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Quantitative relationships between phytoplankton, bacteria and protists in an Aegean semi-enclosed embayment (Maliakos Gulf, Greece)

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ABSTRACT: The seasonal variations of temperature, dissolved organic carbon (DOC), chlorophyll *a* (chl *a*), and bacterial and protistan abundance were investigated in an enclosed Eastern Mediterranean embayment over an 8 mo period. DOC levels in the gulf were high, likely due to allochthonous input through freshwater discharge. However, after the end of spring, when allochthonous input was minimal, bacterial abundance was linearly related to chl *a* and DOC, suggesting that during this period the remaining DOC pool (probably autochthonous DOC) was important. Bacterial abundance was significantly correlated with the biomass of the phytoplankton at the end of spring and throughout summer. A correspondence of protistan abundance with bacteria, especially during the warm months, when the phytoplankton biomass was low, suggests that the microbial loop is the dominant component of the food web structure during the oligotrophic period of the year.

KEY WORDS: Phytoplankton · Bacteria · Protists · Microbial loop · Coastal waters · Mediterranean

INTRODUCTION

The role of the microbial loop (Pomeroy 1974, Azam et al. 1983) in the aquatic environment is now well established. The microbial loop may link microbial biomass with higher trophic levels (Sherr et al. 1986, Sherr & Sherr 1988, Pomeroy 1991) or give rise to an accumulation of microbial biomass (Ducklow et al. 1986). Bacteria, as the first biological component of the microbial loop, are controlled either by substrate limitation (bottom-up control) or by grazing pressure (top-down control), primarily from protists. Thus, the investigation of the relationships between the members of the microbial loop—phytoplankton, bacteria, microzooplankton—allows us to understand better the transfer of energy in those marine ecosystems where the microbial food web is of importance at least for a certain period of the year.

The existence of a linear relationship between chlorophyll and bacterial abundance was first reported more than a decade ago (Bird & Kalff 1984). Since then many studies have supported the concept that bacterial abundance and productivity in aquatic environments are linearly dependent on phytoplankton biomass and productivity (Cole et al. 1988 and references therein), assuming that the major part of organic substrate for bacteria is provided by phytoplankton cells. On the other hand, there are cases, especially in coastal environments, where phytoplankton and bacteria are uncoupled due to temperature and/or substrate limitations (Painchaud & Therriault 1989, Hoch & Kirchman 1993, Cho et al. 1994 and references therein).

There are only few available data regarding the components of the microbial loop and the role of microbes in the trophic webs of Greek coastal waters (Kouvaraki et al. 1993, Tsirtsis 1994). This work is a preliminary study of the relationships between phytoplankton biomass, expressed as chlorophyll *a*, and bacterial and protistan abundance in a semi-enclosed embayment in the western part of the Aegean Sea in the period between March and October when the gulf is characterised by meso- to oligotrophic conditions.

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Some of the most important factors which might influence population abundance and distribution are considered.

MATERIALS AND METHODS

Maliakos Gulf (Fig. 1) covers ca 110 km^2 and it is divided by 2 headlands into 2 parts. In the SW end of the gulf, Spercheios River, one of the major rivers in Greece, meets the sea. The western part forms a basin with a maximum depth of 27 m, while closer to the river mouth, depth does not exceed 10 m. On the eastern side, the gulf is connected to the Aegean Sea through the Orei Channel and the North Evoikos Gulf through the Knimida Channel. This part has an average depth of 36 m.

Sampling was conducted from March to October 1996 on a monthly basis at 3 stations in Maliakos Gulf (Fig. 1): in the shallow southwest area influenced by the Spercheios River (inner compartment, Stn I at 7 m), in the outer gulf influenced more by the Aegean Sea (outer compartment, Stn O at 23 m) and in the intermediate basin (middle compartment, Stn M at 23 m). Samples for dissolved organic carbon (DOC), chlorophyll *a* (chl *a*) and bacterial counts were collected from the surface, 1, 5, 10 and 20 m for the outer and middle stations and the surface, 1 and 5 m for the inner station. Samples for protistan abundance were taken from 1, 10 and 20 m for the outer and middle stations and 1 and 5 m for the inner one. A general description of the seasonal pattern of physical, chemical and biological parameters is in preparation (K. A. Kormas, A. Nicolaidou & M. Thessalou-Legaki unpubl.).

Temperature was measured by a Seabird CTD. Water samples were collected with a 5.5 l Limnos bottle. This volume was adequate for all analyses reported here. Subsamples for DOC were placed in microcentrifuge tubes, immediately frozen and kept frozen until analysis. Prior to use, the tubes were soaked in 20% HCl overnight and then were rinsed 3 times with double distilled water and finally with HPLC purified water (Sharp et al. 1993, Wangersky 1993, F. Azam pers. comm.). The HPLC water was used as the analytical blank and gave 60 to 70 µg C l^{-1}. DOC concentration was determined using high-temperature catalytic oxidation (HTCO; Sugimura & Suzuki 1988) in a Dohrmann DC-190 analyser after filtering the samples with a Mitex 0.5 µm pore size filter (Millipore), which is suitable for the removal of particulate organic substances from aqueous solutions. A volume of 100 µl of sample was used for each injection. For every sample at least 5 replicates were injected. The resulting values varied less than 10% of the average. The same procedure was conducted for the blanks. The calibration was performed by standard solutions of phthalate (1, 10 and 40 mg C l^{-1}) for total carbon provided by Dohrmann Inc. and Na_2CO_3 (analytical grade; 10 and 100 mg C l^{-1}) for inorganic carbon.

Volumes of 0.7 to 1.3 l of seawater were collected in polyethylene bottles for filtration on Whatman GF/C filters (1.2 µm nominal pore size) for the determination of chl *a* using the acetone extraction method as described by Parsons et al. (1984). Although GF/F filters (~0.7 µm retention capacity) would have been more appropriate for the purpose of this study, GF/C filters (~1.2 µm retention capacity) were used to enable comparison with data from our previous studies. In addition, tests were conducted using both filters in February, May, August and November and no statistical difference was found (Kormas unpubl.). GF/C filters have been used for microbial studies by other authors (Hewes et al. 1990) who considered them reliable for chl *a* measurements.

Bacterial abundance was determined after fixing 10 ml of seawater with 0.5 ml of borax buffered 37%

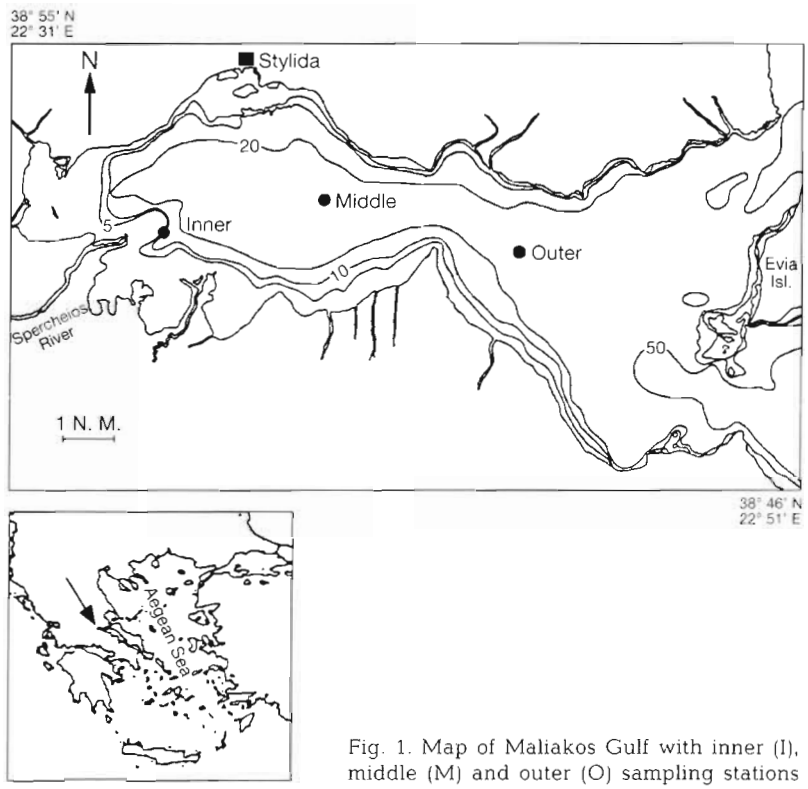

Fig. 1. Map of Maliakos Gulf with inner (I), middle (M) and outer (O) sampling stations

formaldehyde after a few days of fixation. Samples were filtered, onto a 0.2 µm diameter pore size Millipore black isopore polycarbonate filter and stored frozen until counting to prevent cell loss due to storage as recommended by Turley & Hughes (1992). Bacterial cells were counted using the fluorochrome DAPI on a Leitz invertoscope under UV excitation (Hobbie et al. 1977, Porter & Feig 1980).

A 2 l subsample was used for protistan enumeration and identification down to the family level. Specimens were preserved by adding borax buffered formalin to a final concentration of 4%. The sample was concentrated to a volume of 100 ml by filtering at low pressure (ca 150 mm Hg) through membrane filters (Millipore white isopore polycarbonate) of 5 µm diameter pore size. The organisms remaining on the filter were then washed very gently into a 100 ml settling chamber using a fine tipped glass pipette. After fixation and concentration the sample was allowed to settle for 24 to 48 h using the Utermöhl (1958) method. The samples were examined with an inverted-phase Zeiss microscope at a magnification of 100×. For the identification of the organisms the work of Tregouboff & Rose (1957) was used.

The effect of sampling time and depth was investigated using 2-way ANOVA on log-transformed data. Correlations between the parameters measured were tested using a Spearman rank correlation coefficient while the relationship between bacteria and protists with the remaining parameters was examined by stepwise multiple regression. The above analyses were carried out with the Statistica software package. For the Principal Components Analysis (PCA) the package Statgraphics was used.

RESULTS

Temperature (Fig. 2) showed the expected seasonal pattern with lowest values (11.28°C) in March and highest in June (27.00°C). No thermocline developed at the inner station. At the other 2 stations, a thermocline was developed from April until June with a maximum difference of 8°C between bottom (20 m) and surface waters. DOC (Fig. 2) showed significant seasonal variation, mainly at the outer station (2.37 to 23.3 mg l^{-1}). At the other 2 stations, the range was similar (2.04 to 20.33 mg l^{-1} for the middle and 2.62 to 15.55 mg l^{-1} for the inner compartment) although the seasonal variability was smaller. At all stations higher values occurred between March and July. To investigate the possible allochthonous origin of DOC, regressions of DOC versus salinity were carried out (Fig. 3). A decrease of DOC concentrations with increasing salinity (conservative mixing) were detected only at the inner station, suggesting the riverine origin of DOC.

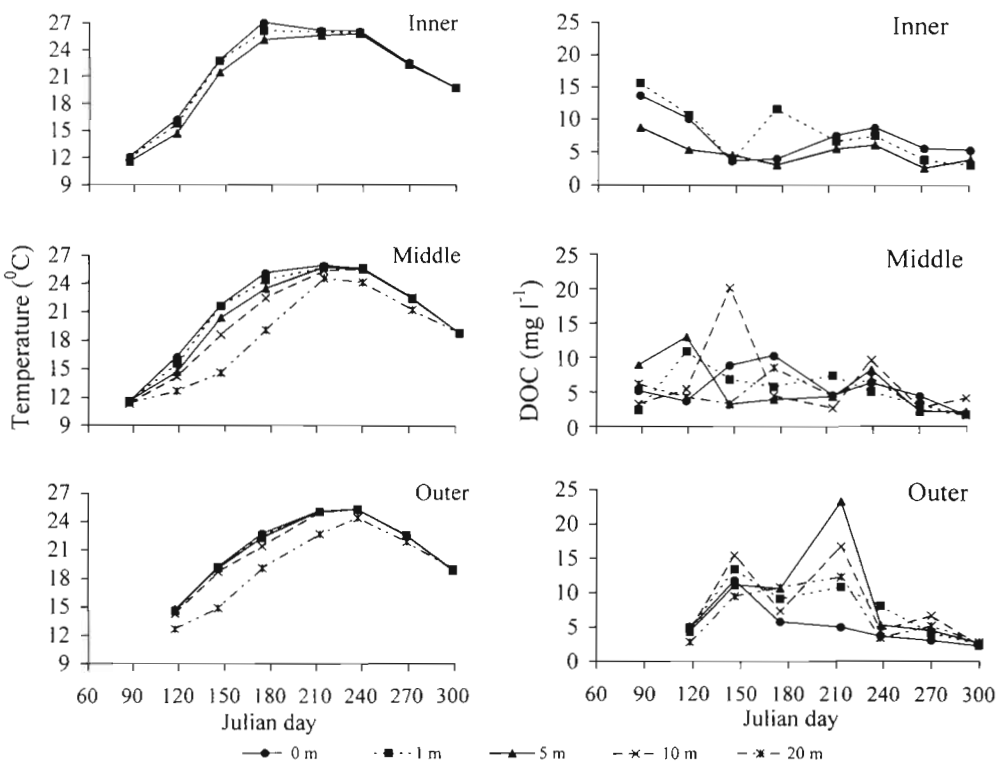

Fig. 2. Seasonal variations of temperature and dissolved organic carbon (DOC) at all stations and depths in Maliakos Gulf

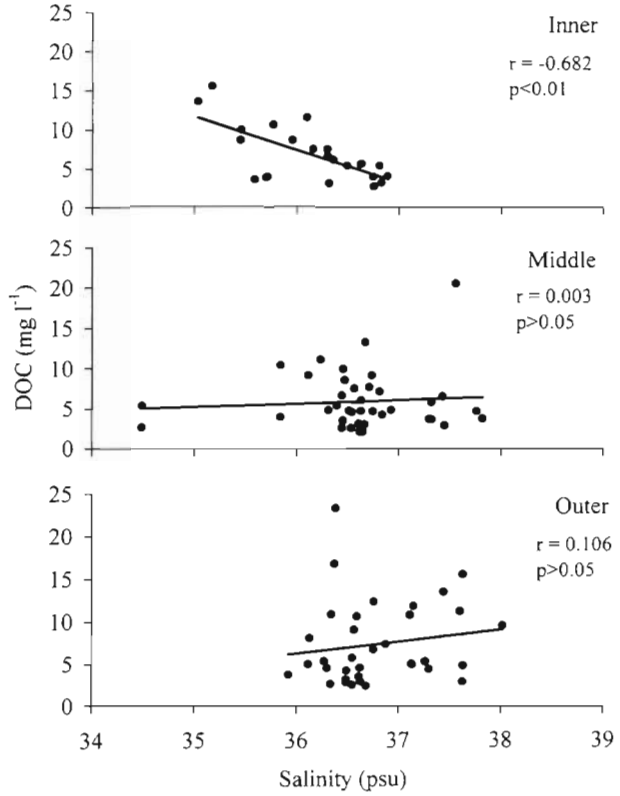

Fig. 3. Regression of DOC versus salinity at the 3 stations in Maliakos Gulf

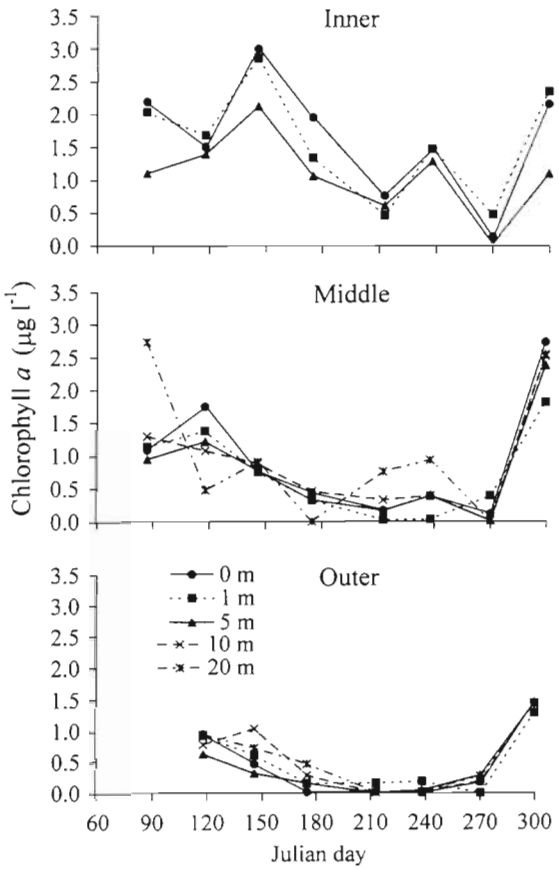

Fig. 4. Seasonal variations of chlorophyll *a* at all stations and depths in Maliakos Gulf

Chl *a* (Fig. 4) was always higher at the inner station, with the maximum in May (3.01 µg l^{-1}), and lower at the middle and outer stations (<0.001 µg l^{-1}). Bacterial abundance (Fig. 5) closely followed the seasonal and spatial variation of chl *a*. Maximum values occurred at the inner station (2.54×10^6 cells ml^{-1}) in May while for the rest of the year bacterial abundance remained at lower levels (less than 10^6 cells ml^{-1}). At the middle and outer stations, the seasonal variation of bacteria was lower (up to ca 10^6 cells ml^{-1}). The seasonal variation of protistan abundance (Fig. 6) followed different patterns at the 3 stations, with highest values always found at the inner station. At the outer station, maximum abundance occurred in September (137 ind. l^{-1}), whereas at the middle and inner stations maxima of 216 to 221/208 ind. l^{-1} occurred in March and May/September, respectively. In total, 6 phyla with 27 families of Protista were found: Ciliata, Dinoflagellata, Radiolaria, Foraminifera, Heliozoa and Acantharia; the latter 2 appeared only in September. The most abundant were the Dinoflagellata, which dominated from May to August, and the Ciliata, which prevailed in March and showed similar relative abundance to Dinoflagellata in September (Fig. 7).

Two-way ANOVA (Table 1) revealed that temperature, DOC, chl *a*, and bacterial and protistan abundance differed significantly with time ($p < 0.01$) while a depth effect was detected only for temperature. The results of PCA for the biotic parameters only (chl *a*, bacteria, protists) showed that samples from the inner station form a completely separate group from the samples of the middle and outer stations (Fig. 8).

Chl *a* (Table 2) was positively correlated with bacteria ($r = 0.64$, $p < 0.01$). Temperature was negatively correlated ($p < 0.01$) with all biotic parameters (Table 2) which was expected since all these parameters de-

Table 1 Temporal and spatial comparison (2-way ANOVA of log-transformed data) for temperature, dissolved organic carbon (DOC), chlorophyll *a* (chl *a*), and bacterial and protistan abundance (* $p < 0.01$)

		Temperature	DOC	Chl *a*	Bacteria	Protista
Time	Test *F*	432.763*	5.489*	14.594*	25.527*	10.298*
	df	7	7	7	7	5
Depth	Test *F*	28.068*	0.284	0.627	0.820	2.602
	df	4	4	4	4	2

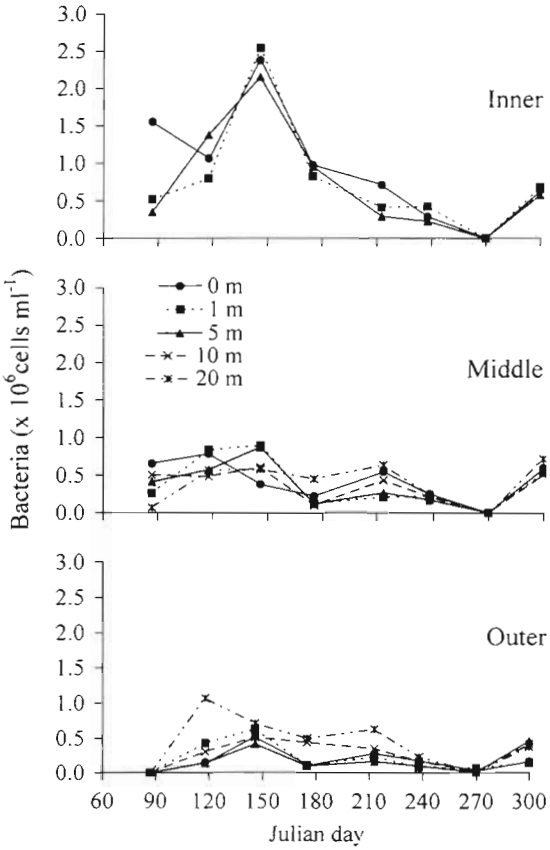

Fig. 5. Seasonal variations of bacterial abundance at all stations and depths in Maliakos Gulf

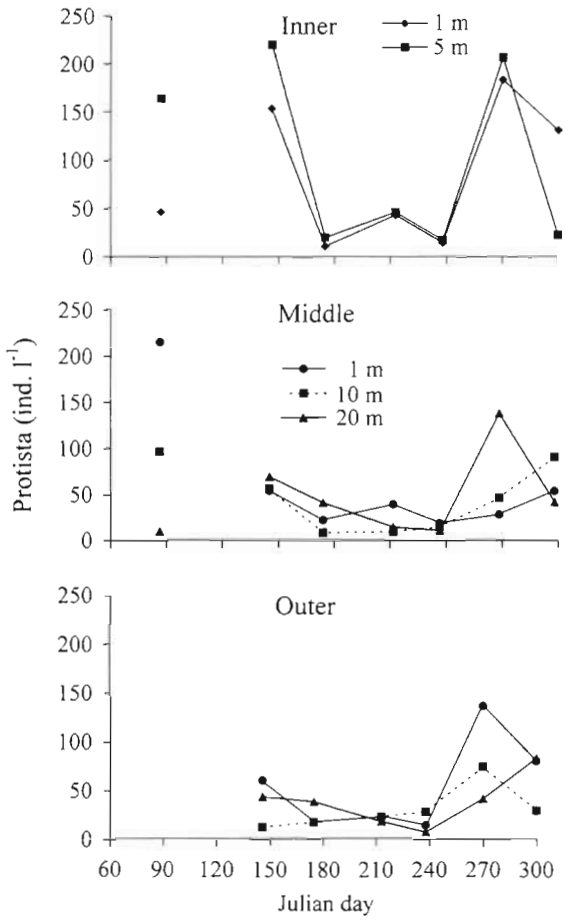

Fig. 6. Seasonal variations of protistan abundance at all stations and depths in Maliakos Gulf

creased from spring to summer. Protista were negatively correlated with DOC ($p < 0.05$).

Stepwise multiple regression (Table 3) was applied to investigate the relationships of (a) bacterial abundance to temperature, DOC, chl *a* and Protista, and (b) protistan abundance to temperature, chl *a* and bacterial abundance. Bacterial abundance was found to be related to temperature, DOC, chl *a* and Protista. Protista were related only to temperature, but after exclusion of the outlier values (8 in total) the model showed higher r^2 and Protista were related to bacteria. Most of the outlier values in both cases came from periods and locations of higher chl *a* levels.

Table 2. Spearman rank correlation coefficients (for all groups n = 99 except for Protista n = 53; $^{**}p < 0.01$, $^{*}p < 0.05$). NS: not significant

	Temperature	DOC	Chl *a*	Bacteria	Protista
Temperature	–				
DOC	NS	–			
Chl *a*	−0.457**	NS	–		
Bacteria	−0.271**	NS	0.644**	–	
Protista	−0.461**	−0.338*	NS	NS	–

Preliminary plotting of bacteria versus chl *a* and protists versus bacteria showed closer relationships for the months April to September and May to August respectively (Fig. 9). When data of these periods were used, highly significant ($p < 0.01$) correlations were obtained with $r^2 = 68\%$ for bacteria versus chl *a* and $r^2 = 72\%$ for protists versus bacteria. The respective values for the whole data set were $r^2 = 40\%$, $p < 0.01$, and $r^2 = 7\%$, $p > 0.05$.

DISCUSSION

DOC concentrations were high but still within the range of those given for coastal environments (1 to 5 mg C l^{-1} for inshore waters; Artemyev 1996). This is likely due to the input of allochthonous organic matter, mainly through the Spercheios River but also through many other small and temporal streams and non-point sources. Autochthonous DOC could be a source for the increase of DOC in late spring. The maximum DOC levels in April and May coincide with the maximum

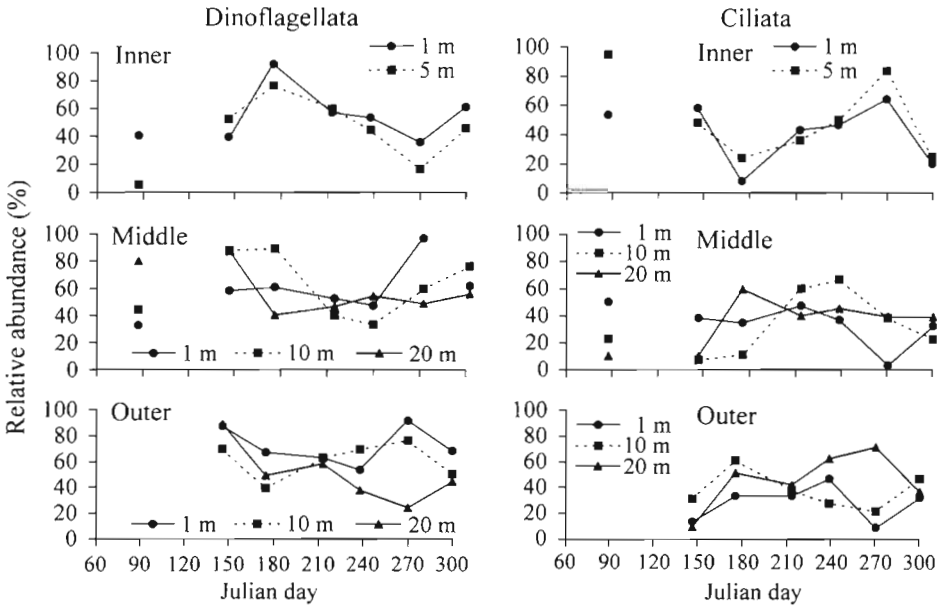

Fig. 7. Relative abundance of Dinoflagellata and Ciliata at all stations and depths in Maliakos Gulf

mesozooplankton abundance and biomass in the gulf which also appears in May (Kormas et al. unpubl.). This concurrence may signify heterotrophic production of DOC (Newell & Turley 1987, Jumars et al. 1989) mainly through exudation and sloppy feeding of mesozooplankton (Legendre & Rassoulzadegan 1995).

The decreasing gradient of chl *a* concentration from the inner end of the gulf (closer to the river mouth) to the outer part (influenced by the open sea) seems to be a permanent characteristic of the gulf as it has also been reported in the past (Christou et al. 1995, Kormas et al. 1997a). This may also be attributed to the inflow of the Spercheios River in the inner gulf. This trend is also maintained in the bacterial and protistan abundance. Based on these parameters and the results of PCA, the gulf can be separated into 2 distinct areas: the inner, shallower part closer to the Spercheios River

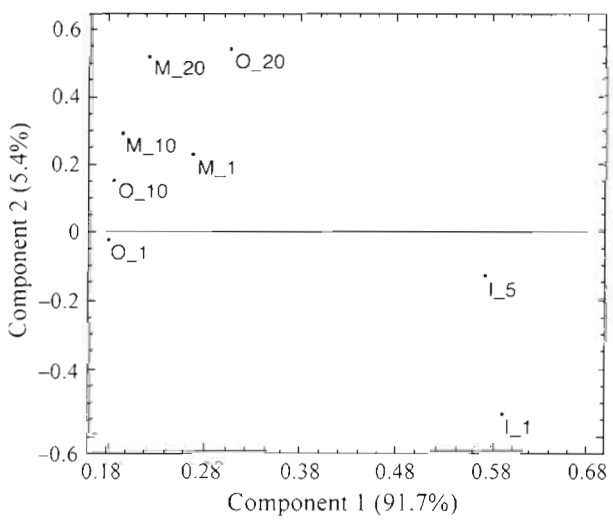

Fig. 8. Principal Component Analysis (PCA) of chlorophyll *a*, bacterial and protistan abundance at inner (I), middle (M), and outer (O) stations and 1, 5, 10, 20 m sampling depths. The % variability of the 2 components is shown in parentheses

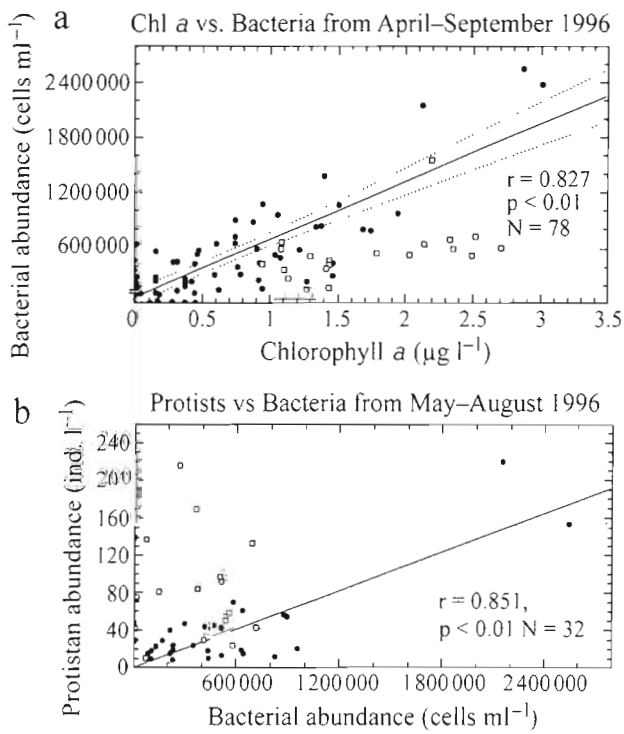

Fig. 9. Regression analyses of (a) chlorophyll *a* versus bacterial abundance and (b) bacterial versus protistan abundance. (□) Values from the rest of the months, excluded from the regression

Table 3. Results of stepwise multiple regression. Italics indicate significant β and bold indicates highest r^2 for $p < 0.01$

		Model	r^2	N	p
(a) Temperature (tem), dissolved organic carbon (DOC), chlorophyll *a* (chla) and protista (pro) versus bacteria (bac)					
	Step 1	bac = 169545 + *0.560 chla*	0.300	53	<0.01
	Step 2	bac = −429892 + *0.668 chla* + 0.237 tem	0.332	53	<0.01
	Step 3	bac = −686238 + *0.649 chla* + *0.307 tem* + 0.221 pro	0.364	53	<0.01
	Step 4	bac = −1131822 + *0.702 chla* + *0.375 tem* + *0.323 pro* + *0.290 doc*	**0.429**	53	<0.01
(b) Temperature (tem), chlorophyll *a* (chla) and bacteria (bac) versus Protista (pro)					
Raw data	Step 1	pro = 151 − *0.350 tem*	0.107	53	0.010
	Step 2	pro = 129 − *0.310 tem* + 0.198 bac	**0.128**	53	0.012
	Step 3	pro = 136 − *0.330 tem* + 0.060 chla + 0.232 bac	0.112	53	0.032
Excluding the outliers	Step 1	pro = 22 + *0.539 bac*	0.274	45	<0.01
	Step 2	pro = 60 + *0.475 bac* − *0.270 tem*	**0.329**	45	<0.01
	Step 3	pro = 57 + *0.446 bac* − 0.250 tem + 0.054 chla	0.314	45	<0.01

estuary and the rest of the gulf (middle and outer compartment) which is deeper and closer to the open sea. Within this group, the 20 m depth forms a separate group. This could be attributed to the thermocline which develops at the middle and outer stations.

The linear relationship between chl *a* and bacteria suggests 2 possibilities: either (1) both chl *a* and bacteria populations respond to common environmental factors, or (2) phytoplankton or material produced by phytoplankton are important substrates for bacterial growth (Cole et al. 1988). Salinity varies insignificantly in the gulf throughout the year (Kormas et al. unpubl.) due to water circulation and mixing (Christou et al. 1995). No statistically significant differences between sampling depths were found for any of the parameters, apart from temperature, indicating that changes happen in a uniform way throughout the water column. Thus, from all the measured parameters, the impact of temperature and DOC on bacterioplankton abundance, and furthermore the relationship between bacteria and protists, remains to be discussed.

In a previous investigation in the same area from October 1995 to February 1996 (Kormas et al. 1996), a correlation between chl *a* and bacterial abundance was not found although the phytoplankton bloom in Maliakos Gulf occurred between January and March (Kormas unpubl.). In other words, bacteria did not seem to respond, at least in terms of abundance, to expected DOM release following the bloom. Since temperature has an important effect on bacterial activity (White et al. 1991), it is possible that an uncoupling between bacterial abundance and phytoplankton biomass during the period of phytoplankton blooms can occur and be related to the low winter temperatures (9 to 11°C) as is often the case in coastal waters (Pomeroy & Deibel 1986, Hoch & Kirchman 1993, Shiah & Ducklow 1994). Soto et al. (1993) found that above 15°C bacterial biomass increased strongly whatever the trophic richness of the water in the plume, frontal and seawater layer of the Rhone River, NW Mediterranean. When water temperature increased in May (14.5 to 22.7°C) the maximum of bacterial abundance occurred. However, this situation seems not to last for long, as, in the summer months, when temperature increased to its maximum, bacterial abundance was declining. This is reflected in the negative correlation between these 2 parameters.

Another possible explanation of the previously observed uncoupling may lie in the allochthonous input of DOC (Ducklow & Kirchman 1983, Hoch & Kirchman 1993, Soto et al. 1993). Estuaries and coastal areas that are close to river mouths or many non-point sources of freshwater input, such as Maliakos Gulf (Kormas et al. unpubl.), usually have high levels of DOC. Most of the riverine organic material is in dissolved form (Artemyev 1996) and can flood DOC from phytoplankton. The riverine origin of DOC in our study was shown by its conservative mixing. However, this pattern is clear only at the inner station. In the middle and outer compartments of the gulf, the pattern is more irregular, probably due to the input from many temporal non-point sources. The coupling of chl *a* and bacteria found from March to October does not negate the importance of allochthonous input of DOC as the latter is important from October to April when the maximum river flow occurs. At the end of spring, the river discharge is used for irrigation purposes around Maliakos Gulf so practically no riverine water enters the sea (Kormas et al. unpubl.). In addition, the rather large diatoms (Christou et al. 1995, authors' pers. obs.) which dominate the phytoplankton during the winter might release less DOC than smaller cells (Krupatkina 1990). Thus, in the spring and summer the major part of DOC available for bacterial growth is probably of algal origin.

In most systems, after the winter or spring bloom (usually consisting of large diatom cells) collapses,

smaller cells (pico- and nanoplankton) tend to dominate (Nielsen & Richardson 1989, Andersson & Rudehäll 1993, Iriarte & Purdie 1994, Rodriguez & Guerrero 1994, Brussaard et al. 1995, Malej et al. 1995). Such a shift, with the potential subsequent development of the microbial loop, seems to be the routine source of fixed carbon to pelagic food webs, if we accept that net phytoplankton blooms (i.e. mainly diatoms) represent a large input of organic matter to the benthos rather than to pelagic organisms (Sherr et al. 1986).

One of the major causes of this succession in cell size of planktonic communities is sedimentation of the bulk of the phytoplankton bloom. Smetacek (1985) argued that rapid sinking of diatom cells following blooms is a feature of diatom life-history. Our sediment trap experiments (Kormas et al. 1997b), conducted in parallel with this study, indicate that the sedimentation rate of chl *a* is considerable in January and February. In addition, the time lag between the phytoplankton bloom and the mesozooplankton abundance and biomass maxima found in earlier studies in Maliakos Gulf (Kormas et al. unpubl.) makes the sedimentation of the winter phytoplankton most probable, as described for other systems by Brussaard et al. (1995), Dagg (1995) and Legendre & Rassoulzadegan (1995). Additionally, mesozooplankton directly interact with the microbial loop food web by grazing upon heterotrophic protists, especially when small phytoplankton dominate the autotrophic community or under oligotrophic conditions (van Wambeke et al. 1996). Summer nutrient depletion, which is also considered as evidence for the dominance of pico- and nanoplanktonic organisms (Anderson & Rudehäll 1993, Iriarte & Purdie 1994, Malej et al. 1995), has been observed in Maliakos Gulf (Kormas et al. unpubl.). According to Krupatkina (1990), oligotrophic waters seem to be a most favourable environment for smaller organisms due to more efficient utilisation of near to analytical zero concentration of nutrients, higher photosynthetic activity and practically zero sinking rate. Christaki et al. (1996) found a strong dominance of the heterotrophic biomass under oligotrophic conditions in the NW Mediterranean.

The linear relationship between the bacterial and protistan abundance from May to August suggests that bacteria can be grazed by protists during this period. No attempt was made to distinguish between auto- and heterotrophic Protista, at least for Dinoflagellata and Ciliata, which are the dominant groups in the present study. Data from the literature, however, suggest that, although many of the former are autotrophic, as many as half of the existing species are heterotrophic or mixotrophic (Schnepf & Elbrächter 1992) Ciliata, the largest protistan group, are often dominant among heterotrophic planktonic protists. They are considered selective grazers which can discriminate prey type based on a variety of mechanisms (Stoecker 1988) and consume a wide range of particles including bacterioplankton (Caron et al. 1991), auto- and heterotrophic pico- and nanoplankton (Bernard & Rassoulzadegan 1990), dinoflagellates (Verity 1988), coccolithophorids (Rassoulzadegan & Etienne 1981), other Protista (Verity 1986) and spermatozoa of metazoans (Galvao et al. 1989). Having a closer look at the seasonal variations in the relative abundance of Dinoflagellata and Ciliata, we can make some assumptions on the trophic status of the protistan population. In most cases there is a clear dominance of 1 of the 2 groups: Dinoflagellata from March until May/June and Ciliata from June/July to October. The autotrophic character of many of the Dinoflagellata might be responsible for the lack of any significant relationship between bacteria and protists during the first months of the sampling period. It is only when Ciliata dominate that the linear relationship becomes more robust, as can be expected from the more clear heterotrophic activity of these organisms.

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