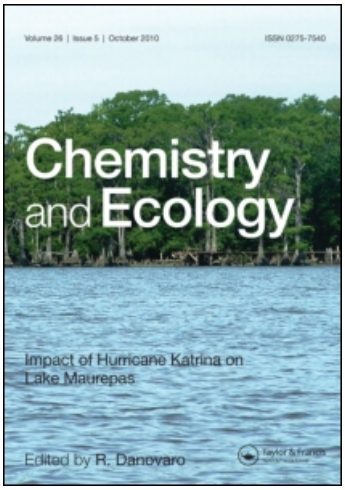

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Prokaryotic activities and abundance in pelagic areas of the Ionian Sea

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The Ionian Sea represents a suitable basin for studying the biogeochemical processes mediated by microbial activities. Because of its characteristics as a crossing region between the western and eastern Mediterranean Sea, it is one of the sites most affected by changes in water mass composition and dynamics, caused by the Eastern Mediterranean Transient (EMT). To date, relatively few data exist on microbial activities in pelagic areas of the Ionian Sea. From 1998 to 2004, during different research cruises, prokaryotic parameters (abundance, extracellular enzyme activities leucine aminopeptidase, β-glucosidase, alkaline phosphatase, bacterial production and respiration) were measured together with culturable bacteria and the main physical, chemical and trophic parameters (temperature, salinity, nutrients, particulated organic matter). The aim of the study was to describe the spatial and temporal variability in microbial activities involved in the carbon and phosphorus cycles, in different layers. Results showed that organic matter transformation mediated by the microbial community displayed a significant increase in autumn, highlighting the occurrence of significant changes at meso- and bathypelagic depths. Unlike the dark ocean, bacterial growth efficiency in the Ionian Sea, which increased with depth, seemed to vary from being a source of carbon in the epipelagic layer to a sink in the meso- and bathypelagic layers. The mechanism of phosphatase regulation showed a weak inverse correlation between specific phosphatase and inorganic P in all seasons except autumn. It is worth mentioning that the reported results constitute, to the best of our knowledge, one of the available datasets giving information about microbial activities in the Ionian Sea.

Keywords: Mediterranean Sea; prokaryotic activities; prokaryotic abundance; growth efficiency; water column; biogeochemical cycles

1. Introduction

The Ionian Sea represents a crossing region between the western and eastern Mediterranean Sea and is one of the sites most affected by changes in water mass composition and dynamics, caused by the climatic event known as the Eastern Mediterranean Transient (EMT) [1]. Since the 1990s, a significant change in the thermohaline deep cell has occurred in the eastern Mediterranean. This change consists of a shift in the source of eastern Mediterranean deep waters from the Adriatic

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to the Aegean Sea [2], which has modified the hydrological structure of the water masses and the distribution of dissolved nutrients in the southern Adriatic and Ionian basins [1,3,4]. Briefly, salinity increased in the intermediate/deep layers of the Mediterranean Sea, whereas temperature increased in the surface layer. Observations in 2003 indicate propagation of the signal from east to west. Newly formed high-temperature and high-salinity water seems to fill the deep layer, replacing the resident waters. So temperature is a key parameter in water mass formation [5] and the EMT influences the characteristics of the biogeochemical cycles and primary production processes in the area [6].

Important changes in the circulation of the water masses have led to changes in some biological ecosystem components, such as increases in organism adaptability or metabolism, connected with the rise in temperature [7].

In general, the epipelagic layer of the Ionian Sea is mainly occupied by Modified Atlantic Water (MAW). This spreads eastward from the Sicily Straits in the surface layer and can usually be identified as a subsurface minimum of salinity between 30 and 200 m depth. The Ionian Surface Water (ISW) is also present in this layer, and can be clearly distinguished from the MAW in summer because it is saltier and warmer [8].

The mesopelagic layer is occupied by the Levantine Intermediate Water (LIW), which joins the Ionian Sea through the Cretan passage, spreading in from its formation sites to the northeastern Levantine basin. The LIW is identified by high salinity levels in the layer between the depths 200 and 600 m. The bathypelagic layer in the depth horizon below 1600 m is occupied by the colder and less saline Eastern Mediterranean Deep Water (EMDW) – the main source of which is the Adriatic Deep Water (ADW). The layer between 700 and 1600 m is occupied by a transitional water mass with properties intermediate between LIW and EMDW.

In addition, the Sicilian Channel, which connects the western and eastern Mediterranean basins, is strongly affected by two water masses (MAW and LIW) flowing in opposite directions [9].

Research over the past two decades has demonstrated that planktonic prokaryotes are the main drivers of marine biogeochemical cycles. Heterotrophic prokaryotes channel about half of the primary production into the microbial loop in the euphotic zone [10] and might compete with eukaryotic phytoplankton for inorganic nutrients [11]. High amounts of organic matter are released into the surrounding water at different trophic levels, providing substrates for heterotrophic prokaryotes. In the euphotic layer, their growth is mainly controlled by flagellates and viruses via a top-down mechanism. The meso- and bathypelagic zones harbour a simpler food web than epipelagic waters, owing to the lack of phytoplankton, which is partly compensated for by a major prokaryotic autotrophic component [12].

In the Mediterranean Sea, many studies have focused mainly on coastal and euphotic zones [13–17], nevertheless little information is available to date on the bacterial activities occurring in the coastal and pelagic zones of the Ionian Sea and Sicily Channel [18–20].

In the pelagic environment, microbial activity over both particulate organic matter (POM) and dissolved organic matter (DOM), produced in the euphotic layer, is the main factor which sustains life at greater depths. In fact, during sinking through the water column, organic polymers are subjected to a gradual degradation by different microbial processes. Bioavailable DOM becomes available to the deep microbial community through the transformation and solubilisation of particles, rather than through the direct export of surface DOM into the dark ocean [21]. Along the water column, the DOM is depleted of N and P but enriched with C which becomes rather refractory [22], as indicated by the increase in the C:N:P ratio of the DOM in layers of different depths. The Mediterranean Sea might be an exception because several studies have identified labile DOC transported into the deep layer [23].

In deep waters, POM might also support the metabolism of the micro-heterotrophic community [18,24]. POM originates mainly from phytoplankton and grazing activities, although it may also derive from the aggregation of organic macromolecules [21]. Few vertical profiles of extracellular

enzymatic activities extending from the euphotic zone to the meso- or bathypelagic realm are available in the Mediterranean Sea [17,18,24–26] and oceans [27,28]. Recent research suggests that in the bathypelagic domain, prokaryotes express highly cell-specific extracellular enzymes and have lower growth yields [28]. Protease and phosphatase activities are much higher than glucosidase activity, producing preferential nitrogen and phosphorus cleavage compared with carbon [21]. Moreover, the lack of long-term time series on enzymatic activity, respiration and bacterial production has limited knowledge of the effects of climatic changes on the microbial marine biocenosis functioning and structure in the epi-, meso- and bathypelagic zones [25].

During several cruises, carried out in the Ionian Sea and Sicily Channel from 1998 to 2004, different biochemical and microbial parameters involved in biogeochemical cycles (C, P, N) were measured. Supported by statistical analysis, we analysed the spatial and seasonal variability in microbial activities involved in organic matter transformation, both in terms of organic matter degradation (exoenzymes production) and utilisation (carbon production and respiration). We paid special attention to bacterial growth efficiency. The aim of this study was to verify whether different microbial processes inside the C and P cycles occur in different layers and seasons in the Ionian Sea.

2. Materials and methods

The data analysed in this study derive from several multidisciplinary field studies carried out in the Ionian Sea (Figure 1). Data were obtained within the framework of the following research projects (see Table 1):

1. Marine Ecosystems, seasonal interannual and decadal variability in the atmosphere, oceans and related marine ecosystems (SINAPSI) project, Task B10: Recent history of the Mediterranean water masses analysed by *in situ* determination of microbial activities. Two cruises were carried out in December 1998 to January 1999 (Sinapsi III) and March to April 2002 (Sinapsi IV).
2. Neutrin Monitoring Observatory (NEMO) project developed from 1999 to 2002, with four cruises named Talastro I–IV (December 1999, October 2000, July 2001, March 2002), to study the hydrobiological characteristics of marine area in front of Capo Passero (Km3 station).

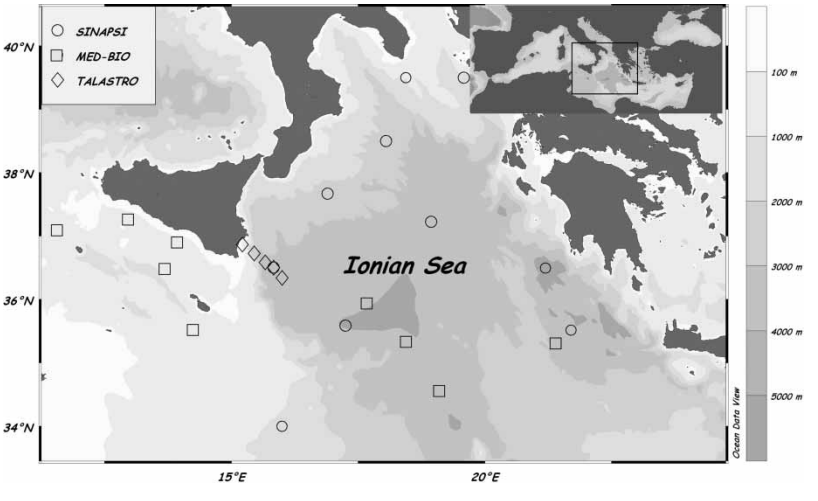

Figure 1. Map of the central Mediterranean Sea and areas investigated over 1998–2004 (Ocean Data View Software, Schlitzer, 2003. Available at http://odv.awi.de): Sinapsi cruises ◯; Talastro cruises ◇; MED-BIO cruises □.

Table 1. Sampling periods, size, depths, parameters and names of cruises and projects.

Area	Year	Months	N samples	Max. depth (m)	Parameters	Oceanographic Surveys	Ref.
Pelagic	1998–1999	December–January	105	4985	T, S, O_2, PPP, PA, POC, PON, BCP, EEA, CB, CDPR, NO_3, PO_4	Sinapsi-III	[18]
	1999	December	48	3400	T, S, O_2, PPP, PA, POC, PON, EEA, CHL, CB, CDPR, NO_3, PO_4	Talastro-I	This study
	2000	October	50	3400	T, S, O_2, PPP, PA, POC, PON, BCP, EEA, CHL, CB, CDPR, NO_3, PO_4	Talastro-II	[25]
	2001	July	49	3400	T, S, O_2, PPP, PA, POC, PON, EEA, CHL, CB, CDPR, NO_3, PO_4	Talastro-III	This study
	2002	March	48	3400	T, S, O_2, PPP, PA, POC, PON, EEA, CHL, CB, CDPR, NO_3, PO_4	Talastro-IV	[25]
	2002	March–April	114	4000	T, S, O_2, PPP, PA, POC, PON, EEA, CB, CDPR, NO_3, PO_4	Sinapsi-IV	[29]
	2004	November	90	3780	T, S, O_2, PPP, PA, POC, PON, BCP, EEA, CHL, CB, CDPR, NO_3, PO_4	MED-BIO	[30]
	2004	November	28	1690	T, S, O_2, PPP, PA, POC, PON, BCP, EEA, CHL, CB, CDPR, NO_3, PO_4	MED-BIO Sicily Channel	This study

Notes: T, temperature; S, salinity; O_2, oxygen; PPP, picophytoplankton abundance; PA, total prokaryotic abundance; POC, particulate organic carbon; PON, particulate organic nitrogen; BCP, bacterial production; EEA, esoenzymatic activities; CB, culturable bacteria; CHL, total chlorophyll; NO_3, nitrates; PO_4, phosphates; CDPR, carbon dioxide production rates.

3. FIRB-MIUR project: Biodiversity and organisation of the communities in different environmental contexts, MED-BIO cruise (Mediterranean biodiversity) carried out in November 2004.

The surveys discussed here had similar scientific objectives, research approaches, sampling strategies and methods. Details on the data collection and sampling strategies (Table 1) have been described previously [18,25,29,30].

The cruises were carried out on board the *R/V Urania* and *R/V Thetis* of the Italian CNR. Water samples were collected at different depths from the surface to the bottom (5 to 4900 m), using a rosette sampler equipped with 10 L acid-rinsed Niskin bottles. Samples were either processed immediately for specific measurements aboard the R/V or stored for subsequent analyses in the laboratory.

The studied parameters, analytical procedures and specific references are reported in Table 2 [14,31–40]. Regarding bacterial carbon production (BCP) analysis, *in situ* determination of leucine isotopical dilution was calculated for each cruise and water layer [34,41]. The conversion factors used for enzymatic activities (calculated as V_{max}) were 72 for leucine aminopeptidase (LAP) and β-glucosidase (GLU) activities, and 31 to convert alkaline phosphatase (AP) activity into P [35]. Also, the results of electron transport system (ETS) activity were converted into C and expressed as carbon dioxide production rates (CDPR), according to La Ferla et al. [42] in the euphotic zone and according to Packard et al. [43] and Azzaro et al. [44] in the aphotic zone.

Bacterial growth efficiency (BGE), which indicated the fraction of carbon utilised by bacteria for their growth, was also calculated as the percentage ratio between bacterial carbon production (BCP) and bacterial carbon demand (BCP + CDPR) [45].

2.1. *Statistical analysis*

Differences in each dependent variable of the dataset (see Table 2 for list of abbreviations) were established using analysis of variance (ANOVA) and considering the following fixed factors: layers (epi- vs meso- vs bathypelagic zone) and seasons (cold vs warm periods – summer and autumn). All variables were logarithmically transformed to comply with the assumption of ANOVA (normal distribution). Pearson's correlation coefficient was also calculated among the parameters. Analyses were performed with SigmaStat software v3.0.

Table 2. Methods and instrumentation utilised for analyses.

	Parameters	Method	Instrumentation	Ref.
T	Temperature	CTD multiparametric sensor	(SBE 19 Plus)	
S	Salinity	CTD multiparametric sensor	(SBE 19 Plus)	
O_2	Oxygen	CTD multiparametric sensor		
PPP	Picophytoplankton abundance	Microscopic count	Zeiss AXIOPLAN 2	[31]
PA	Total prokaryotic abundance	Microscopic count	Zeiss AXIOPLAN 2	[32]
BCP	Bacterial carbon production	[3H]-Leucine uptake	Beta-Counter Wallac 1414	[33,34]
CB	Culturable bacteria	Colony forming units	Marine Agar 2216	[14]
LAP	Leucine–aminopeptidase	Fluorogenic substrates	Turner TD 700 fluorimeter	[35]
GLU	$\beta-$Glucosidase	Fluorogenic substrates	Turner TD 700	[35]
AP	Alkaline phosphatase	Fluorogenic substrates	Turner TD 700	[35]
CHL	Chlorophyll a	Fluorimetric measurement		[36]
POC	Particulate organic carbon		CHN-Autoanalyzer 2400	[37]
PON	Particulate organic nitrogen		CHN-Autoanalyzer 2400	[37]
NO_3	Nitrates		Spectrophotometer	[38]
PO_4	Orthophosphate		Spectrophotometer	[38]
CDPR	Carbon dioxide production rates	ETS activity	Varian Spectrophotometer	[39,40]

In order to identify eventual differences among epi-, meso- and bathypelagic zones of each considered cruise, the principal components analysis (PCA) was computed on normalised data. This multivariate analysis generates new variables, called principal components (linear components of the original variables) which explain the highest dispersion of the samples.

Analysis of similarities (ANOSIM) was carried out to assess statistical differences between clusters into the multivariate datasets. ANOSIM is a nonparametric permutation procedure that tests whether differences in dissimilarity between groups exceed differences within groups [46]. ANOSIM results produce a sample statistic, R, which represents the degree of separation between test groups: a value close to 1 indicates that all replicates of a sampling site or a treatment are more similar to each other than any replicates of another site or treatment, whereas a value of 0 indicates no difference.

The contribution of each parameter to the dissimilarity among clusters, as well as to the similarity within each cluster, was identified using SIMPER analysis [47].

PCA, ANOSIM and SIMPER were carried out using Primer 6 software, Version 6β R6 (Copyright 2004, PRIMER-E Ltd).

3. Results

In order to compare the microbial processes occurring in the Ionian Sea with other marine areas of the world, the results were grouped by layers as follows: epipelagos (10–200 m), mesopelagos (200–1000 m) and bathypelagos (>1000 m).

3.1. *Analysis of dataset*

For PCA, each cruise was subdivided into epi-, meso- and bathypelagic zones (Figure 2). The first four principal components (PCs) explained 75.9% of the total variability, with the first two PCs representing 30.1 and 21.1% of the total variability, respectively. The first PC was dominated by salinity (S), temperature (T), CDPR and PO_4, whereas the second PC was mainly a mixture of several parameters (AP, ETS, C/N, POC and PON) with a greater weight for POC and PON.

Overall, the PCA plots highlighted that the meso- and bathypelagic layers were closely grouped, because their values fell into a single cluster with an Euclidean distance of 5.2 (not shown). By contrast, the epipelagic layer was very dispersed in the PCA plot, because its values fell into various clusters (Euclidean distance of 5.2), reflecting the seasonality of the sampling. Additional ANOSIM, computed with epi-, meso- and bathypelagic zones as factors, revealed a significant difference (Global $R = 0.345$, $p < 0.01$) among all the three layers. In particular, ANOSIM Pairwise Test highlighted a statistical difference between the epi- and mesopelagic zones (R Statistic $= 0.587$, $p < 0.02$) and between the epi- and bathypelagic zones (R Statistic $= 0.556$, $p < 0.02$). Any statistical difference was retrieved between meso- and bathypelagic layers.

A new PCA plot was computed considering only the epipelagic zone (Figure 3a). The first four PCs explained 90.6% of the total variability, whereas the first two PCs represented 36.4 and 32.8% of the total variability, respectively. Both PCs were a mixture of several parameters, with greater weight for S, POC, PON, AP and ETS for PC1 and Total PA and PPP mainly for PC2.

Stations were grouped into a large cluster (Euclidean Distance 5, grey line) including winter and spring, whereas the autumn sample was included in a larger cluster (Euclidean Cluster of 6.7, black line). Two out-group clusters were identified; one corresponding to the Sicily Channel Med-Bio survey (Nov-E-Sc) and the second corresponding to the unique summer cruise (Jul-E). ANOSIM – computed with seasons as factors – highlighted a statistical difference among the stations (Global $R = 0.279$, $p = 0.02$). Moreover, the ANOSIM Pairwise Test underlined a great

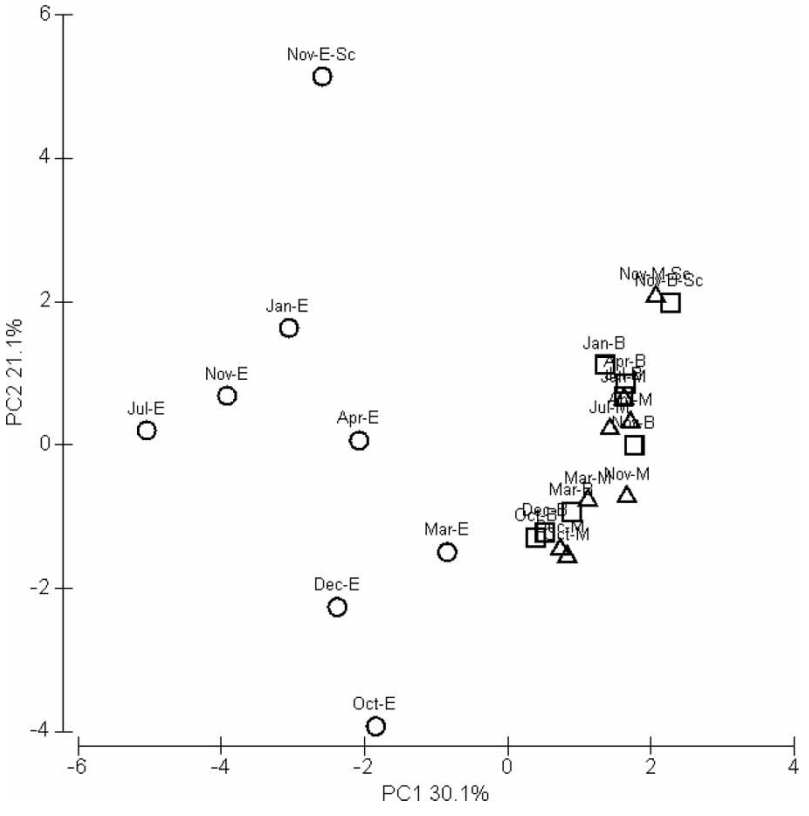

Figure 2. Principal components analysis applied to the biological, chemical and physical parameters dataset. Circles, epipelagic zone; triangles, mesopelagic zone; squares, bathypelagic zone.

difference between seasons (with the R Statistic ranging from 0.5 to 1), with the exception of winter versus spring (R Statistic $= -0.25$). Finally, the Sicily Channel Med-Bio survey (Nov-E-Sc) resulted in a statistically significant difference (Global $R = 0.578$, $p < 0.02$), even though it was performed during winter and the stations were geographically separated from the others (ANOSIM, computed with geographic locations as factors).

The meso- and bathypelagic zones were jointly considered for the PCA, because of their relative homogeneity (Figure 3b) when computed with epi-, meso- and bathypelagic zones as factors. In this case, the first four PCs determined 90.3% of the total variability and the two first PCs accounted for 31.2 and 29.7% of the variability, respectively. The first PC was only dominated by O_2, PON, POC, LAP and BCP parameters, whereas PC2 was an association of trophic parameters (POC, PON and C/N) and microbial activities (LAP, BCP, AP and GLU). The PCA plot did not show a well-defined pattern for such samples, most of them belonging to the same large cluster (Euclidean Distance of 4, black line) with two out-groups. The first is composed by the two Sicily Channel layers (Nov-M-SC and Nov-B-SC) and the second by the July-M sample only.

Inside the large cluster, four sub-clusters (Euclidean Distance of 3, grey line) were recognisable. In particular, it was possible to see that meso- and bathypelagic zones of the campaigns carried out during spring and autumn were closely (in pairs) related. Conversely, the two aphotic layers of each campaign (winter and summer) were more disperse on the PCA plot.

ANOSIM results, computed with seasons as factors, revealed a Global R of 0.231 ($p = 0.05$) and the pairwise tests evidenced that there was a statistical difference between the winter and autumn surveys (R Statistic $= 0.302$), but there was no statistical difference between the winter

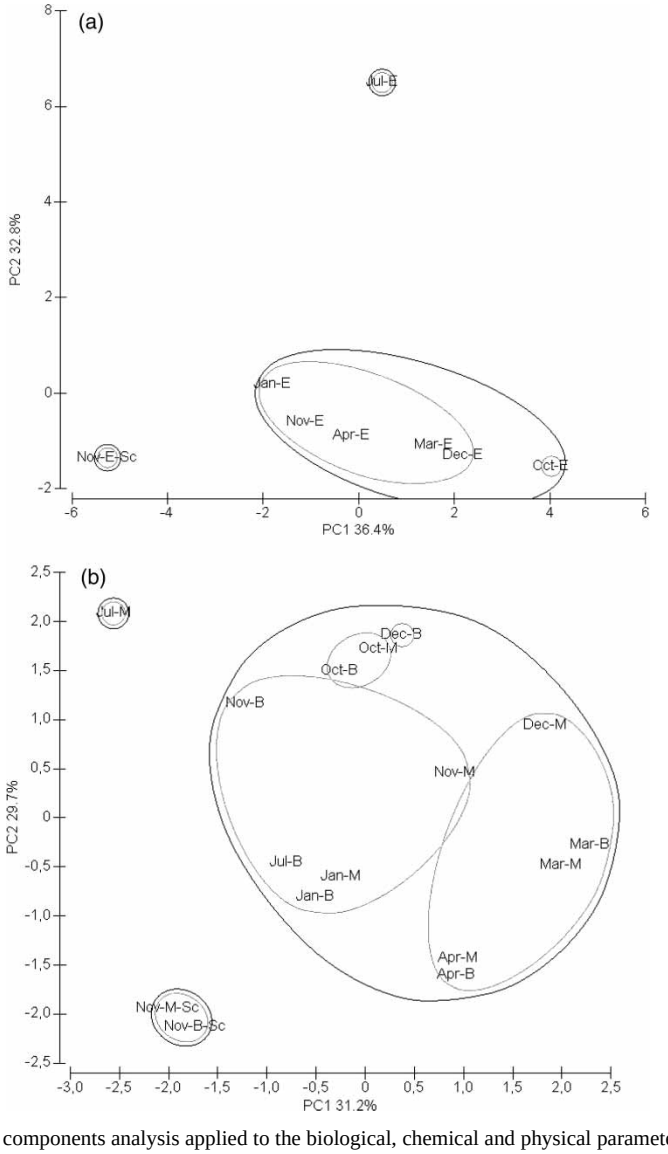

Figure 3. Principal components analysis applied to the biological, chemical and physical parameters of the epipelagic (a), meso- and bathypelagic (b) datasets in different periods.

and both summer and spring surveys. The other pairwise tests (autumn vs summer, autumn vs spring, summer vs spring) were statistically different (*R* Statistic ranging from 0.5 to 1).

SIMPER analysis was used to determine parameter dominance and showed that the most important parameters separating the epi- and meso- groups were S, ETS, PO_4 and GLU (contribution percentage ranging from 13.2 to 8.9, and a cumulative percentage of 53.5), whereas those separating the epi- and bathy- groups were S, PO_4, ETS and T (contribution percentage ranging from 11.4 to 9.2, and a cumulative percentage of 53.9). However, the meso- and bathypelagic layers were separated only by C/N, POC and BCP (contribution percentage ranging from 28.9 to 14.0, and a cumulative percentage of 58.5).

SIMPER analysis was also carried out to individuate parameters explaining the distances among campaigns with 'Seasons' as factors. The distance between winter and autumn groups was mainly

due to AP, PON, GLU and BCP (from 28.1 to 8.5%; cumulative 54.3%), whereas between the winter and spring group the distance was mainly due to C/N, BCP, ETS, POC and PON (from 11.9 to 9.1%; cumulative 51.6%). Finally, the winter and summer groups were separated by three main parameters (cumulative 60.1%): PPP (23.1%), PA (23.1%) and LAP (13.8%). Conversely, autumn and spring were separated by AP (40%), BCP (25.4%) and GLU (10.5%), whereas autumn and summer were separated by AP (20.6%), PPP (22.2%) and Total PA (20.1%). Finally, the summer and spring groups were separated by PA (22.9%), PPP (22.9%) and LAP (18.8%).

Based on the PCA, in the following section, results are reported by considering the epipelagic layer alone, and the meso- and bathypelagos together.

3.2. *Epipelagic layer*

This part of the water column is generally characterised by high variability in chemical, physical and biological parameters, owing to seasonal variations, trophic processes and exchanges with the atmosphere. Results from the ANOSIM showed a great difference among seasons, with the exception of winter versus spring. For this reason, we grouped data into summer, autumn and cold period (all months with the exception of July and October).

Surface water temperature showed a seasonal fluctuation with marked differences between cold and warm periods, ranging from 15.17 °C (January) to 17.47 °C (October). Salinity showed small differences in the Ionian Sea during the examined periods with the exception of the epipelagic layer in the Sicily Channel (Table 3).

In the surface layer, maximum mean values of nitrate and phosphate concentrations were observed in March and November, respectively (Table 3), and the minimum in April (0.45 μM NO_3 and 0.06 μM PO_4). In this layer, nitrate and phosphate distributions were characterised by large standard deviations, particularly for nitrate, which indicated wide variations in nutrient concentrations at these depths, because of biological processes.

POC amounts in the Ionian pelagic waters were generally low, and showed significant decreases with depth (Table 4a). In the epipelagic layer, the highest POC and PON incidences and noticeable seasonal fluctuations were recorded (Table 3). Peaks were registered in October (48.32 μg C·L^{-1} and 7.37 μg N·L^{-1}), whereas the lowest values were in January (20.05 μg C·L^{-1} and 3.54 μg N·L^{-1}).

Prokaryotic abundance (PA) in the Ionian Sea was in the order of 10^5 cells·mL^{-1} and declined significantly with depth (Figures 4–6); the mean values ranged from 3.3×10^5 cells· mL^{-1} in April to 6.3×10^4 cells·mL^{-1} in July. In these layers, a slight increase over time was observed. Seasonal fluctuations in PA were also evident when comparing the cold period versus July and October (Table 4b).

Picophytoplankton abundance (PPP) showed values 1 or 2 orders of magnitude lower than those of PA. The highest mean value was recorded in January (1.79×10^4 cells·mL^{-1}) and the lowest in October (1.2×10^3 cells·mL^{-1}).

CB showed wide variations within each layer and seasonal fluctuations (Figure 4); the highest values were observed in October sampling, and the lowest in March and December. Owing to high standard deviations, the differences in depth were not significant (Table 4a). Seasonal fluctuations in CB were confirmed by ANOVA among cold and warm periods (Table 4b).

BCP, analysed in three periods only, showed values ranging from 0.0166 (January) to 0.0191 μg C·L^{-1}·h^{-1} (October); an intermediate value of 0.0178 μg C·L^{-1}·h^{-1} was observed in November.

Greater variability in enzymatic activities was observed, compared with PA. LAP activity showed high variability in the epipelagic layer with mean values ranging from 0.11 to

Table 3. Mean (SD) values for physical, chemical and trophic properties, characterizing the different layers sampled during the cruises.

Sampling	N	T (°C)	S	O_2 μM	NO_3 μM	PO_4 μM	CHL $\mu g \cdot L^{-1}$	POC $\mu g\ C \cdot L^{-1}$	PON $\mu g\ l^{-1}$	C/N
Epipelagic layer										
January 99	50	15.17 (0.76)	38.59 (0.21)	236.06 (17.01)	1.06 (1.57)	0.06 (0.03)	–	20.05 (8.41)	3.54 (1.65)	8.97 (13.69)
December 99	24	15.79 (1,33)	38.65 (0.13)	233.83 (25.75)	1.06 (1.23)	0.15 (0.03)	0.09 (0.06)	41.85 (15.36)	7.02 (2.25)	7.03 (1.3)
October 2000	24	17.47 (4.05)	38.68 (0.13)	233.03 (22.60)	1.14 (1.01)	0.15 (0.08)	0.08 (0.05)	48.32 (13.08)	7.37 (3.42)	8.36 (3.42)
July 2001	24	17.38 (3.62)	38.62 (0.15)	207.59 (17.33)	0.94 (1.17)	0.13 (0.11)	0.08 (0.1)	32.43 (12.82)	6.87 (2.41)	6.87 (7.11)
March 2002	18	15.19 (0.59)	38.63 (0.20)	268.65 (19.78)	2.02 (1.63)	0.12 (0.10)	0.08 (0.08)	38.40 (11.39)	5.41 (1.95)	8.85 (2.43)
April 2002	48	15.49 (0.54)	38.58 (0.27)	240.17 (10.13)	0.45 (0.75)	0.06 (0.11)	–	29.76 (9.58)	5.52 (1.63)	6.29 (1.03)
November 2004	50	17.14 (2.28)	38.44 (0.42)	224.58 (10.69)	1.37 (1.06)	0.17 (0.09)	0.039 (0.02)	36.42 (15.49)	5.44 (1.99)	7.76 (0.97)
Sicily Channel 2004	18	16.35 (1.66)	37.99 (0.50)	218.57 (14.38)	1.63 (0.94)	0.21 (0.11)	0.09 (0.08)	–	–	–
Mesopelagic layer										
January 99	27	13.98 (0.47)	38.80 (0.09)	198.8 (12.09)	5.09 (1.12)	0.19 (0.05)	–	5.57 (1.42)	0.82 (0.42)	10.94 (8.16)
December 99	9	13.62 (0.15)	38.74 (0.03)	205.26 (5.24)	3.14 (0.67)	0.19 (0.02)		36.97 (21.71)	5.08 (2.01)	8.44 (2.59)
October 2000	12	13.77 (0.11)	38.76 (0.03)	198.98 (7.92)	3.76 (0.69)	0.25 (0.07)		31.38 (9.9)	4.90 (2.08)	9.26 (5.61)
July 2001	11	13.81 (0.16)	38.76 (0.03)	176.05 (5.31)	4.44 (0.49)	0.24 (0.11)		19.69 (13.14)	2.72 (0.84)	8.23 (5.0)
March 2002	9	13.82 (0.14)	38.76 (0.03)	220.95 (4.85)	5.27 (0.69)	0.29 (0.15)		25.97 (1.98)	4.53 (1.99)	6.68 (2.07)
April 2002	43	14.02 (0.42)	38.79 (0.06)	200.57 (14.37)	4.17 (1.22)	0.20 (0.06)	–	11.79 (5.74)	2.61 (1.26)	5.46 (1.50)
November 2004	21	13.96 (0.25)	38.80 (0.05)	196.45 (7.23)	4.33 (1.24)	0.21 (0.11)		19.84 (9.32)	2.51 (2.30)	12.47 (4.79)
Sicily Channel 2004	9	14.08 (0.09)	38.81 (0.01)	187.88 (3.6)	3.39 (0.62)	0.22 (0.15)		–	–	–
Bathypelagic layer										
January 99	36	13.92 (0.19)	38.75 (0.04)	200.97 (5.71)	4.74 (0.20)	0.16 (0.02)	–	5.44 (2.6)	1.01 (1.11)	9.58 (5.35)
December 99	9	13.39 (0.06)	38.71 (0.02)	216.68 (3.43)	3.64 (0.46)	0.17 (0.04)		34.61 (9.78)	5.92 (1.20)	6.84 (1.24)
October 2000	18	13.79 (0.06)	38.73 (0.01)	203.08 (2.49)	4.04 (0.38)	0.24 (0.08)		23.1 (7.37)	5.01 (2.91)	7.18 (4.07)
July 2001	7	13.79 (0.05)	38.73 (0.01)	182.17 (3.41)	4.23 (0.40)	0.19 (0.12)		14.13 (11.53)	2.96 (1.23)	5.29 (2.10)
March 2002	21	13.82 (0.05)	38.71 (0.01)	228.59 (2.16)	4.86 (0.40)	0.34 (0.20)		29.54 (18.65)	4.58 (1.40)	7.36 (3.27)
April 2002	41	13.84 (0.12)	38.74 (0.02)	199.82 (4.92)	4.39 (0.66)	0.22 (0.04)	–	10.39 (5.05)	1.96 (0.83)	6.19 (1.45)
November 2004	32	13.85 (0.06)	38.74 (0.02)	205.31 (4.34)	4.24 (0.99)	0.20 (0.08)		19.74 (14.14)	1.93 (0.71)	11.51 (4.35)
Sicily Channel 2004	2	13.99	38.78		4.35	0.12				

Notes: T, temperature; S, salinity; O_2, oxygen; POC, particulate organic carbon; PON, particulate organic nitrogen; CHL, total chlorophyll; NO_3, nitrates; PO_4, phosphates.

Table 4a. Results of ANOVA (F-test and p) on values for chemical, physical and biological parameters among: F1 (epi vs meso), F2 (meso vs bathy).

Variable	Abbrevation	F1		F2	
		F	p	F	p
Temperature	T	475.2	1%	13.5	1%
Salinity	S	79.5	1%	90.3	1%
Oxygen	O_2	2.0	ns	2.5	ns
Picophytoplankton	PPP	35.2	1%	0.99	ns
Prokaryotic abundance	PA	50.9	1%	6.9	1%
Culturable bacteria	CB	0.16	ns	0.06	ns
Leucine–aminopeptidase	LAP	5.6	5%	0.3	ns
$\beta-$Glucosidase	GLU	7.0	1%	1.5	ns
Alkaline phosphatase	AP	1.7	ns	0.08	ns
Particulate organic carbon	POC	102.2	1%	0.01	ns
Particulate organic nitrogen	PON	137.2	1%	0.4	ns
C/N		1.03	ns	0.9	ns
Carbon dioxide production rates	CDPR	89.5	1%	3.6	ns
Bacterial carbon production	BCP	58.1	1%	6.1	5%
Phosphates	PO_4	108.5	1%	14.0	1%
Nitrates	NO_3	499.0	1%	0.6	ns

Note: ns, Not significant.

Table 4b. Results of ANOVA (F-test and p) on values of chemical, physical and biological parameters among: F1 (cold period vs July); F2 (cold period vs October); F3 (July vs October).

Variable	Abbreviation	F1		F2		F3	
		F	p	F	p	F	p
Temperature	T	21.7	0.01	5.6	0.05	1.3	ns
Salinity	S	0.2	ns	1.7	ns	5.7	0.05
Oxygen	O_2	9.7	0.01	17	0.01	16.1	0.01
Picophytoplankton	PPP	0.1	ns	14.1	0.01	29.1	0.01
Prokaryotic abundance	PA	10.9	0.01	21.1	0.01	0.98	ns
Culturable bacteria	CB	31.9	0.01	85.2	0.01	1.1	ns
Leucine–aminopeptidase	LAP	0.4	ns	1.8	ns	0.4	ns
β-Glucosidase	GLU	1.5	ns	5.4	0.05	6.2	0.05
Alkaline phosphatase	AP	1.9	ns	144.7	0.01	14.2	0.01
Particulate organic carbon	POC	4.1	0.05	33.6	0.01	10.8	0.01
Particulate organic nitrogen	PON	25.4	0.01	22.5	0.01	0.69	ns
C/N		3	ns	0.004	ns	1.4	ns
Carbon dioxide production rates	CDPR	4.2	0.05	18.3	0.01	34.3	0.01
Bacterial carbon production	BCP	–		3.3	ns	–	
Phosphates	PO_4	0.9	ns	5.8	0.05	6.1	0.05
Nitrates	NO_3	5.9	0.05	0.11	ns	3.3	ns

Note: ns, Not significant.

$0.39\,\mu g\,C{\cdot}L^{-1}{\cdot}h^{-1}$ during the different periods; the highest values were observed in December and the lowest in October (Figure 4).

Low values were observed for GLU activity with differences among the seasons. The values ranged from 0.014 in January 1999 to $0.35\,\mu g\,C{\cdot}L^{-1}{\cdot}h^{-1}$ in December 1999. LAP exceeded GLU activity (LAP/GLU ratio, from 1.2 to 10.1) in all months, with the exception of October (LAP/GLU ratio, 0.8).

Also, AP values were highly variable over time. The highest values were observed in October in all layers (Figure 4 and Table 4b). In the epipelagic layer P potentially released by enzymatic activity ranged from $0.04\,\mu g\,P{\cdot}L^{-1}{\cdot}h^{-1}$ in January to $3.1\,\mu g\,P{\cdot}L^{-1}{\cdot}h^{-1}$ in October.

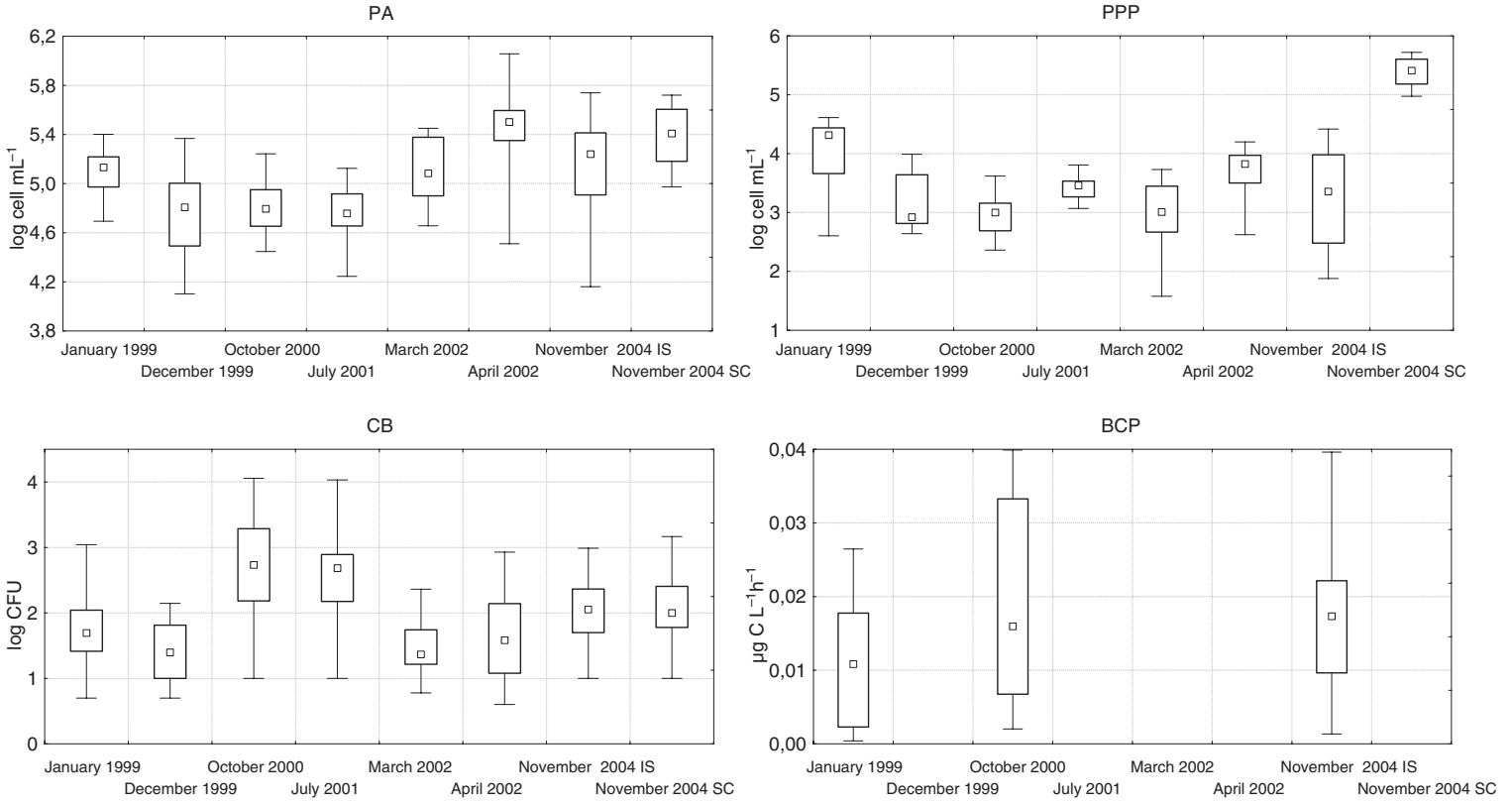

Figure 4. Box plot of prokaryotic abundance (PA), picophytoplankton abundance (PPP), culturable bacteria (CB), bacterial carbon production (BCP), leucine aminopeptidase (LAP), β-glucosidase (GLU), alkaline phosphatase (AP) and respiration (CDPR) in epipelagic layer during the different cruises. The statistical distribution in each box plot is used: the small square represents the median, the large square encloses the 25 and 75% percentiles of the data and the vertical bars indicate the max and min of the data.

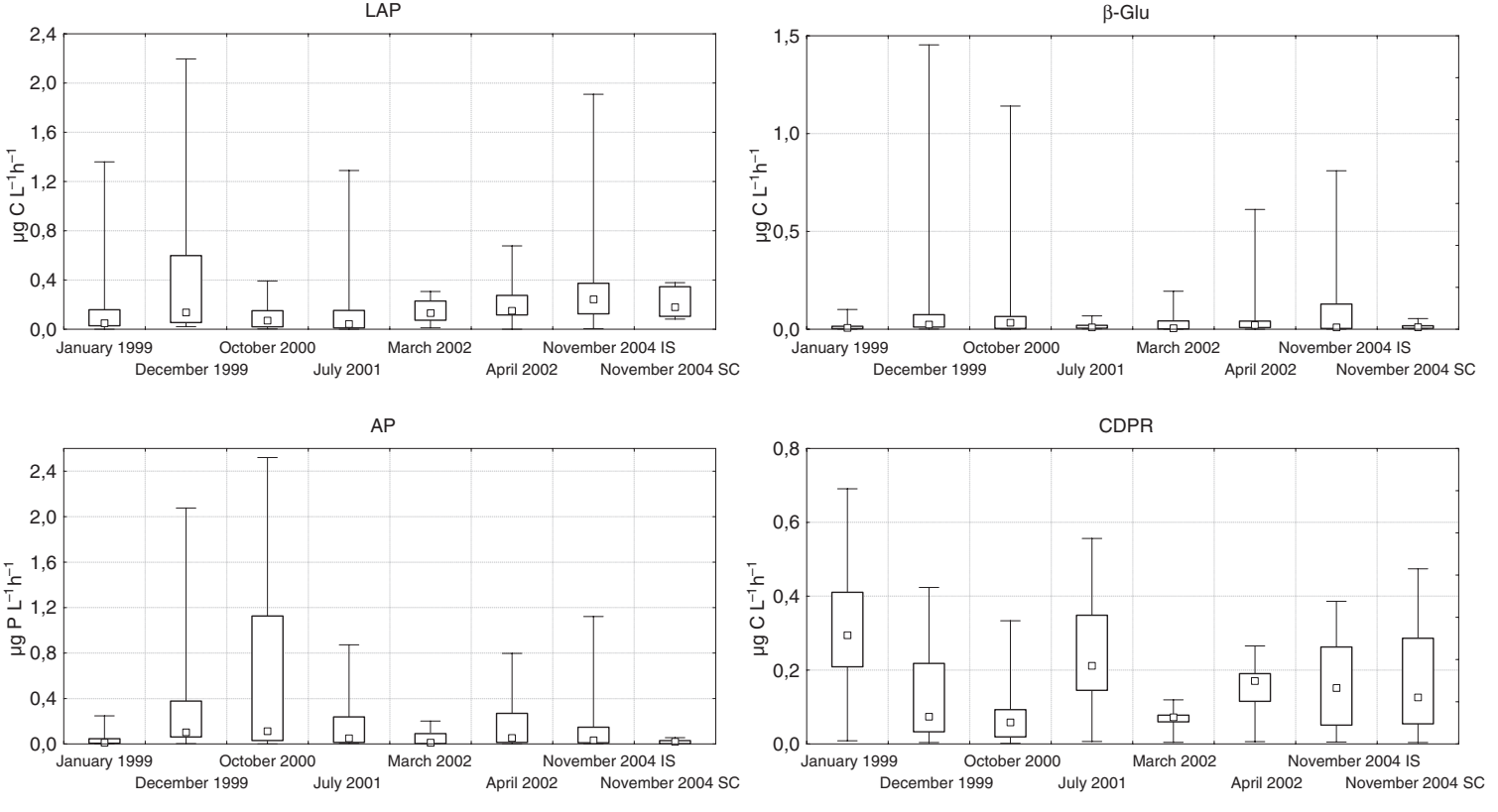

Figure 4. Continued.

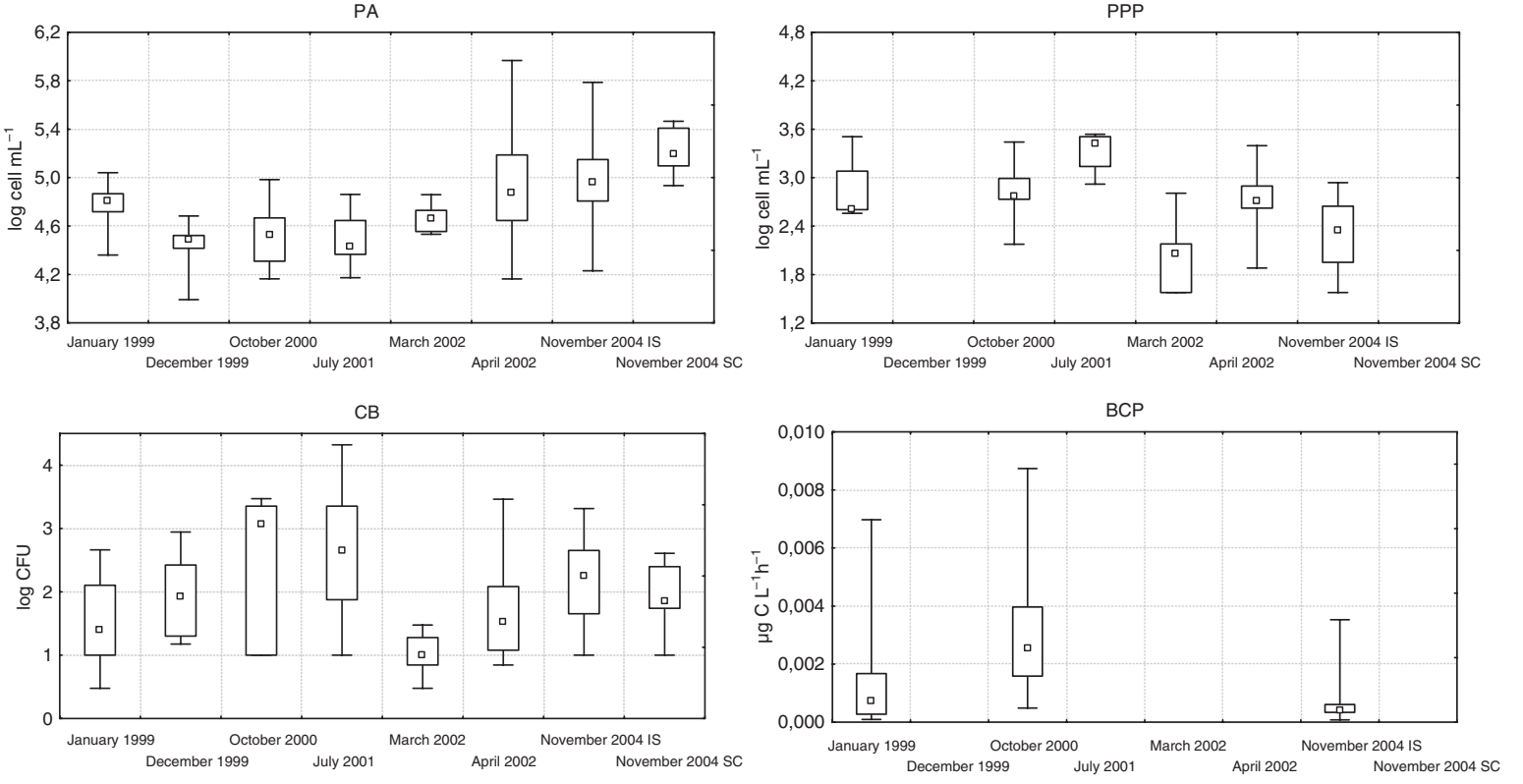

Figure 5. Box plot of prokaryotic abundance (PA), picophytoplankton abundance (PPP), culturable bacteria (CB), bacterial carbon production (BCP), leucine aminopeptidase (LAP), β-glucosidase (GLU), alkaline phosphatase (AP) and respiration (CDPR) in mesopelagic layer during the different cruises. For statistical explanation see Figure 4.

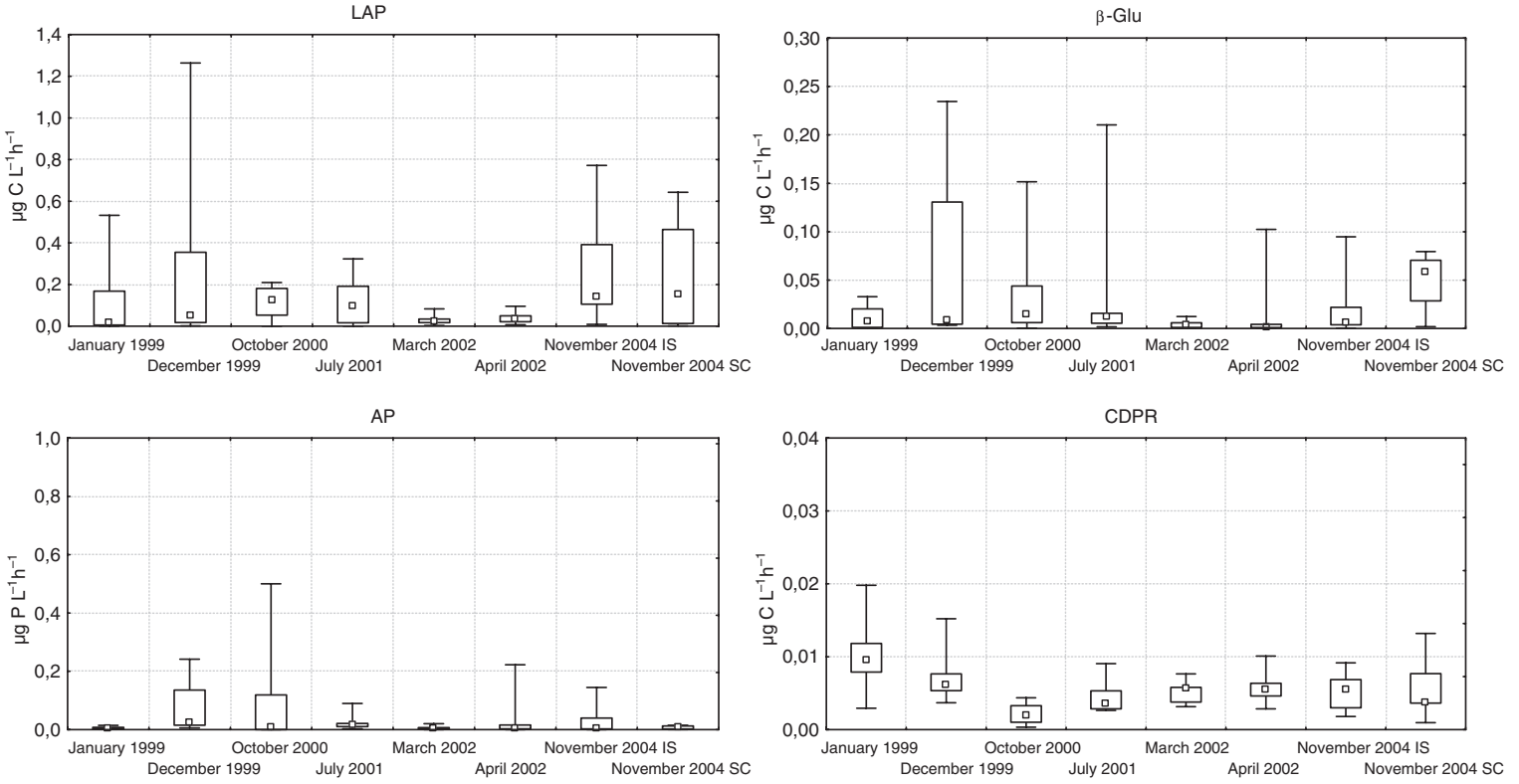

Figure 5. Continued.

LAP/AP ratio was generally >1, except in October when AP was the dominant enzymatic activity (LAP/AP ratio, 0.05).

CDPR greatly varied both over time (months and years) and in relation to depth. High variability occurred in January, July and November. Its distribution showed important decreases along the water column, particularly between the epi- versus mesopelagic layers (Table 4a). In the epipelagic zone the CDPR varied from 0.07 (March) to 0.36 $\mu g\,C{\cdot}L^{-1}{\cdot}h^{-1}$ (January).

3.3. *Meso- and bathypelagic layers*

In the meso- and bathypelagic zones, T decreased significantly compared with the epipelagic layer with a minimum of 13.39 °C in December; the values showed lower standard deviations and variations over seasons. Higher S values with narrow variations in the meso- and bathypelagic layers than in the epipelagic layer were observed (Table 3).

The mean values of nutrients (NO_3 and PO_4) were significantly higher in the mesopelagic than in the other layers. Seasonal variations were found in the meso- and bathypelagic layers for NO_3 and PO_4, reaching maximum values in March. In the deepest layer, nutrient concentrations were relatively constant, as indicated by weak standard deviations (Table 3).

ANOVA results for testing the null hypothesis of equality among layers showed differences from epi- to mesopelagic layers for most of the analysed biological parameters (PA, PPP, BCP, LAP, GLU, POC, PON and CDPR). Only a few biological variables were significantly different between the meso- and bathypelagic layers (PA, BCP) (Table 4a).

POC and PON amounts in the mesopelagic layer were very low, never exceeding 36.97 $\mu g\,C \cdot L^{-1}$ and 0.82 $\mu g\,N{\cdot}L^{-1}$, respectively. In this layer, the most significant decreases, ~44 and 46% of the epipelagic values, were recorded in summer (Tables 3 and 4a).

In the bathypelagic layer, POC and PON showed further decreases respecting the epi- and mesopelagic layers. In particular, POC decreases from the epi- to bathypelagic layers were observed in summer and autumn, whereas in winter the lowest POC values were recorded in the mesopelagic layer (Figure 7). The same patterns were observed for PON, which was significantly correlated with POC (Table 5).

C/N ratios higher than those recorded in the other layers were observed in the mesopelagic layer for all the investigated seasons (except spring), indicating more refractory organic matter in this layer.

The PA decrease was 50% in the mesopelagic layer and ~10% in the bathypelagic layer, with respect to former layer abundance. Nevertheless, the epipelagic trend was reflected at deeper layers although to a lower extent, with an increase in values over time. In the bathypelagic layer, a wide range of PA values was gathered from April to November; the mean values varied from 3.13×10^4 to 1.88×10^5 cells·mL^{-1} (Figures 5 and 6).

PPP cells were also observed repeatedly in the meso- and bathypelagic zones, with a reduction of one order of magnitude (Figure 5). In both the deep layers, higher values were registered in July than in the other periods.

CB showed high variability with the highest values observed in October sampling, whereas the lowest values were observed in March and December in both layers.

BCP showed significant differences among layers with higher values measured in the epipelagic rather than the meso- and bathypelagic layers (Table 4a). Mesopelagic layer displayed mean values ranging from 0.0006 (November) to 0.0033 $\mu g\,C{\cdot}L^{-1}{\cdot}h^{-1}$ (October). Such a layer was less productive. ANOVA showed significant differences among the sampling periods ($F = 0.364$, $p < 0.01$). A slight decrease between meso- and bathypelagic layers was observed. In the bathypelagic zone, the highest mean values were observed in October (0.0041 $\mu g\,C{\cdot}L^{-1}{\cdot}h^{-1}$), followed by January and November (0.0029 and

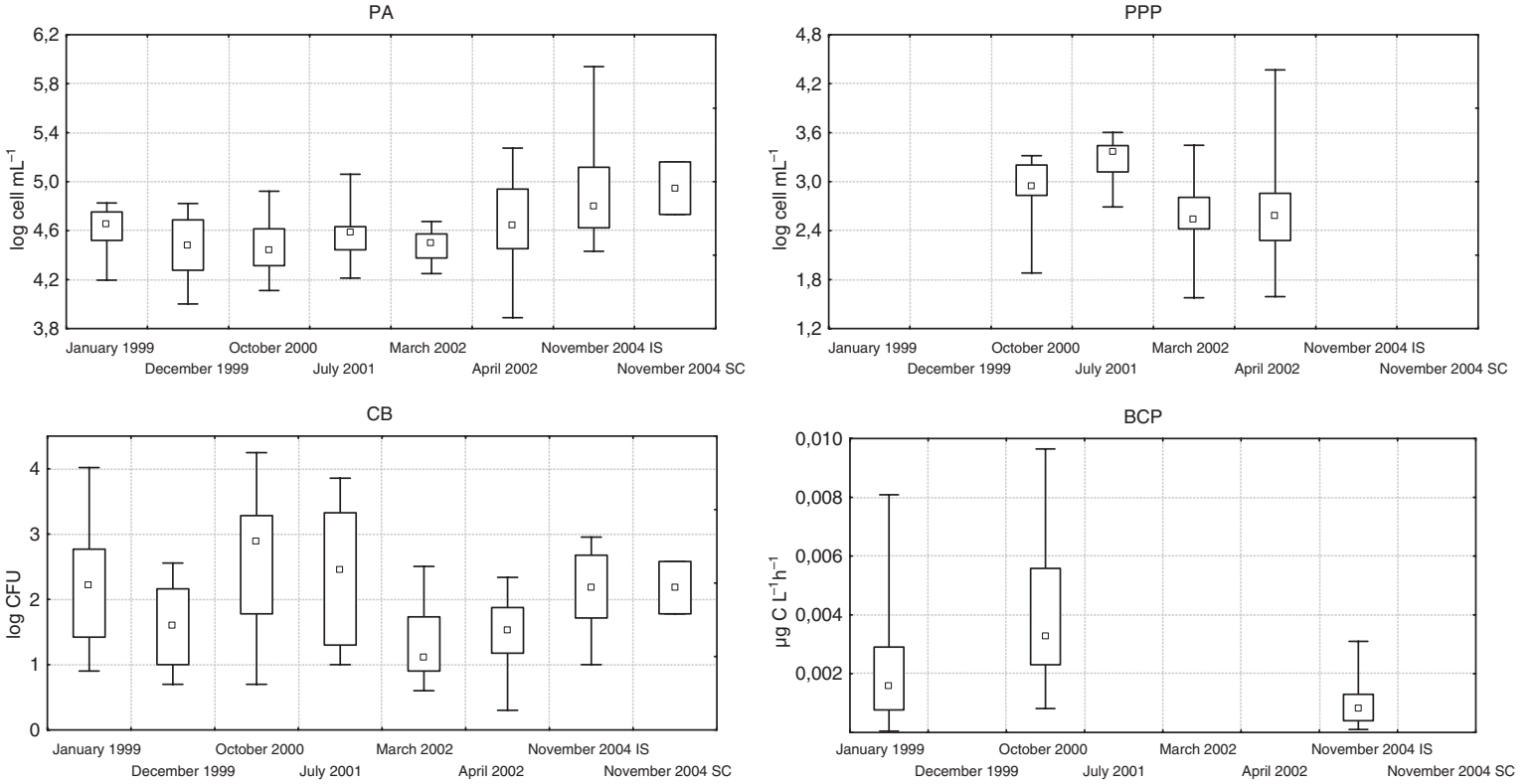

Figure 6. Box plot of prokaryotic abundance (PA), picophytoplankton abundance (PPP), culturable bacteria (CB), bacterial carbon production (BCP), leucine aminopeptidase (LAP), β-glucosidase (GLU), alkaline phosphatase (AP) and respiration (CDPR) in bathypelagic layer during the different cruises. For statistical explanation see Figure 4.

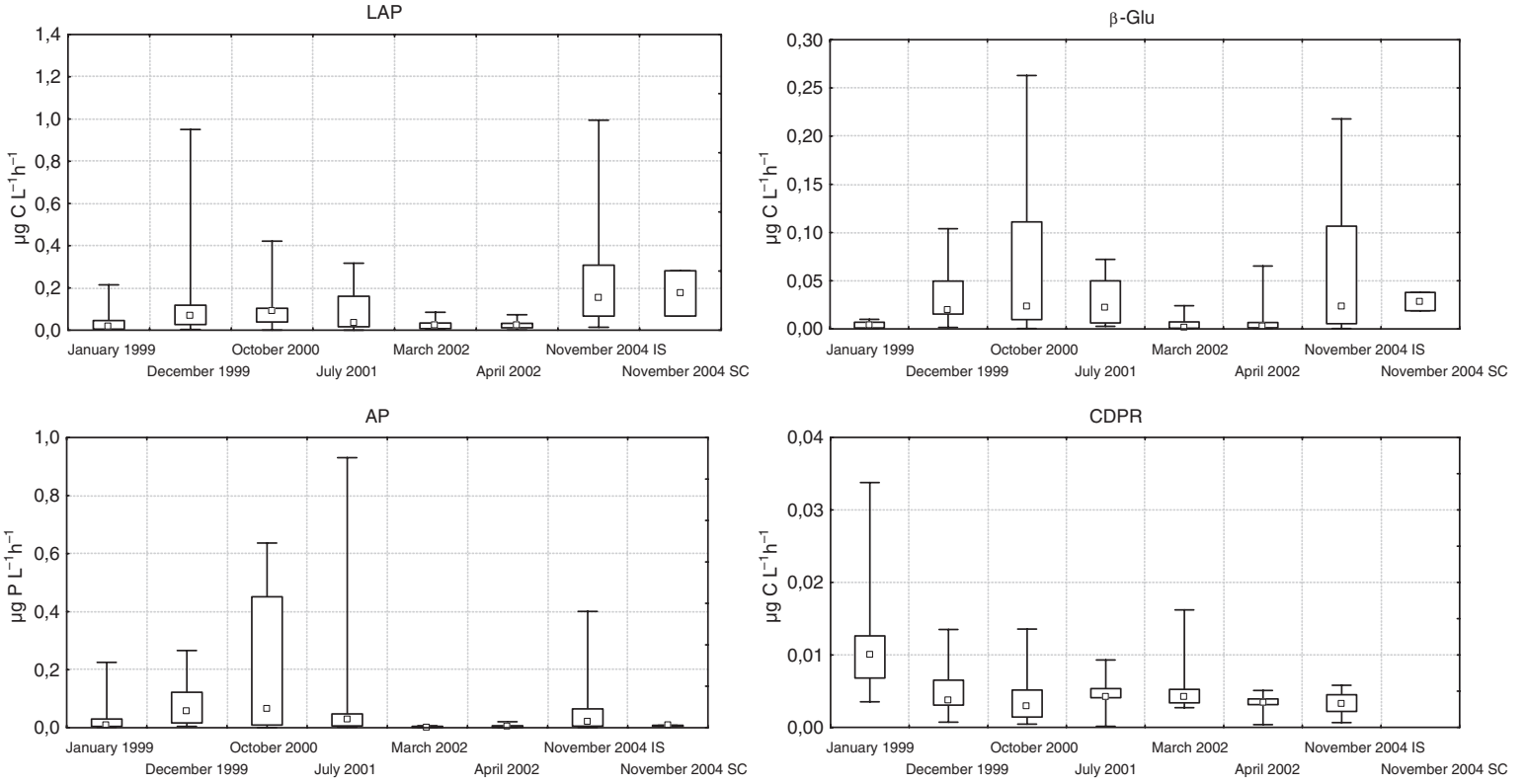

Figure 6. Continued.

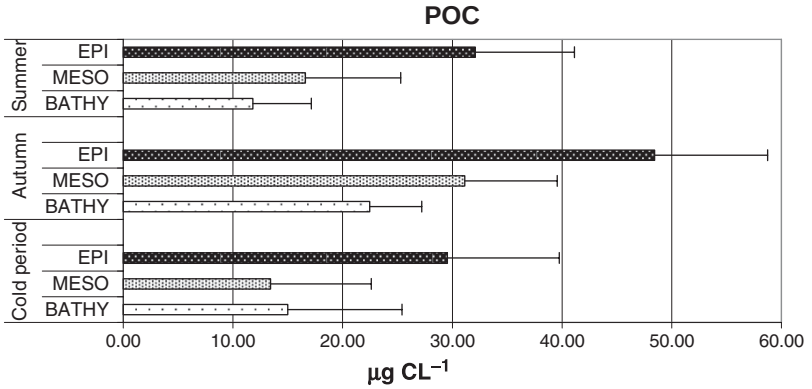

Figure 7. Distribution of particulate organic carbon (POC) in the epi-, meso- and bathypelagic layers (mean values with standard deviation) during cold and warm periods.

$0.001\,\mu g\,C{\cdot}L^{-1}{\cdot}h^{-1}$, respectively). Significant differences among seasons were observed ($F = 8.54$, $p < 0.001$).

The distribution of BCP per cell followed a decreasing trend from epi- to bathy- and mesopelagos (0.161, 0.080, $0.050\,fg\,C{\cdot}cell^{-1}{\cdot}h^{-1}$, respectively).

As other biological parameters, LAP displayed a decreasing pattern from the epi- to mesopelagic layers (Table 4a); the reduction was ∼21%. No seasonal differences were observed, probably because of the high variability, however, the lowest mean values were observed in March and the highest in December (0.032–$0.27\,\mu g\,C{\cdot}L^{-1}{\cdot}h^{-1}$, respectively). In the bathypelagic layer LAP showed similar or lower values than in the mesopelagic layer, varying from $0.02\,\mu g\,C{\cdot}L^{-1}{\cdot}h^{-1}$ in April to $0.33\,\mu g\,C{\cdot}L^{-1}{\cdot}h^{-1}$ in November (Figure 5).

A greater reduction of 66% was observed for GLU between the epi- and mesopelagos (Table 4a), where the values varied from 0.005 (April) to $0.05\,\mu g\,C{\cdot}L^{-1}{\cdot}h^{-1}$ (December). As a consequence, the importance of LAP over GLU increased in mesopelagos (LAP/GLU ratio ranging from 3.1 to 10.5). In the bathypelagic layer, an increase in GLU compared with the upper layer was observed in October, when the highest values were reached ($0.21\,\mu g\,C{\cdot}L^{-1}{\cdot}h^{-1}$). In fact, GLU activity in October exceeded the LAP activity as indicated by the LAP/GLU ratio (0.24). Conversely, the lowest GLU mean value was registered in April ($0.004\,\mu g\,C{\cdot}L^{-1}{\cdot}h^{-1}$).

In the mesopelagic layer, AP showed low values in all the studied periods, with the exception of the October sampling. In this month, AP exceeded LAP activity as indicated by LAP/AP ratio (0.07). High differences in AP activity were observed in the bathypelagic layer in relation to different periods (Figure 6), ranging from 0.003 in March to $0.50\,\mu g\,P{\cdot}L^{-1}{\cdot}h^{-1}$ in October. In fact, the LAP/AP ratio was <1 in October and July.

CDPR in the mesopelagic zones ranged from 0.002 to $0.01\,\mu g\,C{\cdot}L^{-1}{\cdot}h^{-1}$. CDPR variability in the mesopelagic layer showed a trend over time similar to the upper horizon, with high values and wide dispersion in January, low rates and narrow variability in October.

CDPR in the bathypelagic zones varied from 0.0035 to $0.01\,\mu g\,C{\cdot}L^{-1}\,h^{-1}$, in April and January, respectively. The trend with time was quite similar to the mesopelagic one from January to March.

3.4. *Sicily Channel*

Surface waters were characterised by lower temperature and salinity than the Ionian basin (Table 3). PA was characterised by decreasing mean values from epi- to meso- and bathypelagic zones (2.8×10^5, 1.8×10^5, 9.9×10^4 cells·mL^{-1} respectively). In a similar way, CB decreased with depth. BCP was measured only in one station and varied from 0.029 to $0.0002\,\mu g\,C{\cdot}L^{-1}{\cdot}h^{-1}$.

Table 5. Significant correlations computed among the studied parameters (Epipelagos, Mesopelagos, Bathypelagos).

Parameter	No. observations (max–min)	*r* (Pearson correlation) Positive with:	Negative with:
Epipelagos			
Depth	258	**S**, **PO_4**, **NO_3**	**T**, **O_2**, **PPP**, **PA**, **CHL**, **POC**, **LAP**, **AP**, **CDPR**, **PON**, **BCP**
T	258	**PPP**, **CDPR**, POC, **PON**, **BCP, CB, PO_4**	**S**, **O_2**, **NO_3**,
S	256	**CHL**, **NO_3**	**O_2**, **PPP**, **PA**, **POC**, **CDPR**, **PON**, **PO_4**, **BCP**
O_2	256–255	**PPP, PA, CHL, POC, BCP**	**CB**, **PO_4**, **NO_3**
PPP	200–198	**PA**, **CHL**, **CDPR**	POC, **PO_4**, **NO_3**
PA	231–200		**CB, PO_4**, **NO_3**
CB	239–193	**AP, PON**	LAP
LAP	220–125	**GLU, PO_4**	
GLU	226–135	**AP**	
AP	228–131	**POC, PON**	
POC	192–102	**PON, PO_4, BCP**	**NO_3**
PON	192–102	**BCP, PO_4**	**NO_3**
CHL	154–110	**POC, PON, BCP**	**NO_3**
CDPR	232-136	**BCP**	**PO_4, NO_3**
BCP	107–81	**POC, PON, LAP, GLU**	
PO_4	251–188	NO_3	
NO_3	202–148		**BCP**
Mesopelagos			
Depth	153		**T, S, PA, O_2**
T	153	**S, PA, CDPR,**	**POC, PO_4, NO_3**
S	153	**CDPR**	POC, **PO_4, NO_3**
O_2	152–151		**PPP, CB, PO_4**
PPP	86–66	**GLU**	
PA	121–64		**POC, PON**
CB	135–63	**AP**	NO_3
LAP	112–60	**POC**	
GLU	107–58	**AP,** BCP	
AP			
POC	104–54	**PON, PO_4**, BCP	CDPR, **NO_3**
PON	103–89	**PO_4**	**CDPR,** NO_3
CDPR	129–64		**BCP**
BCP	46–29	**GLU, AP**	
PO_4	137–63	**NO_3**	
Bathypelagos			
Depth	181	**T, O_2**	**S**
T	181	**S, NO_3**, CDPR	**O_2**, **POC, PON, AP**
S	180		**O_2, POC, PON**
O_2	168	**POC, PON, PO_4**	**CB**
PA	152–141	**LAP,** GLU, **NO_3**	**PON, POC**
CB	152–139	LAP, **GLU, AP, CDPR, BCP**	
LAP	151–115	**AP**	
GLU	150–115	**AP**	
AP	146–111	**BCP**	
POC	139–115	**PON, PO_4**	CDPR
PON	139–114	**GLU, PO_4**	**NO_3**, **CDPR**
CDPR	173–137	**NO_3**	**PO_4**
BCP	51–33	**AP**	
PO_4	169–130		
NO_3	135–103		

Note: $p < 5\%$ (normal type); $p < 1\%$ (bold type).

Table 6. C potentially mobilised by enzymatic activities, $\mu gC \cdot L^{-1} \cdot h^{-1}$ (LAP + GLU) and ratio with BCP; bacterial growth efficiency (BGE) as % calculated for the epi-, meso- and bathypelagic zones.

		LAP + GLU	EEA/BCP	BGE
January 99	EPI	0.16	13.8	7.84
	MESO	0.13	53.9	14:09
	BATHY	0.04	13.4	18.97
October 00	EPI	0.24	11.6	41.94
	MESO	0.15	42.5	59.3
	BATHY	0.32	62.6	55.1
November 04	EPI	0.50	28.9	17.89
	MESO	0.31	374.0	11.1
	BATHY	0.39	359.2	21.9

EEA showed lower or similar values than those recorded during the November cruise in the Ionian Sea. LAP prevailed over the other activities and AP reached the lowest values in all layers. Respiration as CDPR was similar or lower than the Ionian basin at all depths (Figures 4–6).

4. Discussion

The processes we considered are part of three basic biogeochemical cycles: the carbon, the phosphorus and the nitrogen cycles. We focus the discussion on the spatial (mainly over depth) and seasonal differences in the carbon (even if LAP activity may be used as a source for both C and N by prokaryotes) and the phosphorus cycles in the Ionian Sea. The Ionian Sea has been classified as an oligotrophic area of the eastern Mediterranean Sea, because of low levels of chlorophyll and POC [48]. It is assumed that the microbial community (both auto- and heterotrophic) plays a key role in oligotrophic environments [17,49]; cycling within the microbial loop is reported as the dominant mode of nutrient transfer; C, N and P are stored in DOM and POM which are exported at end of the summer stratified period [50].

4.1. *Depth variability*

In the present study, major differences both in prokaryotic abundance and activities were noticed, mainly between the epi- and both meso- and bathypelagic layers, suggesting the occurrence of different processes in different layers; PCA and ANOVA confirmed these differences. SIMPER analysis showed that the most important parameters which distinguished the epi- and meso- groups were S, ETS, PO_4 and GLU, whereas the meso- and bathypelagic layers were separated only by C/N, POC and BCP.

Vertical profiles of nutrient concentrations also showed differences in NO_3/PO_4 ratio, which increased from 9 at surface waters to ∼19 in the mesopelagic zone and ∼20 in the bathypelagic zone. This was due to different rates of recycling for phosphate and nitrate [21,22,51]. According to other AA, bacteria were mainly P limited in the oligotrophic environment, whereas phytoplankton was P and N limited [52]. In the epipelagic layer, wide variations in nutrient concentrations could be related to increasing mineralisation of organic matter produced in the surface waters. Nitrates and phosphates were related positively to depth and negatively to most microbial and trophic parameters (Table 5), indicating their utilisation by planktonic organisms. Moreover, PPP was positively related to T and CHL and negatively related to nutrients, confirming the positive effect of T on the autotrophic components of plankton [25].

In the epipelagic environment, other biological parameters (CB, POC, BCP and CDPR) followed the temperature trends (Table 5); BCP in the epipelagic layer was also related to LAP and GLU. Although a correlative approach, by itself, is not sufficient to identify a cause–effect relationship, all these findings led us to hypothesise that the increase in microbial processes on organic matter is caused by the water warming. Other investigations in the Mediterranean Sea [25,53,54] found similar correlations. The meaning of this finding could be that the rising temperatures, as expected by water warming, may cause an increase in remineralisation – at the cost of photosynthetic production – with a consequent predominance of a heterotrophic regime.

In the epipelagic area, the greater amount of POC and low values of the C/N ratio were associated with high enzymatic activities; the value of the C/N ratio, recognised as an indicator of organic matter quality [55], suggested the availability of fresh, labile compounds, prone to prokaryotic decomposition.

In the Ionian Sea, GLU activity in the epipelagic layer was in a similar range, whereas LAP values were lower than those reported for the Gulf of Genoa [17]. LAP activity was significantly higher than GLU except in October, as observed in other oligotrophic environments [26,56].

Moreover the amounts of carbon mobilised by EEA and taken up by BCP were quite balanced (see Table 6, EEA/BCP ratio). This coupling suggested a quick turnover of carbon, driving an increase in PA and biomass. By contrast, in the more eutrophic waters of the Mediterranean Sea, the EEA/BCP ratio was >20 [57].

Comparison of BCP and EEA with other areas of the Mediterranean Sea, showed values in a similar range to those found in previous studies [15,18,19,58], but higher than those measured in the eastern Mediterranean Sea [59].

All biological and trophic components decreased significantly in the mesopelagic layer. The reduction in POC content resulted in an increasing C/N ratio, indicating a more refractory organic matter.

In this layer, the lowest BCP values were observed. The EEA/BCP ratio displayed the highest value (Table 6) indicating greater enzyme production with respect to the needs of prokaryotes. This finding has been observed previously in the north Adriatic Sea and in prokaryotes living in aggregates [57,60]. Recently, the increasing expression of extracellular enzymes associated with a decrease in biomass has also been found in the deep subtropical Atlantic Ocean [28]. The free-living prokaryotes usually showed a tight hydrolysis–uptake coupling and the hydrolysis occurred next to the site of monomers uptake [61]. By contrast, loss of EEA–BCP coupling was suggested in particle-associated prokaryotes, with EEA released into the environment [62]. These findings allowed us to hypothesise that the high EEA/BCP ratio occurring in the deep layer (Table 6) could indicate particle-associated microbes. Other authors have hypothesised that EEA was associated with colloidal and microparticulate organic matter in the deep ocean highly colonised by microbes; in this microenvironment, high EEA was produced by particle-associated prokaryotes [63].

BCP was positively correlated with GLU, AP and POC (Table 5) indicating a coupling of organic matter hydrolysis and incorporation of monomers by prokaryotes, similar to processes in the epipelagic zone, although to a lesser extent. In the bathypelagic layer, positive correlations between all EEA and BCP were also observed, as well as negative correlations between PA and POC and PON (Table 5). This suggested bacterial growth supported by EEA and the mineralisation of particulate matter. Our PCA confirmed the coupling of microbial metabolism with organic matter. Strong positive correlations between EEA and BCP and POC were observed in the deep ocean [63], suggesting an active response to the organic matter pool by prokaryotes. The association of microbial metabolism with the organic matter trophic quality was also stated [64].

Assuming that enzymatic activities were mainly due to the prokaryotic fraction and that all cells have similar activity levels, the cell-specific activity was calculated to compare surface cell activity with deep cell activity. The LAP activity per cell in the Ionian Sea (16.29–78.78 amol·cell^{-1}·h^{-1})

was similar or higher than values obtained for the Tyrrhenian Sea [64]. By contrast, very high GLU activity levels per cell were obtained in the Ionian Sea (5.11–23.43 amol·cell^{-1}·h^{-1}), compared with the former study [64]. In the deep zone, the importance of specific GLU activity, compared with LAP, indicated the hydrolysis of more refractory material, such as mucopolysaccharides, with respect to the more labile protein fraction, which was hydrolysed in the surface layers. This may be the response of prokaryotes to changes in organic matter quality along the water column. Baltar et al. [28], in the subtropical Atlantic ocean, showed LAP values similar to our enzyme activity values at the surface and higher in the deep layer, although very low GLU per cell values were reported. Any variation in the rate of the cell-specific activities was expected to depend on variations in the composition of the prokaryotic community or its physiological characteristics [14,65].

Finally, spatial differences were observed between the pelagic station of the Ionian Sea and the Sicily Channel. PCA indicated that this last area was geographically separated from the rest of the Ionian stations, probably linked to different water masses of Atlantic origin with lower temperature and salinity [9].

4.2. *Seasonal variability*

Variability over the seasons was less significant than that among layers. As expected, seasonal patterns were marked in the epipelagic zone, but were also present in the deep waters, especially during the October survey.

Traditionally, meso- and bathypelagic prokaryotes have been considered less active than those living in the epipelagos, because of low temperatures and substrate availability [66]. In the Mediterranean Sea, large seasonal changes have been observed, particularly in the mesopelagic realm, indicating that cells were active [67]. Seasonal variation in BCP in deep water was also observed in the oceans [21].

Significant differences over time were observed for PA, CB, POC, PON, NO_3 and ETS between the cold period and summer. More parameters with an autotrophic component (such as PPP, GLU, AP and PO_4) showed differences between the cold period and autumn. The values of PPP, POC, GLU, AP and ETS were also different between summer and autumn (Table 4b).

Changes in prokaryotic structure or at least in prokaryotic metabolisms were hypothesised in cold and warm periods. During cold periods, enzyme activities were in the order LAP > AP > GLU. The vertical distribution of activities calculated per cell showed a different trend for the three enzymes: LAP prevailed in the epi- and bathypelagic layers, whereas the importance of GLU increased at mesopelagic layer. An increase in EEA values with depths was also observed by Baltar et al. [28] who explained this result as an adaptation of enzyme activity patterns to the refractory nature of deeper organic matter.

During warm periods, enzyme levels showed different patterns of distribution: in summer a prevalence of AP over LAP and GLU was observed in the epipelagic layer, whereas in the meso- and bathypelagos LAP prevailed. In autumn, AP assumed a great importance over the other enzymes in all layers (Figure 8). The high cell-specific activity in the meso- and bathypelagic layers was a common feature of other pelagic zones. Thus, deep sea prokaryotes might express more enzyme activity than those living in the epipelagic layer to achieve the same amounts of organic polymers [28,68,69]. In the present study, this trend was evident only in autumn.

4.3. *Bacterial growth efficiency*

The BGE values reported in Table 6 were generally low and similar to other values observed in the Mediterranean Sea [49,70,71]. The values obtained during January and November were

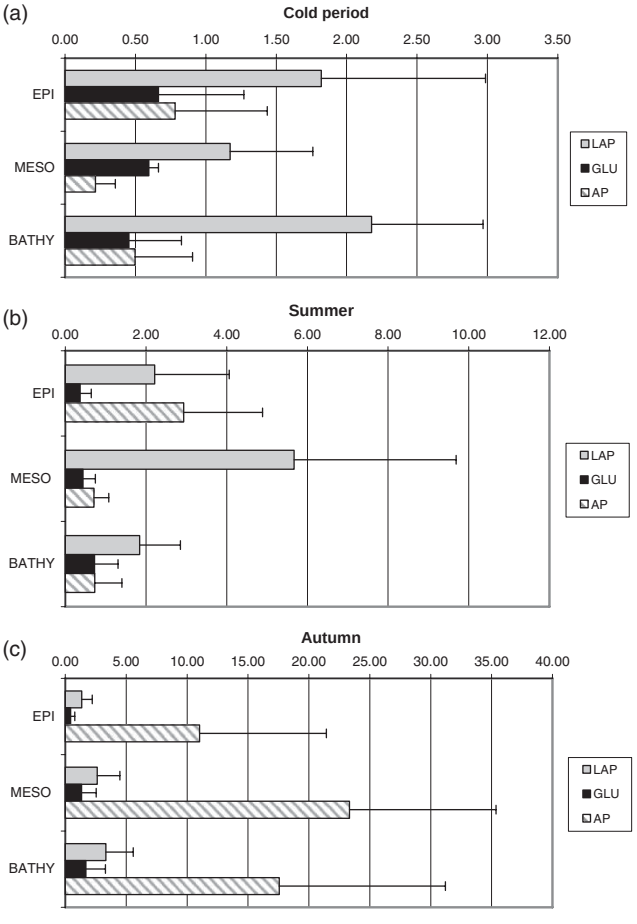

Figure 8. Cell-specific activities of leucine aminopeptidase (LAP) and β-glucosidase (GLU) (fg C·cell^{-1}·h^{-1}) and alkaline phosphatase (AP) (fg P·cell^{-1}·h^{-1}), in different layers during cold and warm periods.

comparable with each other, although the October values were significantly higher in all layers ($F = 109.8$, $p < 0.001$).

In all periods, a significant increase in BGE values between the epi- and mesopelagic layers was observed ($F = 23.3$, $p < 0.001$), whereas no differences between the meso- and bathypelagic layers were shown. The increasing gradient over depth was in opposition to low BGE observed in the dark ocean [21] and suggested that the deep Ionian Sea is more dynamic than other oceans or that organic matter has still not been degraded [23]. Highly cell-specific EEA in the bathypelagic layer, together with the higher temperature of deep Mediterranean compared with open ocean waters, contribute to explain higher BGE at deep waters.

Changes in the trophic conditions (POC values) observed in the autumn sampling affected the BGE because both the availability of organic matter and high enzymatic activities stimulated the increase of the microbial growth efficiency. A relationship between BGE and enzymatic activities had already been reported [28]. Concerning this, the significant correlations between AP and BGE ($r = 0.268$, $n = 172$, $p < 0.001$), highlighted the important role of AP in supporting microbial growth and also in the C cycle [27]. Moreover, BGE was related to POC ($r = 0.223$, $n = 138$, $p < 0.01$), indicating the presence of labile substrate for microbial growth.

At low BGE, more DOM is remineralised, keeping the nutrient recycling within the microbial cycle. In the epipelagic layer, low BGE values indicated higher CO_2 release into the ecosystem

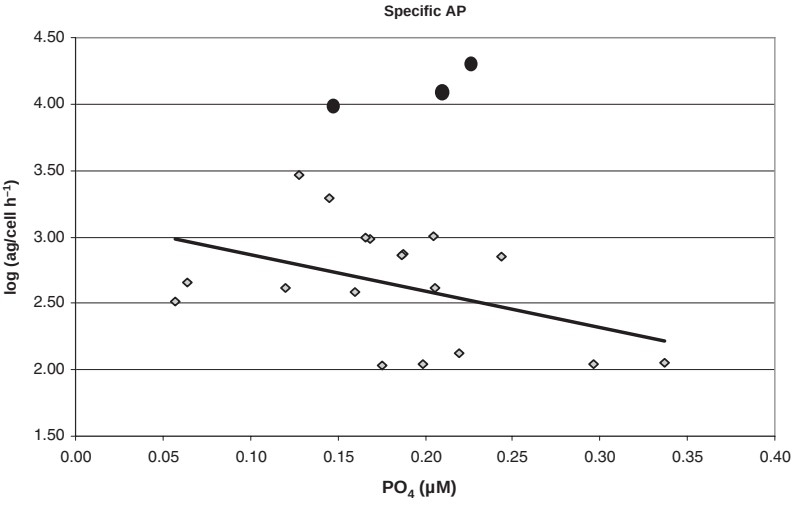

Figure 9. Alkaline phosphatase (AP) cell-specific activity (ag P·cell^{-1}·h^{-1}) in relation to inorganic P concentration in the different layers. The autumn values (circle) were out of linear regression.

and the presence of bacteria, without a good increase in biomass, so the Ionian Sea seemed to act as a CO_2 source.

By contrast, at high BGE, the organic matter is transferred from the dissolved to the particulate phase and, probably, into the larger size trophic fractions [45,72,73]. Conversely, during autumn only, the increase in BGE with the increase in all microbial activities (particularly in meso–bathypelagic waters) indicated that bacteria seem to make a sink of CO_2.

4.4. *P cycle*

AP has been used as a sensitive indicator of P limitation or P starvation [59,74]. It is generally known that the expression of some enzymes is regulated by the end product of a reaction [75,76]. In this study, a weak inverse correlation was found considering all layers and periods except for the autumn sampling (Figure 9). Moreover, very high values, one order of magnitude higher in autumn than in the other seasons, were observed. The mechanism of AP regulation probably changes over time, showing a greater importance of organic matter in regulating AP in autumn in the meso- and bathypelagic waters. Another hypothesis, according to Hoppe [76], was that high AP coinciding with high phosphate concentrations might be caused by the transport of these enzymes which are attached to sinking particles from euphotic zones. The important changes along the water column observed in autumn support this hypothesis. In deep pelagic waters it has been shown that AP was not regulated by the concentration of end products, but the increase in AP was related to C limitation more than P limitation [27]. The decrease in AP with depth, corresponding to an increase in inorganic P has previously been found in the Mediterranean Sea [18]. In the deep Atlantic waters an increase in cell-specific AP activity with depth was observed [28].

5. Conclusions

This study was aimed mainly at improving current knowledge of the spatial and temporal variations in the microbial activities involved in the C and P cycles occurring in the Ionian Sea.

Based on the results obtained during a period of seven years (1998–2004), three main findings can be highlighted:

1. Concerning the vertical distribution of the microbiological parameters, the meso- and bathypelagic layers appeared to be quite similar to each other and, in turn, different from the upper epipelagic waters, in terms of prokaryotic abundance and activities. This suggested that different processes could be performed by prokaryotes at different depths of the water column, even if microscale patchness cannot be excluded. In the epipelagic zone, a positive effect of water warming on microbial activity was observed, indicating a preferential flux of organic matter towards mineralisation. EEA and BCP were coupled, resulting in an increase in biomass. In the meso- and bathypelagic layers, a loose of coupling between these two processes led us to hypothesise the presence of particle-associated prokaryotes. This was also confirmed by the significant relationship found between BCP and POC.
2. Seasonality in the investigated microbiological parameters was generally observed. This was particularly evident in the epipelagos, which was characterised by a high microbiological heterogeneity, as well as in the meso- and bathypelagic layers in which a shift in prevailing enzymatic activity was recorded. Seasonal changes in the rates of organic matter transformation were found, with a significant increase in autumn.
3. Unlike the dark ocean, BGE values showed an increasing trend with depth, reaching higher values in the bathypelagic layer. This could be dependent on the well-known occurrence of the higher temperature in the deep Mediterranean, and therefore in the Ionian Sea, compared with open ocean waters. This corresponds with the high activity rates per cell determined in the bathypelagic layer. Therefore, the quality of organic carbon material supplied in the sea interior by EMT is the likely cause of observed differences. According to BGE variations, the Ionian Sea could act as a source of C in the epipelagic layer and conversely, as a sink of this element in the meso- and bathypelagic layers.

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