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Microbial Food Web Structure and Its Impact on Primary Production in a Meso-Oligotrophic Coastal Area (Pagasitikos Gulf, Aegean Sea)

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Abstract

Seasonal structure and dynamics of the planktonic microbial food web (phytoplankton, bacteria, nanoflagellates and ciliates) were studied in the meso-oligotrophic Pagasitikos Gulf, NW Aegean Sea. Pagasitikos Gulf is exposed to anthropogenic activity and for this, the sampling covered different parts of the system (city of Volos, sewage effluents, central gulf, Trikeri channel, outer gulf). The standing stocks of all the microbial components fell within the range of other similar coastal systems. Depth integrated primary production (PP) indicated a spring phytoplankton bloom in April/May in all sampling sites. A second phytoplankton bloom was recorded in the western and the outer part of the gulf in the fall. An estimation of bacterial carbon demand indicated that in several cases the percentage of PP that is routed through heterotrophic bacteria is close or over 100%. This is consistent with the excess bacterial biomass relative to autotrophic biomass found in the system. Hence recycling processes mediated by heterotrophs and particularly bacteria are crucial for maintaining the structure and functioning of the planktonic community in Pagasitikos Gulf.

Keywords: Pagasitikos Gulf, Aegean, microbial food web, coastal ecosystem.

Introduction

In aquatic environments autotrophic and heterotrophic prokaryotes and unicellular eukaryotes form 'microbial webs' which are an integral part of planktonic food webs. However, the structure of the microbial food web and its contribution to biological fluxes depend significantly on the environmental trophic status. For example, the contribution of heterotrophic bacteria to the total planktonic biomass has been found to increase with the level of oligotrophy (Karayanni *et al*., 2008). Similarly, the smallest autotrophs (<2 μm) are considered the central primary producers in many, mostly oligotrophic, aquatic systems (Mihalatou and Moustaka-Gouni, 2002; Siokou-Frangou *et al*., 2002). In the last two decades several studies have investigated the effects of these microorganisms on biogeochemical cycles in the sea. However, interest on their role as key players in the ecosystems carbon and energy flux has increased lately due to global environmental problems such as coastal eutrophication and global climate change (Azam and Malfatti, 2007).

Pagasitikos Gulf is a semi-enclosed system on the west coast of the Aegean Sea. Its mean depth of 69 m characterizes the system as a shallow gulf, while the deepest area (108 m) is found at the eastern part where more pronounced gradients are observed. It covers 520 km^2 and its total water volume is 36 km^3. It is connected with the Aegean Sea and north Evoikos through the narrow (5.5 km) and relatively deep (80 m) Trikeri channel. Its interaction with the land includes the inflow of agricultural run-off (mostly on its west coast), sewage treatment effluent from Volos city (ca. 120,000 inhabitants, north), and several temporal streams (throughout its perimeter). Since the beginning of the 1980s, a few incidents of mass gelatinous mucilage accumulation in the north part of the gulf have been noticed, possibly related to anthropogenic eutrophication (Gotsis-Skretas, 1995; Petihakis *et al*., 2005). It has been suggested that the appearances of such phenomena, as well as their fate, are related to the functioning of the microbial component of the pelagic food web (Serratore *et al*., 1995; Vanucci, 2003). As an additional point, the Pagasitikos Gulf harbours aquaculture activities, mostly in the form of caged sea bream farms.

In spite of the wealth of literature on the investigation of the structure and function of the microbial loop and the microbial food web in different aquatic habitats across the world, in Greek waters little information on whole/near complete

microbial food webs is available, especially in coastal systems (Giannakourou *et al*., 2005). In the case of Pagasitikos Gulf, although there is a reasonable amount of information on several physical, chemical, and biological factors of its water column processes (Friligos, 1987; Friligos and Gotsis-Skretas, 1989; Triantafyllou *et al*., 2001; Petihakis *et al*., 2012), no data exist on its water column microbial food web. This paper is an investigation of the pelagic microbial food web of this coastal semi-enclosed system and its impact on primary production, on a spatiotemporal scale related to the system's different trophic states and its interaction with land.

Materials and Methods

Eight sampling stations covering the entire gulf area (Figure 1, Table 1) were visited in April, May and September 1999 with the R/V 'AEGAEO'. The stations represented different parts of the gulf: the city/port of Volos, the sewage outfall, the central gulf, the Trikeri channel, and the outer area influenced by the Aegean Sea and Evoikos gulf (Koliou-Mitsou, 2000). Sea water was collected using a Go-Flow

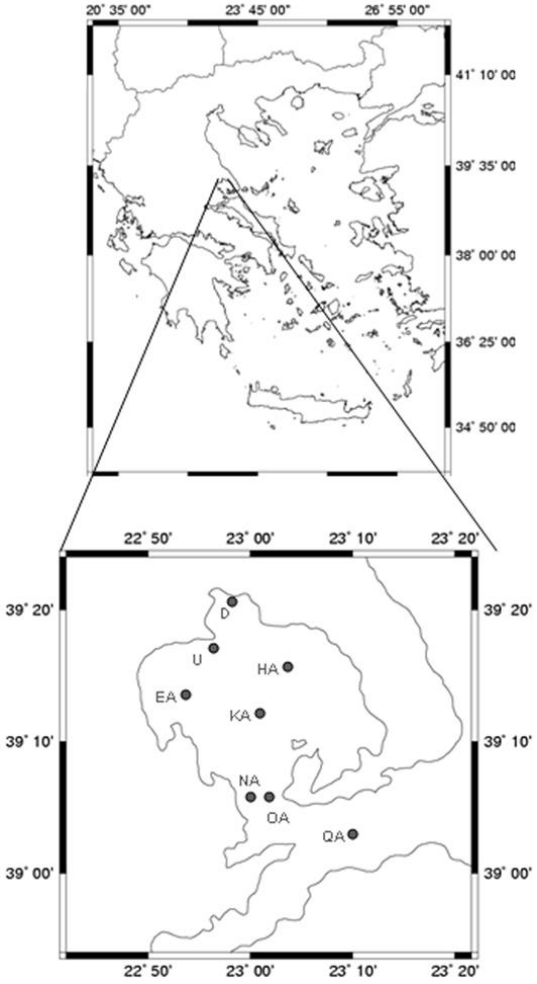

Figure 1. Map of sampling sites in Pagasitikos Gulf, Greece.

Table 1. Sampling stations in the Pagasitikos Gulf

Sampling Station	Maximum depth (m)	Location
D	30	City of Volos
U	75	Sewage effluents
EA	60	Sewage effluents
HA	95	Central gulf
KA	75	Central gulf
NA	85	Trikeri channel
OA	75	Trikeri channel
QA	85	Outer area

rosette from four to nine depths (1, 10, 20, 30, 40, 50, 60, 75, 90 m) between the sea's surface and its bottom, depending on the depth of each station (Table 1). Vertical profiles of temperature and salinity were obtained by CTD casts.

Phytoplankton chlorophyll-*a* (chl-*a*) was determined by fluorometric measurements of acetone extracts (Yentsch and Menzel, 1963) in two fractions, 0.2 to 2 μm and >2 μm (hereafter referred as picoplankton and phytoplankton > 2 μm respectively). Appropriate volume of seawater (until the filter was almost clogged, 2-3 l) was filtered through Millipore polycarbonate filters of 0.2 and 2 μm pore size and was frozen at -20°C until analysis. The fluorescence of the extracted chlorophyll was measured using a Turner AU-10 fluorometer, previously calibrated against chlorophyll-*a* standard solution. An acidification step was not used, but according to Turner Designs Instructions Manual, a procedure for measuring extracted chlorophyll-*a* free from the errors associated with chlorophyll-*b* and phaeopigments was followed by using the proper excitation, emission and reference filters, and lamp, according to (Welschmeyer, 1994).

Phytoplankton production (PP) was measured at four characteristic/representative stations (D, HA, EA, OA) by the ^{14}C-technique (Steemann-Nielsen, 1952). Samples from surface to the bottom (at 10 m increments) were used to fill 130ml plexiglass (transparent, 2 mm thickness) bottles (two light and one dark, for each depth). Each bottle was inoculated with 1ml of 5μCi (185 kBq) of $NaH^{14}CO_3$ (sodium bicarbonate, Amersham), and incubated *in situ* using a free-floating buoyed which allowed incubation at the depth from which samples were taken. The incubation rig was deployed at midday and recovered after ~ 2 hours. At the end of incubations samples were filtrated through 0.2μm polycarbonate Millipore filters under low-vacuum pressure (<100mmHg). Filters were placed in (20ml) scintillation vials, acidified with 1ml 0.5N HCL to remove dissolved ^{14}C (inorganic C). The sample vials left uncapped in a fume hood to dry overnight to the remove the unfixed inorganic ^{14}C. Upon evaporation of the acid, 10ml liquid scintillation cocktail was added. The mixtures were refrigerated until analysis. Radiation of ^{14}C - taken up by phytoplankton was measured in a scintillation counter (Beckman Liquid Scintillation Counter, BECKMAN LS 6500), that used an internal standard for quenching. The conversion of radioactive counts to carbon turnover followed the method described by Steemann-Nielsen (1952).

For heterotrophic bacteria and nanoflagellate counts, seawater samples of 10 and 30ml, respectively, were preserved with formaldehyde (2% final concentration). Samples were kept at 4°C in the dark until filtered on black Nuclepore filters (pore size: 0.8 mm and 0.2 mm for nanoflagellates and bacteria respectively) and stained with DAPI (Porter and Feig, 1980) within a few hours of sampling. Filters were stored at -20°C until counting and measured by using an Olympus AX-70 PROVIS epifluorescence microscope at 1000X under UV excitation. Cyanobacteria and phototrophic nanoflagellates (PNF) were counted under blue light excitation. Both heterotrophic (HNF) and phototrophic nanoflagellates were sorted in three size classses: <3, 3-5 and >5 μm. Bacterial biomass was estimated by applying a conversion factor of 30.2 fg cell^{-1} (Fukuda *et al*., 1998). Bacterial production was estimated using the ^{3}H-leucine uptake method as described by Kirchman et al. (1985). For each depth, duplicate samples (10-20 ml) and a control fixed with formol (2% final concentration) were incubated in the dark at in situ temperature with 1 nM L-[4,5-^{3}H]-leucine and 19 nM non radioactive leucine. After 2h incubation was terminated by addition of formalin (2% final concentration). Radioactivity was measured using a Packard LS 1600 Liquid Scintillation Counter scintillation analyser. Concentration kinetic experiments showed that isotopic dilution was negligible and bacterial production was calculated according to Kirchman (1993) from ^{3}H-leucine incorporation rates.

For ciliate enumeration, 500 ml of surface (2 m) sea water were preserved with borax-buffered formaldehyde (final concentration 2%). The samples were stored at 4°C in the dark and examined within 3 months of collection. Before examination samples were left to settle in their bottles in the dark at 4°C, and after 48 h the top 400 ml of the sample was slowly siphoned off. The bottom 100 ml of the sample was transferred to settling chambers and allowed to settle for 24 h. Finally, the sample was examined using an Olympus IX-70 inverted microscope at 200X. Examination of the supernatant (top 400 ml of sample siphoned) showed minimal cell loss (0 to 6%) during the above sample concentration process.

Results

Physical Environment

Mean temperature (Figure 2) ranged from 12.9±0.2ºC (April) to 18.5±5.1ºC (September). In May, temperature increased in the upper 40 m of the water column and a thermocline (ca. 25 – 31 m) further developed in September. Salinity (Figure 2) ranged between 35.7 and 38.9 with a strong halocline ca. 23 – 30 m in September.

Chlorophyll

Chorophyll-*a* (Figure 3) ranged between <0.01 (HA: Central gulf, May) and 2.26μg l^{-1} (D: City of Volos, April). The highest variation in chl-*a* values was found at station D (0.63–2.26μg l^{-1}). In September, chl-*a* reached 0.83μg l^{-1} (OA: Trikeri channel, near the entrance of the gulf). Mean depth integrated values of chl-*a* in the study area were

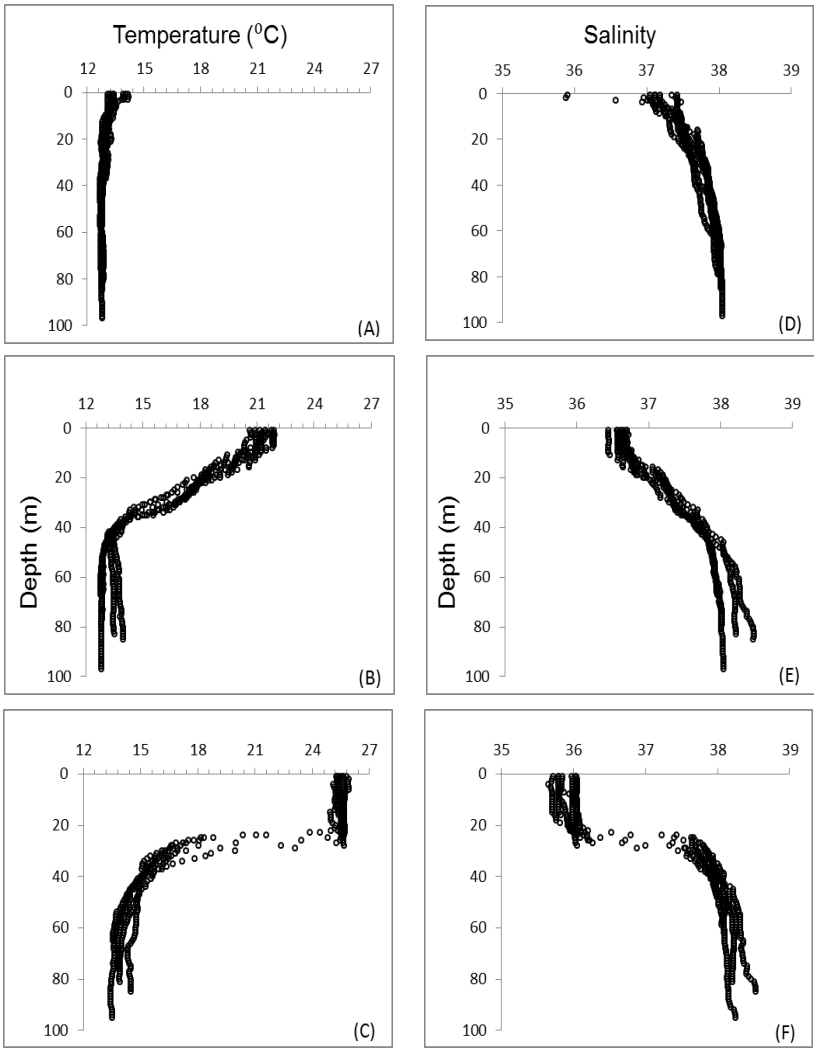

Figure 2. Depth profile of temperature and salinity in Pagasitikos Gulf, Greece in April (A, D), May (B, E) and September (C, F).

similar in April and May (~24±8 mg m^{-2}) and decreased in September (18±8 mg m^{-2}). In general, phytoplankton >2μm depth integrated chl-*a* represented >51% of total chl-*a* except at the stations located at the inner (D) and the central gulf (HA and KA) in April and/or May and near the gulf entrance (OA) in September (36 – 44 %). Phytoplankton >2μm contribution to total chl-*a* increased or remain constant at the inner/central stations (D, HA, U, EA) and decreased at the outer stations (KA, OA, QA) between April and September.

Heterotrophic Bacteria and Cyanobacteria

Bacterial abundance ranged between 1.2 and 9.6 x 10^5 cells ml^{-1} (April). Maximum September values did not exceed 5.9 x 10^5 cells ml^{-1}. The highest variation was found at EA (sewage effluent) where values ranged between 4.0 (September) and 7.5 (April) x 10^5 cells ml^{-1}. QA (outer area) presented the lowest variation in bacterial densities (from 4.6 x10^5 in September to 5.7 x10^5 in April cells ml^{-1}). As for bacteria, cyanobacteria abundance showed a decreasing trend from April (0.57 – 11.26 x 10^4 cells ml^{-1}) to September (0.14 – 2.4 x 10^4 cells ml^{-1}). The highest variation occurred at NA (Trikeri channel) where cyanobacteria density ranged from 0.40 (September) to 11.26 (April) x 10^4 cells ml^{-1}. HA presented the narrowest range of values (0.22 in September – 3.32 in May x 10^4 cells ml^{-1}). For both heterotrophic and phototrophic bacteria, peak abundance occurred above 30 m in April and May, while in September depth variations were much lower. Mean depth integrated densities of cyanobacteria (Figure 4) in the study area varied between 0.85±0.16 and 3.48±1.33 x10^{14} cells m^{-2} and presented statistically significant variations between samplings. Bacteria depth integrated densities in the study area ranged from 2.90±0.83 to 4.02±0.93 x10^{14} (Figure 4). Statistically significant differences were found between April and September cruises. In particular, depth integrated abundance of cyanobacteria decreased by a factor >2 in all sampling stations between April and September. For bacteria, depth integrated abundance showed maximum values in April as well, except to HA (central gulf) and EA

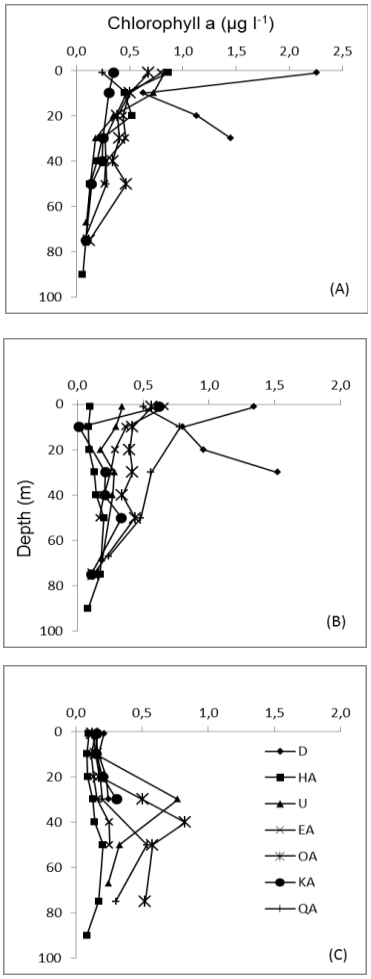

Figure 3. Depth profiles of chlorophyll a in April (A), May (B) and September (C).

(sewage effluent) where maxima occurred in May (Figure 4).

Nanoflagellates and Ciliates

Nanoflagellates (NF) were counted at 5 stations (U, HA, KA, OA, QA) in May and September. Total abundance ranged between 1.70 and 11.34 x 10^3 cells ml^{-1}. HNF densities varied from 1.42 to 10.49 x 10^3 cells ml^{-1}. PNF showed less variation (0.00 – 4.96 x 10^3 cells ml^{-1}). In May, HNF abundance peaked at all stations within the top 10m of the water column, decreasing with depth. PNF peak abundances were observed between 10 and 50 m. In September, the depth profile of both HNF and PNF showed a more uniform pattern. Mean depth integrated nanoflagellate abundance was in the order of 55.06 ± 14.00 and 41.32 ± 11.05 x 10^{10} cells m^{-2} in May and September respectively (Figure 4). HNF always dominated the nanoflagellate assemblage representing ≥57% of it.

Ciliate abundance in surface sea water (Figure 4) was higher at all stations in April (1040 – 4210 cells l^{-1}), while in May and September it decreased sharply (10 – 185 and 60 – 460 cells l^{-1} respectively).

Primary and Bacterial Production

Primary production (PP) ranged between <0.01 (HA: central gulf, September) and 1.89 (D: city of Volos/inner gulf, April) µgC l^{-1} h^{-1} and it occasionally reached 3.23 µgC l^{-1} h^{-1} (at 75 m of HA in April, Figure 5). In September maxima reached 1.11 µgC l^{-1} h^{-1} (OA: Trikeri channel/gulf entrance). During the study, the highest range of values was found at HA (central gulf, <0.01 – 3.23 µgC l^{-1} h^{-1}) followed by D and OA (<0.1 – 1.89 µgC l^{-1} h^{-1}). As for chl-*a* (Figure 3) depth profiles of PP showed their highest values within the top 30m in April and May and a lower variation in September (Figure 5). Depth integrated primary production decreased seasonally at D and HA. In EA (sewage effluent) and OA, PP increased in May and showed low variation in September (Figure 6). In D, HA and EA phytoplankton >2µm contribution to total PP increased from ≤42% to ≥55% from April to September in contrast to OA where its contribution decreased from 81% to 42% respectively.

Maximum bacterial production (BP) occurred at station D in April (1.00 µgC l^{-1} d^{-1}, Figure 5). In May and September, BP reached 0.187 (station D) and 0.05 (station HA) µgC l^{-1} h^{-1} respectively. Mean depth integrated bacterial production decreased from 5.55 ± 0.98 mgC m^{-2} h^{-1} to 3.01 ± 2.27 mgC m^{-2} h^{-1} between

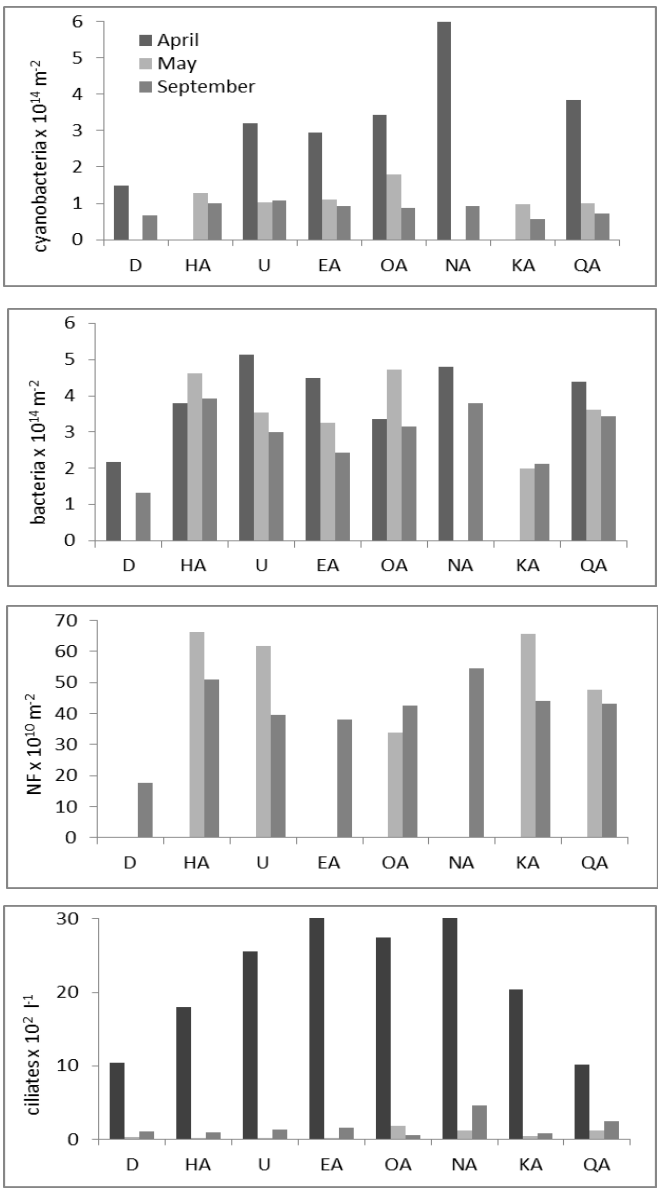

Figure 4. Depth integrated abundance of bacteria, cyanobacteria and nanoflagellates in April, May and September. Ciliate abundance at surface (5m) in volumetric units.

April and September across the entire study area (Figure 6). An exceptionally high value was recorded at HA (central gulf) in September (7.36 mgC m^{-2} h^{-1}).

Discussion

Pagasitikos Gulf, located in the NW Aegean Sea in the E Mediterranean, showed typical temperature and salinity patterns for a temperate coastal system (Wisshak *et al*., 2010; Celik, 2013). Temperature increased between May and September and a strong thermocline, as well as a halocline, developed during the summer and remained undisturbed until early autumn. Ignatiades (2005) suggested that the trophic status in the Aegean Sea could be classified as: open oligotrophic; offshore mesotrophic or inshore eutrophic, based on chlorophyll concentration and primary productivity. According to chlorophyll values, eutrophic conditions prevail at the inner part of the gulf (station D, city of Volos) during spring while the central and outer part of the area can be classified as meso-oligotrophic. In September, Pagasitikos Gulf is characterized as oligotrophic, although a deep chlorophyll maximum of 0.5-0.83 μg l^{-1} corresponding to mesotrophic conditions was found at the external part of the area. High chl-*a* values within the inner gulf (3.5μg l^{-1}) area were also recorded one year earlier, in April 1998 (Petihakis *et al*., 2005). According to this study, these values are related to the shallow depth and nutrient inputs from the city of Volos. Primary production remained relatively low during the study even at the inner station D (<0.8μgC l^{-1} h^{-1}) characterizing a mesotrophic to oligotrophic situation (Ignatiades, 2005). High chlorophyll – low primary production values were also found in the Thermaikos Gulf, a

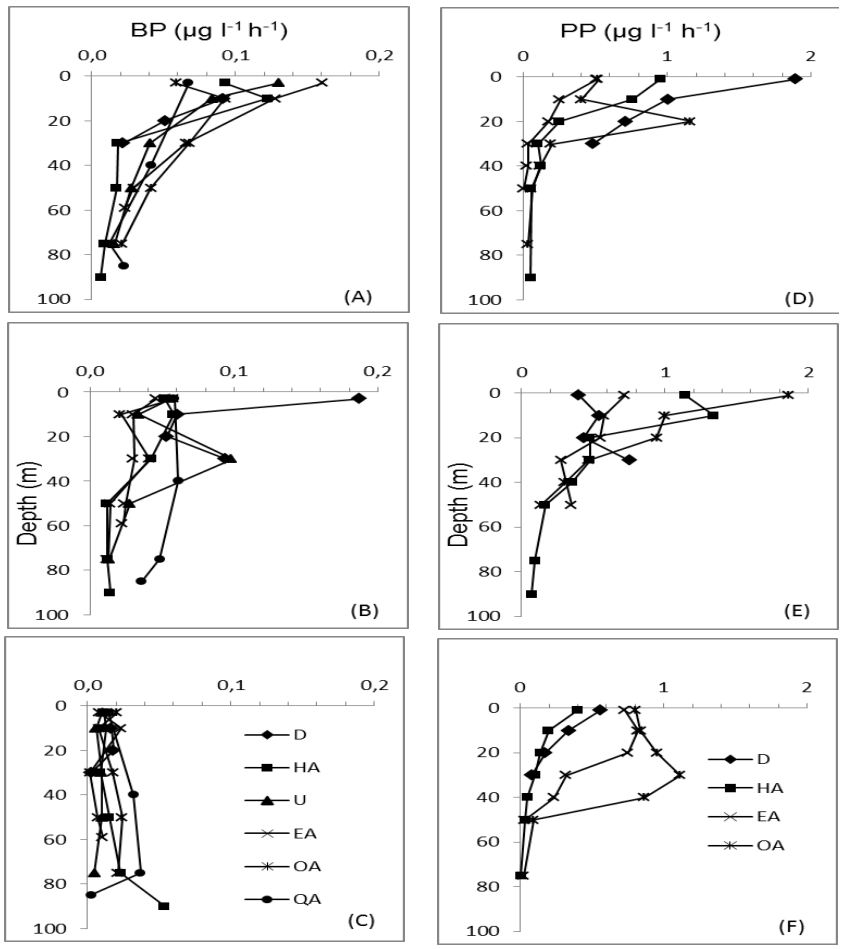

Figure 5. Depth profiles of Bacterial (BP) and Primary Production (PP) in April (A, D), May (B, E) and September (C, F). Maximum BP value (1.00 µgC l^{-1} d^{-1}) recorded at station D (5m) in April is not shown on the graph for simplicity reasons.

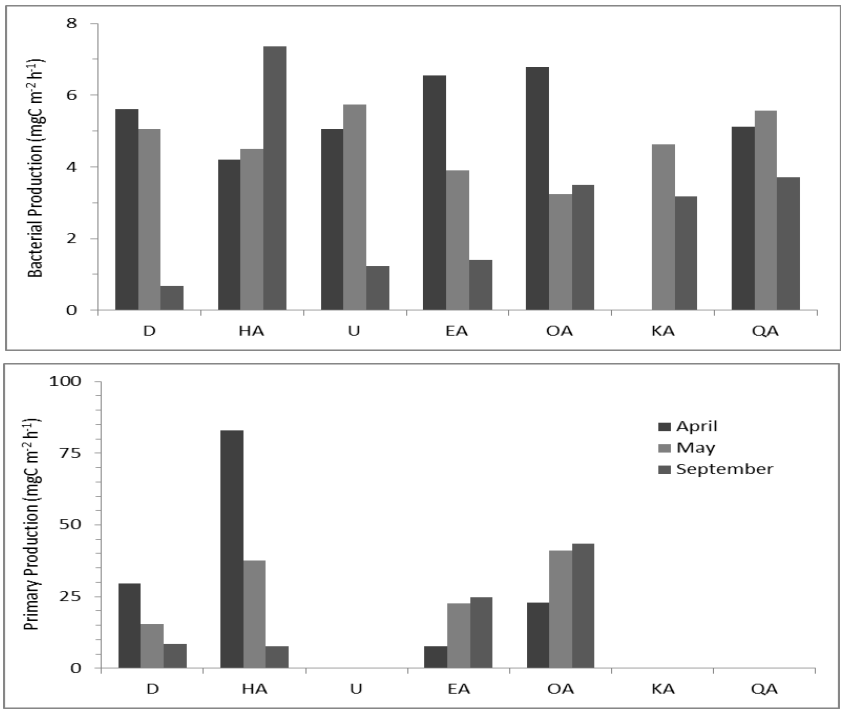

Figure 6. Depth integrated values of Bacterial (BP) and Primary Production (PP) in April, May and September.

eutrophic coastal area of the north Aegean Sea indicating that phytoplankton was most likely not in an active state of growth during the sampling period (Mihalatou and Moustaka-Gouni, 2002).

In the oligotrophic Mediterranean Sea picophytoplankton accounts on average for 59% of total chl-*a* and 65% of the primary production (Siokou-Frangou *et al*., 2010; Celik, 2013). In the Aegean Sea in particular, picophytoplankton was found to represent more than 71% of total chl-*a* (Siokou-Frangou *et al*., 2002; Zervoudaki *et al*., 2007). The percent contribution of pico-sized phytoplankton chlorophyll to total chlorophyll decreases in coastal areas where higher nutrient inputs are recorded (Mihalatou and Moustaka-Gouni, 2002, Bernardi Aubry *et al*., 2006; Šolić *et al*., 2010). In this study, we found that picophytoplankton accounted on average for 40% of total chl-*a*. However, seasonal and spatial variations were observed. For example, at the inner station D located close to the city of Volos, picophytoplankton represented 60% of total chl-*a* in spring. The same percentage was recorded at the outer station OA in autumn. It seems that after the first bloom, dominated by large-sized phytoplankton (nano- and/or microphytoplantkon), picoplankton gained more importance as primary producers of the ecosystem. Unfortunately, data on seasonal changes in species composition or biomass are lacking to confirm or reject this hypothesis.

The abundance of the various microbial components studied during this project fell within the range of values that characterize oligo- and mesotrophic ecosystems (heterotrophic bacteria 10^5-10^6 ml^{-1}, cyanobacteria 10^3-10^4 ml^{-1}, heterotrophic nanoflagellates 10^3 ml^{-1} and ciliates 10^1-10^3 l^{-1}). Selected examples of this range of values in different areas of the Mediterranean Sea are presented in Table 2. Maximal values are higher than those recorded in the open ocean even during eutrophic conditions (Karayanni *et al*.,. 2005; Karayanni *et al*., 2008) but lower than those found along a nutrient gradient of other coastal ecosystems (e.g. Kopuz *et al*., 2012; El-Serehy *et al*., 2012). Bacterial production varied between 0.05 and 4.48 μgC l^{-1} h^{-1}, reaching a maximum of 24.05 μgC l^{-1} d^{-1} at station D (city of Volos) in April. These values compare well with those found in other areas of the Mediterranean (<0.03-3.20 μgC l^{-1} d^{-1}, (Christaki *et al*., 1999; Van Wambeke *et al*., 2000; Van Wambeke *et al*., 2002; Tanaka *et al*., 2007; Zeri *et al*., 2009). Additonally, Umani et al. (2012) reported maximal BP values of 23.33 μgC l^{-1} d^{-1} for the Gulf of Trieste (NE Adriatic Sea).

Maximum depth integrated primary production was measured in April/May indicating a spring bloom over the area. A second phytoplankton bloom was recorded in two out of four stations (EA, OA) in September. Depth integrated BP seems to be out of phase compared to PP, except at the inner station D where the maxima of the two parameters coincide. Bacterial production to primary production ratio (BP:PP) is below 19% during the spring/autumn bloom. These results compare well with data obtained in previous studies conducted in coastal areas during bloom periods (e.g. Lamy *et al*., 2006) and indicate that the two processes are not linked directly. However, a theoretical calculation of bacterial carbon demand based on bacterial growth efficiency equal to 20% (Van Wambeke *et al*., 2002) indicates that in general more than 40% of the PP is routed through bacteria. Moreover, this percentage is close or over 100% in 6 out of 12 samplings. Interestingly, in the outer part of the gulf (OA) as well as in the west, in proximity to sewage effluent (EA), BP maxima

Table 2. Abundance of bacteria, cyanobacteria, nanoflagellates and ciliates from coastal marine systems.

Area	*Bacteria* ($x\ 10^5\ ml^{-1}$)	*Cyanobacteria* ($x\ 10^4\ ml^{-1}$)	*Nanoflagellates* ($x\ 10^3\ ml^{-1}$)	*Ciliates* (L^{-1})	*Reference*
Vranjic Basin (middle Adriatic)	6.1-9.3		1.04±0.03		Šolić *et al.*, 2010
Gulf of Venice (NW Adriatic)		≤18.88*			Bernardi Aubry *et al.*, 2006
Levantine Basin (E Mediterranean)	0.46-4.1	0.013-1.8	9.9-1.0 (PNF) 0.068-0.92 (HNF)	7-325	Tanaka *et al.*, 2007
Thermaikos Gulf (E Mediterranean)	9-68	0.1-1.06*	<0.65	≤156000	Mihalatou and Moustaka-Gouni; 2002
Thau Lagoon (NW Mediterranean)		9*			Bec *et al.*, 2005
Gulf of Lions (NW Mediterranean)	3-12				Van Wambeke *et al.*, 2002
N & S Aegean Sea	≤8.33±3.93**		≤1.43±0.92 (HNF) **	≤417±111**	Siokou-Frangou *et al.*, 2002
Maliakos Gulf (central Aegean Sea)	0.00-25.4				Kormas *et al.*, 1998
Mediterranean Sea (longitudinal transect 34°E-5°E)	0.10-8.63		0.42-4.65	5-157	Christaki *et al.*, 2011
NE Atlantic	0.64-21.7	0.01-9.8	0.02-3.80	12-1900	Karayanni *et al.*, 2005; 2008
SE Black Sea	11.4-36.3	0.04-65.8			Kopuz *et al.*, 2012
Gulf of Aqaba (N Red Sea)	3.53-38.5		2.16-21.66 (HNF) 0.10-2.00 (PNF)	2000-25000	El-Serehy*et al.*, 2012
Pagasitikos Gulf	1.2-9.6	0.14-11.26	1.7-11.30	10-4210	This study

*Total picophytoplankton, **Mean integrated to 100m
PNF: autotrophic nanoflagellates; HNF heterotrophic nanoflagellates

occurred before PP maxima. It is possible that primary production at these sites in May is fueled by regenerated nutrients supplied by microbial decomposition of organic matter. A previous study in the Gulf of Lions in NW Mediterranean (Diaz and Raimbault, 2000) showed that the regenerative nitrogen fluxes are important during spring in this coastal area, and probably controlled phytoplankton growth.

A gradient of nutrients in the area of the sewage effluent was reported in an earlier study (Petihakis *et al*., 2005). However, in this study we did not observe a clear effect of nutrient inputs since all microbial parameters studied in U and EA were within the ranges obtained in the rest of the sampling stations. The early autumn phytoplankton bloom recorded in EA (and in OA as well) seems to be related to physical features that cause erosion of the well-established seasonal thermocline (Figure 2). For example, the bloom in EA was probably associated to the cyclonic circulation recorded in summer in the western part of the gulf (Petihakis *et al*., 2005) and which provided nutrient rich water at surface. On the contrary anticyclonic water movement in the east transfers nutrients and organic matter in depth. This event could explain the high depth integrated BP obtained in HA in September (176 mg C m^{-2} d^{-1}) when maxima in the rest of the stations ranged between 16 and 89 mg C m^{-2} d^{-1}.

At the inner part of the gulf (D) primary and bacterial productions are synchronized since both parameters showed a decreasing trend between April and May. There is accumulating evidence that the limiting nutrient for both phytoplankton and bacterioplankton in the Mediterranean is phosphorous (Van Wambeke *et al*., 2002; Estrada and Vaque, 2014) and that the two groups compete for inorganic nutrients. Vadstein et al. (2003) showed that bacteria experience stronger limitation than phytoplankton in different P concentrations. In an earlier study in Pagasitikos (Petihakis *et al*., 2005), maximal phosphate concentration (0.35μM) was recorded in the inner gulf leading probably to lower N:P ratios and changing the degree of P-limitation for both bacteria and phytoplankton compared to the other sampling sites. Besides industrial and agriculture and city effluents, Lake Karla is probably nourishing the area with nutrients and particularly with P, through a tunnel which outflows at the north part of Pagasitikos. The lake was drained in the 1960s but a small permanent marsh enriched with fertilizers, insecticides and pesticides remained in the area (Oikonomou *et al*., 2012).

In April, ciliate abundance reached its maximum (of the order of 10^3 L^{-1}). Considering that large-sized phytoplankton (>2μm) dominated total chl-*a*, it could be assumed that ciliates, which can efficiently graze on this size class (e.g. Karayanni *et al*., 2005), acted as a direct link between primary production and higher trophic levels. Indeed, copepod abundance, considered as the main ciliate predators, reached its maximum in April as well, and varied slightly in May (Pancucci-Papadopoulou and Christaki, 2000). Although data on heterotrophic nanoflagellate (HNF) abundance in April are lacking, the increased abundance of ciliates suggests a top-down control of HNF by ciliates. In May relaxed grazing pressure of ciliates on HNF results probably in an increase of bacterivory by nanoflagellates. A statistically significant relationship between HNF and bacterial abundance ($R=0.512$, $P<0.05$) and biomass ($R=0.806$, $P<0.001$) in May, suggests such a predator-prey dependence (Krstulovïc *et al*., 1995). The increase of temperature in May (Figure 2) is another factor that probably enhances HNF bacterivory (Šolić *et al*., 2009). In late summer-autumn conditions, when the abundance of different heterotrophic microbial components as well as of mesozooplankton decreases, hydrography probably played a more significant role in the fate of primary production compared to the April/May sampling.

Conclusion

Pagasitikos Gulf, although influenced by anthropogenic activities (rural and industrial), is characterized as meso-/oligotrophic based on chl-*a* and primary production data. This finding is further supported by the high heterotrophic (bacterial) to autotrophic biomass ratio (H:A>4) which was calculated based on a chl-*a* to carbon ratio equal to 100, which is now suggested for oligotrophic ecosystems (Gasol *et al*., 1997). Hence, the results presented in this study indicated a typical microbial food web with recycling processes mediated by heterotrophs and particularly bacteria, crucial for maintaining the structure and functioning of the planktonic community of the gulf.

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