cell^{-1}), then increased to 3.3 pgC cell^{-1} at day 8. On the other hand, protein content per cell (Fig. 2b) decreased from day 1 (10.3 pgC cell^{-1}) to day 8 (2.9 pgC cell^{-1}). The relative significance of carbohydrates, proteins and lipids percentage values as CPOM during the growth phase, is shown in Figure 2c. The percentage of particulate carbohydrates (as C equivalents) reached values as high as 40% at the end of the log phase, while

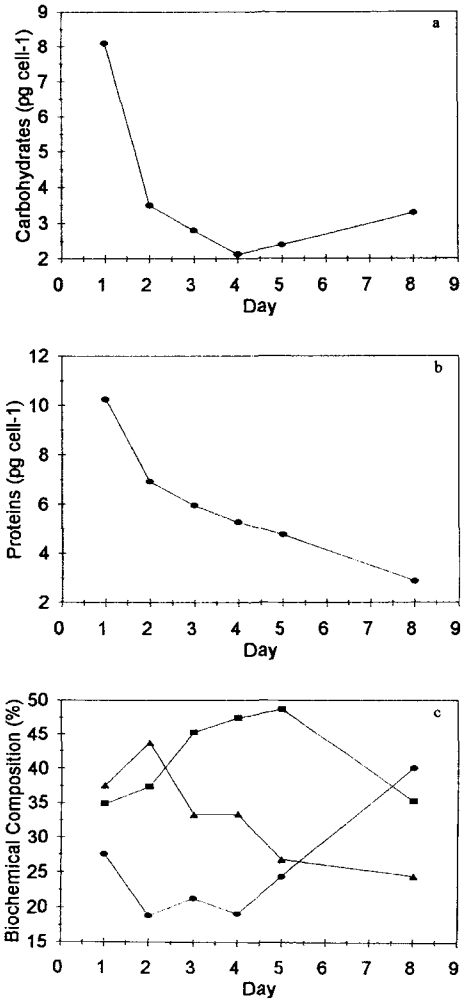

Figure 2 The growth phase of *Tetraselmis suecica*; Carbohydrate (a) and protein (b) content of cells and variation in the biochemical composition of particulate matter (c), expressed as percentage of carbohydrates (●), proteins (■) and lipids (Δ).

proteins increased from day 1 (35%) to day 5 (49%) and strongly decreased to 35% at day 8. The percentage of lipidis, except day 1, decreased until day 8 (25%).

Decomposition Phase

Chlorophyll *a* concentrations in the algal detritus of *Tetraselmis suecica*, continuously decreased until day 40(0.9 μg ml^{-1}) whilst the phaeopigments fluctuated significantly (Fig. 3a). The decomposition rates of the biochemical constituents of the particulate matter were calculated as the constant of decomposition (K, see Taguchi *et al.*, 1993) following the equation

$$P_t = P_0 \, e^{-kt}$$

where P_t is the concentration (μg ml^{-1}) at the end of the experiment, P_0 is the concentration (μg ml^{-1}) at the beginning of the decomposition phase and t is time in days. The decomposition rates are reported in Table I.

The relative significances of carbohydrates, proteins and lipids during the decomposition of phytodetritus of *Tetraselmis suecica*, are shown in Figure 3b.

The percentage of carbohydrates decreased in a few days (from 40% at day 8 to 17% at day 17), being relatively constant in the later decomposition phase (15–28 days). In

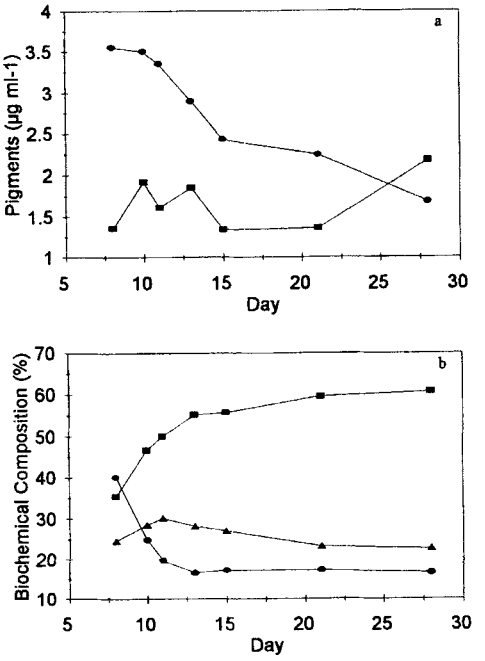

Figure 3 The decomposition phase of *Tetraselmis suecica*; a) decay of Chlorophyll *a* (●) and Phaeopigments ■); b) variation in the biochemical composition of particulate matter, expressed as percentage of carbohydrates (●), proteins (■) and lipids (Δ).

Table I Decomposition rates, expressed as K (see the text for explanation), of the major biochemical components of *Tetraselmis suecica*. The value indicated as "tot" was calculated using the first (day 8) and last (day 28) data. When negative, the K is represented with*.

Day	*Chl-a*	*CCHO*	*CPRT*	*CLIP*
10	0.007	0.288	*	*
11	0.044	0.411	0.109	0.124
13	0.072	0.087	*	0.037
15	0.088	0.003	0.016	0.040
21	0.014	0.045	0.034	0.071
28	0.041	0.061	0.053	0.059
avg	0.044	0.149	0.053	0.066
std	0.029	0.148	0.035	0.031
min	0.007	0.003	0.016	0.037
max	0.088	0.411	0.109	0.124
tot	0.037	0.093	0.022	0.053

contrast, the percentage of proteins increased from day 8 (35%) to day 28 (60%). Lipids percentage increased till day 11(30%) then slightly decreased until day 28 (22%).

Experiments on Isochrysis galbana

Growth Phase

Chlorophyll *a* (Fig. 4a) increased from day 1(0.03 μg ml^{-1}) to day 8(1.96 μg ml^{-1}). The phaeopigments slightly decreased till day 5 (from 0.45 to 0.24 μg ml^{-1}), then increased to 0.76 μg ml^{-1} at day 8. The chlorophyll *a* content of cells increased, until day 5, from 0.05 to 0.2 pg $cell^{-1}$, whilst phaeopigment consistently decreased from 0.91 pg $cell^{-1}$ (day 1) to 0.03 pg $cell^{-1}$ (day 8). CPOM and POC (Fig. 4b) varied in parallel with cell concentration (Fig. 4c) until day 8. At day 10, the number of cells decreased to 20056 cell μl^{-1}, whereas CPOM and POC reached their maximum values: 31.6 μgC ml^{-1} and 109.3 μgC ml^{-1} respectively. Protein content of the cells (Fig. 5a) continuously decreased from 8.8 pgC $cell^{-1}$ at day 1 to 0.4 pgC $cell^{-1}$ at day 8, whereas, carbohydrate (Fig. 5b) showed a strong peak at day 3 (2.4 pgC $cell^{-1}$). Carbohydrates, as a percentage of CPOM (Fig. 5c), increased until day 4 (33%), then remained quite constant. By contrast, the percentages of proteins and lipids slightly decreased from day 1 to day 8.

Decomposition Phase

The chlorophyll *a* and the phaeopigments decreased from day 10 to day 40, respectively from 1.06 to 0.47 μg ml^{-1} and from 1.03 to 0.70 μg ml^{-1} (Fig. 6a). The decomposition constant (K) of the biochemical constituents of the phytodetritus deriving from *Isochrysis galbana* are reported in Table II. As shown in Figure 6b, the carbohydrate and protein percentages as CPOM showed opposite trends, whilst lipids accounted for a smaller fraction of CPOM.

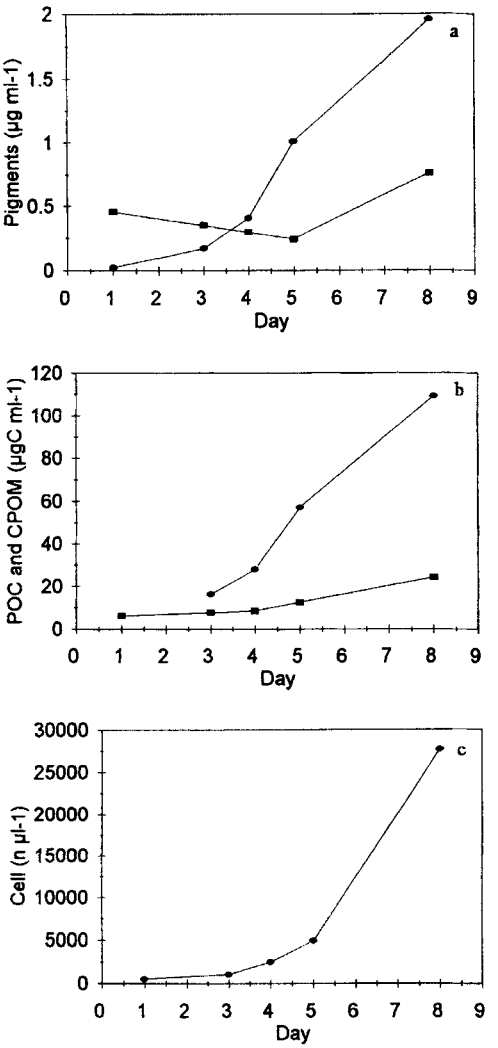

Figure 4 The growth phase of *Isochrysis galbana*: a) Chlorphyll *a* (●) and Phaeopigments (■); b) particulate organic carbon (POC, ●) and particulate organic matter expressed in carbon equivalents (CPOM; ■); c) number of cells.

DISCUSSION

The Biochemical Composition of Phytoplankton during Growth

The results from the growth experiments of the algae are consistent with those generally observed during blooms in natural environments. The parameters usually utilized to measure the phytoplankton biomass, i.e. chlorophyll *a*, number of cells, particulate organic carbon showed similar increasing patterns, reaching the highest concentrations all at the same time (day 8). However, the chlorophyll *a* content of cells decreased

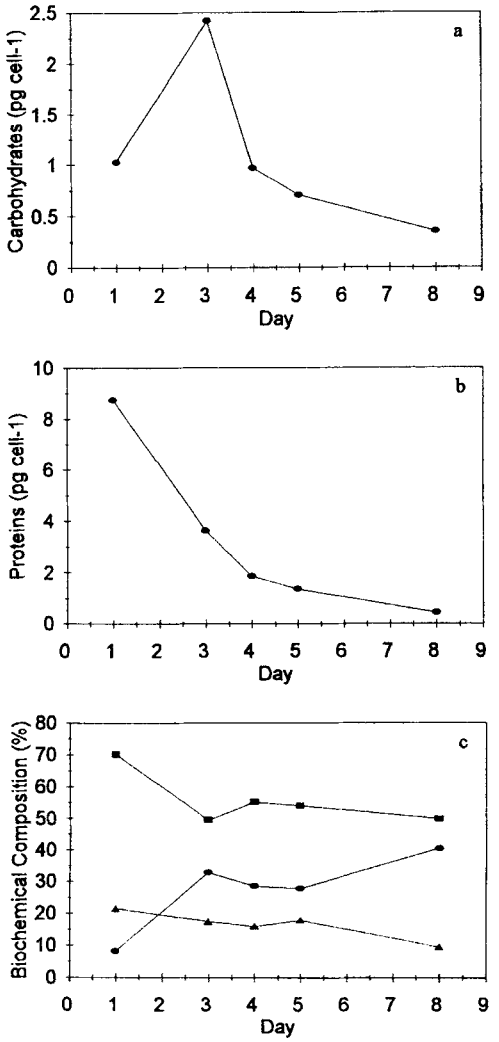

Figure 5 The growth phase of *Isochrysis galbana*; Carbohydrate (a) and protein (b) content of cells and variation in the biochemical composition of particulate matter (c), expressed as percentage of carbohydrates (●), proteins (■) and lipids (Δ).

till the end of the logarithmic growth phase. This result confirms that chlorophyll *a* may not always provide an accurate estimate of phytoplankton biomass. Moreover, since the chlorophyll is often used to estimate the food available for suspension-feeders, we observe that this measure, drastically underestimating the actual phytoplankton biomass, may fail if used as a tool to measure the availability of food for consumers.

Although the amounts of particulate carbon increased with the cell number, several differences occurred in the biochemical composition of the algae. *Isochrysis galbana* was characterized by a protein content higher than *Tetraselmis suecica* (on average

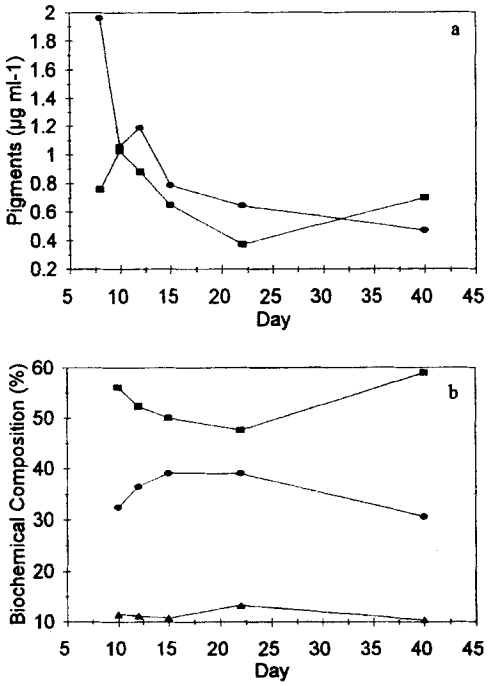

Figure 6 The decomposition phase of *Isochrysis galbana*; a) decay of Chlorophyll *a* (●) and Phaeopigments (■); b) variation in the biochemical composition of particulate matter, expressed as percentage of carbohydrates (●), proteins (■) and lipids (Δ).

Table II Decomposition rates, expressed as K (see the text for explanation), of the major biochemical components of *Isochrysis galbana*. The value indicated as "tot" was calculated using the first (day 8) and last (day 28) data. When negative, the K is represented with*.

Day	*Chl-a*	*Phe*	*CCHO*	*CPRT*	*CLIP*
10	0.308	*	*	*	*
12	*	0.076	0.022	0.115	0.094
15	0.205	0.151	0.013	0.050	0.047
22	0.101	0.281	0.002	0.009	*
40	0.018	*	0.060	0.034	0.060
avg	0.158	0.169	0.024	0.052	0.067
std	0.109	0.084	0.022	0.039	0.020
min	0.018	0.076	0.002	0.009	0.047
max	0.308	0.281	0.060	0.115	0.094
tot	0.027	0.013	0.039	0.036	0.041

54% and 42% respectively), that, by contrast, was richer in the carbohydrate fraction. This is attributable to the structural differences between the two algae: *Isochrysis galbana* is a nude cell whilst *Tetraselmis suecica* is a scale-armed cell.

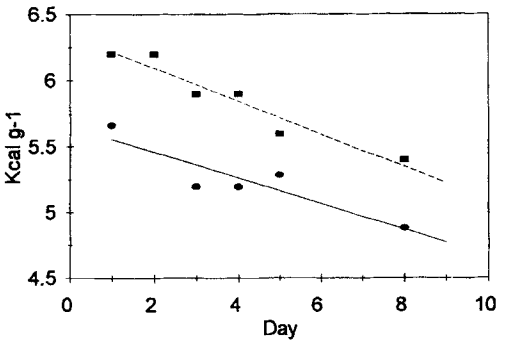

Figure 7 The decreasing pattern of caloric content of particulate matter during the growth phase ■ and dashed line are data from *Tetraselmis suecica*; ● and continuous line are data from *Isochrysis galbana*.

We observed that the contribution of the labile fraction to the bulk of particulate carbon, expressed as CPOM/POC ratio, significantly decreased during the growth phase of *Isochrysis galbana* from 47% (day 1) to 22% (day 8); in *Tetraselmis suecica* the ratio fluctuated but at day 1 was 29% and decreased to 24% at day 8. This decreasing pattern is due to the carbohydrate accumulation in the algae. In fact, particulate carbohydrates, being the more important product of photosynthesis, increased during the growth phase, whilst the percentage of proteins decreased, possibly as a result of the nitrogen depletion during the later growth phase.

A decreasing pattern of the energy content of phytoplankton was observed (Fig. 7) and related to the decrease of the protein content of the cells. Proteins are generally considered a limiting factor for the consumers (Buchsbaum *et al.*, 1991; Fichez, 1991; Tenore, 1983; Tenore *et al.*, 1984) and account for a significant amount to the caloric value of the cells. Therefore, even though the biochemical composition of the phytoplankton communities, at the beginning of the bloom, are different because of their species or taxa composition, similar processes could apply to the natural environments: 1) at the beginning of the bloom, the particulate organic matter, mostly composed of living phytoplankton, is characterized by relatively low quantities but of high quality (i.e. high protein content, high values of the CPOM/POC ratio and high energy content); 2) at the end of the growth phase, when highest number of cells occurs, the particulate matter, composed of senescent cells, is characterized by higher concentrations of organic matter of lower quality and nutritive value.

Table III Averaged decomposition rates, expressed as K, of *Tetraselmis suecica* and *Isochrysis galbana* (rates given as per day).

	CCHO	*CLIP*	*CPRT*	*Chl-a*
Tetraselmis suecica	0.149	0.066	0.053	0.044
Isochrysis galbana	0.024	0.067	0.052	0.158

Table IV Values of the protein/carbohydrate (PRT/CHO), Carbon/Nitrogen (C/N) and Carbon/Chlorophyll a (POC/ Chl-a) ratios during the growth phase (Ig and IIg) and the decomposition phase (Id and IId) of *Tetraselmis suecica* and *Isochrysis garbana.*

Tetraselmis suecica

Phase (Days)	*PRT/CHO*	*C/N*	*POC/Chl-a*
Ig(1–5)	1.8 ± 0.7	6.7 ± 1.2	31.3 ± 5.5
IIg(8)	0.9 ± n.d.	8.9 ± n.d.	30.8 ± n.d.
Id(10)	1.9 ± n.d.	7.7 ± n.d.	30.8 ± n.d.
IId(11–28)	3.4 ± 0.4	7.1 ± 0.2	32.4 ± 1.8

Isochrysis galbana

Phase (Days)	*PRT/CHO*	*C/N*	*POC/Chl-a*
Ig(1–5)	2.2 ± 0.1	4.2 ± 1.0	73.9 ± 16.3
IIg(8–12)	1.3 ± 0.2	5.3 ± 0.1	87.9 ± 22.8
Id(15–40)	1.0 ± 0.1	5.2 ± 0.1	188.8 ± 31.1

The Biochemical Composition of the Phytodetritus during the Decomposition Processes

The decomposition constants (K) of the major biochemical components of the phytodetritus are reported in Table III. In the experiment with *Tetraselmis suecica*, the carbohydrates showed on average a faster loss rate ($K = 0.149$), followed by lipids ($K = 0.066$), proteins ($K = 0.053$) and chlorophyll *a* ($K = 0.044$). In the experiment with *Isochrysis galbana*, while proteins ($K = 0.052$) and lipids ($K = 0.067$) showed loss rates similar to those reported for *Tetraselmis*, the chlorophyll *a* showed a faster loss rate ($K = 0.158$) and carbohydrates the slowest ($K = 0.024$). The differences between the loss rates of both algae were reflected also by the changes in the biochemical composition of the phytodetritus.

Several variations of the protein/carbohydrate (PRT/CHO), Carbon/Nitrogen (C/N) and Carbon/Chlorophyll *a* ratios (Table IV) occurred from the early stage of the bloom until the later decomposition phase. In our experiments, the PRT/CHO ratio decreased in both species during the growth phase, while, during the decomposition phase, the trends were different: in *Tetraselmis* experiments it continuously increased from 1.8 (Ig) to 3.4 (IIdays), whereas, in *Isochrysis* it drastically decreased from 2.2 (Ig) to 1.0 (Id) days. During the short-term decomposition phase of phytoplankton most of the leachate is composed of dissolved carbohydrates (Rice and Tenore, 1981; Krog *et al.*, 1986) and the PRT/CHO ratio has been reported to increase. In this sense the PRT/CHO ratio in our experiments presents an expected trend only in *Tetraselmis*, in which the scale-armed cellular wall is mostly composed of carbohydrates that rapidly entered the dissolved pool. This was also confirmed by the high loss rate of the carbohydrates ($K = 0.149$). Conversely, in *Isochrysis* the PRT/CHO ratio decreased during the decomposition phase: this was probably due to the low size of the nude cell that allowed a faster loss of labile proteins than for carbohydrates. During the

phytoplankton blooms the PRT/CHO ratio has been reported to be lower than 1 (Nival *et al.*, 1976; Daumas, 1974), whilst, during grazing activities or during the decay of blooms, it reaches values > 2 (Roy *et al.*, 1991; Fabiano and Povero, 1992). Fabiano *et al.*, (1991) using the PRT/CHO ratio as a measure of the "age" of particulate organic matter demonstrated that this ratio decreases with depth (on average from 1.6 in the photic layers to 0.6 in the aphotic layers).

The C/N ratio has been used extensively as a measure of the detrital to living fraction of particulate organic matter in natural ecosystems (Bodungen *et al.*, 1986; Copin-Montegut and Copin-Montegut, 1978; Nelson and Smith, 1986; Nelson *et al.*, 1987; Treguer *et al.*, 1988; Fabiano *et al.*, 1991), and has been reported to decrease during the decomposition phase of cultured *Chlorella* (Fukami *et al.*, 1981). In natural environments the ratio has been reported to decrease with depth (Posedel and Faganeli, 1991) and to be negatively correlated with the PRT/CHO ratio (Fabiano *et al.*, 1991) as a result of the preferential loss of nitrogen during particulate sedimentation (Bodungen *et al.*, 1986; Muller *et al.*, 1986). In *Tetraselmis* we observed a similar trend of the C/N ratio, which was higher during the bloom, ranging from 6.7 (Ig) to 8.9 (IIg) days and decreased, during the decomposition processes, to 7.7 (Id) and 7.1 (IId) days in *Isochrysis*, as a result of the faster loss rate of proteins compared to carbohydrates, the C/N ratio increased from 4.2 (Ig) to 5.2 (Id) days. This suggests that, during decomposition in absence of grazers, the phytodetritus deriving from low-size phytoplankton cells (like *Isochrysis*) loses its labile nitrogen pool (i.e. proteins) more rapidly than high-size phytoplankton cells (like *Tetraselmis*) and consequently may furnish a lower energy to consumers.

The POC/Chl-a of a ratio has been used extensively in natural environments to detect the detrital cf. algal fraction of the particulate organic matter. In *Tetraselmis*, the ratio was quite constant (31 ± 3.8 μg cell^{-1}) and comparable to the POC/Chl-a ratio of natural occurring phytoplankton blooms. This provides a further confirmation of the lower degradability of this algae. Data on various phytoplankton communities indicate that POC/Chl-a ratio higher than 50 is typical of nutrient, light or self-shading stressed environments (Smith and Nelson, 1985; Smith and Sakshaug, 1990) and of the presence of phytodetritus (Treguer *et al.*, 1990). In *Isochrysis* the ratio increased significantly from 73.9 (Ig) to 87.9 (IIg) days and to 188.8 (Id) days, demonstrating its higher degradability.

Although inferences from our experiments to the field must be considered with caution, the results reported in this paper suggest that, despite some differences encountered in the biochemical composition of different species of living phytoplankton, similar processes and chemical changes occur during the development of the bloom. By contrast, during decay, the changes in the biochemical composition of phytodetritus are related to the size, composition and structural differences between the different algae.

Acknowledgements

This work was financied by the Ministero Università Ricerca Scientifica Technologica of the Italian Government (60 and 40%). Dr. A. Pusceddu was financially supported by a grant from Credito Industriale Sardo (Italy). We are grateful to Prof. M. Cottiglia and M. L. Masala (University of Cagliari) for laboratory facilities, to Dr. C. Misic (Univer-

sity of Genoa) for POC, PON and lipids analyses and to Dr. R. Dolfi for sampling. Full thanks are due also to Dr. M. Giordano (University of Ancona) for the criticism and the important suggestions for improvement of the manuscript.

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