The dark portion of the Mediterranean Sea is a bioreactor of organic matter cycling

Luna G.M.[1, #], Bianchelli S.[1], Decembrini F. [2] , De Domenico E. [3], Danovaro R. [1,*], Dell'Anno A.[1]

[1] *Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italia*

[2] *Istituto per l'Ambiente Marino Costiero – Consiglio Nazionale delle Ricerche (IAMC-CNR) sez. Messina, Sp.ta S. Raineri, 86, 98122 Messina, Italia*

[3] *Dipartimento di Biologia Animale ed Ecologia Marina, Salita Sperone 31, Università di Messina, 98166 Messina, Italia*

[#] *Present address: Istituto di Scienze Marine – Consiglio Nazionale delle Ricerche (ISMAR-CNR), Castello 1364/a, 30122 Venezia, Italia*

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* Corresponding author:

Prof. Roberto Danovaro

Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche

Via Brecce Bianche, 60131 Ancona (Italy)

Tel: +39-071-2204654

Fax: +39-071-2204650

email: r.danovaro@univpm.it

Abstract

Total prokaryotic abundance, prokaryotic heterotrophic production and enzymatic activities were investigated in epi-, meso- and bathypelagic waters along a longitudinal transect covering the entire Mediterranean Sea. The prokaryotic production and enzymatic activities in deep waters were among the highest reported worldwide at similar depths, indicating that the peculiar physico-chemical characteristics of the Mediterranean Sea, characterized by warm temperatures (typically 13°C also at abyssal depths), support high rates of organic carbon degradation and incorporation by prokaryotic assemblages. The higher trophic conditions in the epipelagic waters of the Western basin resulted in significantly higher prokaryotic production and enzymatic activities rates than in the Central-Eastern basin. While all of the variables decreased significantly from epi- to meso- and bathypelagic waters, cell-specific hydrolytic activity and cell-specific carbon production significantly increased. In addition, the deep-water layers were characterised by low half-saturation constants (K_m) of all enzymatic activities. These findings suggest that prokaryotic assemblages inhabiting the dark portion of the Mediterranean Sea are able to channel degraded carbon into biomass in a very efficient way, and that prokaryotic assemblages of the deep Mediterranean waters work as a "bioreactor" of organic matter cycling. Since prokaryotic production and enzymatic activities in deep water masses were inversely related with oxygen concentration, we hypothesise a tight link between prokaryotic metabolism and oxygen consumption. As climate change is increasing deep-water temperatures, the predicted positive response of prokaryotic metabolism to temperature increases may accelerate oxygen depletion of deep Mediterranean waters, with cascade consequences on carbon cycling and biogeochemical processes on the entire deep basin.

1. Introduction

Global oceans play a key role in global carbon cycling [Del Giorgio and Duarte, 2002] and it is now widely recognised that marine prokaryotes drive the functioning of marine ecosystems, acting at different spatial scales [Azam and Malfatti, 2007]. The deep sea represents more than 95% of the global biosphere, and host the largest fraction of prokaryotes on Earth [Whitman *et al.*, 1998]. However, information on prokaryotic activity and metabolism has mainly been confined to epipelagic waters [Ducklow, 1999; Hoppe *et al.*, 2002; Misic *et al.*, 2006; Alonso-Saez *et al.*, 2007; Vàsquez-Domìnguez *et al.*, 2008]. Although several studies have reported exponential decreases of microbial activity with increasing water depth [see review by Aristegui *et al.*, 2009 and references therein), recent studies have reported metabolic activities in meso- and bathypelagic waters much higher than previously expected [Reinthaler *et al.*, 2006; Baltar *et al.*, 2009; Fonda-Umani *et al.*, 2010]. Accordingly, high levels of prokaryotic metabolism have been reported also in deep-sea sediments, likely as a result of a strong viral-induced mortality [Danovaro *et al.*, 2008]. These findings let to hypothesize that deep-sea prokaryotes can be a highly dynamic component of the ocean interior [Danovaro *et al.,* 2000; Buesseler *et al.*, 2007; Arìstegui *et al.*, 2009].

Prokaryotic heterotrophic metabolism is primarily controlled by temperature [Apple *et al*., 2006] and by the availability of organic substrates [Del Giorgio and Cole, 1998]. When compared to other oceanic systems, the deep Mediterranean Sea is characterised by warm temperatures (typically 13°C also at abyssal depths), that are approximately 10°C higher than in any oceanic system at similar depths. It can therefore be expected that these conditions promote high prokaryotic growth rates and metabolism [Rivkin and Legendre, 2001].

Investigating prokaryotic metabolism in the deep Mediterranean is crucial not only for providing essential information on the contribution of this basin as a source (or sink) of CO_2 [La Ferla *et al.*, 2003], but also for predicting the potential response of deep-sea prokaryotes

to deep-water warming, which is expected to occur in large oceanic sectors in the next decades [Masuda *et al.*, 2010]. In the Mediterranean Sea, deep-water warming has been already documented over the last few decades [Bethoux *et al.*, 1990], and it is expected that this trend will further increase in the near future [Somot *et al.*, 2006; Herrmann *et al.*, 2008]. Information on prokaryotic metabolism in the deep-sea is therefore essential also for acquiring a benchmark to allow the determination of potential prokaryotic responses to deep-sea warming, and the functional consequences for the whole Mediterranean basin.

Prokaryotic metabolism in the deep sea can be investigated in terms of: i) prokaryotic heterotrophic carbon production [Kirchman *et al.*, 1985], ii) enzymatic activities [Hoppe *et al.*, 1993]; and iii) prokaryotic respiration [Del Giorgio and Duarte, 2002]. An uncoupling between prokaryote-mediated production and degradation processes can have important consequences on the functioning, carbon sequestration and ecological efficiency of deep-sea ecosystems [Cottrell *et al.,* 2006; Taylor *et al.,* 2009]. In the present study we have investigated the key functional variables of prokaryotic metabolism (prokaryotic heterotrophic carbon production and enzymatic degradation potential) across the deep-water masses of the entire Mediterranean basin and we have explored the ecological role of prokaryotic assemblages in the carbon cycling at the basin scale.

2. Materials and Methods

2.1. Study area and sampling activities

The Mediterranean Sea accounts for 0.82% of the world ocean surface and has an average depth of ca. 1,450 m (approximately 1/3 of the average depth of the oceans). In terms of its hydrographic characteristics, the Mediterranean Sea is generally divided into a Western and a Central-Eastern basin, connected by the Strait of Sicily. The former is subdivided into three main basins: the Alboran Basin, the Algero-Provencal (or Balearic) Basin and the Tyrrhenian

105 Basin. The Central-Eastern basin is divided into the Ionian and the Cretan and Levantine
106 sectors [Sardà *et al.*, 2004]. The Mediterranean Sea is also characterised by an eastward
107 decreasing gradient of trophic conditions, and the Eastern basin is defined as an ultra-
108 oligotrophic, phosphorus-depleted system [Sarmiento *et al.*, 1988; Danovaro *et al.,* 1999;
109 Thingstad *et al.*, 2005].

110 Water samples were collected across the Mediterranean Sea, from 06°22'W to
111 26°41'E, during two oceanographic cruises carried out from 31 March to 19 April 2006 in the
112 Central-Eastern sector and from 4 to 25 October 2006 in the Western sector onboard the R/V
113 *Urania* (CNR). Sampled stations crossed the gradient of trophic conditions, from the more
114 productive western basin (e.g. the Alboran Sea; [Magagnini *et al.*, 2007]) to the highly
115 oligotrophic Cretan Sea (Central-Eastern Mediterranean; Figure 1). Water samples were
116 collected across the entire basin from 18 sites using a carousel sampler equipped with 24
117 Niskin bottles (each of 10 L). Only station M25 was located in the Atlantic Ocean close to the
118 Strait of Gibraltar (Figure 1). Water samples were collected at standard depths from
119 epipelagic waters (surface, 25, 50, 75, 100 and 200 m depth), mesopelagic waters (300, 500
120 and 1000 m depth) and bathypelagic waters (1500, 2000, 2500 and 3000 m depth); in
121 addition, at all of the stations, water samples were collected at 5–20 m above the sediment
122 surface. At each station, a Sea Bird Electronics SBE 9/11 plus CTDO (SBE43) profiler
123 equipped with a Turner Aquatracka fluorometer was employed to measure temperature,
124 fluorescence (as a proxy for chlorophyll-a concentrations), salinity and oxygen content
125 throughout the water column.

126

127 **2.2. Total prokaryotic abundance**

128 Total prokaryotic abundance was determined, for the seawater samples collected at nine
129 selected stations located in the Western Mediterranean basin (stations M25, M23, G1, J8, A4,

M14, M11, R11and M4), according to the protocol described by Noble and Fuhrman [1998] with minor modifications. Sub-samples (1 to 3 ml) were filtered onto 0.2µm Anodisc filters (diameter 25 mm) and stained with 20 µL of SYBR Green I (stock solution diluted 1:20). The filters were incubated in the dark for 15 min, washed twice with 3 mL of Milli-Q water and mounted onto glass slides with a drop of 50% phosphate buffer (6.7mM phosphate, pH 7.8) and 50% glycerol (containing 0.5% ascorbic acid). Prokaryote counts were obtained by epifluorescence microscopy (Zeiss Axioplan, magnification 1000×) by examining at least 20 fields (at least 400 prokaryotes per replicate).

2.3. Enzymatic activities

Enzymatic activities were determined for aminopeptidase, ß-glucosidase and alkaline phosphatase by the analysis of the cleavage rates of their artificial fluorogenic substrates: L-leucine-4-methylcoumarinyl-7-amide (Leu-MCA); 4-methylumbelliferyl (MUF)-ß-D-glucopyranoside (Glu-MUF), and 4-MUF-P-phosphate (MUF-P), respectively (all from Sigma Chemicals), as described in Corinaldesi *et al.* [2003] and Danovaro *et al.* [2005]. Kinetics experiments were conducted at selected water depths and stations using a range of fluorogenic substrate concentrations as described by Hoppe [1993] and Tamburini *et al.* [2002]. Briefly, each substrate was added at seven different concentrations (0.05, 0.25, 0.5, 1.0, 5.0, 10 and 20 µM) to the unfiltered seawater (final volume 5 mL, n = 3) and incubated in the dark at the *in situ* temperature for 1–3 hours. The substrate concentrations were selected to cover the entire range of potential enzymatic activities (up to saturation for the enzymes tested). The increases in fluorescence were measured fluorometrically: at 380 nm excitation, 440 nm emission (for Leu-MCA) and 365 nm excitation, 455 nm emission (for Glu-MUF and MUF-P). The maximum velocity (V_{max}) and the half-saturation constant (K_m) were calculated

154 using Lineweaver-Burke plots of the reaction velocities *versus* the substrate concentrations
155 [Tamburini *et al.,* 2002; Dell'Anno and Corinaldesi, 2004].

156 The fluorescence was converted into enzymatic activity using standard curves of 7-
157 amino-4-methylcoumarin (Sigma Chemicals) for Leu-MCA and of 4-methylumbelliferone
158 (Sigma Chemicals) for both Glu-MUF and MUF-P. All of the assays were carried out in
159 triplicate, and enzymatic activities were expressed as μmol of substrate hydrolysed L^{-1} d^{-1}.
160

161 **2.4. Prokaryotic heterotrophic production**

162 Prokaryotic heterotrophic production was determined by [^{3}H]-leucine incorporation [Smith
163 and Azam, 1992]. Preliminary experiments were carried out to determine the leucine
164 concentration for reaching substrate saturation in the surface and deep water samples
165 collected at different stations, by using increasing concentrations (from 2 nM to 100 nM) of
166 L-[4,5-^{3}H] leucine (Amersham). Here, three replicates and two controls (sample volume 1.7
167 ml) were used for each concentration and each water sample. All of the incubations were
168 conducted in the dark at *in situ* temperature for 1 hour and stopped with 5% trichloroacetic
169 acid (final concentration). Time course experiments, performed at different stations and
170 sampling depths, consistently indicated that [^{3}H]-leucine incorporation rates increased linearly
171 over time up to 6 h (data not shown). The samples were then stored at 4°C and transferred to
172 the laboratory, where the pellets were washed with 5% trichloroacetic acid and 80% ethanol,
173 and supplemented with 1 mL of Hionic-Fluor scintillation fluid (Packard Bioscience). The
174 radioactivity was measured in a liquid scintillation counter (Packard Tri-Carb 300), with the
175 counts per minute (CPM) converted to disintegrations per minute (DPM). The incorporation
176 rates of [^{3}H]-leucine into protein were fitted to hyperbolic functions for Michaelis–Menten
177 type kinetics using nonlinear regression analysis. Our data demonstrated that 20 nM of [^{3}H]-
178 leucine saturated the prokaryotic incorporation in both surface and bottom waters (data not

179 shown). This concentration of 20 nM of [^{3}H]-leucine was then used for the analysis of all of
180 the water samples, performed according to the procedure described above. The amount of
181 incorporated [^{3}H]-leucine was then converted into prokaryotic heterotrophic carbon
182 production per hour per liter of water according to Simon and Azam [1989] and Smith and
183 Azam [1992]. An intracellular isotopic dilution of leucine of 2 was assumed.
184
185 **2.5. Statistical analyses**
186 To test large-scale differences in prokaryotic heterotrophic production and enzymatic
187 activities between the two basins (Western and Central-Eastern Mediterranean basins) we
188 used one-way analysis of variance (ANOVA). Two-way analysis of variance (station × water
189 depth) was used to test for differences in all of the variables investigated, separately for the
190 Western and Central-Eastern Mediterranean basins. Before the analyses, the homogeneity of
191 variances was checked using Cochran's test on appropriately transformed data, whenever
192 necessary. For each of the investigated enzymes, the differences between the mean K_m values
193 at the different depths were tested using a t-test.
194 To assess the statistical differences between basins and layers, we used the analysis of
195 similarity (ANOSIM) tool. When significant differences were observed, non-metric
196 multidimensional scaling ordination (nMDS) was carried out, to visualise the similarities
197 between the Mediterranean basins (Western vs. Central-Eastern) and layers (epipelagic,
198 mesopelagic and bathypelagic). nMDS was performed on a dataset composed of all of the
199 measured enzymatic activities (aminopeptidase, ß-glucosidase and alkaline phosphatase) and
200 based on a Bray-Curtis similarity matrix. SIMPER analysis was then applied to quantify the
201 observed dissimilarities (as percentages) and to identify which, among the variables
202 investigated, contributed most to the similarities between the basins and layers. ANOSIM,

MDS and SIMPER analyses were performed using the PRIMER v5 software (Plymouth Marine Laboratory).

3. Results

3.1. Environmental variables

The main environmental variables that were measured in the water column of the Western and Central-Eastern Mediterranean basins are shown in Figure 2. In the epipelagic waters, the temperatures ranged from 20.59°C to 23.56°C in the Western Mediterranean and from 15.40°C to 17.05°C in the Central-Eastern Mediterranean. The salinity showed an increasing eastward gradient, with lower values in the Western Mediterranean (range 36.55 – 38.24) and higher values in the Central-Eastern Mediterranean (range 37.82 – 38.85). The oxygen content ranged from 148.3 to 260.4 μmol kg^{-1} in the Western basin and from 200.5 to 255.2 μmol kg^{-1} in the Central-Eastern basin. Fluorescence was, on average, 8-fold higher in the Western than in the Central-Eastern basin (0.13 vs. 0.017 Arbitrary Units, respectively).

In the mesopelagic and bathypelagic waters of the Western and the Central-Eastern basin, the oxygen content decreased with increasing water depth, with lowest concentrations at depths between 500 and 1000 m in the Western basin and between 1000 and 2000 m in the Central-Eastern basin (Figure 2). Generally, the deep water masses in the Central-Eastern basin were characterized by higher oxygen concentrations than observed in the Western basin. In mesopelagic waters, in the Western basin temperature ranged from 13.09°C to 13.98°C and in the Central-Eastern basin from 13.58°C to 15.38°C. In the same water layer, the salinity ranged from 35.98 to 38.70 in the Western basin and from 38.23 to 38.90 in the Central-Eastern basin. In the bathypelagic waters, the temperature ranged from 13.14°C to 13.29°C in the Western basin and from 13.39°C to 13.98°C in the Central-Eastern basin, while salinity ranged from 38.45 to 38.47 in the Western basin and from 38.71 to 38.78 in the Central-

228 Eastern basin.

229

230 **3.2 Enzymatic activities**

231 For the enzymatic activities investigated, saturation occurred at substrate concentrations from
232 5 μM to 10 μM (data not shown). The half-saturation constants (K_m) for aminopeptidase
233 (Leu-MCA), ß-glucosidase (Glu-MUF) and alkaline phosphatase (MUF-P) for the selected
234 stations are shown in Table 1. The K_m values of Leu-MCA ranged from 0.5 ± 0.1 μM to 4.7 ±
235 0.1 μM in the surface waters and from 3.6 ± 1.7 μM to 22.2 ± 9.2 μM in the bottom waters.
236 The K_m values of Glu-MUF were similar between the surface and deep waters (0.3–1.9 μM
237 and 0.1–2.0 μM, in surface and deep waters, respectively) and, analogously, the K_m values of
238 MUF-P were similar in surface waters (1.5–17.7 μM) and bottom waters (1.5–14.9 μM).

239 The depth profiles of the aminopeptidase activities in the Western and Central-Eastern
240 Mediterranean basin are shown in Figures 3 and the spatial distribution of the three enzymatic
241 activities is shown in Figure 4. Overall, the maximum rates of hydrolysis (Vmax) of the
242 investigated enzymatic activities significantly decreased with increasing depth (ANOVA,
243 $p<0.01$; Table 2). The activities of aminopeptidase, ß-glucosidase and alkaline phosphatase
244 decreased significantly from the Western to the Central-Eastern basin (ANOVA, $p<0.01$),
245 with the Western Mediterranean showing values on average 12–28-fold higher for
246 aminopeptidase, 2.9–4.4-fold higher for ß-glucosidase and 2.5–3.4-fold higher for alkaline
247 phosphatase (Table 3). The significant differences between Western and Central-Eastern basin
248 were confirmed by multivariate analysis (ANOSIM, Table 4), and the SIMPER analysis
249 revealed that aminopeptidase activity always explained >42% of the total variance,
250 independently of the water layer (Table 4). The nMDS indicated a clear segregation between
251 the stations located in the Western Basin and those located in the Central-Eastern Basin
252 (Figure 5).

When normalized per prokaryotic cells, the enzymatic activity rates in the Western basin showed a significant increase in the cell-specific hydrolytic activity with increasing water depth, with patterns similar to those seen when the prokaryotic production was normalized per cell (highest in the mesopelagic layer).

3.3. Total prokaryotic abundance and heterotrophic production

The total prokaryotic abundance ranged from 0.15 ± 0.01 to 14.1 ± 2.5 $\times 10^8$ cell L^{-1} in the Western Mediterranean and decreased significantly from the surface to the deeper layers (Figure 6, ANOVA, $p<0.01$). Data for the Central-Eastern Mediterranean basin are unfortunately not available.

As with the total prokaryotic abundance, the prokaryotic heterotrophic production also decreased significantly from the surface to the deeper layers when the two basins were considered together ($p<0.05$, Table 2). However, this depth-decreasing pattern was not evident in different stations especially of the Western Mediterranean basin (e.g. at stations J8, A4, M4, 557 and Crete; Figure 7). Prokaryotic production in the Western Mediterranean basin (range: 16.3±4.8 to 962.4±140.9 ngC L^{-1} h^{-1}) was significantly higher (ANOVA, $p<0.01$) than in the Central-Eastern basin (range: 0.4±0.0-387.5±44.2 ngC L^{-1} h^{-1}; Figure 8). On average, these production values were always ca. 4-6-fold higher in the Western than in the Central-Eastern basin, independent of the water layer (epipelagic, mesopelagic and bathypelagic; Table 3).

When the prokaryotic production rates were normalised per cell (using prokaryotic counts available only for the Western basin), there was a significant increase in the cell-specific carbon production with increasing water depth in the Western Mediterranean Sea (Figure 9). The cell-specific production rates averaged from 1.44±0.16 (in the epipelagic layer) to 5.21±0.88 fgC $cell^{-1}$ h^{-1} (in the mesopelagic layer). In the bathypelagic, cell-specific

278 rates were on average 3.84±0.63 fgC $cell^{-1}$ h^{-1}.

279

280 **4. Discussion**

281 It has been repeatedly demonstrated that the Western and Eastern Mediterranean basins have
282 clear differences in trophic conditions, that reflect the well-known eastward-decreasing
283 gradient pattern for primary productivity [Turley *et al.,* 2000; Koppelmann *et al.*, 2004] and
284 the gradient in particle fluxes to the sea bottom [Danovaro *et al.*, 1999]. Here, we show for
285 the first time that, at all water depths (from epipelagic to mesopelagic and bathypelagic
286 waters), this gradient is reflected by a clear decreasing pattern of prokaryotic heterotrophic
287 production and exo-enzymatic activities moving from the Western to the Eastern basin. This
288 spatial pattern led us to hypothesize, as expected, that degradation potential and prokaryotic
289 production in the Mediterranean Sea are coupled with the trophic gradients and with the
290 organic inputs coming from the photic zone.

291 Total prokaryotic abundance decreased significantly with increasing water depth,
292 reaching at meso- and bathypelagic depths values within the range of those reported
293 worldwide for the same bathymetric range [Tanaka and Rassoulzadegan, 2002; Magagnini *et*
294 *al.*, 2007; Arìstegui *et al.*, 2009; La Ferla *et al.*, 2010]. Also enzymatic activities displayed a
295 similar significant decrease with increasing water depth, but values observed at mesopelagic
296 and bathyal depths of the Mediterranean Sea were one order of magnitude higher than those
297 reported in other oceanic regions worldwide at comparable depths [Hoppe *et al.*, 1993; Koike
298 and Nagata, 1997; Hoppe and Ullrich, 1999; Hoppe, 2003; Baltar *et al.,* 2009; Taylor *et al.,*
299 2009, Baltar *et al.,* 2010; Nagata *et al.*, 2010].

300 Although the Mediterranean Sea often sees large inputs of organic matter (continental
301 shelf export, through dense shelf-water cascading events; [Canals *et al.*, 2006; Heussner *et al.*,
302 2006]), these episodic events influence water characteristics only at a regional scale (i.e. the

303 Aegean Sea, the Adriatic Sea and the Gulf of Lion; [Canals *et al.*, 2009]). Deep-water
304 convection mechanisms can facilitate the downward displacement of surface waters into
305 deeper layers, although these processes occur at the mesoscale [Millot, 1990; Manca *et al.,*
306 2004]. At the same time, the deep Mediterranean waters are characterised by high
307 temperatures, with values approximately 10°C higher than in other oceanic systems at depths
308 between 1000 m and 4000 m. When determining enzymatic activities we used universally
309 standardised protocols and incubated the samples at the *in situ* temperature [Hoppe *et al.*,
310 2002; Misic *et al.*, 2006; Baltar *et al.*, 2009; 2010; Taylor *et al.,* 2009]. Thus, these results
311 clearly suggest a stimulating effect of the higher temperatures on prokaryotic metabolism. In
312 addition, Mediterranean waters and the associated pool of dissolved organic carbon are
313 younger than those of other oceanic systems, thus potentially representing a more labile and
314 available food source for prokaryotes [Santinelli *et al.*, 2010].

315 Information on microbial metabolism in the deep Mediterranean waters is limited
316 [Tamburini *et al.,* 2002; Zaccone *et al.,* 2003; La Ferla et al. 2005; Tamburini *et al.* 2009].
317 Previous studies have demonstrated that enzymatic activities in the deep waters of different
318 areas of the Mediterranean Sea show wide temporal and spatial variability suggesting that the
319 deep Mediterranean ecosystems are far from steady-state conditions [La Ferla *et al.,* 2010].
320 Such a high variability is likely dependent also upon changes in the quantity, distribution and
321 bioavailability of the organic substrates [Santinelli *et al.,* 2010], which can greatly influence
322 the degradation activities of deep-sea prokaryotes.

323 The nMDS analysis based on the enzymatic activities showed clear segregation
324 between the Western and Central-Eastern basins. Our data also indicate that most of the
325 variance between these two basins is explained by the aminopeptidase activity.

326 The kinetic experiments revealed that the half-saturation constants (K_m) of alkaline
327 phosphatase are comparable with those previously reported for other oceanic systems

(Atlantic Ocean; [Baltar *et al.,* 2009; 2010]). Our data also revealed low half-saturation constants (K_m) for aminopeptidase in the bathypelagic waters. Such low values, consistent with previous studies of the deep Mediterranean [Tamburini *et al.,* 2002], are typically at least one order of magnitude lower than those reported in the deep Atlantic Ocean at similar depths [Baltar *et al.,* 2009; Baltar *et al.*, 2010]. The much higher K_m values of aminopeptidase in the deep Atlantic waters (up to 1,000 μM vs 11 μM in Mediterranean waters) indicate an adaptation of the deep-Mediterranean prokaryotic assemblages to cope with limited amounts of organic resources [Davey *et al.,* 2001; Baltar *et al.,* 2010]. In the Mediterranean Sea, the K_m of aminopeptidase increased with increasing water depth as previously reported from the Atlantic Ocean [Baltar *et al.*, 2009], but data reported here indicate that deep-sea prokaryotic assemblages of the Mediterranean sea have a much higher affinity for the target substrate, and thus a much higher efficiency for exploiting the organic substrate at very low concentrations. This is also supported by the very high cell-specific hydrolytic rates in the mesopelagic and bathypelagic waters of the Western Mediterranean Sea, which are amongst the highest reported to date in deep-sea waters worldwide (see [Baltar *et al.*, 2009] and references therein). The cell-specific hydrolytic rates, as well as cell-specific carbon production rates reported here, should be considered with caution since the normalization to total cell counts does not take into account the fraction of dormant/inactive cells and the different metabolic activities of different prokaryotic taxa (Smith and del Giorgio, 2003; Sintes and Herndl, 2006). The lack of literature data for the K_m of ß-glucosidase in deep waters does not allow comparisons with other oceanic systems, but the K_m of alkaline phosphatase in the deep Mediterranean waters is similar to that reported in the deep Atlantic waters [Baltar *et al.*, 2009]. Thus, our data suggest that the main differences between the Atlantic Ocean and the Mediterranean Sea are linked to the availability and utilization of organic nitrogen sources by prokaryotes.

353 The high degradation potential in the deep Mediterranean Sea is consistent with the
354 high values of prokaryotic heterotrophic production, which are significantly higher than that
355 reported to date for deep-sea waters worldwide [Ducklow *et al.*, 1993; Baltar *et al.*, 2009;
356 Nagata *et al.*, 2010]. These high values of prokaryotic heterotrophic production were also
357 reflected in the high cell-specific production rates observed in meso- and bathypelagic waters
358 of the Western Mediterranean basin, much higher than those reported in other oceanic
359 systems worldwide at similar depths [Baltar *et al.*, 2009; Gasol *et al.*, 2009]. Production and
360 growth rates of prokaryotes in marine ecosystems are extremely sensitive to changes in
361 temperature. Since microbial metabolism shows Q_{10} values ranging from 2 to 3 [Pomeroy and
362 Wiebe, 2001; Rivkin and Legendre, 2001; Apple *et al.*, 2006], the higher deep-water
363 temperature in the Mediterranean Sea can certainly contribute to explain a large proportion of
364 these differences, although other factors can certainly contribute. We cannot exclude the
365 possibility that the values of prokaryotic heterotrophic production might be influenced by
366 decompression, although both positive or negative effects have been reported in previous
367 experiments conducted in the deep sea. Previous studies have shown that, during the
368 stratification period, decompression may induce an underestimation of the measured rates as
369 compared with those measured at *in situ* pressure conditions [Tamburini *et al.*, 2002 and
370 references therein]. Conversely, other studies have shown stimulation of prokaryotic
371 metabolism by decompression in different hydrological conditions (i.e. in winter mixed
372 waters; [Bianchi and Garcin, 1994]) or no changes between decompressed and non-
373 decompressed samples at the sediment-water interface [Danovaro *et al.*, 2008].

374 The comparison of prokaryotic heterotrophic production reported in this study with
375 those reported in the literature from the deep Mediterranean waters reveals a wide spatial and
376 temporal variability. Values reported here, indeed, are higher than those reported by some
377 authors in the deep Mediterranean [Tamburini *et al.*, 2002; Pitta *et al.*, 2005; Magagnini *et al.*,

2007], but similar to or lower than those observed in different sectors of the Central and Eastern Mediterranean basins (La Ferla *et al.,* 2005; Yokokawa *et al.,* 2010). Such differences can also be dependent upon the different methodological approaches and conversion factors used. For instance, we used a centrifugation-based procedure, that has been used previously for the analysis of deep water samples [Pedros-Aliò *et al.*, 1999; Tanaka and Rassoulzadegan, 2004; Gasol *et al.*, 2009], while others utilized a filtration-based approach (both procedures are described in Smith and Azam [1992]). We have estimated prokaryotic heterotrophic production rates assuming an intracellular isotopic dilution of leucine of 2 (Simon and Azam, 1989), while others neglected the dilution factor (Tamburini *et al.*, 2002; Tanaka and Rassoulzadegan, 2004; Tamburini *et al.*, 2009). In addition, we have performed one hour incubations (La Ferla *et al.*, 2005; Gasol *et al.*, 2009), while previous studies used longer incubation times (from 2 to 24 hours, [Zaccone *et al.*, 2003; Tanaka and Rassoulzadegan, 2004; Yokokawa *et al.,*, 2010]). Caution should be posed when comparing data obtained using a single incubation time (especially after 1–3 hours), as deviation from linearity of incorporation rates of radiolabeled substrates occurs over time, leading to a significant underestimation of prokaryotic metabolism in deep–sea environments (Dixon and Turley, 2001). This was not the case of our study, as time-course experiments revealed linear incorporation of [^{3}H]-leucine up to 6 h. Also, the use of non-saturating concentrations of [^{3}H]–leucine can determine a significant underestimation of the prokaryotic heterotrophic production rates, which can be ruled out in our study as assessed by the dedicated kinetic experiments.

One potential explanation of the high deep-sea prokaryotic metabolism observed in the Mediterranean Sea might rely on the high efficiency in the degradation and use of organic matter. The ratio of prokaryotic heterotrophic production to the carbon potentially mobilised enzymatically by aminopeptidase (assuming that 1 nmol of substrate hydrolysed

403 enzymatically corresponds to 72 ng of mobilised carbon, according to Pusceddu *et al.* [2009])
404 can be used to provide insights into the efficiency of the prokaryotic assemblages for the
405 conversion of degraded organic matter into biomass. In both basins, the ratio of prokaryotic
406 heterotrophic production to carbon mobilised enzymatically by aminopeptidase increased
407 with water depth, with the highest values seen in the deeper water layers (0.081 and 0.275 in
408 the Western and Central-Eastern basins, respectively) when compared with the epipelagic
409 layer (0.048 and 0.237, respectively). Such ratios are consistent with those estimated from
410 literature where synoptic measurements were made of prokaryotic heterotrophic production
411 and aminopeptidase activity in deep water layers of the Mediterranean Sea [Tamburini *et al.,*
412 2002; Zaccone *et al.,* 2003]. These data provide supporting evidence of the high efficiency of
413 the prokaryotic assemblages of the deep Mediterranean Sea in the exploitation of the available
414 organic pools. Indeed, the deep Mediterranean waters, being much younger than oceanic ones,
415 with renewal times estimated between 11 and 100 years, are expected to be characterized by a
416 higher content of bioavailable organic compounds, which can determine a higher efficiency of
417 prokaryotic assemblages in degrading organic resources [Seritti *et al.*, 2003; Santinelli *et al.*,
418 2009].

419 The data reported from the deep-Mediterranean sea support the hypothesis that this
420 warm miniature ocean is a "bioreactor" of organic matter cycling, i.e. a system in which
421 biological processes are accelerated. The high rates of organic matter degradation and
422 prokaryotic production, in the face of the relatively low prokaryotic abundance and biomass
423 in deep Mediterranean waters, are consistent with previous findings reporting respiration and
424 consumption rates of organic carbon (2.2–14.4 μM C yr^{-1} [Christensen *et al.*, 1989; Santinelli
425 *et al.*, 2010]) ca. 10 times higher than values reported in other oceanic systems (0.1–0.9 μM C
426 yr^{-1} [Carlson *et al.*, 2010]).

The concentration of oxygen in deep water masses is controlled by a balance between supply from ventilation processes and consumption due to microbial remineralization of organic matter [Sarmiento *et al.*, 2004]. Correlation analyses revealed that enzymatic activities and prokaryotic heterotrophic production were inversely related to oxygen concentration in meso- (n=102, R^2=0.183, P<0.001 for aminopeptidase and n=66, R^2=0.156, P<0.01 for prokaryotic production) and bathypelagic waters (n=51, R^2=0.606, P<0.001 for aminopeptidase and n=45, R^2=0.245, P<0.001 for prokaryotic production). Data reported here let us to hypothesize that prokaryotic-mediated degradation and subsequent utilization of organic sources could play a key role in oxygen consumption in Mediterranean deep waters. However, the variance explained by these relationships accounted for 16 – 61% of the total variance in oxygen concentration, indicating that other factors also contribute to determining the patterns of oxygen concentrations in the Mediterranean deep waters [Keeling *et al.*, 2010].

The Mediterranean Sea is sensitive to climate changes, and minor changes in the physico-chemical characteristics of the deep-water masses have already been shown to have a significantly impact on the biodiversity and functioning of its benthic ecosystems [Danovaro *et al.*, 2001; 2004]. Here we showed that the deep Mediterranean Sea acts as an accelerated bioreactor for the cycling of the organic matter, in which the biogeochemical cycling of organic matter at the basin scale is accelerated when compared to equally deep oceanic systems. There is increasing evidence that the present climate changes are altering the physico-chemical conditions in the deep Mediterranean Sea [Bethoux *et al.*, 1990] and recent studies predicted, for the end of the 21st century, an increase in temperature (up to 1.5°C) and a reduction of ventilation processes in deep Mediterranean waters [Somot *et al.*, 2006; Herrmann *et al.*, 2008]. Data presented here lead us to hypothesize that the predicted positive response of prokaryotic metabolism to temperature increase [Rivkin and Legendre, 2001] may further accelerate the processes leading to a progressive oxygen depletion of Mediterranean

452 deep waters, with potentially important implications on biogeochemical cycles and ecosystem
453 functions at the basin scale.
454
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465

References

Alonso-Saez, L., J.M. Gasol, J. Arìstegui, J.C. Vilas, D. Vaquè, C.M. Duarte, and S. Agustì (2007), Large-scale variability in surface bacterial carbon demand and growth efficiency in the subtropical northeast Atlantic Ocean, *Limnol. Oceanogr.*, 52: 533–546.

Apple, J.K., P.A. del Giorgio, and W.M. Kemp (2006), Temperature regulation of bacterial production, respiration, and growth efficiency in a temperate salt-marsh estuary, *Aquat. Microb. Ecol.*, 43: 243–254.

Arìstegui, J., S. Agustì, J. Middelburg, and C.M. Duarte (2005), Respiration in the mesopelagic and bathypelagic zones of the oceans, In "Respiration in Aquatic Ecosystems" (Eds. del Giorgio, P.A., Williams, P.J.B.). Oxford University Press, Oxford, NY. pp. 181-205

Arìstegui, J., J.M. Gasol, C.M. Duarte, and G.J. Herndl (2009), Microbial oceanography of the dark ocean's pelagic realm, *Limnol. Oceanogr.*, 54:1501-1529.

Azam, F., and F. Malfatti (2007), Microbial structuring of marine ecosystems, *Nat. Rev. Microbiol.*, 5: 782-791.

Baltar, F., J. Arístegui, E. Sintes, H.M. van Aken, J.M. Gasol, and G.J. Herndl (2009), Prokaryotic extracellular enzymatic activity in relation to biomass production and respiration in the meso and bathypelagic waters of the (sub)tropical Atlantic, *Environ. Microbiol.*, 11: 1998–2014.

Baltar, F., J. Arístegui, J.M. Gasol, E. Sintes, H.M. van Aken, and G.J. Herndl (2010), High dissolved extracellular enzymatic activity in the deep central Atlantic Ocean, *Aquat. Microb. Ecol.*, 58: 287–302.

Bethoux, J.P., B. Gentili, D. Tailliez, and J. Raunet (1990), Warming trend in the western Mediterranean deep water, *Nature*, 347: 660–662.

490 Bianchi, A., and J. Garcin (1994), Bacterial response to hydrostatic pressure in seawater
491 samples collected in mixed-water and stratified-water conditions. *Mar. Ecol. Progr. Ser.*,
492 111: 137-141.
493 Bosc, E., A. Bricaud, and D. Antoine (2004), Seasonal and interannual variability in algal
494 biomass and primary production in the Mediterranean Sea, as derived from 4 years of
495 SeaWiFS observations, *Global Biogeochem. Cycles*, 18, GB1005,
496 doi:10.1029/2003GB002034
497 Buesseler, K.O., C.H. Lamborg, P.W. Boyd, P.J. Lam, T.W. Trull, R.R. Bidigare, J.K.B.
498 Bishop, K.L. Casciotti, F. Dehairs, M. Elskens, M. Honda, D.M. Karl, D.A. Siegel, M.W.
499 Silver, D.K. Steinberg, J. Valdes, B. Van Mooy, and S. Wilson (2007), Revisiting carbon
500 flux through the Ocean's twilight zone, *Science,* 316: 567-570.
501 Canals, M., R. Danovaro, S. Heussner, V. Lykousis, P. Puig, F. Trincardi, A.M. Calafat, X.
502 Durrieu de Madron, A. Palanques, and A. Sànchez-Vidal (2009), Cascades in
503 Mediterranean submarine grand canyons, *Oceanogr.*, 22: 26–43.
504 Canals, M., P. Puig, X. Durrieu de Madron, S. Heussner, A. Palanques, and J. Fabres (2006),
505 Flushing submarine canyons, *Nature*, 444: 354-357.
506 Carlson, C.A., D.A. Hansell, N.B. Nelson, D.A. Siegel, W.M. Smethie, S. Khatiwala, M.M.
507 Meyers, E. Wallner (2010), Dissolved organic carbon export and subsequent
508 remineralization in the mesopelagic and bathypelagic realms of the North Atlantic basin.
509 *Deep-Sea Res.* II, 57: 1433–1445.
510 Christensen, J.P., Packard T.T., Dortch F.Q., Minas H.J., Gascard J.C., Richez C., and
511 Garfield, P.C. (1989), Carbon oxidation in the deep Mediterranean Sea: evidence for
512 dissolved organic carbon source, *Global Biogeochem. Cycles*, 3: 315–335.

513 Corinaldesi, C., E. Crevatin, P. Del Negro, M. Marini, A. Russo, S. Fonda Umani, and R.
514 Danovaro (2003), Large-scale spatial distribution of virioplankton in the Adriatic sea:
515 testing the trophic state control hypothesis, *Appl. Environ. Microbiol.*, 69: 2664-2673.
516 Cottrell, M.T., R.R. Malmstrom, V. Hill, A.E. Parkera, and D.L. Kirchman (2006), The
517 metabolic balance between autotrophy and heterotrophy in the western Arctic Ocean,
518 *Deep-Sea Res. I*, 53:1831–1844.
519 Danovaro, R., A. Dinet, G. Duineveld, and A. Tselepides (1999), Benthic response to
520 particulate fluxes in different trophic environments: a comparison between the Gulf of
521 Lions–Catalan Sea (western-Mediterranean) and the Cretan Sea (eastern-Mediterranean),
522 *Progr. Oceanogr.*, 44: 287-312.
523 Danovaro, R., A. Dell'Anno, A. Pusceddu, D. Marrale, N. Della Croce, M. Fabiano, and A.
524 Tselepides (2000), Biochemical composition of pico-, nano- and micro-particulate organic
525 matter and bacterioplankton biomass in the oligotrophic Cretan Sea (NE Mediterranean),
526 *Progr. Oceanogr.*, 46: 279-310.
527 Danovaro, R., A. Dell'Anno, and A. Pusceddu (2004), Biodiversity response to climate
528 change in a warm deep sea, *Ecol. Lett.*, 7: 821–828.
529 Danovaro, R., M. Armeni, G.M. Luna, C. Corinaldesi, A. Dell'Anno, C.R. Ferrari,
530 Fiordelmondo, C. Gambi, M. Gismondi, E. Manini, M. Mecozzi, F.M. Perrone, A.
531 Pusceddu, and M. Giani (2005), Exo-enzymatic activities and dissolved organic pools in
532 relation with mucilage development in the Northern Adriatic Sea, *Sci. Tot. Environ.*, 353:
533 189-203.
534 Danovaro R., A. Dell'Anno, C. Corinaldesi, M. Magagnini, R. Noble, C. Tamburini, and M.
535 Weinbauer (2008), Major viral impact on the functioning of benthic deep-sea ecosystems,
536 *Nature*, 454: 1084-1087.

537 Davey, K.E., R.R. Kirby, C.M. Turley, A.J. Weightman, and J.C. Fry (2001), Depth variation
538 of bacterial extracellular enzyme activity and population diversity in the northeastern
539 North Atlantic Ocean, *Deep Sea Res. II*, 48: 1003-1017.
540 Dell'Anno, A., and C. Corinaldesi (2004), Degradation and turnover of extracellular DNA in
541 marine sediments: ecological and methodological considerations, *Appl. Environ.*
542 *Microbiol.*, 70: 4384-4386.
543 Del Giorgio, P.A., and J.J. Cole (1998), Bacterioplankton growth efficiency in aquatic
544 systems, *Ann. Rev. Ecol. System.*, 29: 503-541.
545 Del Giorgio, P.A., C.M. Duarte (2002), Respiration in the open ocean, *Nature,* 420: 379–384.
546 Dixon, J.L., and C.M. Turley (2001), Measuring bacterial production in deep-sea sediments
547 using ^{3}H-thymidine incorporation: ecological significance, *Microb. Ecol.*, 42: 549-561.
548 Ducklow, H.W., D.L. Kirchman, H.L. Quinby, C.A. Carlson, and H.G. Dam (1993), Stocks
549 and dynamics of bacterioplankton carbon during the spring bloom in the Central-Eastern
550 North Atlantic Ocean, *Deep Sea Res. II*, 40: 245-263.
551 Ducklow, H.W. (1999), The bacterial component of the oceanic euphotic zone, *FEMS*
552 *Microbiol. Ecol.*, 30: 1–10.
553 Fonda Umani, S., E. Malisana, F. Focaracci, M. Magagnini, C. Corinaldesi, and R. Danovaro
554 (2010), Disentangling the impact of viruses and nanoflagellates on prokaryotes in
555 bathypelagic waters of the Mediterranean Sea, *Mar. Ecol. Progr. Ser.*, 418: 73-85.
556 Keeling R.F., A. Kortzinger, and N. Gruber (2010), Ocean deoxygenation in a warming
557 world, *Annu. Rev. Mar. Sci.,* 2: 199–229.
558 Kirchman, D.L., E. K'Nees, and R. Hodson (1985), Leucine incorporation and its potential as
559 a measure of protein synthesis by bacteria in natural aquatic systems, *Appl. Environ.*
560 *Microbiol.*, 49: 599-607.

561 Koike, I., and T. Nagata (1997), High potential activity of extracellular alkaline phosphatase
562 in deep waters of the central Pacific, *Deep Sea Res. II*, 44: 2283-2294.
563 Koppelmann R., H. Weikert, C. Halsband-Lenk, and T. Jennerjahn (2004), Mesozooplankton
564 community respiration and its relation to particle flux in the oligotrophic eastern
565 Mediterranean, *Global Biogeochem. Cycles*, 18, GB1039, doi:10.1029/2003GB002121
566 Gasol J.M., L. Alonso-Sáez, D. Vaqué, F. Baltar, M.J. Calleja, C.M. Duarte, and J. Arístegui
567 (2009), Mesopelagic prokaryotic bulk and single-cell heterotrophic activity and community
568 composition in the NW Africa–Canary Islands coastal-transition zone, *Progr. Oceanog.*,
569 83: 189-196.
570 Herrmann, M., S. Somot, F. Sevault, C. Estournel, M. Déqué (2008), Modeling the deep
571 convection in the northwestern Mediterranean Sea using an eddy-permitting and an eddy-
572 resolving model: case study of winter 1986–1987, *J. Geophys. Res.*, 113, C04011,
573 doi:10.1029/2006JC003991
574 Heussner, S., X. Durrieu De Madron, A. Calafat, M. Canals, J. Carbonne, N. Delsaut, and G.
575 Saragoni (2006), Spatial and temporal variability of downward particle fluxes on a
576 continental slope: lessons from an 8-yr experiment in the Gulf of Lions (NW
577 Mediterranean), *Mar. Geol.*, 234: 63-92.
578 Hoppe, H.G. (1993), Use of fluorogenic model substrates for extracellular enzyme activity
579 (EEA) measurements of bacteria, In "Handbook of Methods in Aquatic Microbial
580 Ecology" (Eds. Kemp, P.F., Sherr, B.F., Sherr, E.B., Cole, J.J.) Lewis, London, pp. 509–
581 512.
582 Hoppe, H.G., H. Ducklow, and B. Karrasch (1993), Evidence for dependency of bacterial
583 growth on enzymatic hydrolysis of particulate organic matter in the mesopelagic ocean,
584 *Mar. Ecol. Progr. Ser.*, 93: 277-283.

585 Hoppe, H.G., and S. Ullrich (1999), Profiles of ectoenzymes in the Indian Ocean: phenomena
586 of phosphatase activity in the mesopelagic zone, *Aquat. Microb. Ecol.*, 19: 139–148.
587 Hoppe, H.G., K. Gocke, R. Koppe, and C. Begler (2002), Bacterial growth and primary
588 production along a north–south transect of the Atlantic Ocean, *Nature,* 416: 168-171.
589 Hoppe, H.G. (2003), Phosphatase activity in the sea, *Hydrobiologia,* 493: 187-200.
590 La Ferla, R., M. Azzaro, G. Civitarese, M. Ribera d'Alcalà (2003), Distribution patterns of
591 carbon oxidation in the eastern Mediterranean Sea: evidence of changes in the
592 remineralization processes, *J. Geophys. Res.* 108 (C9), 8111, doi: 10.1029/2002JC001602.
593 La Ferla, R., F. Azzaro, M. Azzaro, G. Caruso, F. Decembrini, M. Leonardi, G. Maimone,
594 L.S. Monticelli, F. Raffa, C. Santinelli, R. Zaccone, and M. Ribera d'Alcalà (2005),
595 Microbial contribution to carbon biogeochemistry in the Mediterranean Sea: variability of
596 activities and biomass, *J. Mar. Syst.*, 57: 146-166.
597 La Ferla, R., M. Azzaro, G. Caruso, L.S. Monticelli, G. Maimone, R. Zaccone, and T.T.
598 Packard (2010), Prokaryotic abundance and heterotrophic metabolism in the deep
599 Mediterranean Sea, *Adv. Ocean. Limnol.,* 1: 103-118.
600 Magagnini, M., C. Corinaldesi, L.S. Monticelli, E. De Domenico, and R. Danovaro (2007),
601 Viral abundance and distribution in mesopelagic and bathypelagic waters of the
602 Mediterranean Sea, *Deep Sea Res. I*, 54: 1209-1220.
603 Manca, B., M. Burca, A. Giorgetti, C. Coatanoan, M.-J. Garcia, and A. Iona (2004), Physical
604 and biochemical averaged vertical profiles in the Mediterranean regions: an important tool
605 to trace the climatology of water masses and to validate incoming data from operational
606 oceanography, *J. Mar. Syst.*, 48: 83-116.
607 Masuda S., T. Awaji, N. Sugiura, J.P. Matthews, T. Toyoda, Y. Kawai, T. Doi, S. Kouketsu,
608 H. Igarashi, K. Katsumata, H. Uchida, T. Kawano, and M. Fukasawa (2010), Simulated
609 rapid warming of abyssal North Pacific waters, *Science*, 329: 319-322

610 Millot, C. (1999), Circulation in the Western Mediterranean Sea, *J. Mar. Syst.*, 20: 432-442.
611 Misic, C., M. Castellano, M. Fabiano, N. Ruggieri, V. Saggiomo, and P. Povero (2006),
612 Ectoenzymatic activity in surface waters: a transect from the Mediterranean Sea across the
613 Indian Ocean to Australia, *Deep Sea Res. I*, 53: 1517-1532.
614 Nagata, T., C. Tamburini, J. Arístegui, F. Baltar, A. Bochdansky, S. Fonda-Umani, H.
615 Fukuda, A. Gogou, D.A. Hansell, R.L. Hansman, G. Herndl, C. Panagiotopoulos, T.
616 Reinthaler, R. Sohrin, P. Perdugo, N. Yamada, Y. Yamashita, T. Yokokawa, and D.H.
617 Bartlett (2010), Emerging concepts on microbial processes in the bathypelagic ocean –
618 ecology, biogeochemistry and genomics, *Deep Sea Res. II,* 57: 1519-1536.
619 Noble, R.T., and J.A. Fuhrman (1998), Use of Sybr Green I for rapid epifluorescence counts
620 of marine viruses and bacteria, *Aquat. Microb. Ecol.*, 14: 113-118.
621 Pedrós-Alió, C., J.I. Calderón-Paz, N. Guixa-Boixereu, M. Estrada, and J.M. Gasol (1999),
622 Bacterioplankton and phytoplankton biomass and production during summer stratification
623 in the northwestern Mediterranean Sea, *Deep Sea Res I*, 46: 985–1019.
624 Pitta, P., N. Stambler, T. Tanaka, T. Zohary, A. Tselepides, and F. Rassoulzadegan (2005),
625 Biological response to P addition in the Central-Eastern Mediterranean Sea. The microbial
626 race against time, *Deep Sea Res. II*, 52: 2961-2974.
627 Pusceddu, A., A. Dell'Anno, L. Vezzulli, M. Fabiano, V. Saggiomo, S. Cozzi, G. Catalano,
628 G., and L. Guglielmo (2009), Microbial loop malfunctioning in the annual sea ice at Terra
629 Nova Bay (Antarctica), *Pol. Biol.*, 32: 337-346.
630 Reinthaler, T., H. van Aken, C. Veth, J. Aristegui, C. Robinson, P.J.B. Williams, P. Lebaron,
631 and G.J. Herndl (2006), Prokaryotic respiration and production in the meso- and
632 bathypelagic realm of the Central-Eastern and western North Atlantic basin, *Limnol.*
633 *Oceanogr.*, 51: 1262–1273.
634 Rivkin, R.B., and L. Legendre (2001), Biogenic carbon cycling in the upper ocean: effects of

635 microbial respiration, *Science*, 291: 2398-2400.
636 Santinelli, C., L. Nannicini, and A. Seritti (2010), DOC dynamics in the meso and
637 bathypelagic layers of the Mediterranean Sea, *Deep Sea Res. II*, 57: 1446-1459.
638 Sardà, F., A. Calafat, M.M. Flexas, A. Tselepides, M. Canals, M. Espino, and A. Tursi
639 (2004), An introduction to Mediterranean deep-sea biology, *Sci. Mar.*, 68: 7-38.
640 Sarmiento, J.L., R. Slater, R. Barber, L. Bopp, S.C. Doney, A.C. Hirst, J. Kleypas, R. Matear,
641 U. Mikolajewicz, P. Monfray, V. Soldatov, S.A. Spall, and R.J. Stouffer (2004), Response
642 of ocean ecosystems to global warming, *Global Biogeochem. Cycles*, 18, GB3003,
643 doi:10.1029/2003GB002134.
644 Sarmiento, J.L., T.D. Herbert, and J.R. Toggweiler (1988), Mediterranean nutrient balance
645 and episodes of anoxia, *Global Biogeochem. Cycles*, 2: 427-444.
646 Seritti, A., B.B. Manca, C. Santinelli, E. Murru, A. Boldrin, and L. Nannicini (2003),
647 Relationships between dissolved organic carbon (DOC) and water mass structures in the
648 Ionian Sea (Winter 1999), *J. Geophys. Res.,* 108(C9), 8112, doi:10.1029/2002JC001345.
649 Simon, M., and F. Azam (1989), Protein content and protein synthesis rates of marine
650 bacterial assemblages, *Mar. Ecol. Prog. Ser.*, 34: 41-49.
651 Sintes, E., and Herndl, G.J. (2006) Quantifying substrate uptake of individual cells of marine
652 bacterioplankton populations by catalyzed reporter deposition fluorescence *in situ*
653 hybridization combined with microautoradiography. *Appl. Environ. Microbiol.* 72: 7022–
654 7028.
655 Smith, D.C., and F. Azam (1992), A simple economical method for measuring bacterial
656 protein synthesis rates in seawater using ^{3}H-leucine, *Mar. Microb. Food Webs*, 6: 107-114.
657 Smith, E.M., and P.A. del Giorgio (2003) Low fractions of active bacteria in natural aquatic
658 communities?, *Aquat. Microb. Ecol.* 31: 203–208
659 Somot, S., F. Sevault, and M. Déquè (2006), Transient climate change scenario simulation of

660 the Mediterranean Sea for the twenty-first century using a high-resolution ocean
661 circulation model, C*lim. Dyn.*, 27:851–879.
662 Tamburini, C., J. Garcin, M. Ragot, and A. Bianchi (2002), Biopolymer hydrolysis and
663 bacterial production under ambient hydrostatic pressure through a 2000 m water column in
664 the NW Mediterranean, *Deep Sea Res. II*, 49: 2109-2123.
665 Tamburini, C., M. Garel, B.A. Ali, B. Mérigot, P. Kriwy, B. Charrière, and G. Budillon
666 (2009), Distribution and activity of Bacteria and Archaea in the different water masses of
667 the Tyrrhenian Sea, *Deep Sea Res. II*, 56: 700–712.
668 Tanaka, T. and F. Rassoulzadegan (2002), Full-depth profile (0–2000 m) of bacteria,
669 heterotrophic nanoflagellates and ciliates in the NW Mediterranean Sea: vertical
670 partitioning of microbial trophic structures, *Deep Sea Res. II*, 49: 2093-2107.
671 Tanaka, T., and F. Rassoulzadegan (2004), Vertical and seasonal variations of bacterial
672 abundance and production in the mesopelagic layer of the NW Mediterranean Sea: bottom-
673 up and top-down controls, *Deep Sea Res. I,* 51: 531-544.
674 Taylor, G.T., R. Thunell, R. Varela, C. Benitez-Nelson, and M.I. Scranton (2009), Hydrolytic
675 ectoenzyme activity associated with suspended and sinking organic particles within the
676 anoxic Cariaco Basin, *Deep Sea Res. I*, 56: 1266-1283.
677 Thingstad, T.F., M.D. Krom, R.F.C. Mantoura, G.A.F. Flaten, S. Groom, B. Herut, N. Kress,
678 C.S. Law, A. Pasternak, P. Pitta, S. Psarra, F. Rassoulzadegan, T. Tanaka, A. Tselepides,
679 P. Wassmann, E.M.S. Woodward, C. Wexels Riser, G. Zodiatis, and T. Zohary (2005),
680 Nature of phosphorus limitation in the ultraoligotrophic Central-Eastern Mediterranean,
681 *Science*, 309: 1068–1071.
682 Turley, C.M., M. Bianchi, U. Christaki, P. Conan, J.R.W. Harris, S. Psarra, G. Ruddy, E.D.
683 Stutt, A. Tselepides, and F. Van Wambeke (2000), Relationship between primary
684 producers and bacteria in an oligotrophic sea: the Mediterranean and biogeochemical

implications, *Mar. Ecol. Progr. Ser.*, 193:11-18.

Vázquez-Domínguez, E., C.M. Duarte, S. Agustí, K. Jürgens, D. Vaqué, and J.M. Gasol (2008), Microbial plankton abundance and heterotrophic activity across the Central Atlantic Ocean, *Prog. Oceanogr.*, 79: 83-94.

Yokokawa, T, D. De Corte, E. Sintes, and G.J. Herndl (2010), Spatial patterns of bacterial abundance, activity and community composition in relation to water masses in the eastern Mediterranean Sea, *Aquat. Microb. Ecol.*, 59: 185–195.

Zaccone, R., L.S. Monticelli, A. Seritti, C. Santinelli, M. Azzaro, A. Boldrin, R. La Ferla, and M. Ribera d'Alcalà (2003), Bacterial processes in the intermediate and deep layers of the Ionian Sea in winter 1999: vertical profiles and their relationship to the different water masses, *J. Geophys. Res.,* 108: 8117-8128.

Figures legends.

Figure 1. The locations of the sampling stations in the Mediterranean Sea.

Figure 2. Isoclines of temperature (°C), salinity, oxygen concentration (μmol kg^{-1}) and fluorescence (as Arbitrary Units) distributions in the stations investigated across the Mediterranean Sea (Left panels, Western basin; right panels, Central-Eastern basin). Black dots indicate sampling depths and locations. The salinity and fluorescence scales differ for the two basins.

Figure 3. Vertical patterns of aminopeptidase in the stations investigated across the Mediterranean Sea.

Figure 4. Spatial distribution of enzyme activity for aminopeptidase (top panels), ß-glucosidase (middle panels) and alkaline phosphatase (bottom panels) activities in the stations investigated across the Mediterranean Sea. Left panels, Western basin; right panels, Central-Eastern basin. Black dots indicate sampling depths and locations.

Figure 5. Multi-Dimensional Scaling (MDS) plot based on the enzymatic activities (aminopeptidase, ß-glucosidase and alkaline phosphatase) at the depth layers investigated (epipelagic, mesopelagic and bathypelagic). Stress value is indicated.

Figure 6. Vertical patterns of total prokaryotic abundance in the stations investigated across the Western basin of the Mediterranean Sea. The equivalent data for the Central-Eastern Mediterranean basin are not available.

Figure 7. Vertical patterns of prokaryotic heterotrophic production in the stations investigated across the Mediterranean Sea.

Figure 8. Spatial distribution of prokaryotic heterotrophic production across the Mediterranean Sea. Top panel, Western basin; bottom panel, Central-Eastern basin. Black dots indicate sampling depths and locations.

Figure 9. Vertical pattern of cell-specific prokaryotic heterotrophic production. Reported are typical profiles for some selected stations.

Table 1. K_m values for the enzymatic activities in the surface and bottom waters for the specified stations in the Mediterranean Sea. Standard deviations are reported in parentheses. N.A. = not available.

Station	K_m (µM)					
	Aminopeptidase		β-Glucosidase		Alkaline phosphatase	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
J8	5.2 (1.8)	11.9 (0.4)	0.4 (0.02)	0.6 (0.01)	3.9 (0.1)	4.8 (1.2)
M14	0.5 (0.1)	3.6 (1.7)	0.7 (0.1)	0.5 (0.01)	11.6 (0.7)	10.7 (6.2)
M4	4.7 (0.9)	10.8 (1.3)	1.9 (0.01)	N.A.	14.9 (2.3)	17.7 (10.1)
M25	8.0 (3.5)	22.2 (9.2)	0.5 (0.01)	0.2 (0.02)	3.8 (2.5)	3.0 (0.3)
R11	1.8 (0.1)	6.5 (3.8)	0.3 (0.02)	2.0 (0.6)	1.5 (0.2)	1.5 (0.1)
Means	**4.0**	**11.0**	**0.76**	**0.80**	**7.14**	**7.54**

Table 2. Output of the 1-way ANOVA analysis carried out to test for changes in the microbial variables between: A) the epipelagic and the mesopelagic and bathypelagic layers and B) the mesopelagic and the bathypelagic layer. MS=Mean Square; F=F statistic; P=probability level; SNK=output of the Student–Newman–Keuls *post-hoc* test; ***=P<0.001; *=P<0.05; ns=not significant.

		MS	F	P	SNK
A)	Aminopeptidase	1.51	15.91	***	epipelagic > mesopelagic, bathypelagic
	β-glucosidase	0.12	25.96	***	epipelagic > mesopelagic, bathypelagic
	Alkaline phosphatase	0.04	9.35	***	epipelagic > mesopelagic, bathypelagic
	Prokaryotic het. production	0.28	3.23	*	epipelagic > mesopelagic, bathypelagic
B)	Aminopeptidase	0.22	31.53	***	mesopelagic > bathypelagic
	β-glucosidase	0.04	6.87	***	mesopelagic > bathypelagic
	Alkaline phosphatase	0.02	1.14	ns	ns
	Prokaryotic het. production	0.22	5.24	*	mesopelagic > bathypelagic

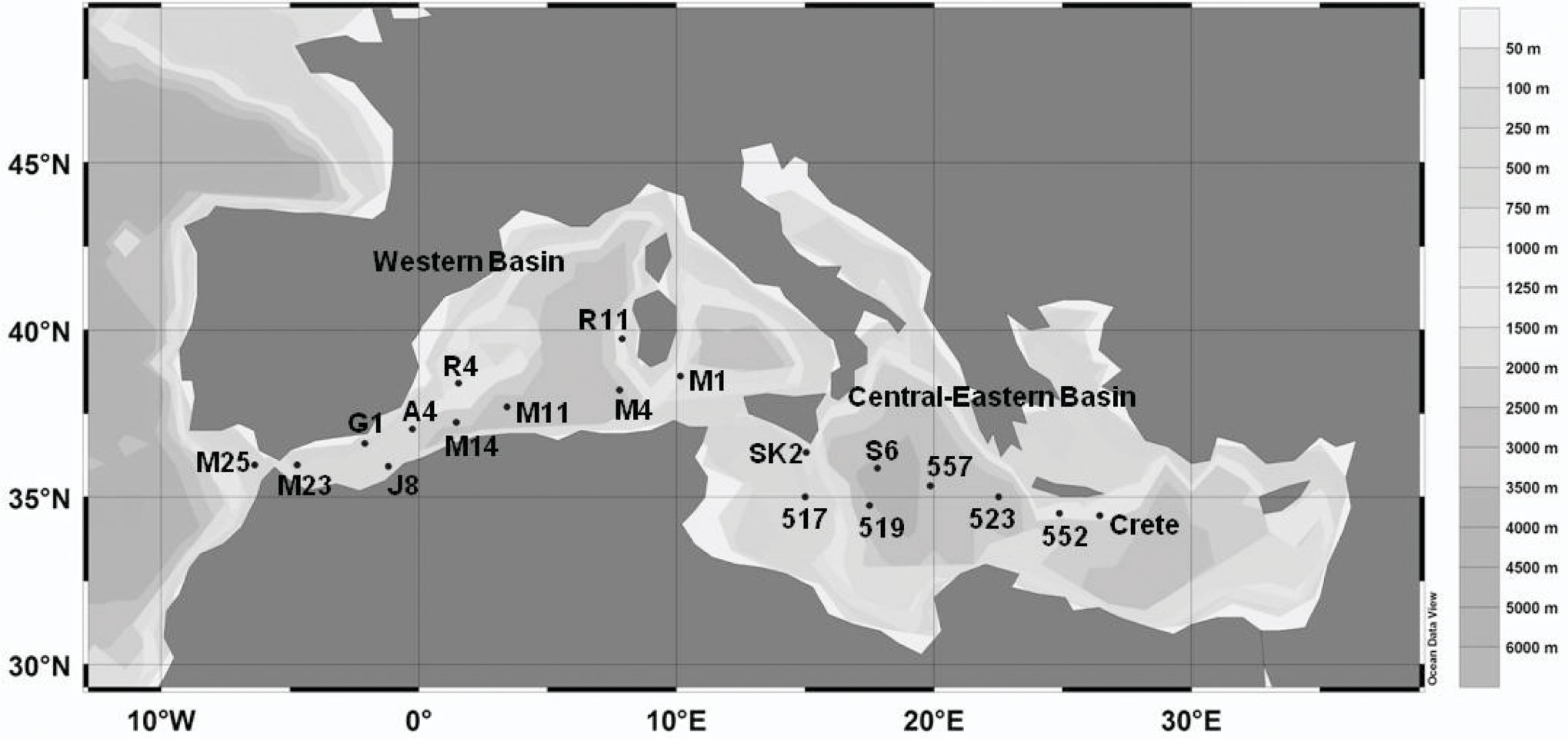
Table 3. Comparisons of prokaryotic heterotrophic production and enzymatic activities in the epipelagic, mesopelagic and bathypelagic layers of the Western and Central-Eastern Mediterranean Sea.

Layer	Prokaryotic heterotrophic production (ngC L^{-1} h^{-1})				
	Western basin		Central-eastern basin		Ratio W/E
	Mean	SD (n)	Mean	SD (n)	
Epipelagic	317.16	37.81 (35)	56.96	12.83 (35)	5.6
Mesopelagic	201.77	109.42 (25)	37.25	12.44 (11)	5.4
Bathypelagic	159.75	37.70 (11)	41.21	16.74 (13)	3.9
	Aminopeptidase (µmol L^{-1} d^{-1})				
	Western basin		Central-eastern basin		Ratio W/E
	Mean	SD (n)	Mean	SD (n)	
Epipelagic	2.18	0.26 (43)	0.08	0.01 (29)	26.1
Mesopelagic	1.04	0.13 (25)	0.09	0.05 (11)	12.0
Bathypelagic	0.66	0.13 (13)	0.05	0.01 (14)	14.3
	ß-Glucosidase (µmol L^{-1} d^{-1})				
	Western basin		Central-eastern basin		Ratio W/E
	Mean	SD (n)	Mean	SD (n)	
Epipelagic	0.88	0.09 (43)	0.31	0.05 (29)	2.8
Mesopelagic	0.51	0.31 (25)	0.10	0.03 (12)	4.8
Bathypelagic	0.41	0.10 (13)	0.13	0.04 (13)	3.1
	Alkaline phosphatase (µmol L^{-1} d^{-1})				
	Western basin		Central-eastern basin		Ratio W/E
	Mean	SD (n)	Mean	SD (n)	
Epipelagic	0.51	0.04 (43)	0.20	0.03 (34)	2.5
Mesopelagic	0.35	0.15 (25)	0.13	0.04 (13)	2.6
Bathypelagic	0.31	0.18 (13)	0.09	0.03 (13)	3.4

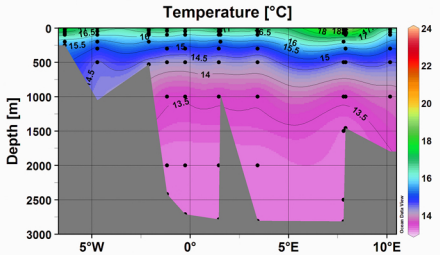
792 Table 4. Results of the multivariate ANOSIM (R and P) and SIMPER (dissimilarity %,
793 explanatory variable and explained variance) analyses carried out to assess the differences
794 in the enzymatic activities in the Western and Central-Eastern Mediterranean basin. *
795 $p<0.05$, ** $p<0.01$, *** $p<0.001$.
796

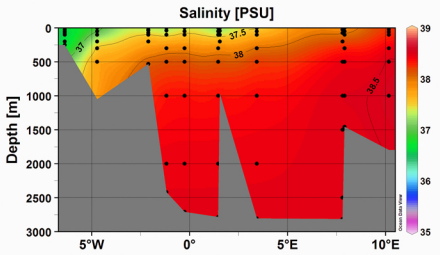
Comparison	ANOSIM		SIMPER		
	R	P	Dissimilarity (%)	Explanatory variable	Explained variance (%)
West vs. Central-East	0.796	***	24.75	aminopeptidase	46.8
				β-glucosidase	27.7
				alkaline phosphatase	25.5
Epipelagic West vs. Central-East	0.597	***	28.48	aminopeptidase	47.2
				β-glucosidase	26.8
				alkaline phosphatase	26.0
Mesopelagic West vs. Central-East	0.284	ns	16.52	aminopeptidase	45.4
				β-glucosidase	28.4
				alkaline phosphatase	26.2
Bathypelagic West vs. Central-East	0.648	***	18.52	aminopeptidase	42.5
				β-glucosidase	32.1
				alkaline phosphatase	25.3

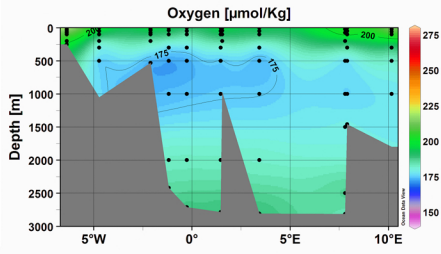
797
798
799
800

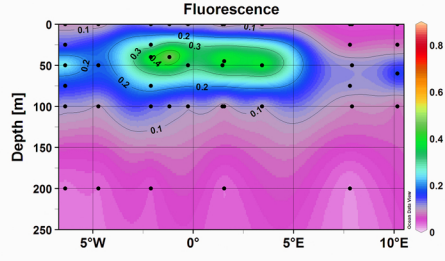

Western Basin
Central-Eastern Basin
R11
R4
M1
G1
A4
M11
M4
M14
M25
M23
J8
SK2
S6
557
517
519
523
552
Crete
45°N
40°N
35°N
30°N
10°W
0°
10°E
20°E
30°E
50 m
100 m
250 m
500 m
750 m
1000 m
1250 m
1500 m
2000 m
2500 m
3000 m
3500 m
4000 m
4500 m
5000 m
6000 m
Ocean Data View

Western Basin

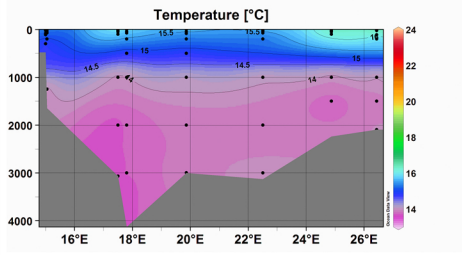


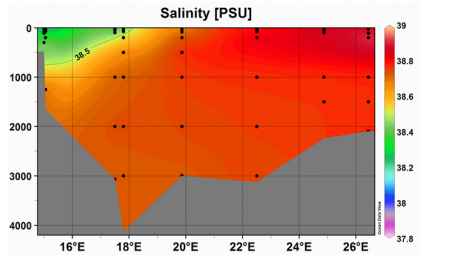


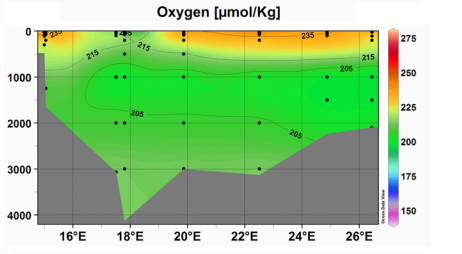


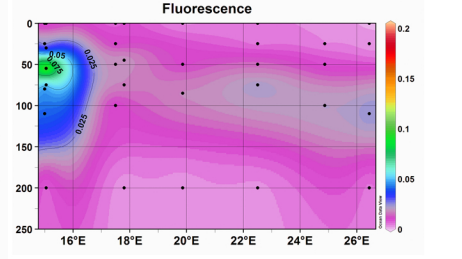


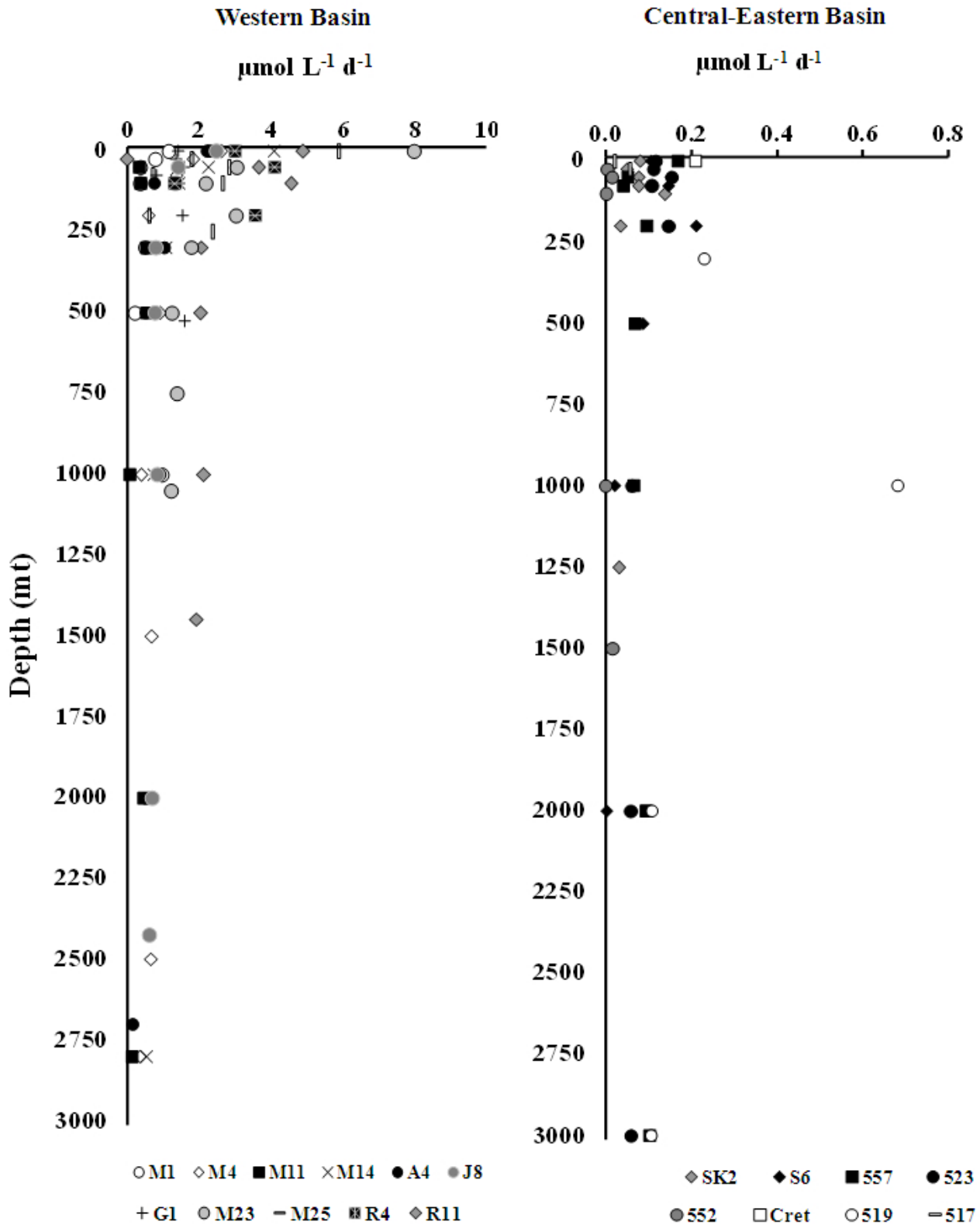
Central-Eastern Basin





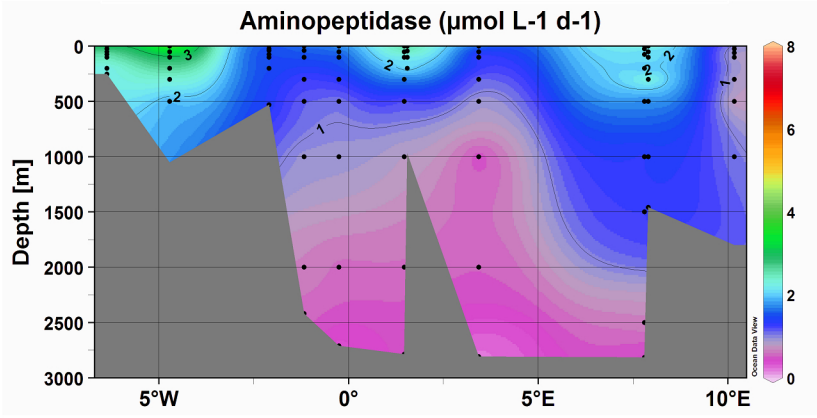


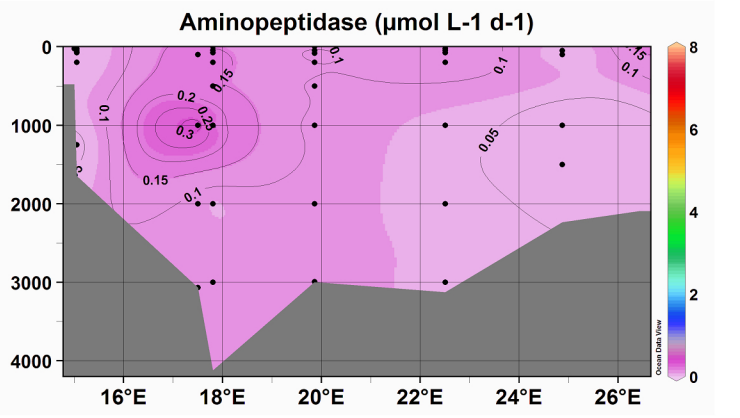


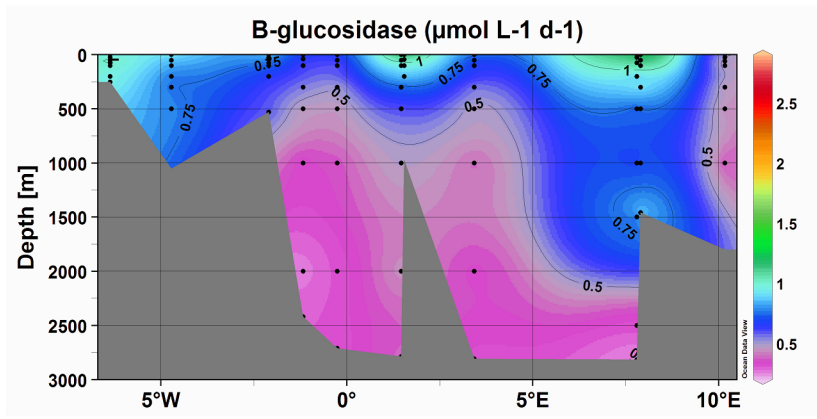

Western Basin
µmol L-1 d-1
Central-Eastern Basin
µmol L-1 d-1
Depth (mt)
0
250
500
750
1000
1250
1500
1750
2000
2250
2500
2750
3000
0 2 4 6 8 10
0.0 0.2 0.4 0.6 0.8
M1 M4 M11 M14 A4 J8
G1 M23 M25 R4 R11
SK2 S6 557 523
552 Cret 519 517

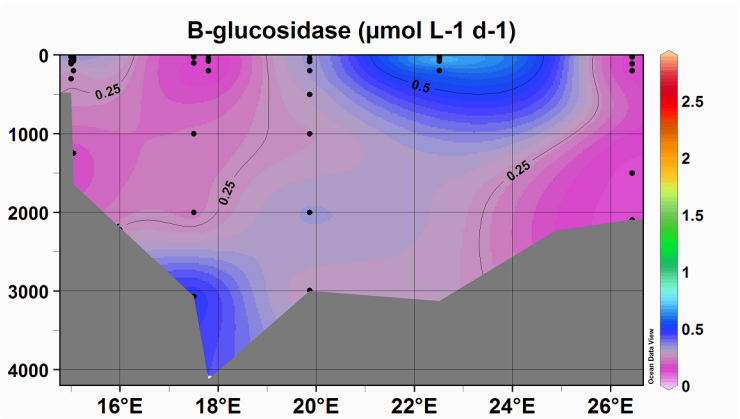
Western Basin

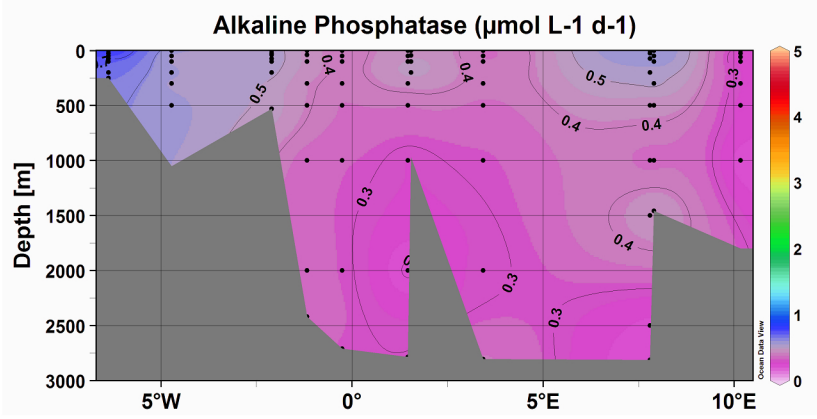
Central-Eastern Basin

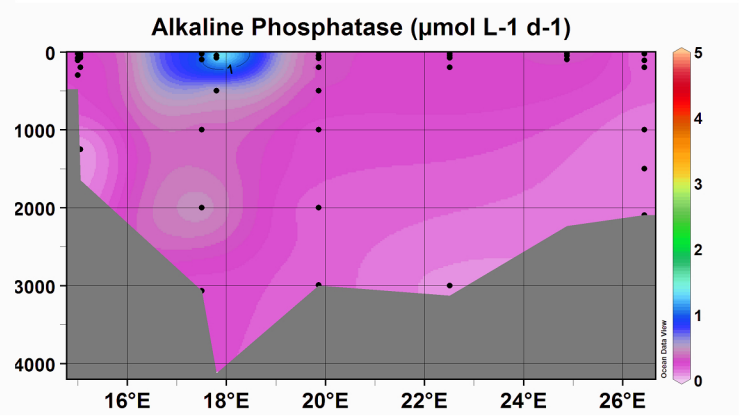


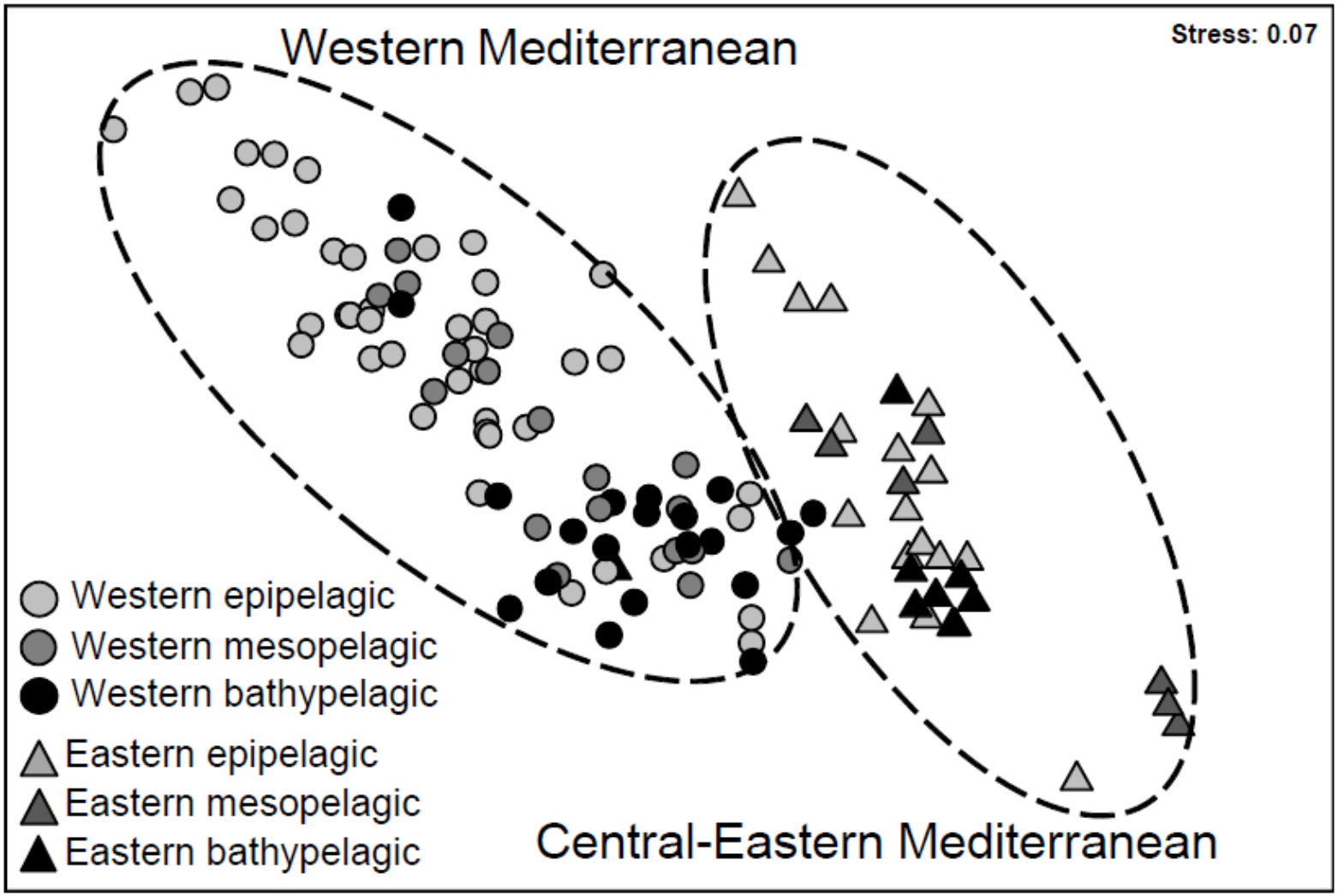


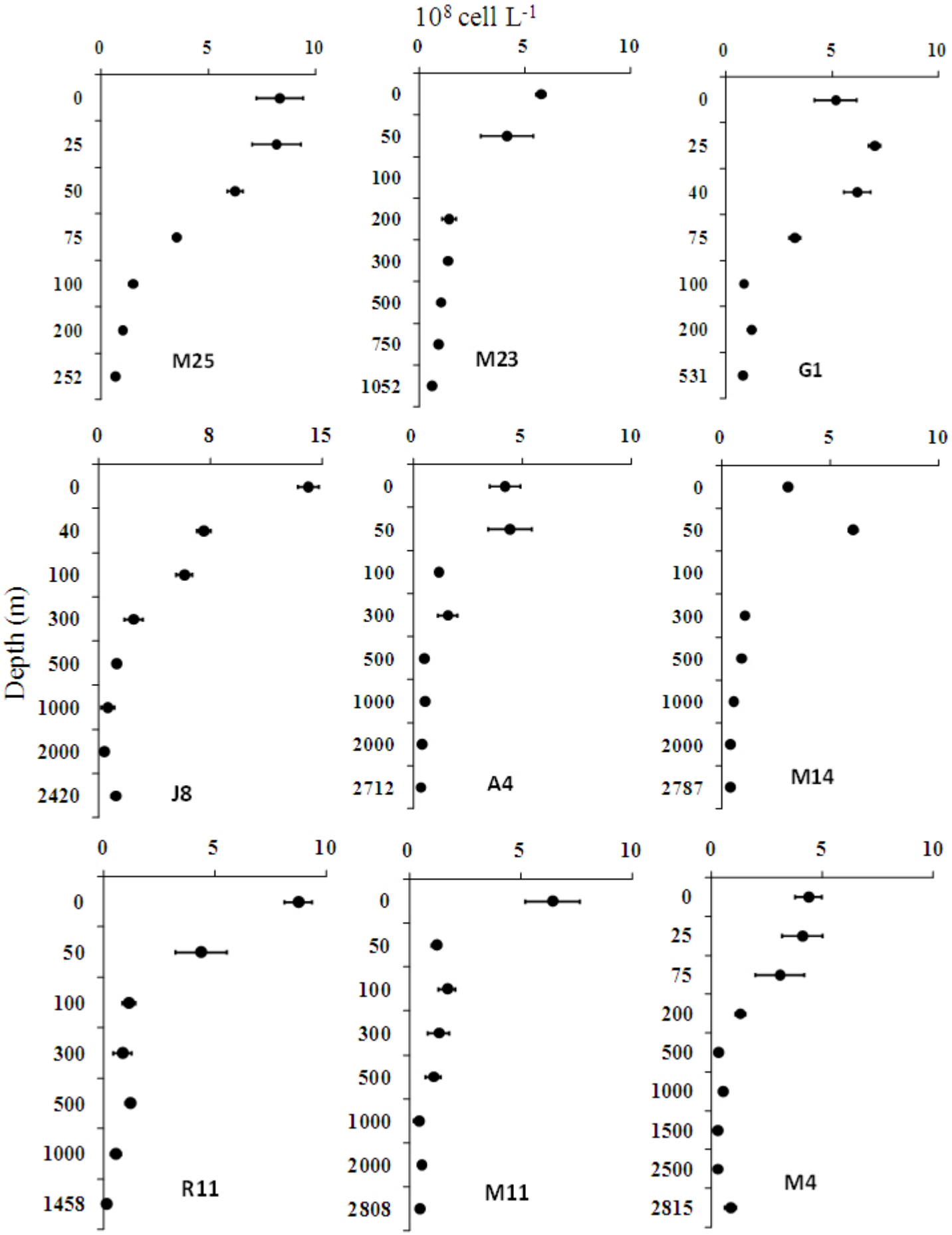


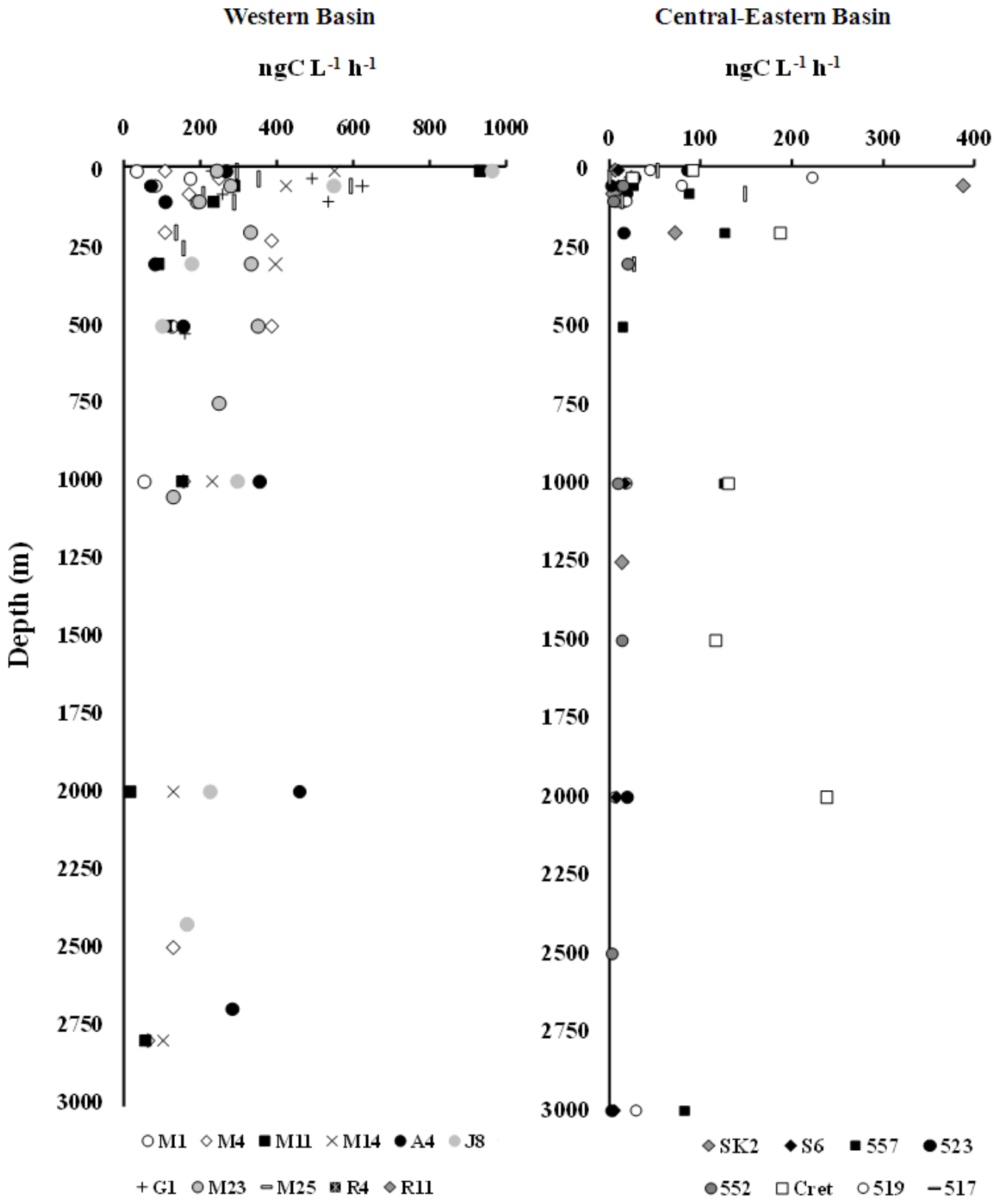






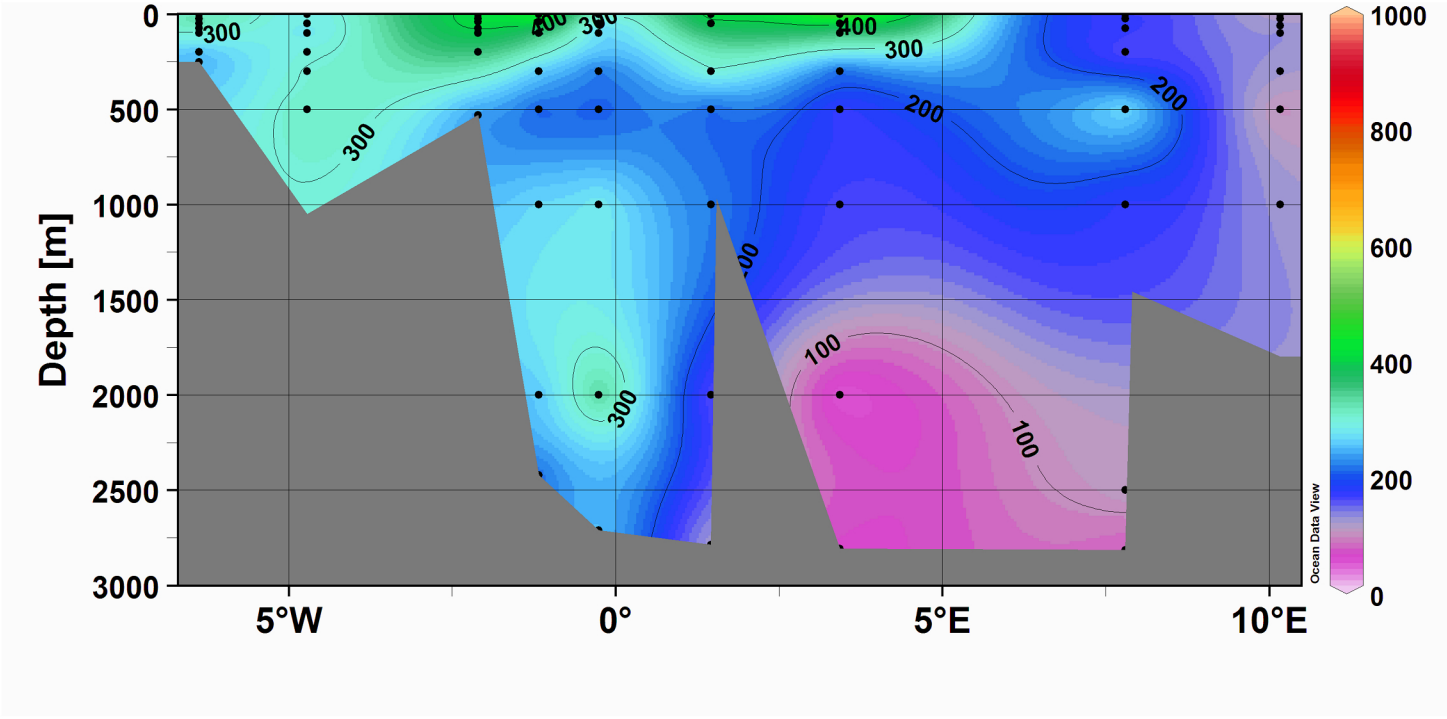

Western Mediterranean
Stress: 0.07
Western epipelagic
Western mesopelagic
Western bathypelagic
Eastern epipelagic
Eastern mesopelagic
Eastern bathypelagic
Central-Eastern Mediterranean


10^8 cell L^{-1}
Depth (m)
M25
M23
G1
J8
A4
M14
R11
M11
M4

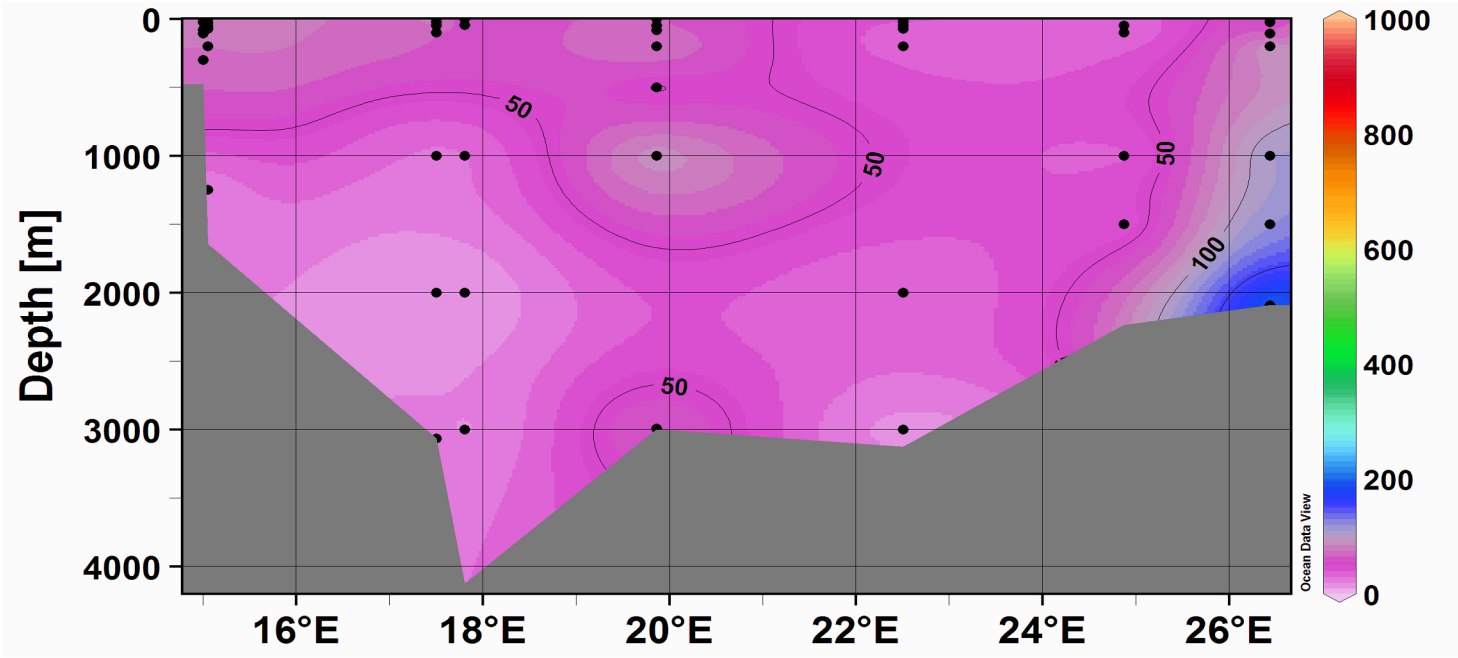

Western Basin
ngC L-1 h-1
0
200
400
600
800
1000
Depth (m)
0
250
500
750
1000
1250
1500
1750
2000
2250
2500
2750
3000
M1
M4
M11
M14
A4
J8
G1
M23
M25
R4
R11
Central-Eastern Basin
ngC L-1 h-1
0
100
200
300
400
SK2
S6
557
523
552
Cret
519
517

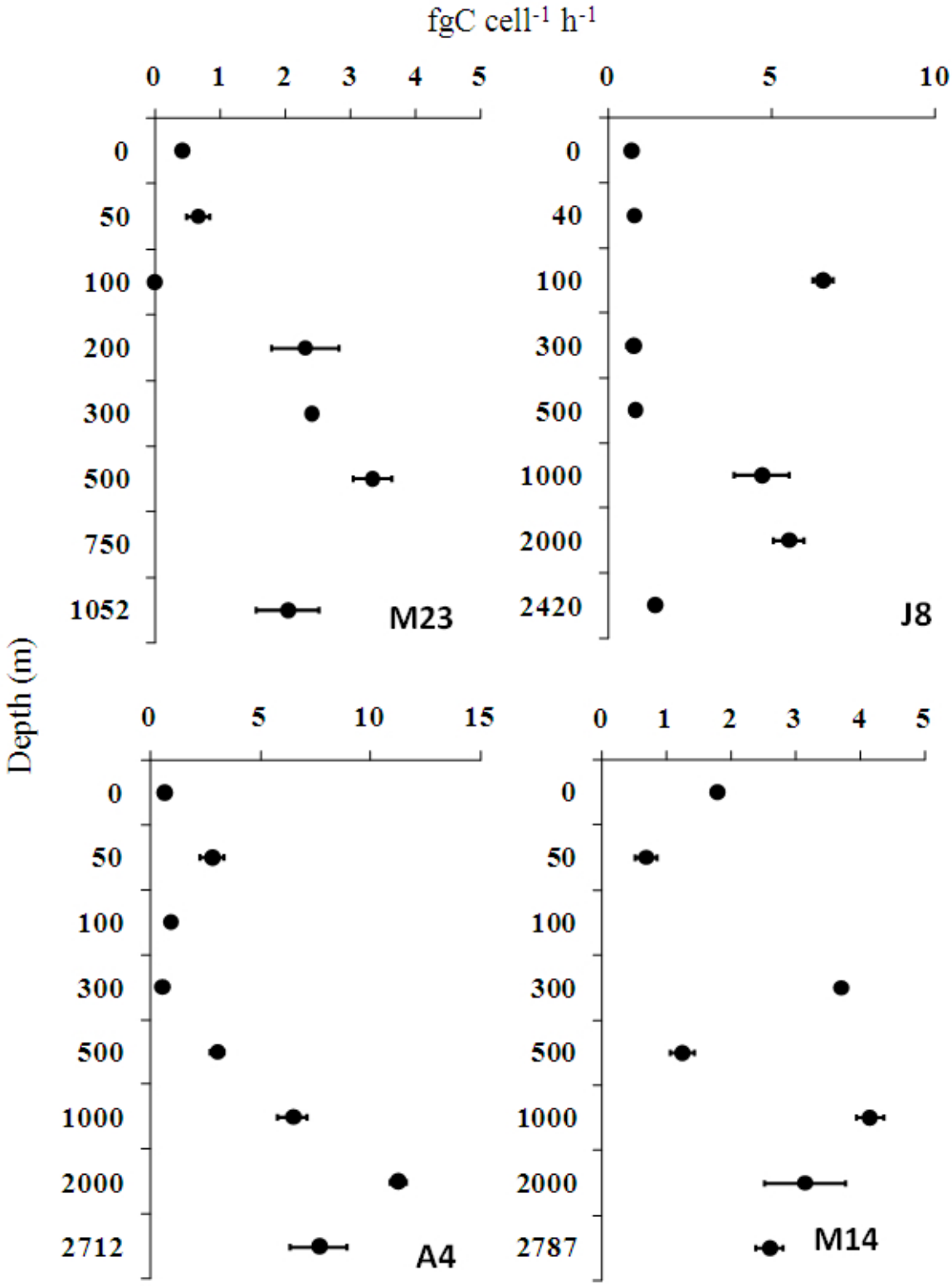
Prokaryotic Heterotrophic Production (ngC L^{-1} h^{-1})

Western Basin



Central-Eastern Basin




fgC cell-1 h-1
Depth (m)
M23
J8
A4
M14