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Particulate DNA and protein relative to microorganism biomass and detritus in the Catalano-Balearic Sea (NW Mediterranean) during stratification

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Abstract. Using microscopic and biochemical approaches, the relative contribution of the main groups of pelagic microorganisms (bacteria, heterotrophic nanoflagellates, phytoplankton and ciliates) and detritus (<150 μm) to total particulate protein and DNA was investigated at two stations of the Catalano-Balearic Sea (NW Mediterranean) during the stratified period. The two stations, one located in the shelf break front (S) and the other in the open sea, above the central divergence zone (D), were sampled twice in early summer 1993. Both of them showed a well-developed deep chlorophyll maximum (DCM). Maximum DNA concentrations were observed close to the DCM, while protein concentrations were fairly homogeneous from the surface to 60 m depth in all samplings. In general, the microorganism distribution showed maximum concentrations at or near the DCM depths. At both stations, bacteria were the most important contributors to living particulate DNA (22.5–32.6%), while phytoplankton and heterotrophic nanoflagellates were the main contributors to living particulate protein (3.8–24.4 and 2.9–29.1%, respectively). In addition, an important amount of detrital DNA and protein was estimated to occur at both stations. Detrital DNA accounted for 23.9–42.9% of the particulate DNA, while detrital protein represented from 63.5 to 84.7% of the particulate protein. Because both protein and DNA contain nitrogen and DNA is also a phosphorus source, these results indicate that heterotrophic organisms and detrital particles play an important role in the nitrogen and phosphorus cycles in the open sea waters of the NW Mediterranean.

Introduction

The proportion between heterotrophic and autotrophic organisms, the contribution of detrital material to the suspended organic matter, and the partitioning between the classic trophic chain and the microbial food web are basic questions about the structure and plankton dynamics in oceans. During the last 20 years, many studies have changed some tenets about these topics. For instance, in contrast to the classical idea of a plankton community sustained by a broad base of autotrophs, there is now increasing evidence that the microbial food web dominates in oligotrophic waters (Cho and Azam, 1987, 1990) and most biomass is heterotrophic (e.g. Dortch and Packard, 1989; Gasol *et al*., 1997). This suggests that, in oligotrophic ecosystems, the plankton community would have a higher trophic efficiency than in eutrophic ecosystems.

In addition, the proportion of detrital organic matter appears to be relatively high in oligotrophic areas (Winn and Karl, 1986; Boehme *et al.*, 1993; Caron *et al*., 1995). Detrital matter may play an important role in aquatic ecosystems because it serves as food for filter-feeding animals (Wotton, 1984; Posch and Arndt, 1996), contributes to the vertical transport of organic matter (Passow and Alldredge, 1994), and provides important substrates for nutrient remineralization and

carbon cycling (Long and Azam, 1996). However, data on the proportion of detritus to total particulate organic matter are still scarce.

In the oligotrophic open waters of the Catalano-Balearic Sea (NW Mediterranean), there are few data on the contribution of autotrophic and heterotrophic organisms and detritus to total suspended matter. In this area, the water column is stratified from early spring to late autumn and a deep chlorophyll maximum (DCM) is found at depths ranging from 40 to 90 m, coinciding with the nutricline (Estrada, 1985a,b; Estrada *et al.*, 1993). Several studies have provided data on the distribution of different groups of planktonic organisms in relation to the DCM and the associated structures of the water column (Alcaraz *et al.*, 1985; Estrada, 1985a; Algarra and Vaqué, 1989; Delgado *et al.*, 1992; Dolan and Marrasé, 1995; Calbet *et al.*, 1996). However, the relative contribution of particular planktonic groups to total particulate matter (TPM) has only been approached partially by Alcaraz *et al.* (1985). These researchers found that the ratio of phytoplankton biomass to TPM and phytoplankton to mesozooplankton biomass (in terms of particulate nitrogen and carbon) was lower in the Mediterranean than in upwelling areas, but there are still no data on the relative contribution of the components of the microbial community and detritus to the TPM in the Mediterranean Sea.

Particulate DNA and protein concentrations are often used as indicators of biomass (i.e. living organisms), but there is evidence that in natural environments DNA and protein represent both living and non-living (detrital or non-replicating) particulate matter (Holm-Hansen, 1969b; Paul *et al.*, 1985; Winn and Karl, 1986; Bailiff and Karl, 1991; Boehme *et al.*, 1993). DNA and protein are two important macromolecules in the cell. DNA constitutes the genetic material and varies within a factor of two in eukaryotic cells (Berdalet *et al.*, 1992, 1994); proteins, which constitute >50% of the dry weight in cells (Lehninger, 1974), vary with nutrient (fundamentally nitrogen) availability (Dortch, 1982; Dortch *et al.*, 1984, 1985). Furthermore, because of their chemical composition, both molecules participate in the dynamics of nitrogen in the aquatic environment and DNA participates, in addition, in that of phosphorus. Additionally, particulate protein has nutritional implications for animal feeding, because this compound provides a potential nutritive value.

The objective of the present work was to investigate the relative contribution of the autotrophic and heterotrophic components of the microbial community and of detritus to the suspended DNA and protein. This objective was approached by a combination of microscopic and biochemical techniques. The importance of the different DNA and protein partitioning is discussed in the context of nutrient cycles.

Method

Field sampling

The study was carried out in the Catalano-Balearic Sea, during the VARIMED 1993 cruise on board the RV 'Hespérides'. A transect, perpendicular to the coast of Catalonia (Figure 1), was repeatedly surveyed between 1 and 8 June 1993 for the estimation of hydrographic parameters and chlorophyll *a* concentration. Two

stations, one (S) located in the shelf break front (41°06′N, 2°23′E) and another (D) in the open sea (40°38′N, 2°45′E), above the central divergence zone of the Catalano-Balearic Sea, were sampled twice to perform biochemical profiling and other biological studies (Figure 1). Stations S and D were located ~30 and 85 km offshore, respectively; sampling dates were 10 and 14 June for station S, and 11 and 15 June for station D.

At each station, a CTD cast was performed with a Neil Brown Mark III probe down to 1000 m, to obtain profiles of temperature, salinity and sigma-t. Water samples for major inorganic nutrients, oxygen and chlorophyll *a* were obtained with a rosette of Niskin bottles, at 10 m intervals between 0 and 100 m, and at larger intervals between 100 and 400 m. Aliquots for phytoplankton identification and estimation of ciliates, autotrophic and heterotrophic flagellates, and bacterial abundance were taken down to 100 m (except for heterotrophic flagellates on 11 June). Samples for protein and DNA analyses were obtained at six selected depths (between 0 and 100 m). More details on the sampling strategy and the basic data of the cruise can be found in Masó and Grupo VARIMED (1995).

Chlorophyll analysis and plankton counts

Chlorophyll *a* concentration was estimated fluorometrically (Yentsch and Menzel, 1963). Water samples (50–100 ml) were filtered through Whatman GF/F (25 mm) glass fiber filters, which were ground in 90% acetone and left to extract for 30 min in the dark at room temperature. The fluorescence of the extract was measured with a Turner Designs fluorometer.

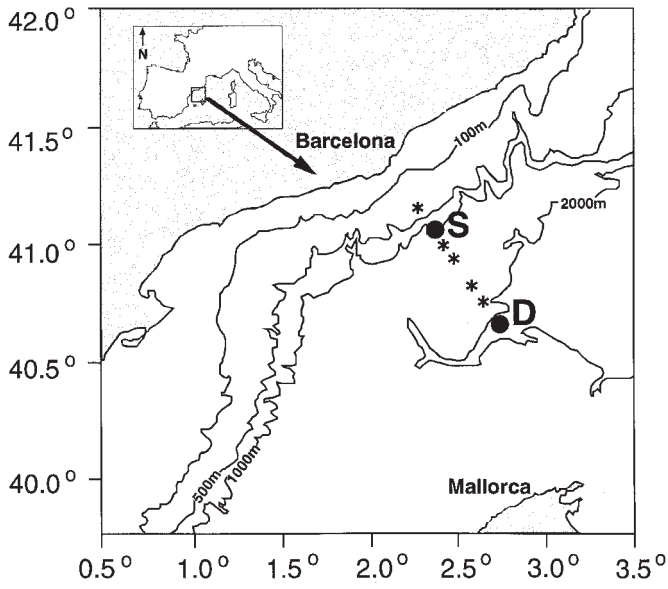

Fig. 1. Main transect studied in the VARIMED 93 cruise during June. S and D indicate the location of the shelf break front and the open sea station, respectively.

Bacterial samples were fixed with formaldehyde (4% final concentration) and flagellate samples with glutaraldehyde (1% final concentration). Samples for determination of bacterial (15 ml) and flagellate (50 ml) abundance were filtered through 0.2 and 0.8 μm pore diameter black polycarbonate filters, respectively. Both types of samples were stained with the DNA-specific fluorescent stain DAPI and counted with an epifluorescence microscope (Porter and Feig, 1980). Phototrophic and heterotrophic nanoflagellates (PNF and HNF, respectively) were counted separately. Samples for phytoplankton counts (100 ml) were fixed with a formalin–hexamine solution (Throndsen, 1978) and examined in an inverted microscope (Utermöhl, 1958) as described in Estrada (1985a). The observed organisms (mainly diatoms, dinoflagellates and coccolithophorids) were classified to the lowest possible taxonomic level and sorted into three size classes (<10 μm, 10–20 μm and >20 μm). Ciliate samples were preserved with acid Lugol's (1–2% final concentration). Information regarding the ciliate population can be found in Dolan and Marrasé (1995).

Table I. Mean DNA concentration per cell (± SE) for the main groups of microorganisms. The average DNA value (using all studies for a given group) was used for the calculations

Group		DNA (pg cell^{-1})	*N*	Reference
Bacteria		0.0025		Simon and Azam, 1989
Phytoplankton	PNF	0.6 ± 0.2	12	Holm-Hansen, 1969a Wallen and Geen, 1971 Cattolico and Gibbs, 1975 de Madariaga and Joint, 1990 Boucher *et al.*, 1991 A.Fara and E.Berdalet (unpublished)
	<10 μm	1.1 ± 0.6	3	Wallen and Geen, 1971 Holm-Hansen, 1969a* A.Fara and E.Berdalet (unpublished)
	10–20 μm	8.5 ± 2.4	13	Rizzo and Noodén, 1973 Holm-Hansen, 1969a Galleron and Durrand, 1978 Berdalet *et al.*, 1994 Oku and Kamatawi, 1995 A.Fara and E.Berdalet (unpublished) Boucher *et al.*, 1991
	20–150 μm	82.1 ± 16.4	14	Strickland *et al.*, 1969 Holm-Hansen, 1969a Werner, 1971 Rizzo and Noodén, 1973, 1974 Haapala and Soyer, 1974 Tappan, 1980 Cembella and Taylor, 1985 Boucher *et al.*, 1991
HNF		0.6 ± 0.2	12	Idem references as PNF
Ciliates		10	1	Mandel, 1967

N, number of data points; PNF, phototrophic nanoflagellates; HNF, heterotrophic nanoflagellates.
*Data from *Navicula pelliculosa* were excessively low compared to the average and were not included for our calculations.

Table II. Mean protein concentration per cell (± SE) for the main groups of microorganisms. The average protein value (using all studies for a given group) was used for the calculations

Group		Protein (pg cell^{-1})	*N*	Reference
Bacteria		0.0177		Simon and Azam, 1989
Phytoplankton	PNF	25.9 ± 6.7	29	Moal *et al*., 1987*
				Thompson *et al.*, 1992
				Madariaga and Joint, 1990
				Montagnes *et al.*, 1994
	<10 μm	16.6 ± 6.6	15	Thompson *et al.*, 1992
				Dortch, 1982
				Moal *et al.*, 1987
				Montagnes *et al.*, 1994
	10–20 μm	271.2 ± 38.4	24	Berdalet *et al.*, 1994
				Conover, 1975
				Moal *et al.*, 1987
				Montagnes *et al.*, 1994
				A.Fara and E.Berdalet (unpublished)
	20–150 μm	933.5 ± 330.6	10	Moal *et al.*, 1987*
				Montagnes *et al*., 1994
HNF		25.9 ± 6.7	29	Idem references as PNF

N, number of data points; PNF, phototrophic nanoflagellates; HNF, heterotrophic nanoflagellates.
*Data from *Cryptomonas* sp. and *Coscinodiscus wailesii* were excessively high compared to the average and were not included for our calculations.

Protein and DNA determination

For particulate protein and DNA determinations, 4 l of sea water were passed through a 150 μm mesh and then vacuum filtered (at a pressure of <100 mmHg) on pre-combusted (350°C for 24 h) Whatman GF/F (47 mm) glass fiber filters which were immediately frozen in liquid nitrogen. Protein quantification was carried out using the Lowry *et al.* (1951) method. DNA was determined using a DNA-specific stain (Hoechst 33258) as described in Berdalet and Dortch (1991); DNA values were divided by 1.46, the conversion factor proposed by Fara *et al*. (1996), to correct for the overestimation of DNA resulting from the use of this method. Bovine serum albumin and DNA from calf thymus were used as protein and DNA standards, respectively.

To investigate the contribution of the different groups of microorganisms to total DNA and protein, values of DNA and protein content per cell taken from the literature (Tables I and II) were multiplied by the average abundance of each group at three layers of the water column [above the DCM, at the DCM (chlorophyll > 0.6 μg l^{-1}) and below the DCM], on the two sampling days at each station.

Four groups of microorganisms were evaluated: bacteria, phytoplankton (including the PNF counted by epifluorescence), HNF and ciliates (fundamentally heterotrophic; see Dolan and Marrasé, 1995).

For bacterial DNA and protein content per cell, we used the conversion factors given by Simon and Azam (1989) for 0.05 μm^3 bacteria, which corresponds to the average bacterial volume found in this cruise (Massana *et al.*, 1997). The phytoplankton group was classified into four classes (PNF, <10 μm, 10–20 μm and 20–150 μm), and the average DNA and protein content per cell of each class (using all values for a given class listed in Tables I and II) was used for the

calculations. Owing to the lack of DNA and protein data for HNF, we assumed values of DNA and protein per cell equal to PNF. No data are available on the protein content of ciliates and only one value was found on DNA content.

Detrital DNA and protein were estimated by the difference between the calculated and the analyzed DNA and protein respectively. Hereafter, the terms calculated DNA and protein will be used for the estimated amounts of DNA and protein in the different groups of microorganisms, and analyzed DNA and protein to refer to the results of the biochemical analyses of seston.

As some bacteria can pass through GF/F filters (0.7 μm nominal pore size), we carried out tests to estimate possible bacterial losses. Mediterranean water samples were filtered through GF/F filters and the bacterial abundance in the filtrates were estimated by flow cytometry (Gasol and Morán, 1999) and epifluorescence microscopy. We determined that an average of 14.3% of the total bacteria passed through the GF/F filters and we used this result to correct the bacterial contribution to the analyzed DNA and protein.

Results

Hydrographic conditions

The S and D stations presented a similar vertical distribution of temperature, salinity and sigma-t during the sampling period (Figure 2). Thermal stratification was observed at both stations, but was stronger at D; the maximum temperature gradient occurred between 10 and 25 m depth (Figure 2A and B).

Distribution of the biochemical parameters and the main groups of microorganisms

A DCM was observed between 40 and 50 m depth at the two stations throughout the sampling period (Figure 3A). At both stations, the chlorophyll *a* concentration at the DCM was >1 μg l^{-1} and maximum DNA values were observed close to the DCM and generally above it (Figure 3B). DNA concentrations ranged from 0.45 to 3.30 μg l^{-1} at station S, and from 0.72 to 4.22 μg l^{-1} at station D. The highest protein concentrations were recorded between the surface and 60 m depth at both stations (Figure 3C), without a deep maximum (station S) or with only a slight increase at or above the DCM depth (station D). Protein concentration ranges were higher at station S (21.1–215.9 μg l^{-1}) than at station D (7.8–133.7 μg l^{-1}). Phytoplankton, bacteria, heterotrophic flagellates and ciliates tended to present population peaks at or above the DCM (Figure 4). However, phytoplankton vertical distribution differed from others. Phytoplankton abundance in surface waters was much lower than at the DCM, while heterotrophic organisms were abundant not only at or near the DCM depth, but also in the upper layers.

Contribution of microorganisms and detritus to DNA and protein

The distribution of calculated and analyzed DNA values observed at the three layers of the water column (above, at the DCM and below it) was similar at

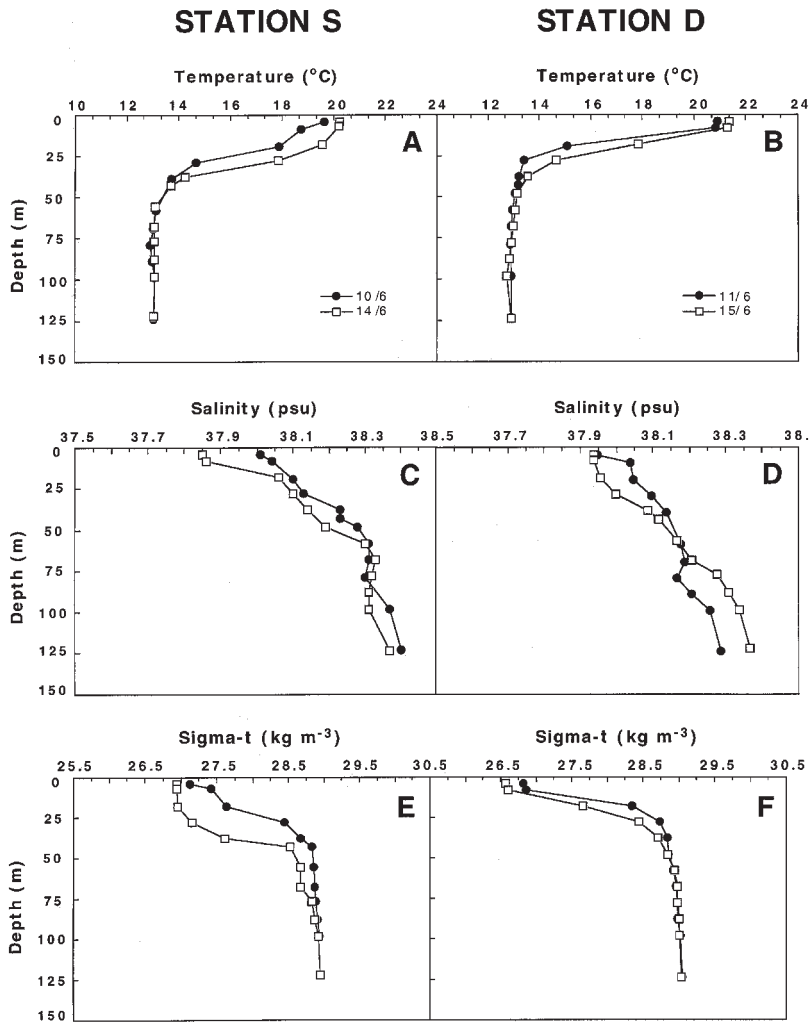

Fig. 2. Vertical distribution of temperature, salinity and sigma-t at stations S (**A**, **C**, **E**) and D (**B**, **D**, **F**).

stations S and D (Table III). Both analyzed and calculated DNA showed a slight increase at the DCM level and the lowest values of them were found below this level. The highest calculated protein at station S (Table IV) was found at the DCM, while the highest analyzed protein at this station was found above the DCM. At station D, the maximum of analyzed and calculated protein was observed at the DCM level.

At both stations, the heterotrophic (i.e. bacteria + HNF + ciliates) and detrital fraction accounted for 70–87 and 77–96% of the analyzed DNA and protein, respectively (Tables III and IV). In the case of DNA, the main contributor was the bacterial group (23–33%), while for protein the detrital fraction represented

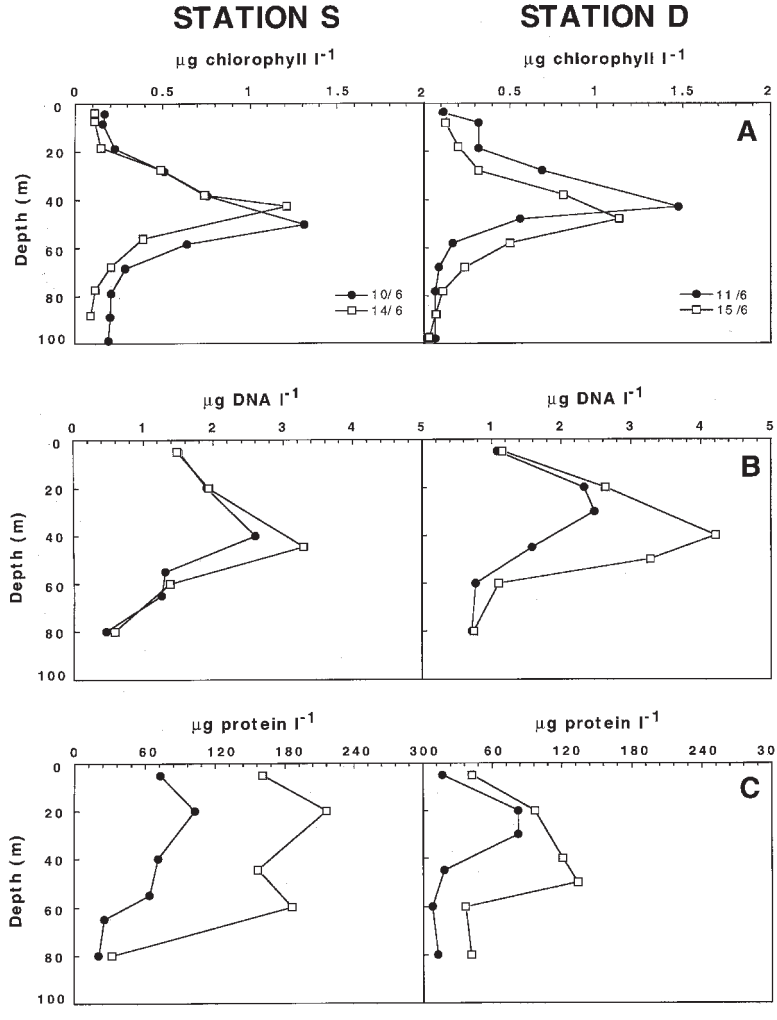

Fig. 3. Vertical distribution of chlorophyll *a* (**A**), DNA (**B**) and protein (**C**) (all in µg l^{-1}) at stations S and D.

the main protein pool (51–85%) at the three layers of the water column. Less than 30% of the analyzed DNA and protein corresponded to the HNF group, and the ciliate contribution to analyzed DNA was only between 0.2 and 0.5%. The contribution from the autotrophic fraction in terms of DNA and protein was <30% and <25%, respectively.

Discussion

The hydrographic conditions (Figure 2) and the vertical chlorophyll profiles (Figure 3A), which showed a well-developed DCM, were characteristic of the stratification period in the study area (Delgado *et al.*, 1992; Estrada *et al.*, 1993).

Table III. Analyzed and calculated DNA (μg l^{-1}) and DNA contribution (as a percentage) of the main groups of microorganisms and detrital DNA to the total analyzed DNA at stations S and D. In all cases, the given data constitute the average of the parameters obtained at the depths above, at and below the DCM, on the two sampling dates at each station. Phototrophic nanoflagellates (PNF) were included in the phytoplankton group

	DNA (μg l^{-1})		% DNA				
	Analyzed	Calculated	Bacteria	Phytoplankton	HNF	Ciliates	Detritus
Station S							
Above DCM	1.71	1.02	30.3	12.9	16.4	0.2	40.3
DCM	2.40	1.51	23.9	26.7	12.1	0.3	37.1
Below DCM	0.91	0.55	25.7	30.0	4.8	0.5	39.0
Station D							
Above DCM	1.80	1.37	32.6	20.8	22.4	0.3	23.9
DCM	2.89	1.79	22.5	30.4	8.8	0.2	38.2
Below DCM	0.84	0.48	23.7	28.6	4.6	0.2	42.9

HNF, heterotrophic nanoflagellates.

Table IV. Analyzed and calculated protein (μg l^{-1}) and protein contribution (as a percentage) of the main groups of microorganisms and detrital protein to the total analyzed protein at stations S and D. In all cases, the given data constitute the average of the parameters obtained at the depths above, at and below the DCM, on the two sampling dates at each station. Phototrophic nanoflagellates (PNF) were included in the phytoplankton group

	Protein (μg l^{-1})		% protein			
	Analyzed	Calculated	Bacteria	Phytoplankton	HNF	Detritus
Station S						
Above DCM	137.56	21.01	2.7	3.8	8.8	84.7
DCM	97.17	35.50	4.2	19.5	12.9	63.5
Below DCM	66.05	10.29	2.5	10.2	2.9	84.4
Station D						
Above DCM	59.80	30.74	6.9	12.8	29.1	51.1
DCM	88.73	37.19	5.2	24.4	12.3	58.1
Below DCM	24.73	9.44	5.7	23.6	6.9	63.9

HNF, heterotrophic nanoflagellates.

The DCM results not only from a higher content of chlorophyll *a* per cell, due to photoacclimation (Jensen and Sakshaug, 1973; Latasa *et al.*, 1992), but also from an increase in phytoplankton abundance (Figure 4A). Our measures of DNA were in the range reported in other oligotrophic areas (i.e. mean chlorophyll < 1.5 μg l^{-1}; mean DNA values < 2 μg l^{-1}), including previous studies conducted in the Catalano-Balearic Sea (Berdalet and Estrada, 1993; Table V). Measured protein values, reported here for the first time in the Catalano-Balearic Sea, were close to those found in other oligotrophic marine areas (i.e. mean chlorophyll < 1.5 μg l^{-1}; mean protein between 30 and 130 μg l^{-1}) including other oligotrophic NW Mediterranean waters (Nival *et al.*, 1976; Table VI).

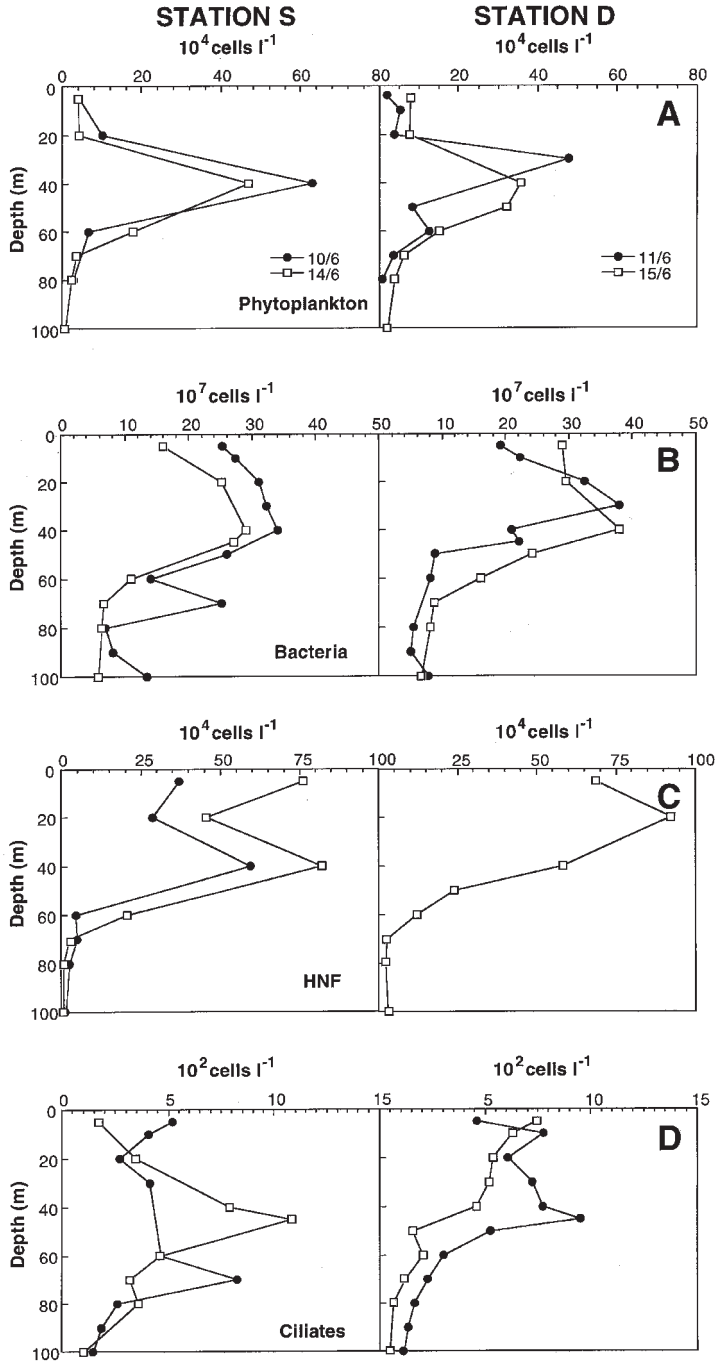

Fig. 4. Vertical profiles of phytoplankton (**A**), bacteria (**B**), heterotrophic nanoflagellates (HNF) (**C**) and ciliates (**D**) (cells l^{-1}) at stations S and D.

Table V. Mean (± SE) and range of chlorophyll and DNA concentrations (both in μg l^{-1}) in different marine ecosystems and in this study. Note that when mean chlorophyll is <1.5 μg l^{-1}, mean DNA values are <2 μg l^{-1}

Source	Location	Chl mean ± SE	Chl range	DNA mean ± SE	DNA range	*N*
Takahashi *et al.*, 1974	North Pacific Ocean	0.45 ± 0.28	0.02–7.11	1.65 ± 0.22	0.13–5.24	25
Dortch *et al.*, 1985	NE Pacific Ocean	5.10 ± 1.10	1.62–11.40	4.88 ± 0.71	2.10–10.6	10
Paul *et al.*, 1985	Gulf of Mexico (nearshore, interm. and offshore stations)	2.24 ± 0.95	0.002–12.80	10.24 ± 2.72	0.16–42.6	16
Fabiano *et al.*, 1993	Ross Sea (Antarctica) photic layer	1.87 ± 0.60	0.46–4.66	31.59 ± 6.46	10.90–63.20	9
Berdalet and Estrada, 1993	NW Mediterranean Sea May 1989	0.65 ± 0.14	0.15–2.18	1.09 ± 0.13	0.46–2.55	21
	February 1990	0.86 ± 0.07	0.07–1.62	1.32 ± 0.09	0.16–2.77	30
This study	NW Mediterranean Sea	0.40 ± 0.09	0.04–1.43	1.73 ± 0.21	0.45–4.22	23

N, number of data points.

Table VI. Mean (± SE) and range of chlorophyll and protein concentrations (both in μg l^{-1}) in different marine ecosystems and in this study. Note that when mean chlorophyll is <1.5 μg l^{-1}, mean protein ranges between 30 and 130 μg l^{-1}

Source	Location	Chl mean ± SE	Chl range	Protein mean ± SE	Protein range	*N*
Takahashi *et al.*, 1974	North Pacific Ocean	0.45 ± 0.28	0.02–7.11	48.70 ± 9.01	11.40–230.00	25
Packard and Dortch, 1975	North Atlantic Ocean					
	Oceanic stations	0.23 ± 0.03	0.02–0.64	39.98 ± 5.58	5.04–135.24	30
	Upwelling stations	2.42 ± 0.31	0.43–4.02	141.68 ± 19.37	36.12–297.36	12
Nival *et al.*, 1976	NW Mediterranean Sea (Villefranche-Sur-Mer Bay)	0.66 ± 0.12	0.12–1.60	107.19 ± 10.01	60.00–200.00	16
Mayzaud and Taguchi, 1979	Bedford Basin	3.27 ± 0.72	0.30–7.06	437.38 ± 48.18	185.00–604.00	8
Hendrickson *et al.*, 1982	Peruvian upwelling	2.25 ± 0.35	0.50–5.77	306.9 ± 37.53	82.00–764.00	20
Fabiano *et al.*, 1984	Ligurian Sea	0.40 ± 0.04	0.17–0.49	95.82 ± 6.00	72.00–121.20	8
Dortch *et al.*, 1985	NE Pacific Ocean	5.10 ± 1.10	1.62–11.4	104.20 ± 19.30	28.56–204.12	10
Dortch and Packard, 1989	Washington coast					
	Oligotrophic stations	0.52 ± 0.09	0.15–1.03	133.63 ± 27.05	64.29–386.42	11
	Eutrophic stations	21.88 ± 8.62	7.72–63.74	496.60 ± 136.55	266.21–1158.16	6
Bode *et al.*, 1990	Cantabrian Sea	1.12 ± 0.15	0.13–2.68	31.79 ± 3.43	13.15–79.28	18
Fabiano *et al.*, 1993	Ross Sea (Antarctica)					
	photic layer	1.71 ± 0.56	0.23–4.66	169.6 ± 49.18	41.00–485.20	10
This study	NW Mediterranean Sea	0.40 ± 0.09	0.04–1.43	78.29 ± 12.48	7.80–215.90	23

N, number of data points.

Compared to chlorophyll, DNA presented a smoother deep maximum near the DCM (Figure 3A and B). Nevertheless, phytoplankton DNA contribution was also high below the DCM (Table III). This was due to a different size distribution of the phytoplankton cells in the water column. At the DCM level, around 80% of the phytoplankton cells corresponded to PNF (with the lowest DNA cell content; Table I), while below this level bigger cells (>10 μm) were more abundant (>36%). Analyzed protein showed a different vertical distribution than chlorophyll (Figure 3A and C) with high values throughout the euphotic zone, suggesting a low protein contribution from autotrophic organisms in the shallow waters. In addition, above the DCM, the phytoplankton protein contribution was the lowest compared with the other levels of the water column (Table IV).

We estimated that bacteria were the main contributors to the living DNA at the three layers of the water column, while most living protein was accounted for by phytoplankton or HNF, depending on the level of the water column (Tables III and IV). In other systems, a major contribution of bacteria to total DNA has been reported by Paul and Myers (1982), Paul and Carlson (1984) and Paul *et al.* (1985). In addition, Boehme *et al.* (1993) found that bacterioplankton provided the largest contribution (>50%) to the particulate (>0.2 μm) DNA pool, whereas phytoplankton accounted for an average of 8% in the subtropical waters from the Southeastern Gulf of Mexico. In contrast, in terms of protein, we found that bacterioplankton would be a small contributor (3–7% of the total protein), probably as a result of the high nucleic acid:protein ratio found in small organisms (Skjoldal, 1993).

Thus, depending on which biochemical component is considered as an indicator of biomass, the contribution of the different groups of microorganisms varied. However, the heterotrophic biomass (bacteria + HNF + ciliates), in terms of both DNA and protein, generally exceeded that of the autotrophs. This result supports the idea that heterotrophy and the microbial food web dominate in oligotrophic planktonic ecosystems, in contrast to eutrophic systems with a much higher proportion of autotrophic biomass (Cho and Azam, 1987; Dortch and Packard, 1989; Gasol *et al*., 1997).

Assuming the difference between analyzed and calculated protein as detritus, our approach also revealed a high fraction of detrital protein (>50% of the total), whereas the detrital DNA fraction constituted between 24 and 43% of the total. In eukaryotic cells, protein content is variable and depends on parameters such as nutrient concentration—particularly nitrogen (Dortch *et al.*, 1984, 1985; Berdalet *et al.*, 1994)—light (Wallen and Geen, 1971) or stage of growth (Moal *et al.*, 1987). On average, the protein content ranges within a factor of two under different physiological or environmental conditions (Moal *et al.*, 1987; Berdalet *et al.*, 1994). Even using twice the amount of protein concentration per cell reported in Table IV for phytoplankton and HNF groups (although values in this table reflect different physiological stages of cells), the detrital protein would still account for a very high percentage of the analyzed protein (41% on average). In addition, given the lack of data on the protein content of ciliates, assuming values similar to those reported for dinoflagellates, ciliates would only account for <0.5% of the protein. For cyanobacteria (data not available in our study), we have

estimated that they would have contributed up to 7 and 1.5% of the particulate DNA and protein, respectively, based on the data of a previous cruise in this area (Algarra and Vaqué, 1989) and assuming the same cellular content as bacteria.

The fact that detrital protein may be an important part of the suspended organic matter has recently been considered by Long and Azam (1996). Using a protein stain, they visualized proteinaceous particles which appeared to constitute an important fraction of the marine snow in natural sea water. With regard to the detrital DNA, its existence was noted as early as this compound was measured in sea water (Holm-Hansen, 1969b; Falkowski and Owens, 1982; Winn and Karl, 1986; Boehme *et al*., 1993). Most of these studies were concerned with biomass, but Winn and Karl (1986) found that non-replicating DNA comprised 75–90% of the total DNA in various locations in the oligotrophic Pacific Ocean. Our data suggest that detritus, in this case in the form of either DNA or protein, constitutes an important component of particulate matter in the oligotrophic NW Mediterranean in summer. Whether the proportion of detrital protein and DNA is higher in the NW Mediterranean than in other oligotrophic areas is not known. Using a similar indirect approach (i.e. based on particulate carbon and nitrogen conversion factors), in the Sargasso Sea, Caron *et al.* (1995) estimated that the detrital fraction accounted for 76 and 70% (August 1989) and 45 and 37% (March–April 1990) of the total carbon and nitrogen, respectively. Both this study and our present work reveal the importance of detritus in the oligotrophic food webs, indicating that a major effort is required in quantifying this fraction.

Both detrital protein and detrital DNA may be an important source of organic nitrogen and phosphorus to bacteria and zooplankton in the open sea waters of the NW Mediterranean, where inorganic nutrients are almost undetectable in the euphotic zone during the stratification period. Thingstad and Rassoulzadegan (1995) and Thingstad *et al.* (1998) argued that the microbial food web in Mediterranean surface waters is characterized by phosphorus limitation of both phytoplankton and bacteria during the stratified period. In this cruise, very low bacterial production values (between 0.5 and 1.2 μg C l^{-1} day^{-1}; Pedrós-Alió *et al.*, 1999) were found. Thus, we can hypothesize that the utilization of detrital material by the heterotrophic bacteria, limited by phosphorus, occurs at low rates, and therefore a major part of this material is available to grazers; this food source could account for the high zooplankton:phytoplankton biomass ratio found by Alcaraz *et al.* (1985).

In other words, in the stratification period, the detrital fraction with low sedimentation rates, and degraded slowly by bacteria, can constitute a reservoir of nutritive particles, which can be used by predators. Therefore, we conclude that quantification of the detrital fraction, especially in periods of low nutrient availability, should be routinely considered in studies addressing nutrient and carbon fluxes in marine systems.

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