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Chemical composition of phytoplankton and Particulate Organic Matter in the Ría de Vigo (NW Spain)*

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SUMMARY: Elemental (C, H, O, N, Si, P) and biochemical composition (proteins, carbohydrates, lipids, phosphorus compounds, chlorophyll and opal) in particulate organic matter, diatoms, other autotrophs, heterotrophs and detritus from natural plankton were established simultaneously by measuring relatively few components. Using standard techniques in marine chemistry on board ship, it is possible to infer a great deal about the composition and condition of the plankton. In addition, the organic matter content in terms of cell volume was determined for each group of plankton. Variation of chemical composition with depth was also considered. The ratio carbohydrates/lipids (Cbh/Lip) was used as an indicator of the chemical quality of the plankton.

Key words: elemental composition, biochemical composition, carbon, nitrogen, phosphorus, chlorophyll, silica, protein, carbohydrate, lipids, biovolume, phytoplankton

INTRODUCTION

Shellfish aquaculture can be improved if the chemical quality of the plankton is known. To achieve this purpose, it is essential to determine the elemental and biochemical compositions of plankton and detritus which in its turn is important in models of oceanic processes, ecosystem flux, and elemental cycling. The goal of this study is to simultaneously obtain the elemental composition (C, H, O, N, Si, P) of the particulate matter and its biochemical composition (proteins, carbohydrates, lipids, phosphorus compounds, chlorophyll and opal) from C, N, P, Si, Chl*a* and carbohydrate analyses, and to differentiate the various groups forming the particulate organic matter (diatoms, other autotrophs, heterotrophs and detritus) in order to determine the elemental and biochemical composition of each group. The purpose is to identify the biochemical quality of the particulate matter in Ría de Vigo where plankton is typical of the coastal zone due to the upwelling influence and the prevailing hydrographic conditions. A knowledge of the chemical quality of the plankton the Ría de Vigo is of major significance because of the importance of plancton in the growth of larvae and adult shellfish species which are cultured there.

The Ría de Vigo is a partially mixed positive estuary which is subjected to the influence of oceanic water affected by coastal seasonal upwelling. The water which upwells in the contiguous shelf is Eastern North Atlantic Water (ENAW), bringing nutrients to the ría (Otto, 1975; Fraga 1981; Blanton *et al*., 1987). These nutrients favour the development of dense phytoplankton populations (Estrada, 1984; Figueiras and Pazos, 1991; Figueiras and Ríos, 1993; Fraga and Bakun, 1993). These populations

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evolve with the seasons and their elemental and biochemical composition is modified by this evolution in an annual cycle (Smetacek and Hendrikson, 1979; Veldhuis *et al*., 1986, Figueiras and Niell, 1987). As a result, the ratios between carbon, nitrogen, phosphorus and oxygen are always changing and cannot be replaced by a global constant such as Redfield *et al*. (1963) reported.

A large number of studies on the chemical composition of plankton in different geographical zones has been made, but most are focused on qualitative studies and do not include the total elemental or biochemical composition. Measurements of carbon, nitrogen and phosphorus have been numerous (Menzel and Ryther, 1964; Fraga, 1966; Perry, 1976; Bishop *et al*., 1977; Eppley *et al*., 1977; Yanada and Maita, 1978; Armas, 1981; Herbland and Le Bouteiller, 1981; Nøst-Heseth, 1982; Copin-Montegut and Copin-Montegut, 1983; Sakshaug *et al*., 1983; Youakim and Reiswig, 1984). A few works report measurements of silicon (Copin-Montegut and Copin-Montegut, 1978; Lahdes and Leppänen, 1988; Nelson *et al*., 1988; Tréguer *et al*., 1988; Leynaert *et al*., 1991) and a few others have only reported the variations between chlorophyll and carbon and nitrogen (Roman *et al*., 1983; Hager *et al*., 1984; Shim and Shin, 1989; Furuya, 1990).

The elemental composition of natural plankton groups is poorly known except for a few studies of monospecific blooms (Eppley *et al*., 1977; Hendrikson *et al*., 1982; Sakshaug *et al*., 1983). Studies referring to the composition of cultivated species are more numerous (Ketchum and Redfield, 1949; Vinogradov, 1953; Parsons *et al*., 1961; Herbert, 1961; Redalje and Laws, 1983; Lirdwitayaprasit *et al*., 1990). On the other hand, Varela *et al*. (1988) and Andersson and Rudehäll (1993) have obtained the proportion of particulate organic carbon from different classes of particulate organic matter in seawater. Given the importance of modelling the flow of carbon, nitrogen, and phosphorus through marine ecosystems it seems necessary to know the variability in the elemental composition of the various plankton groups. The elemental composition of detritus is also poorly known and considering its ubiquity as a POM component this composition and its variability should be determined. In this work we have considered detritus as a fraction of the plankton, visible by light microscopy and presumeable produced as a result of the decomposition of the plankton population, including material produced by grazing by secondary producers and indigenous bacterial population.

The biochemical composition (lipids, carbohydrates, and proteins/amino acids) of natural populations (Haug *et al*., 1973; Smetacek and Hendrikson, 1979; Hendrikson *et al*., 1982; Tanoue, 1985) is poorly reported though there is a greater number of measurements relating to cultivated species (Parsons *et al*., 1961; Langdon and Waldock, 1981; Ben-Amotz *et al*., 1985, 1987; Whyte, 1987; Fernández-Reiriz *et al*., 1989; Fernández *et al*., 1992). The biochemical composition measured from natural populations, compiled from the literature (see Table 7), gives average proportions 40±7, 26±14 and 15±8 for proteins, carbohydrates, and lipids, respectively. The typical average C/N ratio according to Redfield *et al*. (1963) is 6.6. However, when phytoplankton has a high growth rate, the C/N ratio decreases because the proportion of proteins increases. In contrast, the proportion of storage compounds (carbohydrates and lipids) decreases. Conversely, the protein proportion decreases when the phytoplankton population is in stationary phase or cells become degraded. Since production and regeneration processes takes place at different levels of the water column, the changes in chemical composition with depth are also examined in the present paper.

MATERIAL AND METHODS

Sampling

From February 1988 to February 1989, the three stations shown in Fig. 1 were sampled on 26 occasions coinciding with neap tide conditions of the first and last quarter to minimize variations in the results. Samples were taken with 5-l Niskin bottles at 0, 2, 5, 10, 20, 30 and 40 m depth.

Analytical Methods

Particulate organic carbon and nitrogen

One litre sea water samples, containing between 100 and 2000 μg of organic matter, were filtered through glass fibre filters (Whatman GF/F, 25 mm diameter). Carbon and nitrogen content was determined using a Perkin-Elmer 240 CHN analyzer. The filters were dried at 110°C for 15 minutes. Combustion was carried out at 740°C using the method of

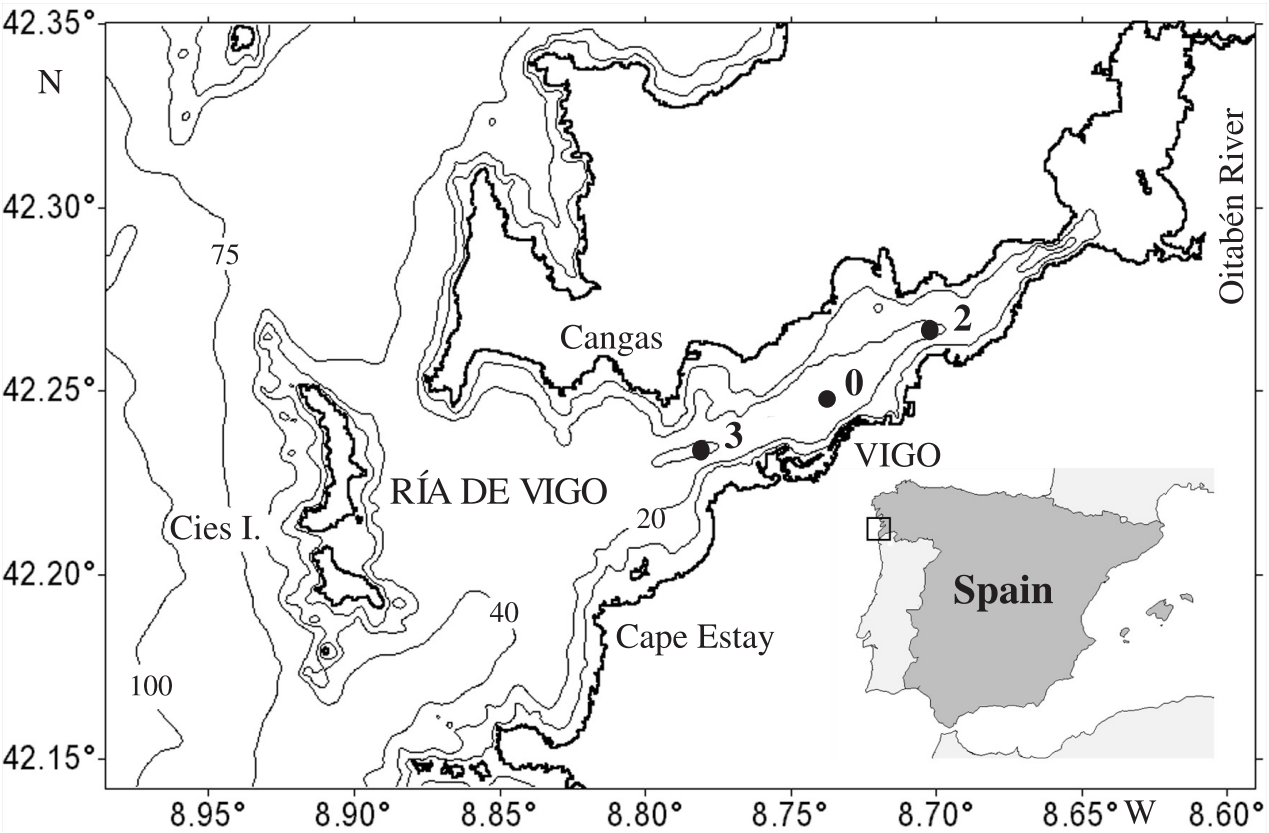

FIG. 1. – The Ría de Vigo and the position of three stations 3, 0 and 2 sampled between February 1988-1989. Isobaths in metres.

Fraga (1976). The reproducibility of the method was ±0.1 μM for C and ±0.02 μM for N.

Particulate organic phosphorus

200 ml samples of sea water were filtered through 25 mm diameter Millipore AAWP02500 cellulose acetate filters, 0.8 μm pore size. The filters were digested with 0.5 ml of 60% perchloric acid and concentrated sulphuric acid (8:1, V/V) by boiling until the samples lost colour. The final concentration of the acid mixture was 12.12N. to reduce this acidity, 50 ml of 0.12 N NH_4OH were added. Phosphate was measured according to the method of Grasshoff *et al*. (1983). The reproducibility of the method was ±0.02 μM.

Particulate biogenic silicon

200 ml samples of sea water were filtered through 25 mm diameter Millipore AAWP02500 cellulose acetate filters, 0.8 μm pore size. The filters were placed on plastic flask bottoms and 2 ml of 0.05 N NaOH added. They were left 24 hours at 45°C in an oven, and then diluted with varying volumes of distilled water according to the amount of plankton in the sample. Silicate was analysed using a Technicon autoanalyser according to the method of Grasshoff *et al*. (1983). The reproducibility of the method was ±0.10 μM.

Carbohydrates

100 ml samples of sea water were filtered through glass fibre filters (Whatman GF/F, 25 mm diameter). To analyse for carbohydrates, anthrone reagent was used. This reagent was made by pouring 670 ml of concentrated sulphuric acid into 335 ml of distilled water. When the solution cooled, 0.67 g of anthrone were added. Filter edges were cut off and the trimmed filter placed in a 16x160 mm test tube, 4 ml of anthrone reagent added, and heated to 90°C for exactly 16 minutes. The absorption was measured in 1 cm cuvettes at 625 nm in a "Beckman

DU" spectrophotometer. Soluble "Merck" starch was used as standard. About 0.16 g were vacuum dried over silica gel, weighed and dissolved in 100 ml of water, and heated without boiling. This solution was diluted to the appropriate volume. The standards and blanks were treated in the same way as the samples. The reproducibility of the method was ±0.006 μM of carbon.

Chlorophyll a

100 ml samples of sea water were filtered through 25 mm diameter Whatman GF/F glass filter. Chlorophyll was extracted with 90% acetone and the fluorescence of the extract measured using a Turner Designs Model 10.000R fluorometer. Chlorophyll concentrations without correction for phaeopigments were calculated according to the method of Yentsch and Menzel (1963).

Plankton counts

Sedimented plankton samples, fixed in Lugol's solution, were examined using an inverted microscope. The organisms of 468 samples were counted and identified at 600x magnification. A species list was published by Ríos (1992). The detrital particles were also counted and classified with regard to their diameter and geometrical shape, although it was difficult to assign dimensions and shapes to irregular particles

The phytoplankton species were grouped according to their physiology and chemical composition into i) diatoms (Dia) which includes also chrysophytes, both with siliceous skeletons; ii) other autotrophs (Other autotrophs) which includes Euglena, autotrophic dinoflagellates and autotrophic ciliates; iii) heterotrophs (Het) which includes zooplankton, heterotrophic dinoflagellates and ciliates, Ebria and Foraminifera; and iv) detritus (Det).

Nanoflagellates were divided into autotrophs (69%) and heterotrophs (31%) according to the epifluorescence counts made in the Ría de Vigo during 1990-91 at station 3 (Fig. 1). Because the samples were fixed with Lugol's solution, the dinoflagellate species, once identified, were grouped into autotrophs and heterotrophs according to the existing literature (Schiller, 1933, 1937; Dodge, 1982; Lessard and Swift, 1986). The ciliate *Mesodinium rubrum* was included among the autotrophs because it is capable of photosynthesis (Lindholm, 1981).

Cell volume

About 10 to 50 individuals of each species fixed with Lugol were measured to calculate cell volumes. The volumes were calculated by assigning one or several geometrical shapes to each species, following the recommendations of Edler (1979). The mean volumes for each species were reported by Ríos (1992).

The total cell volume is frequently an inadequate estimation of biomass because it includes the cell vacuole, which contains relatively non-nutritious cell sap. Sicko-Goad *et al.* (1977) proposed that plasma volume was equal to cell volume for all phytoplankton species except diatoms. In this group, it is mainly the larger species which have large vacuoles. The diatom plasma volume was calculated using the simplified procedure ($V_p = S * C + 0.1 * V$) of Smayda (1978), where Vp is the plasma volume (μm^3), S the cell surface area (μm^2), C the cytoplasmic-layer thickness (μm), and V the total cell volume (μm^3). The cytoplasmic-layer thickness (2, 1.5, 1 μm), used in the calculation of plasma volume is based on the surface-volume ratio (<0.35, 0.35 to 0.50, 0.51 to 0.89 respectively) given by Smayda (1978). When surface-volume ratios were higher than 0.90, plasma volume equaled total volume. Shrinkage of the cells caused by Lugol fixation (Montagnes *et al.*, 1994) was not taken into account. In this study, we have used the plasma volume for diatoms and total volume for the other groups, although in the text we refer only to volume.

Data Processing

Chemical content and cell volume relationship

Data shown in Table 1, indicating the content of each element and compound analysed per unit cell volume, were obtained in two ways: i) POM data are the mean values of the 468 data sets analysed, ii) plankton group data are the result of splitting the content of each element or compound analysed as the sum of the content of each element or compound in the different groups (Dia, Oau, Het, Det) by fitting to minimum squares the following multivariate equation using the 468 data sets:

$$X = x_1 \text{ Dia} + x_2 \text{ Oau} + x_3 \text{ Het} + x_4 \text{ Det} \qquad \text{(I)}$$

where X is variable measured here C, N, P, Si, Chl*a*, or Cbh. The coefficients x_1, x_2, x_3 and x_4 are the coefficients calculated by multivariate fitting

TABLE 1. – Content of each element and compound analysed per cell volume unit for the mean particulate organic matter (POM) and for each plankton group (**Dia**toms, **O**ther **au**totrophs, **Het**erotrophs and **Det**ritus). Units expressed in mol/dm^3 of cell volume, except for carbohydrates which are expressed in mol of C by dm^3 of cell volume and chlorophyll a expressed in g/dm^3 of cell volume. The values of POM were directly analysed. The values of the other groups of plankton were calculated using the multivariate equation (I) in function of the elemental analysis and the cell volumes which were calculated from the plankton counts. The error for the POM is the STD/N$^{1/2}$ and for the other groups of plankton is the multivariate regression error. The determination coefficient (r^2) was obtained from the multivariate fitting.

	C mol/dm^3	Error	N mol/dm^3	Error	P mol/dm^3	Error	Si mol/dm^3	Error	Cbh mol-C/dm^3	Error	Chla g/dm^3	Error
POM	9.0	0.3	1.31	0.05	0.101	0.004	0.53	0.02	1.93	0.09	1.93	0.13
Dia	6.2	0.3	0.91	0.05	0.068	0.003	0.36	0.02	1.38	0.08	2.11	0.09
Oau	10.9	3.0	1.97	0.44	0.205	0.031	0.00	0.20	3.04	0.73	1.01	0.91
Het	0.8	0.7	0.12	0.10	0.014	0.007	0.02	0.04	0.29	0.16	0.00	0.21
Det	5.5	0.6	0.70	0.09	0.057	0.007	0.44	0.04	1.02	0.16	0.68	0.20
r^2	0.60		0.61		0.66		0.55		0.57		0.55	

that represent the content of each variable per unit cell volume in each plankton group. Dia, Oau, Het and Det are given as volume. Data of elements C, N, P and Si are expressed in mol/dm^3, chlorophyll *a* (Chl*a*) in g/dm^3 and carbohydrates (Cbh) in mol of C/dm^3. So, for instance, in the case of carbon, and considering the carbon coefficients given in Table 1, the equation (I) is:

$$C = 6.2\ Dia + 10.9\ Oau + 0.8\ Het + 5.5\ Det$$

During the course of this experiment, we were unable to use the epifluorescence technique. Therefore the cyanobacteria, bacteria and picoplankton (autotroph and heterotroph) groups were not taken into account. However other resaerch in the Ría de Vigo on counts and volumes has shown that these bacteria, cyanobacteria, and both autotrophic and heterotrophic picoplankton together represent only up to 8% of the total biovolume.

Chemical composition

The main chemical components of the phytoplankton are proteins (Prt), carbohydrates (Cbh), lipids (Lip), phosphorus compounds (Pho), opal (Opa) and total chlorophylls (Chl). The mean elemental composition of this group of biomolecules for natural phytoplankton as given by Fraga and Pérez (1990), is shown in Table 2. Opal ($Si_2O_5H_2$), with a 13% water content, is the major component of diatom frustules.

From the mean composition of biomolecules (Table 2), we have established a series of equations, following the proposal of Fraga and Pérez (1990), which allows the mutual transformation between

TABLE 2. – Mean elemental formulae for each group of molecules (Fraga and Pérez. 1990)

	Formula weight	grams
Proteins	$[C_{139}\ H_{217}\ O_{45}\ N_{39}\ S]$	3186.6
Chlorophylls	$[C_{46}\ H_{52}\ O_5\ N_4\ Mg]$	765.3
Proteins+Chlorophylls	$[C_{148}\ H_{225}\ O_{44}\ N_{40}\ S]$	3300.7
Carbohydrates (1)	$[C_{17}\ H_{28}\ O_{14}]$	456.4
Lipids (2)	$[C_{53}\ H_{89}\ O_6]$	822.2
Phosphorus compounds (3)	$[C_{45}\ H_{76}\ O_{31}\ N_{12}\ P_5]$	1436.0
Opal	$[Si_2\ H_2\ O_5]$	138.2

(1) Ribose and deoxyribose nucleic acids are excluded.
(2) Phospholipids are excluded.
(3) All phosphorus compounds. both organic and inorganic are included.

elemental and biochemical composition of phytoplankton. Therefore, using the variables measured of C, N, P, Si, Cbh and Chl*a* (Table 1) in the following system of equations

$$\begin{aligned} C &= 139\ Prt + 17\ Cbh + 53\ Lip + 45\ Pho + 46\ Chl \\ N &= 39\ Prt + 12\ Pho + 4\ Chl \\ P &= 5\ Pho \\ Si &= 2\ Opa \end{aligned} \qquad \text{(II)}$$

the unknown variables Prt, Lip, Pho and Opa (opal) can be calculated. All units are expressed in moles. When only chlorophyll *a* was determined, total chlorophyll (Chl) can be calculated by dividing the chlorophyll *a* value by 0.56 (Fraga and Pérez, 1990).

Once the biochemical composition of plankton has been determined, the elemental composition can be completed by calculating the H and O content, expressed in moles, based on the compositions given in Table 2:

TABLE 3.- Elemental and biochemical composition of particulate organic matter (POM) and of each group of plankton (**dia**toms. **o**ther **au**totrophs. **het**erotrophs and **det**ritus) that compose the plankton, calculated from the data of Table 1 by means of the equations (II) and (III) (See text). Content of organic matter, without Opa, (OM) in g dry weight per dm^3 of cell volume for the POM and for each plankton group.

	C	H	O	N	P	Si	OM
				in atoms			g/dm^3
POM	106	177.1 (0.4)	59 (1)	15.4 (0.3)	1.19 (0.04)	6.3 (0.4)	224 (5)
Dia	106	176.1 (0.4)	59 (1)	15.7 (0.3)	1.17 (0.04)	6.2 (0.4)	154 (7)
Oau	106	170.6 (1.4)	53 (5)	19.3 (0.9)	2.00 (0.12)	0.0 (1.4)	269 (68)
Het	106	175.3 (1.3)	64 (4)	16.0 (0.8)	1.88 (0.11)	3.2 (1.3)	21 (15)
Det	106	180.6 (0.4)	60 (1)	13.5 (0.2)	1.09 (0.03)	8.4 (0.4)	137 (14)

	Prt	Cbh	Lip	Pho	Chl	Opa	Cbh/Lip
				in percentages of weight			
POM	35.7 (0.8)	20.8 (0.8)	14.7 (0.9)	12.1 (0.3)	1.44 (0.08)	15.3 (0.8)	1.41
Dia	36.1 (0.8)	21.7 (0.8)	13.0 (0.9)	11.8 (0.3)	2.29 (0.08)	15.1 (0.8)	1.67
Oau	44.5 (2.8)	28.7 (2.8)	4.4 (2.9)	21.8 (1.1)	0.66 (0.26)	0.0 (2.6)	6.49
Het	32.9 (2.5)	34.4 (2.5)	6.1 (2.6)	18.9 (1.1)	0.00 (0.23)	7.7 (2.4)	5.61
Det	30.4 (0.7)	17.5 (0.7)	20.3 (0.8)	10.9 (0.3)	0.81 (0.07)	20.1 (0.7)	0.86

$$H = 217\ Prt + 28\ Cbh + 89\ Lip + 76\ Pho + 52\ Chl + 2\ Opa$$
$$O = 45\ Prt + 14\ Cbh + 6\ Lip + 31\ Pho + 5\ Chl + 5\ Opa \quad \text{(III)}$$

The variables measured were C, N, P, Si, Chl*a*, Cbh and the variables calculated were H, O, Prt, Lip, Pho, Chl, Opa.

RESULTS

Elemental and Biochemical Composition

The elemental and biochemical composition of POM and of each plankton group (Dia, Oau, Het, Det) shown in Table 3 were calculated from the data presented in Table 1 using equations (II) and (III). Table 3 also shows the organic matter content of POM and of each plankton group expressed in g/dm^3 of cell volume. These organic matter contents have been obtained directly by expressing the carbon content data per unit of cell volume (Table 2) and the percentage of carbon in the organic matter (C+H+O+N+P). The organic matter content is expressed in dry weight excluding ash content because biogenic silica was not included in the organic matter. The POM represents 22.4% of dry weight and diatoms, 15.4%, the same values obtained by Eppley *et al*. (1977).

Although chemically it is more correct to refer the elemental composition to the element with lowest atomic value, we have chosen to relate all elements to 106 carbon atoms in order to facilitate comparison with the Redfield ratio (Table 3). It is well known that the variability in the Redfield ratio is higher when the time scale is small. However, on a global scale the variability is lower, producing a relatively constant composition, as Redfield *et al*. (1963) established.

The elemental composition of POM (Table 3) is close to the Redfield ratios although N content was slightly lower and P slightly higher. The elemental composition of diatoms was similar to POM as expected, because this is the dominant group, representing 54% of the total biovolume or 61% of the dry weight. The biochemical composition shows a similar pattern with the diatoms group having a higher value of Chl than that of POM. The N and P composition of other autotrophs is higher than that of the POM. The other autotrophs group comprises motile organisms, which require energy to swim. This may explain the low value of storage compounds (Lip) and consequently the high value of N and P. The higher value of N is also reflected in the biochemical composition which shows the highest value of Prt. Species without silicon were included in the other autotrophs group. The results of these calculations are in agreement with this fact. The ratio of heterotrophs is close to the Redfield ratio, although P content was almost double. The absence of Chl stands out. On the other hand, the small amount of silicon corresponds to the presence of Radiolaria in the samples. The ratio of detritus shows low N, Prt, Cbh, and the highest value of Lip. This is to be expected since detritus rep-

resents the remains of the plankton populations and the most labile molecules have been released during mineralization. The high Si content found in the detritus group was due to sponge spicules and diatom frustules that constitute the detritus. Although the elemental and biochemical composition of each group appear to agree with the biological behaviour of the different organisms of which they are composed, it is necessary to interpret the results of the other autotrophs and heterotrophs groups with caution since they represent only 4% and 12% respectively of the total plankton biovolume or 8% and 2% of the dry weight.

Analysis of the Cbh/Lip ratios (Table 3) show that the different plankton groups have different values. The more energetic groups of organisms (heterotrophs and other autotrophs) exhibit higher Cbh/Lip ratios than diatoms because they contain more Cbh. This storage compound although containing less energy, is easier to mobilise than Lip as an energy source. On the other hand, diatoms have higher Lip contents because this is their main storage product. Given that detritus contains the remains of different plankton groups, its Cbh/Lip ratio is the lowest because lipids are difficult to remineralise.

According to data presented in Table 2, the percentages of nitrogen in protein, chlorophyll and phosphorus compounds are 17.2%, 7.3% and 11.7% respectively. From the biochemical composition calculated for POM (Table 3), the total particulate nitrogen for phytoplankton -average composition- is distributed in the following way: protein nitrogen 80.0%, phosphorus linked nitrogen 18.6% and chlorophyll nitrogen 1.4%.

Elemental and Biochemical composition with depth

The chemical composition of phytoplankton depends on nutrient availability and other environmental conditions. Data presented in Table 4 show nutrient concentrations, salinity, temperature, incident radiation, oxygen saturation, pH, populations of autotrophic organisms, detritus and detritus percentage, and their coefficients of variation from February 1988 to February 1989. The salinity of the deep and surface water shows little variation, although surface salinity is more variable. Temperature follows the same trend with a little more variability through the water column. Nutrients, as with salinity and temperature, are more variable in the surface waters, but the variability through the water column is 5-7 times greater.

Data in Table 5 shows the variability of the mean values of elemental and biochemical composition with depth. The C/N ratio, as expected, increases with depth although between surface and 10 metres, coinciding with the photic zone, the values are relatively constant and close to the Redfield ratio. The mean value for the 1% light level (5.99 μE m^{-2} s^{-1}) was located at 17 metres (Table 4) although the compensation depth, calculated as the depth corre-

TABLE 4. – Average values for salinity, temperature, nutrients (nitrate, nitrite, ammonia, phosphate, silicate) incident light (I), autotrophic organisms, detritus, percentage of detritus, oxygen saturation and pH (referred at 15°C) in the water column for the period February 1988 to February 1989, at the sampling site (see Fig. 1). Numbers in brackets are coefficients of variation (V=100.STD/mean).

metres	S	T	NO_3^- μmol kg^{-1}	NO_2^- μmol kg^{-1}	NH_4^+ μmol kg^{-1}	$PO_4H^=$ μmol kg^{-1}	$Si(OH)_4$ μmol kg^{-1}
0	34.160 (6)	15.04 (13)	2.44 (97)	0.33 (92)	1.56 (90)	0.41 (68)	3.54 (75)
2	34.408 (4)	14.94 (13)	2.40 (98)	0.33 (93)	1.54 (92)	0.40 (70)	3.36 (80)
5	34.754 (2)	14.48 (11)	2.55 (100)	0.33 (96)	1.51 (92)	0.39 (68)	3.25 (81)
10	35.057 (2)	14.09 (11)	3.28 (83)	0.35 (72)	1.69 (88)	0.43 (58)	3.55 (70)
20	35.373 (1)	13.68 (8)	4.79 (62)	0.46 (56)	2.18 (71)	0.56 (35)	5.25 (49)
30	35.474 (0)	13.57 (8)	5.32 (58)	0.48 (55)	2.34 (69)	0.59 (28)	5.97 (40)
40	35.515 (0)	13.49 (8)	5.98 (59)	0.49 (57)	2.17 (65)	0.61 (31)	6.35 (47)

metres	I μE m^{-2} s^{-1}	Aut. μg l^{-1}	Det. μg l^{-1}	%Det.	%O_2	pH_{15}
0	598.9 (50)	288 (165)	75 (85)	21	109 (16)	8.201 (1)
2	342.1 (46)	278 (173)	59 (84)	18	109 (16)	8.199 (1)
5	155.9 (51)	295 (176)	63 (67)	18	105 (14)	8.182 (1)
10	46.8 (67)	278 (161)	70 (85)	20	97 (14)	8.154 (1)
20	5.4 (99)	104 (216)	122 (122)	54	90 (12)	8.116 (1)
30	1.0 (125)	91 (245)	148 (70)	62	87 (14)	8.103 (1)
40	0.2 (174)	72 (290)	260 (176)	78	86 (14)	8.104 (1)

TABLE 5. – Mean elemental and biochemical composition of the particulate organic matter (POM), and the content of organic matter without silica in μg/kg of sea water at each depth. Numbers in brackets are coefficients of variation (V=100.STD/mean) of the measured data used for this study.

metres	C	H	O	N	P	Si	OM
				in atoms			μg/kg
0	106 (71)	177	58.1	15.7 (63)	1.30 (68)	5.75 (88)	566 (69)
2	106 (67)	176	57.5	15.8 (62)	1.23 (64)	5.08 (78)	559 (65)
5	106 (83)	176	55.7	15.5 (78)	1.16 (70)	4.95 (81)	552 (80)
10	106 (98)	176	58.2	16.0 (101)	1.13 (85)	6.16 (111)	525 (98)
20	106 (53)	180	62.1	15.0 (65)	1.16 (63)	8.54 (82)	317 (54)
30	106 (62)	181	63.8	14.1 (78)	1.11 (83)	9.14 (87)	322 (64)
40	106 (46)	181	62.7	13.7 (58)	1.05 (67)	9.13 (66)	321 (47)

metres	Prt	Cbh	Lip	Pho	Chl	Opa	Cbh/Lip
			in percentages of weight				
0	35.7	21.2 (87)	14.1	13.3	1.59 (113)	14.1	1.50
2	37.1	23.0 (83)	13.3	12.7	1.30 (122)	12.6	1.73
5	37.1	22.0 (88)	14.9	12.2	1.41 (139)	12.5	1.48
10	37.7	20.3 (109)	13.6	11.5	1.88 (162)	15.1	1.48
20	33.3	17.7 (65)	16.4	11.3	1.27 (113)	20.0	1.08
30	30.8	18.8 (70)	17.4	10.8	1.06 (144)	21.2	1.08
40	30.6	18.1 (68)	18.9	10.3	0.71 (91)	21.4	0.96

sponding to 100% oxygen saturation (see Table 4), was 8 metres, coinciding with the mean nutricline. The total population of autotrophs and detrital material follows the same pattern with depth as the C/N ratio (Table 5). Biomass production occurs in the upper 10 meters of the water column, as the high O_2 saturation and pH values demonstrate.

Changes with depth are more clearly observed by following changes in biochemical composition. Phosphorus diminishes steadily with increasing depth. This is because phosphorus compounds are labile and phosphate can be released through hydrolysis even if the organic matter is not oxidized. In contrast, and as expected, Opa increases with depth due to the sinking siliceous skeletons. The greatest variation was produced between 10 and 20 metres, as with the detritus (Table 4). Moreover, slight maxima of Opa (Table 5) and detritus were recorded at the surface, which confirms the report of Fraga (1967) that detritus accumulates at the sea surface. The other chemical components (Chl, Prt and Cbh) diminished as depth increased. Conversely lipids, since they are more difficult to remineralise, increased with depth. Therefore, it is important to know the Cbh/Lip ratio (Table 5). This ratio decreased with depth, showing the maximum variation, as with the C/N ratio, between 10 and 20 metres. Detrital material was the main component present at 30 and 40 metres, representing more than 60% (Table 4), and its biochemical composition (Table 5) was very similar to that calculated using the multivariate equation (Table 3).

The Cbh/Lip ratio was inversely correlated with the percentage of detritus dry weight in each sample:

$$\%Det = 149.9 - 83.6 \cdot (Cbh/Lip)$$
$$r^2 = 0.90;\ n=7$$

Thus, when the POM is 50% composed of detrital material and 50% autotrophs, the Cbh/Lip ratio is 1.19. Below this value, detritus dominates. In our study, the Cbh/Lip ratio for the POM was 1.41 and it was composed of 30% detrital material and 68% autotrophs. The remaining 2% corresponded to heterotrophs. On the other hand, if the detrital component was zero, the ratio would be 1.79, a value close to that obtained for the diatoms (1.67) and lower than that found by Lancelot (1980) in the North Sea. In the case of POM without detritus the ratio could reach very high values, for example during a plankton bloom (Haug *et al.*, 1973) or in a population composed of motile species that use carbohydrates as storage compounds.

Seasonal variations

Besides the variation of the biochemical composition with depth, seasonal changes occur. Since sampling was fortnightly, the evolution of the biochemical composition from bloom formation to collapse for the same population was not observed. However, we selected as a case study two sampling times when biochemical composi-

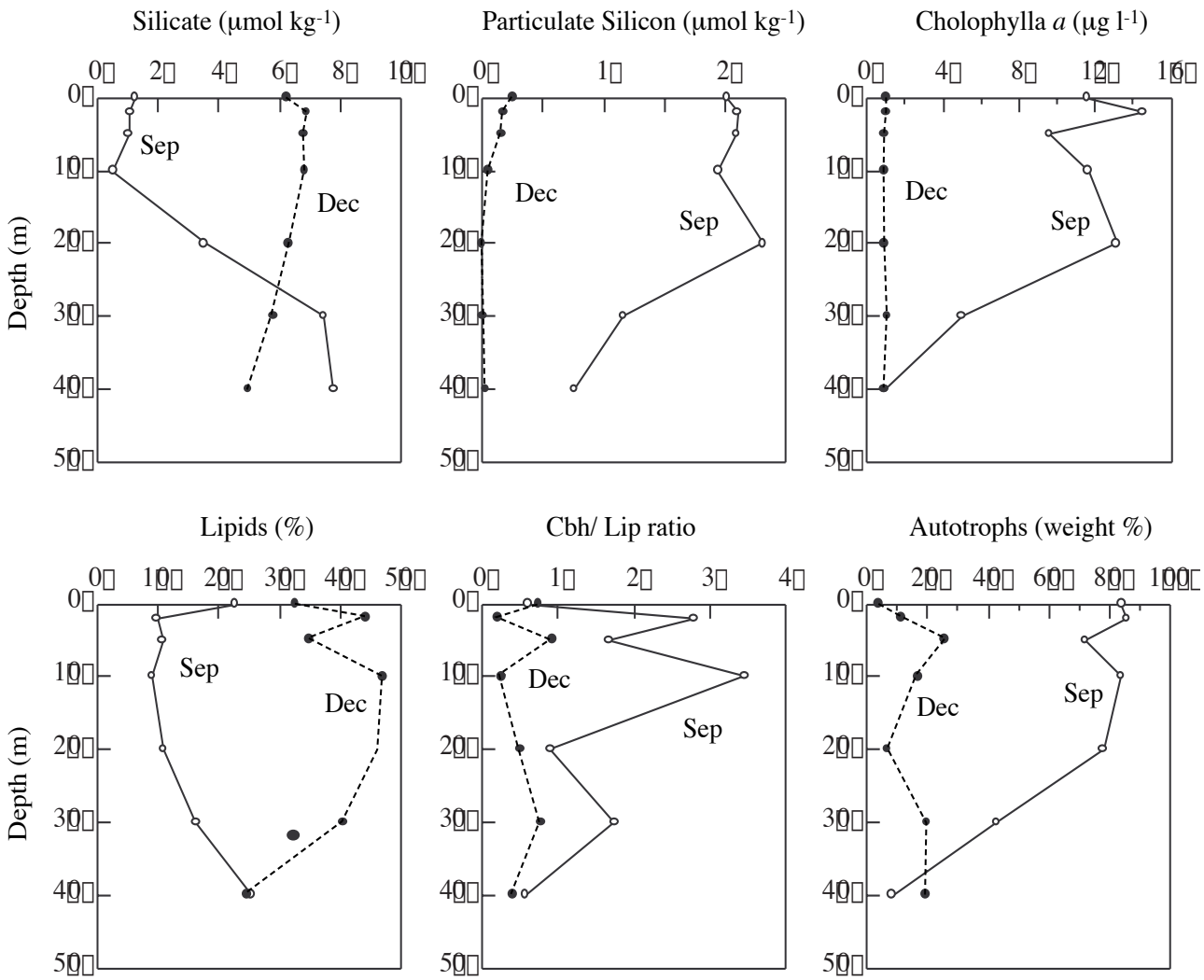

FIG. 2. – Vertical profiles of silicate (μmol kg^{-1}), particulate biogenic silicon (μmol kg^{-1}), chlorophyll a (μg l^{-1}), lipid percentage, Cbh/Lip ratio and autotroph percentage expressed in weight of organic matter, carried out in September and December 1988.

tion was different. Samples were collected at different times (September and December), when the irradiance was 965 and 332 μE m^{-2} s^{-1} respectively. The plankton population in September, although multispecific, was dominated by diatoms while in December detritus was dominant. Data presented in Figure 2 show the vertical profile of silicate, particulate biogenic silicon, percentage lipid, chlorophyll content, Cbh/Lip ratio and percentage of autotrophs for the two periods. In both cases, silicate was not depleted. The higher silicate uptake was recorded in September and this was reflected by the high content of particulate biogenic silicon in the cells. In contrast, in December the converse occurred. These findings are in agreement with the chlorophyll profile (Fig. 2) that shows values higher in September than in December, and also with the integrated weight of total autotrophs throughout the water column (1.49 μg/l in September, 0.13 μg/l in December). On the other hand, the detritus and lipid percentage were higher in December than in September. As might be expected, the Cbh/Lip ratio was higher in September than in December. The maximum value of Cbh/Lip ratio found in September (3.4) exceeds the theoretical value (1.79) given in this work, because at this time the plankton community, although dominated by diatoms, is more heterogeneous, with other autotrophic and heterotrophic species present (Figueiras and Ríos, 1993). Under such conditions the Cbh/Lip ratio increases as a consequence of the presence of dinoflagellates and heterotrophs that require large quantities of carbohydrates as metabolites.

DISCUSSION

The biochemical composition values in this study have been determined by calculation using equation (II), excepting for carbohydrates which were measured directly. Almost all methods used in the measurement of biochemical composition involve some problems. Many investigators have measured protein content using the Lowry method. Most authors calculate protein values from the PON analysis using a conversion factor 6.25. This value is not valid because it corresponds to that of animal protein. The correct conversion factor for phytoplankton is 5.8 reported by Fraga and Pérez (1990), who obtained the value from a data set of 175 amino acid samples and is close to the value of 5.6 recorded by Chuecas and Riley (1969) for diatoms. On the other hand, to estimate Prt using a factor of 5.8, it is necessary to multiply this value by the 80% of total N in order to remove the N-contribution from chlorophyll and nucleic acids. Miyata and Hattori (1986) developed a method for measuring phosphorus components in phytoplankton, fractionating and analyzing eight different P compounds. Data obtained by these authors show that the representative P compounds are orthophosphoric acid (40% with 7.5% of variation coefficient) and nucleic acids (37% with 13% of variation coefficient). Given that the percentage of orthophosphic acid is higher and shows a low variation coefficient, we decided to analyze total phosphorus. Total lipids are typically determined by means of solvent extraction followed by carbonization with sulfuric acid. The values obtained depend on the standard used. Other methods involve the use of gas chromatography which yield quantitative data on fatty acids composition but not total lipids. The method of Bligh and Dyer (1959) modified by Fernández-Reiriz *et al.* (1989) determines total lipids gravimetrically. However, in order to use this method we would need to filter 10 litres of sea water. Thus, although the methodology used in this study has some disadvantages -being an indirect measurement- it is nonetheless superior to other methods.

The ratios between chemical elements show important changes due to the physiological differences of the plankton species which develop over an annual cycle. For this reason and taking into account the problems of seasonal and spatial variability, direct comparison of the chemical composition obtained in this study with that reported by other authors has to be made with caution. Data presented in Table 6 give a short summary of the chemical composition of plankton. These data do not show values for all six elements measured in this study. As expected, the ratios between elements show a variability around the Redfield ratio. The mean values of C, H and O obtained in this study are similar to those of Haug *et al.* (1973) although their N and P values are lower and their N/P ratio is higher. These authors collected plankton samples with a net. The main part of sample for chemical analysis was concentrated by filtration, and transferred to a flask. Those manipulations may account for the low P values since phosphate can be readily removed from phosphorus compounds, thereby resulting in a higher N/P ratio. The C, N and P ratios in this study were similar to those obtained by Eppley *et al.* (1977) in California. The Si analysed in this work for POM and diatoms are similar to those reported by Lahdes and Leppänen (1988) in the Northern Baltic. The Chl/C and Chl/N ratios obtained here are high, and are indicative of the low levels of contamination in the zone studied, although a decrease in the Chl/C ratio may result from a shortage of silicate (Lombardi and Wangersky, 1991).

Several authors (Strickland, 1960; Mullin *et al.*; 1966, Strathmann, 1967; Eppley *et al.*, 1970; Smetacek and Hendrikson, 1979) have calculated phytoplankton carbon content taking into account cell and plasma volume. The values obtained by these authors are 10.8 mol C per dm^3 for dinoflagellates and 9.2 for total plankton. These values are identical with those obtained in this study (Table 1). The diatoms/other autotrophs C content ratio of this study, where other autotrophs are composed mainly of dinoflagellates, was 0.57 (Table 3), higher than the diatom/dinoflagellate ratio (0.32, also in C content) calculated from the data of Vives and Fraga (1961). These authors considered that diatoms and dinoflagellates both contained the same amount of chlorophyll, whereas the chlorophyll content of other autotrophs was 3.5 times lower than for diatoms. This value is higher than the ratio of 2.9 reported by Gillbricht (1952). Margalef *et al.* (1955) found that diatoms contain 2.85 times more chlorophyll than dinoflagellates. The low percentage of organic matter in dry weight for the heterotrophs group is due to the presence of *Noctiluca scintillans*, a large-cell species that formed the dominant species in samples collected on 7 July and a major component on 5 August, although on this occasion the dominant group was detrital material.

TABLE 6. – Summary of the chemical composition of natural plankton populations, in atoms. Chl/C in grams of Chlorophyll/mol C; Chl/N in grams of Chlorophyll/mol N.

Author	Place	C	H	O	N	Si	P	C/N	Chl/C	Chl/N
Menzel and Ryther (1964)	Atlantic(1/62)	106			9		1.8	13		
	" (4/62)	106			20		1.6	5		
Fraga (1966)	Indian 1-5 m day	106			20		1.3	6		0.43
	" " night	106			20		1.4	5		0.89
	" 5-14 m day	106			19		1.4	6		0.80
Haug *et al.* (1973)	Trondheimsfjord	106	171	62	13		0.8	8		
Perry (1976)	Pacific (1-70 m)	106			12		0.7	9		
	" (80-120)	106			14		0.9	8		
Bishop *et al.* (1977)	Atlan.Ec.<1μm	106			15		0.7	7		
	" " 1-53μm	106			14		0.7	7		
	" " >53μm	106			13		0.6	8		
Eppley *et al.* (1977)	S. California	106			18		1.3	6	0.17	1.12
	" " NO_3<1μg/l	106			16		1.2	7	0.16	1.19
	" " Diatoms	106			14		1.4	7	0.29	2.07
	" " Dinofl.	106			13		1.0	8	0.06	0.41
	" " P.micans	106			15		1.0	7	0.15	1.22
Copin-Montegut and Copin-Montegut (1978)	Indian Ocean:									
	Tropical zone	106			16	3	0.9	7		
	Subantarctic	106			17	1	1.0	6		
	Polar front	106			19	20	1.2	6		
	Antarctic	106			18	16	1.6	6		
Yanada and Maita (1978)	Funka Bay	106			11		0.6	10		
Armas (1981)	Canary Islands	106			11		0.6	10		
Herbland and Le Bouteiller (1981)	Atlan.Ec.<200μm	106			13		0.9	8		
	" " < 35μm	106			13		0.8	8		
	" " < 3μm	106			12		0.6	9		
Nøst-Hegseth (1982)	Trondheimfjord	106			11		0.6	10	0.09	0.97
	" (8-25m)	106			13		0.7	9	0.17	1.51
Copin-Montegut and Copin-Montegut (1983)	Indian	106			19		1.1	6		
	Antarctic	106			19		1.3	6		
	NW Africa	106			19		1.5	6		
	Mediterranean	106			17		0.8	7		
	All samples	106			16		1.0	6		
Sakshaug *et al.* (1983)	Trondheimfjord:									
	Bloom Skelet.76	106			14		1.6	7		
	Bloom Skelet.77	106			14		1.1	8		
	Multiespecif.81	106			10		1.3	11		
	Multiespecif.77	106			14		0.8	8		
Youakim and Reiswig (1984)	Bideford. Canada	106			12		0.8	9		
Tanoue (1985)	Antarctic	106			14		1.1	8		
Lahdes and Leppänen (1988)	Baltic North	106			10	6	0.6	10	0.19	
Tréguer *et al.* (1988)	Pacific	106			14	17	0.7	7	0.03	
This paper	total POM	106	177	59	15	6	1.2	7	0.22	1.48
	diatoms	106	176	59	16	6	1.2	7	0.34	2.32
	other autotrophs	106	171	53	19	0	2.0	6	0.09	0.51
	heterotrophs	106	175	64	16	3	1.9	7	0	0
	detritus	106	181	60	13	8	1.1	7	0.12	0.98

Table 7 summarises data obtained by several authors on the biochemical composition of plankton. Variations in the biochemical as well as in the elemental composition are due to the physiological differences in species composition and growth phase. The different biochemical composition found during the most intense upwelling and downwelling events in this study, reflect the composition of the dominant group of phytoplankton species. The development of diatoms (with upwelling) or dinoflagellates (with downwelling) was favoured by the physical structure of the water column and the nutrient availability caused by these events (Figueiras and Ríos, 1993; Ríos *et al.*, 1995). Differences in biochemical composition were also exacerbated by difficulties encountered when using different analytical methods. The biochemical composition data given in Table 7 considers only the four main groups (Prt, Cbh, Lip, Pho). To make comparison between data easier, we have assigned a value of 14% for Pho data of Lancelot (1980) and Hendrikson *et al.* (1982), and recalculated their results since they only considered three components. Our data for the biochemical composition of POM are in agreement with the mean values for the data summarised in Table 7. The data of Takahashi *et al.* (1985) were calculated from

TABLE 7. – Summary of the biochemical composition of plankton obtained by various authors, expressed in percentages.

Author	Prt		Cbh	Lip	Pho	
Haug *et al* (1973)[1]	34.1		55.8	2.1	8.0	
Smatecek and Hendrikson (1979)	35.0	*	23.0	26.0	16.0	ψ
Barlow (1980)			14.1			
Lancelot (1980)	43.6		29.9	12.5	14.0	γ
Franco *et al* (1981)	30.6	+		7.1		
Hendrikson *et al* (1982)	44.7	*	24.5	16.8	14.0	γ
Takahashi *et al* (1985)[2]	43.4		19.0	26.7	10.9	
Tanoue (1985) 0-50 m	50.7	•	16.2	16.7	16.4	ϕ
Tanoue (1985) 0-1500 m	39.0	•	18.5	21.5	21.0	ϕ
This work (POM)	43.9	*f*	24.5	17.4	14.2	

[1] calculated using the equations of Fraga and Pérez (1990)
[2] calculated from Redfield ratio using the equations of Fraga and Pérez (1990)
* calculated from PON using the conversion factor 6.25
\+ Lowry method
• calculated from amino acids
ϕ calculated by difference between total nitrogen and protein nitrogen
f including chlorophyll as protein
γ assuming Pho=14% and recalculating the other 3 components
ψ Pho calculated by difference

the composition of the organic matter oxidised within the thermocline of the Atlantic and Indian oceans. The protein values obtained by these works are similar to the values obtained in this study, however, the other components show some differences because the values are for oxidised organic matter. Therefore Pho is lower, and the Cbh/Lip ratio (0.70) corresponds to a detritus value. The biochemical composition given by Hendrickson *et al*. (1982) is almost identical in terms of percentage values as recorded in this study for POM. It is equally interesting to note that the Cbh/Lip ratios obtained in both studies are similar.

The increase in lipids and decrease in proteins and Cbh/Lip ratio with depth observed in this study (Table 5) are in agreement with the results of Tanoue (1985). During POM sedimentation, phosphorus compounds are initially degraded, followed by chlorophyll, protein and carbohydrates. Lipids and silica, derived principally from diatoms frustules, accumulate with depth since the other elements are released more rapidly and silicon accumulates; this accumulation is accentuated in the Antarctic (DeMaster *et al*., 1992). Therefore, the Cbh/Lip ratio is a good indicator of the state of POM mineralization.

Lombardi and Wangersky (1991) reported an increase in total lipids per cell under nutrient stress, due to the large increase in triglyceride content. Conversely in this study we observed that when the nutrient content was low in the water column, the percentage lipid decreased. However, taking into account the total lipid content in the water column instead of the percentage lipid, we observed that the integrated total lipid content in the water column was 1.5 times higher in September than in December. In contrast, the percentage lipid in the water column was 2 times lower in September than in December (Fig. 2). In other words, there was a significant difference between total lipid content and the proportion of lipid present in the organic matter.

In this study we used the experimental approach of Fraga and Pérez (1990) and Laws (1991) to determine simultaneously the biochemical and elemental composition of the plankton, including hydrogen and oxygen, which cannot be measured directly, using three analytical methods (C&N, P and Cbh), or five if Si and Chl are included. The results obtained using our method compare fairly well with those results obtained by other authors with different analytical methods. It suggests that our equations are robust enough. Using the elemental composition data, a stoichiometric equation can be derived to calculate the ΔO_2 values (Ríos *et al*., 1989). Once the stoichiometric equations have been derived, the ratios $R_C=\Delta O_2/\Delta C$, $R_N=\Delta O_2/\Delta N$ and $R_P=\Delta O_2/\Delta P$ can be obtained. These ratios are of particular interest for primary production studies, especially when production is measured by means of inorganic nutrient uptake. By knowing the elemental composition of the organic matter, primary production can be expressed in the required units, so that direct comparisons can be made with data obtained by other authors. In addition, these R_C, R_N and R_P ratios are essential for the calculation of the conservative parameters of Broecker (1974). These parameters are useful both in studies of water mass characterisation

(Pérez *et al*., 1993) and the detection of red tides produced by the vertical migration of dinoflagellates (Fraga *et al*., 1992). Equally, the biochemical composition enables the physiological state of the plankton populations to be determined and from the Cbh/Lip ratio to predict the percentage of detritus in particular organic matter. This knowledge is useful and of considerable importance for commercial shellfish production.

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