Phytoplankton
Chemical composition
Protein
Carbohydrate
Starch

Phytoplancton
Composition chimique
Protéines
Glucides
Amidon

Interspecific and intraspecific variability of the chemical composition of marine phytoplankton

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ABSTRACT

The following cellular components, carbon, nitrogen, chlorophyll *a*, protein, carbohydrate and starch, were measured in batch culture for 11 phytoplankton species, including members of the Cryptophyceae, a group which has received little previous attention. For equal external cell volume, large differences between species were observed in the concentrations of cellular compounds. Diatoms had the lowest levels per unit cell volume whereas Cryptophyceae had the largest (range 1 to 10). Protein was the major component (40-70% of carbon). Carbohydrates showed large variations, starch being detected in Cryptophyceae, Chlorophyceae, and Haptophyceae, but not in Dinophyceae. The effects of growth phase on changes in chemical content were also analysed. The most important finding was a drop in cellular content as growth slowed. Intraspecific and interspecific differences in chemical composition may have important implications for the value of phytoplankton as food for marine herbivores.

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RÉSUMÉ

Variabilité interspécifique et intraspécifique de la composition chimique du phytoplancton marin

Onze souches phytoplanctoniques, dont les cryptophycées pour lesquelles peu de données existent, ont été cultivées en culture confinée et la composition en carbone, azote, chlorophylle *a*, protéine, glucide et amidon, analysée au cours de deux phases de la croissance. On observe pour tous ces composés de très grandes différences de concentrations cellulaires entre les espèces. Les diatomées présentent les plus faibles niveaux par unité de volume cellulaire et les cryptophycées les plus forts. Les protéines représentent le composé majeur (40 à 70% du carbone). Les glucides sont plus variables, et on trouve de l'amidon chez les cryptophycées, les chlorophycées et les prymnesiophycées, mais on n'en a pas détecté chez les dinophycées. L'influence du stade de croissance de la culture sur la composition est très marquée, et se caractérise par une baisse de concentration de tous les composés lorsque la croissance ralentit. Ces différences intra et interspécifiques jouent un rôle important sur la valeur nutritionnelle des algues pour les consommateurs herbivores.

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INTRODUCTION

An extensive literature exists on the chemical composition of phytoplankton species. Most studies have been concerned with the food value of algae for aquaculture purposes and with the physiology of natural and cultivated phytoplankton species in relation to environmental factors such as temperature, light and nutrients (Darley, 1977; Morris, 1981). Some studies have concentrated on specific chemical components for example: amino acids, fatty acids, sterols, lipids, carbohydrates, starch, pigments and ATP. The influence of

nutrients on chemical composition has also been widely studied: Dortch (1982), Dortch *et al.* (1984) for the nitrogen pool; Myklestad and Haug (1972), Myklestad (1974), Moal *et al.* (1978) for carbohydrates; Harrison *et al.* (1976) and Falkowski (1980) for pigments; Caperon and Meyer (1972) for carbon and chlorophyll content. Verity (1981), Redalje and Laws (1983), Post *et al.* (1984) and Sakshaug and Andresen (1986) have investigated the effect of light and temperature on the biochemical composition of marine diatoms (especially carbon and chlorophyll). Other papers have considered the extracellular metabolites released by phytoplanktonic cells (Vogel *et al.*, 1978; Fogg, 1983; Poulet, Martin-Jezequel, 1983). Most of these studies have been concerned with a small number of species, especially diatoms and dinoflagellates, which form the major component of marine phytoplankton in terms of biomass. Since the works of Parsons *et al.* (1961) and Ricketts (1966), few comparative studies of other classes have been published.

This study is linked to related work on trophic interactions between zooplankton and phytoplankton (Harris *et al.*, 1986; Poulet *et al.*, 1986) and was undertaken to select two phytoplanktonic species among eleven algae, as food for copepods on the basis of their chemical composition: similar carbohydrate/protein ratio but with differing carbohydrate composition. We cultivated eleven different species of phytoplankton in batch culture and screened two characteristic periods of phytoplankton growth with theoretically large chemical differences to choose the best pair of species. Although the sampling strategy was not adapted to obtain exhaustive information on the relations between nutritional status and chemical composition, we were able to report a first set of data on interspecific and intraspecific differences in chemical composition, for which there is a need in the literature. These data also provide information on the chemical composition of some little-studied species, especially for the smaller groups such as the Cryptophyceae. These small cells of the nanoplankton and picoplankton may be widely distributed in some areas and are thought to account for a large proportion of the total phytoplankton production (Joint *et al.*, 1983; Gieskes, Kray, 1986).

In this paper, we present data on the chemical composition (carbon, nitrogen, protein, carbohydrate, starch and chlorophyll *a*) of eleven phytoplankton species belonging to the following classes: Bacillariophyceae, Dinophyceae, Chlorophyceae, Cryptophyceae, Haptophyceae (Prymnesiophyceae). Detailed information on the amino acid composition of these species will be presented separately.

MATERIAL AND METHODS

Eleven phytoplankton strains from the Marine Biological Association, Plymouth, culture collection, representing most major groups, were cultured at 15°C in 3 litres Erlenmeyers of "F/2" medium (Guillard, Ryther, 1962; Tab. 1). Cells were grown under continuous light illumination (cool-white type) at an average light intensity of 100 $\mu E.m^{-2}.s^{-1}$. Samples of these cultures were withdrawn daily and cell counts were made, after fixation in Lugol solution, with Nageotte and Mallassez count-chambers. To allow for any possible diurnal rhythm in the division time of the cells, the cultures were always sampled at approximately the same time of day (1 400 h).

For chemical analyses, two sampling dates were selected for each of the stock cultures, one during the exponential phase, and the other at the start of, or during, the stationary phase when the growth rate was much reduced (Tab. 2). Known volumes of culture were filtered on to pre-ashed GF/C filters (450°C.5 h), in conjunction with cell counts. The samples were replicated and blanks (culture medium) subtracted from each sample value. Total carbon and nitrogen were measured with a Hewlett Packard CHN analyser. Total protein and carbohydrate were determined according to the methods of Lowry *et al.* (1951) and Dubois *et al.* (1956). To permit maximum extraction and solubilization, the filters were ground with distilled water in a Potter homogenizer and the reagents added. Optical density was measured after centrifugation (Scott, 1980; Moal *et al.*, 1985). Possible interference with other compounds such as lipids or sugars was tested and

Table 1
Mean volume of the species studied.
1: from Coulter counter measurements; 2: from inverted microscope measurements.

Volume moyen des espèces phytoplanctoniques étudiées.
1 : mesure au Coulter counter ; 2 : mesure au microscope inversé.

Class	Family	Species	Volume μm^3
Bacillariophyceae	*Coscinodiscaceae*	*Thalassiosira rotula*	14 250(2)
		Thalassiosira weissflogii	1 130(2)
		Skeletonema costatum	402(2)
		Coscinodiscus wailesii	3 328 525(2)
Cryptophyceae	*Cryptomonadaceae*	*Cryptomonas maculata*	395(1)
	Cryptomonadaceae	*Cryptomonas appendiculata*	359(1)
Dinophyceae	*Gymnodiniaceae*	*Gyrodinium aureolum*	6 709(1)
		Gymnodinium simplex	230(1)
	Peridiniaceae	*Scrippsiella trochoidea*	9 600(2)
Chlorophyceae	*Dunaliellaceae*	*Dunaliella tertiolecta*	230(1)
Haptophyceae Prymnesiophyceae	*Isochrysidaceae*	*Isochrysis galbana*	31(1)

found to be less than 10-15%. Only differences higher than these known errors were considered; we applied this routine methodology to relate our results to published ones. For quantitative determination of starch, the filter was ground in 2 ml sodium, potassium phosphate buffer (0.1 M), pH 6.8, and the starch extracted at 100° over a period of 75 min. 200 µl of reagent (IK-I_2 0.005 N) was added to 1.5 ml of supernatant and optical density read at 660 nm. Chlorophyll was analysed in acetone extracts (90%) by the fluorimetric method of Yentsch and Menzel (1963), using the equations of Lorenzen (1966). Cell volumes were determined by direct measurement of cell dimension, using an inverted microscope for all the diatoms and *Scrippsiella trochoidea*. Other cell volumes were estimated directly with a model TA II Coulter counter, cross-calibrated with the inverted microscope.

Table 2

Sampling dates (days from inoculation) and estimated growth rate, at the two sampling times (division/day).

Dates de prélèvements (en jour à partir de l'inoculation) et taux de croissance estimé (division/jour) pour les 2 prélèvements.

	Sampling date	µ Division/day	Sampling date	µ Division/day
Thalassiosira rotula	8	1.10	14	0.08
Thalassiosira weissflogii	8	0.87	15	0
Skeletonema costatum	8	0.69	14	0.04
Coscinodiscus wailesii	8	0.36	14	0.04
Cryptomonas maculata	10	1.02	21	0.04
Cryptomonas appendiculata	12	0.81	21	0.10
Gyrodinium aureolum	13	0.31	26	0.29
Gymnodinium simplex	13	0.43	22	0
Scrippsiella trochoidea	13	0.58	26	0
Dunaliella tertiolecta	10	0.75	14	0.05
Isochrysis galbana	10	0.73	15	0.04

RESULTS

Composition and cell volume

The quantities of the different components are significantly ($P<0.05$) related to the cell volume, by the equation $LnC = ALnV + B$ where C is the chemical composition as pg.cell^{-1} and V the cell volume in µm^3. The correlations have been established for the whole data: all the species and the two growth periods (Tab. 1 and 2). The coefficients of this equation for each parameter are given in Table 3. As the coefficient A is always less than unity, it follows that the concentration (C/V) will decrease as the cell volume increases. Then the small cells exhibit a more concentrated chemical composition compared to larger cells.

Table 3

Relationship between chemical composition and cell volume. Intercept (B) and slope (A) of linear regressions represented by the equation Ln pg/cell = A Ln v (µm³) + B.

Relation entre la composition chimique et le volume cellulaire. Paramètres de la régression linéaire. Ln pg/cellule = A Ln v (µm³) + B.

	Carbon	Nitrogen	Protein	Carbohydrate	Chlorophyll
A	0.829	0.838	0.802	0.847	0.722
B	—0.927	—2.73	—0.715	—1.93	—4.72
r	0.942	0.949	0.926	0.888	0.882
N	22	22	22	22	22

Chemical composition and species

The preceding correlations emphasize the importance of cell size in relation to the quantity of the cellular components per cell. Consequently, in order to compare species of different sizes, we normalized the cellular contents to cell volume (fg/µm^3). Table 4 gives the cellular concentrations of carbon, nitrogen, protein, carbohydrate, starch and chlorophyll *a* for the eleven species. Large differences of up to one order of magnitude were observed between species. Diatoms had the lowest concentrations of all the components, with the exception of *T. weissflogii* (0.2 pg.C/µm^3) which differed from other diatoms (0.017 to 0.064 pgC/µm^3) in having higher levels of cellular components. Among the eleven species, the Cryptophyceae were the most concentrated cells, dinoflagellates and Chlorophyceae occupying an intermediate position. The most abundant cellular constituent was generally protein, ranging from 0.015 pg/µm^3 for *S. costatum*, 0.43 pg/µm^3 for *G. simplex* to 0.805 pg/µm^3 for *C. maculata*. Proteins represented 40-70% of total carbon.

The percentage of carbohydrates was highly variable in Cryptophyceae (1 to 70% of carbon). For other groups, the variability of this parameter was less important and had a mean value of 20-30%. The presence of starch was only detected for Cryptophyceae, Chlorophyceae and Haptophyceae. Starch, a reserve carbohydrate, showed large variations in relation to growth. *D. tertiolecta* exhibited 23-43% starch in carbohydrates, *I. galbana* 0-8% and both *C. maculata* and *C. appendiculata* 0-17%.

Carbon/Chl *a* and protein/Chl *a* ratios are reported in table 5 for all species. The range of variations for these ratios is large, 19 to 833 for the C/chlorophyll *a* ratio and 20 to 740 for protein/chlorophyll *a* ratio. As the variations within groups were as large as the variations between groups, a classification of the families related to these ratios was not possible.

Chemical composition and growth stages

For each species, the variation in chemical parameters was followed during two periods of the growth cycle. Table 2 shows that some species have not reached the stationary phase. more particularly, *G. aureolum* did not exhibit a decrease of growth rate, while for *C. appendiculata*, the stationary phase was only approached by lowering the growth rate. From the exponential to the onset of the stationary phase, we

observed a decrease of all the components, exceptions being *T. rotula*, for which the trend was the reverse, and *D. tertiolecta* in which concentrations remained constant (Tab. 4). For all the species, the range of decrease in carbon and nitrogen was parallel (10-60%). Carbohydrate and protein variations, on the other hand, were not parallel. For *T. weissflogii*, protein showed the largest variation, while carbohydrate exhibited the greatest change for *G. simplex*, *S. trochoidea*, *C. maculata* and *C. appendiculata*. The carbohydrate/protein ratio decreased as growth slowed. Except for some species, this ratio was low. The fluctuations of chlorophyll *a* content showed no trend, for example, a threefold decrease for *G. simplex* and a fourfold increase for *S. trochoidea* were observed.

DISCUSSION

Composition and cell volume

All data considered (taxonomic groups and growth stages), there was a good correlation between chemical content and cell size (Tab. 3). Mullin *et al.* (1966) and Strathmann (1967) have already described the relation between cell size and carbon content. More recently, Hitchcock (1982) demonstrated a similar trend for cellular compounds such as proteins, carbohydrates, lipids and chlorophyll. Generally, these correlations have been established for cultures in a well-defined growth stage (logarithmic growth). Also the correlation between size and nitrogenous compounds has been investigated by Dortch *et al.* (1984), for N-sufficient and N-starved cultures.

Our results generally agree with previous studies. The values for the coefficients of the equation (Ln chemical content/cell = A Ln V + B) agree with those from Mullin *et al.* (1966) and Strathmann (1967) for carbon. The slope values for POC, PON and chl *a* were equivalent to those found by Blasco *et al.* (1982), and those for proteins and carbohydrates to the values found by Hitchcock (1982) for diatoms. We did not observe differences in the slope between diatoms and other groups as reported by the latter author, a discrepancy which may be explained by the different methods used for the estimation of cell volume: we used a Coulter counter and Hitchcock an inverted microscope, assuming a cylindrical morphology. Another explanation would be the lower range in the cell size for diatoms in our study.

Because of the coefficient A value in the relation $LnC = ALnV + B$, the cellular concentrations ($fg/\mu m^3$) are not constant but decrease as the cell volume increases. This size-dependent concentration may be characteristic of marine algae; freshwater algae do not appear to exhibit such a relation (Rocha, Duncan, 1985). Among the diatoms *T. weissflogii* exhibited the highest concentration of all the chemical parameters (Tab. 4). Falkowski *et al.* (1985) also found high carbon values (174-240 $fg/\mu m^3$) for this species.

Chemical composition and species

The major feature is the highly variable organic content depending on species. Cryptophyceae exhibited the highest concentrations of all the chemical parameters, while diatoms showed the lowest organic content by volume unit (Tab. 4). The existence of a large vacuole in diatoms probably explains the fact that among all groups studied, these had the lowest cellular content. For this group, the cytoplasm where all metabolites are concentrated is limited to a thin layer (1 to 3 µm width) within the cell periphery for diameter ranging from 8 to 217 µm. The effective volume compared to total volume is thus less than in other groups which do not have large vacuoles. Moreover diatoms have siliceous walls, in contrast with the organic walls of other species, and may be expected to have less organic content per unit cell volume than the other groups.

Protein was the major component of the carbon biomass (40 to 70%). The proportion of protein was lower (40%) for diatoms than for the other groups, particularly the Dinophyceae and Cryptophyceae. The nitrogen and protein values found in this study agree well with those found by Dortch *et al.* (1984) for *D. tertiolecta* and *S. costatum*.

Carbohydrate in general accounted for 20-30% of the carbon with the exception of the Cryptophyceae, which showed large fluctuations of this parameter (1 to 70%). The type of storage carbohydrate of phytoplankton is known to be species-dependent (Percival, McDowell, 1967). The main polysaccharide is a (β_{1-3}) linkage polyglucan *i.e.* laminarin in diatoms and Chrysophyceae (Myklestad, Haug, 1972) but a (α_{1-4}) linkage polyglucan (starch) in Chlorophyceae and Cryptophyceae. We have only detected starch in *C. maculata*, *C. appendiculata*, *L. galbaba* and *D. tertiolecta*. The absence of starch in dinoflagellates is noteworthy, as both Taylor (1980) and Painter (1983) referred to starch as a reserve polysaccharide in these organisms. In fact, this apparent contradiction may merely result from the stage of the cultures leading to a low level of sugar reserves that allow no detection, the quantity being below the detection threshold of the colorimetric method. The presence of starch is unusual in *L. galbana*, as the major soluble polysaccharide is assumed to be chrysolaminarin in Haptophyceae (Taylor, 1980; Painter, 1983). The proportion of starch in the total carbohydrates was variable. For *C. maculata*, the percentage was relatively constant (17 and 13%), whereas for *C. appendiculata*, starch was only detected on the second sampling date (8.6%). *D. tertiolecta* contained the largest quantity of starch (22.8-33.7%). In this context it is interesting to note that recent studies from the field (Joint, Pipe, 1984) have reported members of the picoplankton measuring less than 1 µm as containing starch-like granules.

Large variations in the carbon/chl.*a* and protein/chl.*a* ratios occurred between the different groups (Tab. 5), dinoflagellates exhibiting the highest values. As emphasized by Chan (1980), "since the chloroplasts are located towards the periphery of the cell, it is likely that the amount of chloroplast material would be propor-

Table 4

Concentration of carbon, nitrogen, protein, carbohydrate, starch, chlorophyll a expressed by fg/μm³ (10^{-15} g/μm³) for the eleven phytoplanktonic algae studied.
For each species, upper value for active growth period, lower value for reduced growth (see *Tab.* 2).
Concentrations du carbone, de l'azote, des protéines, des glucides, de l'amidon et de la chlorophylle *a* exprimées en fg/μm³ (10^{-15} g/μm³) pour les 11 espèces phytoplanctoniques étudiées.
Pour chaque espèce : valeur haute : croissance active; valeur basse : croissance ralentie.

Class	Species	Carbon	Nitrogen	Protein	Carbohydrate	Starch	Chlorophyll *a*
Bacillariophyceae	*Thalassiosira*	22	6	28	4	undetectable	0.3
	rotula	36	12	42	7	»	0.5
	Thalassiosira	182	26	141	174	»	4.8
	weissflogii	146	13	73	196	»	2.9
	Skeletonema	28	5	24	15	»	0.2
	costatum	18	3	15	7	»	0.2
	Coscinodiscus	64	10	47	50	»	0.3
	wailesii	44	8	35	30	»	0.4
Cryptophyceae	*Cryptomonas*	689	99	805	972	164	7.1
	maculata	232	46	347	131	17	4.1
	Cryptomonas	457	110	707	5	undetectable	2.7
	appendiculata	300	50	337	186	16	2.9
Dinophyceae	*Gyrodinium*	214	35	268	45	undetectable	0.7
	aureolum	86	13	80	37	»	0.7
	Gymnodinium	237	45	434	161	»	1.9
	simplex	143	29	191	59	»	0.7
	Scrippsiella	49	7	43	27	»	0.1
	trochoidea	34	6	28	8	»	0.2
Chlorophyceae	*Dunaliella*	136	25	143	69	23	5.0
	tertiolecta	144	25	152	61	14	7.5
Haptophyceae	*Isochrysis*	300	44	258	161	undetectable	4.2
prymnesiophyceae	*galbena*	265	40	225	96	8	5.2

Table 5

Specific value of the ratios: C/N, C/chl. a and protein/chl a and carbohydrate/protein. Upper value: active growth period; Lower value: reduced growth (see *Tab.* 2).
Valeur spécifique des rapports : C/N, C/chl. *a*, protéine/chl. *a* et glucide/protéine. Valeur haute : croissance active; Valeur basse : croissance ralentie.

Class	Species	C/N	C/Chl.	Prot./Chl.	Ch/Prot.
Bacillariophyceae	*Thalassiosira*	3.5	78	102	0.16
	rotula	2.9	68	79	0.16
	Thalassiosira	6.9	38	30	1.23
	weissflogii	10.8	50	25	2.67
	Skeletonema	5.2	133	117	0.61
	costatum	5.4	103	88	0.40
	Coscinodiscus	6.2	232	172	1.08
	wailesii	5.7	108	86	0.85
Cryptophyceae	*Cryptomonas*	6.9	96	113	1.20
	maculata	5.1	55	83	0.38
	Cryptomonas	4.1	167	258	0.01
	appendiculata	5.9	103	115	0.55
Dinophyceae	*Gyrodinium*	6.0	312	385	0.17
	aureolum	6.4	131	123	0.46
	Gymnodinium	5.3	122	222	0.37
	simplex	4.8	200	267	0.30
	Scrippsiella	6.6	833	740	0.63
	trochoidea	5.6	139	114	0.30
Chlorophyceae	*Dunaliella*	5.4	27	29	0.49
	tertiolecta	5.7	19	20	0.40
Haptophyceae	*Isochrysis*	6.8	72	61	0.61
	galbana	6.5	45	39	0.40

tional to the cell surface area"; in contrast, the other chemical components would be proportional to the cell volume, except for cells with large vacuole for which the concentration also would be proportional to cell surface. Diatoms with a low ratio of plasma volume to total volume would consequently have lower carbon/chl. *a* and protein/chl. *a* ratios than dinoflagellates and other species without vacuoles. In respect of these ratios, our results show differences between dinoflagellates and diatoms; but surprisingly diatoms do not differ from other species without a large vacuole. The high proportion of chlorophyll *a* per unit biomass in the Chlorophyceae, Cryptophyceae and Haptophyceae compared to dinoflagellates cannot be related to

a larger number of chloroplasts in these classes, which are known to possess only one or two chloroplasts compared to a variable number in dinoflagellates (Sournia, 1981), nor to a size-dependency of the C/chl. *a* (Blasco *et al.*, 1982; Chan, 1978). If the protein/chl. *a* ratio is closely related to the cell division rate (Chan, 1978), the importance of the productivity of these species with low prot/chl. *a* would be emphasized in the field.

Chemical composition and physiology

The most characteristic feature of our data is the decrease of all cellular components as growth limitation occurred (Tab. 4). However *T. rotula* exhibited an increase of the parameters studied and the amounts in *D. tertiolecta* remained stable. We have checked the cell volumes for the two phases of sampling, no changes in size being observed. It follows that the observed variations of cellular amounts corresponded to physiological changes. Numerous limiting factors may be responsible for the observed variations, as in batch cultures, conditions (nutrients, excretion products, light, growth phase) are continually changing.

The most general effect of nutrient deficiency on the composition of algae is a decrease in protein and photosynthetic pigments and an increase in storage products, carbohydrates and lipids (Healey, 1973; Strickland, 1965; Myklestad, Haug, 1972; Dortch *et al.*, 1984). Consequently, some characteristic chemical ratios have often been used as physiological parameters: for example carbohydrate/protein, C/N, C/chl. *a* and protein/chl. *a*.

Only *T. weissflogii* showed variations of composition characteristic of a nutrient deficiency. The two species that had not reached the stationary phase, *C. appendiculata* and *G. aureolum*, were in an intermediate situation. Their C/N and carbohydrate/protein ratios were increased while remaining at a comparatively low level (*cf.* Paasche *et al.*, 1984). However the C/chl. *a* ratio decreased for the two species and *G. aureolum* did not show an increase in carbohydrate. Sakshaug *et al.* (1984) did not observe glucan accumulation in dinoflagellates associated with nutrient depletion.

For all other species, our data (Tab. 4) are quite different: no carbohydrate accumulation was evidenced and C/N and carbohydrate/protein ratios were low (Tab. 5). Our results are generally characteristic of algae with a good nutritional nitrogen status. Goldman *et al.* (1979), Sakshaug *et al.* (1984) give a C/N ratio of 6.6 for non-limiting cells, and Shifrin and Chisholm (1981) a C/N between 3.7 and 13.6. The range of C/N among the 11 species studied is 2.9 to 10.8. The C/N values for *T. rotula* 2.9 and 3.5 were unusually low. Obviously, optimum storage of carbohydrates was not obtained in our present study (13-17% starch in *C. maculata* and 41.8% in another set of experiment: Harris *et al.*, 1986). Also the preferential storage product may be lipids (Healey, 1973), as in the case of green algae in nitrogen limitation (Shifrin, Chisholm, 1981) and diatoms in silicon limitation (Coombs *et al.*, 1967).

Many of the parameters chosen as indicative of physiological state were especially significant for nitrogen deficiency. Other limiting factors may be responsible for the onset of the stationary phase; their implications for chemical composition are unknown. Little is known about limiting factors such as phosphorus, or trace elements (metals or vitamins). In the case of Fe-deficiency or phosphorous-limitation as found by Sakshaug and Holm-Hansen (1977) in cultures of *S. costatum* and *P. lutheri*, the C/N ratio cannot be a good indicator because of values that resemble those of good nutritional status.

As emphasized by the decrease in the C/chl. *a* and protein/chl. *a* ratios (Tab. 5), a light limitation may have occurred, induced by the high density of cells at the end of the growth curve. The classical pattern for photoadaptation is the increase of cellular chlorophyll, the decrease of C/chl. *a* ratio and polysaccharide synthesis when cells become more shade-adapted (Verity, 1981; Falkowski *et al.*, 1985; Hitchcock *et al.*, 1986). The C/N ratio is not related to light limitation. Tett *et al.* (1985) observed light-limited phytoplankton growing slowly with a C/N near the Redfield ratio (6.6). In our results, the loss of all the intracellular compounds, chlorophyll *a* included, must indicate a more complex limitation than that related only to light.

Nutritional implications for herbivores

In conclusion, our results show major differences in chemical composition between the different taxonomic groups, but also within a taxonomic division depending on the growth phase. These findings, which have important implications for grazing on phytoplankton, suggest that the higher chemical content of smaller cells may to some extent compensate for reduced grazing as cell size decreases (Frost, 1972). There is an effect of cell size in terms both of animal feeding behaviour and the nutritive content of the cell. For example, from our results, an animal feeding on exponential-phase cells of a large diatom such as *C. waillesii* would have to ingest ten times the volume of cellular material compared with a diet of *C. maculata* to achieve the same carbon intake. In addition, the differences in chemical composition between species we have observed are significant in view of recent studies (Huntley *et al.*, 1983; Poulet, Marsot, 1978) suggesting that zooplankton are capable of discriminating between cells of different species when presented with a mixture. Hitchcock (1982) explained the enhanced growth rate of *Calanus helgolandicus* fed on dinoflagellates as opposed to diatoms by the higher cellular content and caloric values of *G. splendens*. On a volume basis, we have found that the Cryptophyceae exhibited higher chemical content than Dinophyceae, suggesting that their nutritive value for grazers should be considered. However the cellular content of compounds such as protein and carbohydrate only provides a potential nutritive value. Other parameters, such as limiting factors (specific amino acids or fatty acids, or vitamins) in the food, digestive ability (enzymes) or the level of satiety of grazers, must also be taken into account.

In this connection, and arising out of the findings of this investigation, we have recently carried out experiments with *Calanus helgolandicus* fed on *C. maculata* and *T. weissflogii* (Harris *et al.*, 1986) in order to study the relations between digestive enzyme activity and ingestion of potential substrates, in particular starch.

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