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Biogeography of planktonic microbial communities across the whole Mediterranean Sea

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Abstract

The M84/3 cruise recently held onboard of R/V Meteor represented a great and rare opportunity for the scientific community to realize a multidisciplinary survey on the whole Mediterranean Sea. In this context, molecular microbiology investigation, realized by applying Automated Ribosomal Intergenic Sequence Analysis (ARISA) and microscope evaluation of prokaryotic abundance, were performed on seawater samples aiming to identify the environmental factors driving planktonic bacterial community composition across both vertical and longitudinal transects. Prokaryotic abundance decreased along with depth in all the stations and presented similar values in sub-surface, meso- and bathypelagic layers across the whole Mediterranean basin. On the contrary, peculiar bacterial assemblages were selected along a longitudinal transect in the surface layers of the eastern and western sub-basins. Sharp vertical profiling of the bacterial communities was observed only considering the boundary of the water column, while the study of bacterial β-diversity at finer scale across the water column displayed higher variability at the intermediate layers. Nonetheless, different physico-chemical factors were significantly related to microbial zonation both according to geographic position and across the water column in the whole Mediterranean Sea. These results demonstrated that bacterial diversity is putatively correlated to different water masses across the water column of the complex hydrographical systems of the eastern and western Mediterranean sub-basins.

1 Introduction

Prokaryotes are key players in sea ecosystems both in terms of biomass and phylogenetic diversity and for their role in biogeochemical cycles. Only recently, due to the development and advance of molecular tools, marine scientist undertook the investigation of microbiome distribution and adaptation to specific environmental conditions in oceans (Agogué et al., 2011; Fuhrman et al., 2008; Galand et al., 2009; Hewson

OSD
10, 291–319, 2013

et al., 2006; Lovejoy et al., 2002; Varela et al., 2008a; Yilmaz et al., 2012). Few reports focused nevertheless on the ecological structuring of prokaryotes in the Mediterranean Sea, mainly by investigating the eastern sub-basin (Feingersch et al., 2010; Moesender et al., 2001; De Corte et al., 2009; Yokokawa et al., 2010). The Mediterranean Sea has been proposed as a marine ecosystem hot spot in term of biodiversity, hosting about 17000 marine species belonging to the *Eukarya* domain and a number of *Bacteria* and *Archaea* species at present impossible to be estimated (Coll et al., 2010). The Mediterranean Sea is a semi-enclosed basin divided by the Sicily channel in two main sub-basins, the western and eastern Mediterranean Sea. A typical trait of Mediterranean seawater is its oligotrophic nature, exceptionally pronounced in the eastern most area, where the condition can be defined ultra-oligotrophic. The low amount of inorganic phosphorous has been reported as a limiting factor of primary productivity in the eastern Mediterranean basin (Thingstad et al., 2005). On the contrary, the lack of thermal confines in the warm deep waters of the Mediterranean makes its bottom layers hospitable for an active microbial community, even though the effect of pressure must be considered. Recent reports indicate that it is possible to correlate specific microbial community structure to different water masses in the ocean (Agogué et al., 2011; Galand et al., 2009; Varela et al., 2008a, b) but similar studies realized on the Mediterranean Sea could not draw unequivocal conclusions (Tamburini et al., 2009; Yokokawa et al., 2010), indicating in some cases a depth related distribution of specific groups of prokaryotes (De Corte et al., 2009; Winter et al., 2009). Indeed, the water masses circulation and dynamics is extremely complex in the Mediterranean Sea (Bensi et al., 2012; Hecht et al., 1988; Pinardi et al., 2000 and references therein; Rubino et al., 2007) and a pronounced spatial and temporal variability of the water masses composition in the different sub-basins can be related to local geographic peculiarity, such as deep water formation in the Adriatic and the Gulf of Lions, and the water input entering the basin from the Strait of Gibraltar, the Black Sea and the Suez channel. Most of the microbiological investigations realized on the Mediterranean Sea focus on a small number of stations, generally located in a narrow area (De Corte et al., 2009;

OSD
10, 291–319, 2013

Tamburini et al., 2009; Yokokawa et al., 2010). Taking advantage of the M83/4 cruise, held onboard of R/V *Meteor* during April 2011, in the present work we studied the vertical and longitudinal distribution of bacterial populations in different sampling stations located across the whole Mediterranean Sea, from the Levantine to the Alboran basins.

Automated Ribosomal Intergenic Sequence Analysis (ARISA) was proved as a valuable tool for describing the bacterial community structure in marine ecosystems (Borin et al., 2009a, b; Fuhrman et al., 2008; Hewson et al., 2006; Zehr et al., 2009) and it was applied here aiming to depict the microbiome structure of surface, meso- and bathypelagic realms. The aims of the present study were (i) to identify distinct or common patterns of bacterial diversity in surface and deep waters sampled from twenty three stations along a longitudinal transect in the whole Mediterranean Sea and (ii) to describe microbiome composition of the seawater collected throughout the water column at eight stations located in the eastern and the western Mediterranean basins.

To the best of our knowledge this is one of the most comprehensive investigations of bacterioplankton diversity realized along a longitudinal transect in surface and deep Mediterranean oligotrophic marine biomes.

2 Materials and methods

2.1 Study site, sample collection and oceanographic data

Sampling was carried out during the cruise M84/3 held on April 2011 on the R/V *Meteor*. Sampling stations were located in the main sub-basins (eastern and western) of the Mediterranean Sea, across a longitudinal transect of about 3500 km (Fig. 1). Water samples were collected from subsurface (0–100 m), meso- (100–1000 m) and bathypelagic (> 1000 m) layers of the water column in order to investigate prokaryotic abundance and diversity (i) in surface and bottom waters along the whole longitudinal transect and (ii) at finer vertical scale on the water column of 8 stations to elucidate the vertical profiling of prokaryotic communities in the Eastern and Western sub-basins. Water

OSD
10, 291–319, 2013

samples were retrieved at different depths (from 5 to a maximum depth of 4190 m) of the water column by using Niskin bottles housed on a cable-connected rosette sampler under the control of a CTD (Conductivity-Temperature-Depth) probe (Seabird 19 Plus) providing the measurements of salinity, temperature and pressure data. Inorganic nutrient concentration were measured on board using a segmented flow Skalar SANplus System Instrument (Rahav et al., 2013; Tanhua et al., 2013). Dissolved oxygen was measured following the Winkler potentiometric method as described by Tanhua et al. (2013).

2.2 Abundance of prokaryotes

Prokaryotic abundance was determined in seawater collected from the Niskin bottles and fixed with sterile formaldehyde (4 % final concentration) in the dark. Thereafter, the samples were filtered on 0.22 µm pore size black polycarbonate filters (Millipore, USA) and frozen and kept at −20 °C until analysis. Prokaryotic abundance was evaluated by 4',6-diamidino-2-phenylindole (DAPI) staining (excitation 340/360 nm, emission 440/470 nm). Prokaryotic cells positively stained by DAPI were counted by epifluorescence microscope. For each sample, 30 microscope field and more than 3000 DAPI-stained cells were counted.

2.3 DNA extraction and ARISA fingerprinting

A volume of 4–10 L of water was filtered through sterile GWSP 0.22 µm pore size filters (Millipore, USA). Thereafter, 1.8 mL of lysis buffer (40 mM EDTA, 50 mM Tris-HCl, 0.75 M sucrose) was immediately added to the filters before to store them at −20 °C until extraction. DNA extraction from the filters was performed following the protocol described in Borin et al. (2009b) by the addition of lysozyme and sodium-dodecyl-sulphate (SDS) to lyse the cells. Proteins were removed from the lysis mixture by proteinase K and chloroform/phenol extraction before DNA precipitation by isopropanol. The pellet was washed with 70 % ethanol and resuspended in sterile TE buffer. ARISA-PCR was

OSD
10, 291–319, 2013

conducted on a standard amount of DNA on each sample by using the primer set ITSF, 5'-GTC GTA ACA AGG TAG GCC GTA-3' and ITSReub, 5'-GCC AAG GCA TCC ACC 3', as previously described (Cardinale et al., 2004). ARISA fragments were separated by using the ABI3730XL genetic analyzer applying the internal standard 1200-LIZ (Macrogen, Korea) or Peak Scanner Software – Applied Biosystems and the internal standard 1500-ROX. The ARISA analyses were realized separately on (i) surface and bathypelagic longitudinal transect and (ii) the vertical profile covering subsurface, meso- and bathypelagic zones of the water column. ARISA fingerprintings were performed respectively at the University of Milan and Instituto Español de Oceanografía (IEO) and the results were analysed as two different datasets. The output peak matrix was transferred to Microsoft Excel for the following analysis. Peaks showing height value < 50 fluorescence units were removed from the output peak matrix before statistical analyses. Each polymorphic ARISA peak is defined as a different operational taxonomic unit (OTU).

2.4 Statistical analysis

Non-metric multidimensional scaling (nMDS) was carried out as visual information to explore similarities between OTUs, based on the resemblance matrix generated using Bray-Curtis similarity on the presence/absence of the OTUs within each sample. Principal components analysis (PCA) was performed on the environmental data matrix (latitude, longitude, pressure, temperature, salinity, oxygen concentration) to visualize the relationship among the samples. The same set of environmental data was used in the distance-based multivariate analysis for a linear model (DistLM, Anderson, 2002) to determine which significant environmental variables explain the observed similarity among the samples. The Akaike Information Criterion (AIC) was used to select the predictor variables. The contribution of each environmental variable was assessed, firstly using "marginal tests" to assess the statistical significance and percentage contribution of each variable taken alone, and secondly a "sequential test" was employed to evaluate the interaction among the environmental variables explaining biotic similarity.

OSD
10, 291–319, 2013

A distance-based redundancy analysis (dbRDA) was used for graphical visualization of the DistLM results. Significant differences in microbial community composition were investigated by permutational analysis of variance (PERMANOVA, Anderson, 2001), considering the sampling area as a fixed and orthogonal factor. Ecological diversity indices were calculated from the matrix of ARISA OTUs. All the statistical tests were performed by PRIMER v. 6.1 (Clarke et al., 2006), PERMANOVA+ for PRIMER routines (Anderson et al., 2008) and PAST software.

3 Results and discussion

3.1 Environmental parameters

There was little variability in temperature and salinity among the stations in the main sub-basins (eastern and western) (Table 1). The temperature differed between subsurface and deep waters with a maximum range of 13.1 to 18.7 °C. Phosphate and nitrate concentrations exhibited the common depth related trend with low concentrations in the surface layer (< 100 m) and increasing with depth (Table 1). Oxygen concentrations remain rather constant with depth, exhibiting no pronounced oxygen minimum layer below the euphotic zone (> 100 m) Environmental parameters results are listed in Supplement Table 1.

3.2 Prokaryotic abundance decrease throughout the water column of eastern and western Mediterranean basins

Prokaryotic abundance was estimated in different stations distributed along the two subbasins (western and eastern) from the subsurface, meso- and bathypelagic waters of the Mediterranean Sea (Fig. 2). Prokaryotic abundance ranged between 1.94×10^8 and 8.15×10^8 cell L^{-1} ($3.24 \times 10^8 \pm 1.40 \times 10^8$, mean ± SE) in the subsurface layer up to 100 m depth and decreased exponentially with depth to $4.82 \times 10^7 - 3.65 \times 10^8$ cell L^{-1} ($9.81 \times 10^7 \pm 6.60 \times 10^7$, mean ± SE) in the mesopelagic layers and to $2.88 \times 10^6 -$

OSD
10, 291–319, 2013

1×10^8 cell L^{-1} ($4.55 \times 10^7 \pm 2.50 \times 10^7$, mean ± SE) in the bathypelagic realm. Total microbial abundance reported in this study are in agreement with previously published data for both subsurface and deeper layers of the water column in different oceanic regions, including the North Atlantic (Varela et al., 2008a; Aristegui et al., 2009) and the eastern Mediterranean Sea (Borin et al., 2009b; Yokokawa et al., 2010).

The PERMANOVA analysis indicated that prokaryote concentrations in the subsurface, meso- and bathypelagic zones are significantly different ($p = 0.0001$). On the contrary, the comparison of the total cell number obtained along the water column between the eastern and western basins of the Mediterranean Sea revealed the absence of significant differences in the subsurface ($p = 0.3215$), meso- ($p = 0.0628$) and bathypelagic ($p = 0.4274$) layers of the two sub-basins. The statistical approach adopted in this study showed that differences in the prokaryotic abundance values are ascribable to a depth-related decline and excluded any correlation between total cell abundance and specific water masses conditions characterizing the different sub-basin of the Mediterranean Sea.

3.3 Surface and deep water layers host significantly different bacterial communities

In the present study, we described qualitatively the bacterial communities inhabiting subsurface and deep water along a transect covering the main basin of the Mediterranean Sea. ARISA fingerprinting has been applied to detect spatial pattern in the structure of bacterial communities, according to the environmental conditions that rule the subsurface and deep realms of the Mediterranean Sea. The use of the molecular methods based on the PCR amplification of regions of the ribosomal operon is particularly suitable to depict the bacterial community composition in marine oligotrophic ecosystems (Brown et al., 2005; Moeseneder et al., 2001) since bacteria growing in nutrient poor water generally have single or few identical operon copies (Brown et al., 2005; Fegatella et al., 1998).

OSD
10, 291–319, 2013

The ARISA profiles of the bacterial communities colonising the subsurface and the deep waters in the eastern and western Mediterranean Sea showed in total 194 polymorphic peaks, ranging between 157–1600 bp, considered as different bacterial taxa (OTUs). None of the detected OTUs was ubiquitously present in the surface and deep layers of the analyzed stations while 35 % of the OTUs were unique. The ARISA profiles of the surface layers comprised 32–68 OTUs (56.1 ± 8.9, mean ± SE) and displayed Shannon diversity index values ranging between 2.91 and 3.73. On the opposite, the ARISA patterns in the deep layers showed a slightly lower numbers of taxa (45 ± 13.7, mean ± SE) and lower phylogenetic diversity (Shannon index values: 1.2–3.67). 139 out of the total 194 retrieved OTUs (71.6 %) were present in the deep layers, where only 3 OTUs (2 %) were shared among all the stations and 31 OTUs (22 %) were singletons. Non-metric Multidimensional Scaling (NMDS) analysis was applied on the ARISA fingerprints, showing a clear separation between the surface and deep layer dwelling microbiome (Fig. 3a). The NMDS analysis, characterized by a low stress value, and PERMANOVA test indicated that the bacterial communities are significantly diverse (p = 0.0001) in seawater samples collected up to 100 m and at depths lower than 1000 m, the latter hosting a more similar bacterial community within the different analyzed stations indicating the deep waters as an environment with stronger selecting forces (Fig. 3a).

A Principal Component Analysis (PCA) has been applied on the available environmental data (Table 1), to illustrate the distribution of the samples according to their physico-chemical and geographical parameters (Fig. 3b). In this case both the surface and abyssal samples were divided according to the longitude and latitude values, whereas the pressure, representing a proxy for depth, mainly determined the segregation of subsurface and deep samples. A distance-based multivariate analysis for a linear model (distLM) was applied aiming to the identification of the environmental variables shaping the bacterial community composition in subsurface and deep waters across a wide transect (about 3500 km) in the Mediterranean Sea. The distLM analysis pointed out that 68.37 % of total variation was related to five significant environmental

OSD
10, 291–319, 2013

variables (Table 2), responsible for the observed spatial distribution of bacterial communities in subsurface and abyssal zones. The distLM analysis indicated that longitude ($p = 0.04$), pressure ($p = 0.0001$), salinity ($p = 0.0001$), temperature ($p = 0.0001$) and oxygen concentration ($p = 0.004$) were significant variables, while the latitude was not significantly related ($p = 0.36$) to the variation in bacterial community composition. The two axes of the distance-based redundancy analysis (db-RDA) displayed 56.39 % of the total variation (Fig. 3c) overlapping the bacterial populations distribution detected in subsurface and deep layer with the environmental variables, represented as vectors. Deep and subsurface samples clustered separately according to pressure, temperature and oxygen values, which represent the primary factors driving bacterial stratification in such different zones of the water column. Microbial zonation according to depth has been previously reported both in the Mediterranean Sea and in the oceans by applying molecular fingerprinting (De Corte et al., 2009; Moeseneder et al., 2001), 16S rRNA pyrotag sequencing (Agogué et al., 2011) and metagenomics (DeLong et al., 2006). In the Mediterranean Sea the water temperature of the deep layer is only slightly lower than measured in the surface waters (see Table 1 in this study). Still, the differences of the microbial community structure in the subsurface and abyssal biomes in the Mediterranean Sea can be ascribed to factors of remarkable influence including the hydrostatic pressure effect (Tamburini et al., 2009), selecting for piezophilic population in the deep water, light penetration and nutrient profiles. Our study confirms, for the first time on a transect covering the whole Mediterranean Sea, microbial stratification data previously reported on single stations and/or shorter transects in different oceanographic regions around the world.

3.4 Surface bacterial community composition is patterned according to longitude

The occurrence of a distribution pattern of planktonic bacterial populations within the surface seawater was gathered from the db-RDA analyses performed on the ARISA profiles of surface and deep waters (Fig. 3c). To investigate in detail the existence

OSD
10, 291–319, 2013

of different bacterial assemblages in the surface water sampled across the Mediterranean Sea transect during the M84-3 cruise, the ARISA profiles of surface samples were analyzed separately adopting the same approach described above for the whole ARISA dataset. ARISA fingerprinting detected 142 OTUs in surface samples, including 15 ubiquitous OTUs (10 %) and 26 OTUs (18 %) present as singleton. NMDS analysis demonstrated the separation of planktonic microbial communities colonising surface waters in two distinct clusters of samples (Fig. 4a), corresponding to the stations located in the eastern and western Mediterranean Sea. The result of NMDS analysis (stress value = 0.07) was strengthened by PERMANOVA test, showing that the bacterial community structure in the eastern and western sub-basins of the Mediterranean Sea was significantly different ($p = 0.0001$). Principal Component Analysis (PCA) of the surface samples according to the environmental variables showed a less clear separation among the surface seawaters (Fig. 4b), nonetheless the distLM analysis indicated that four significant explaining variables accounted up to 76.12 % of the total variation detected in the bacterial community composition (Table 3). Longitude ($p = 0.0001$), latitude ($p = 0.0017$), salinity ($p = 0.0021$) and oxygen concentration ($p = 0.047$) were identified as significant variables while temperature ($p = 0.068$) was not significantly related to the observed biodiversity pattern. The low importance of temperature in determining the distribution of bacterial surface populations could be expected, considering that the recorded temperature values were rather constant (14.83–18.19 °C at approximately 5 m depth) across the surface waters of the eastern and western Mediterranean Sea (Table 1). The two axes of the distance-based redundancy analysis (db-RDA) displayed 58.3 % of the total variation of bacterial distribution (Fig. 4c), indicating that bacterial zonation in surface samples is unambiguously related to longitude, as already inferred by NMDS.

The existence of biogeography patterns in the diversity of planktonic bacterial communities in surface seawater was recently demonstrated in the Mediterranean Sea for specific bacterial taxa. The abundance of aerobic anoxygenic phototrophs resulted inversely linked to the nutrient concentrations in surface waters of the Mediterranean

OSD
10, 291–319, 2013

Sea as shown by Lamy et al. (2011) along a transect covering the whole eastern and part of the western Mediterranean basins. Similarly, two diversity studies on Cyanobacteria showed that *Synechococcus* (Mella-Flores et al., 2011) and *Prochlorohococcus* (Feingersch et al., 2010) clades were differently distributed in the surface layers of the eastern and western Mediterranean Sea, debating the possible influence of water inputs from the Suez channel (Feingersch et al., 2010). About 600 exogenous species belonging to Plantae and Animalia kingdoms have been introduced in the Mediterranean Sea, mainly from the Suez channel (Coll et al., 2010 and references therein). This is a partial estimation since unicellular organisms are not included in the number of allocthonous species constituting the core of a novel biodiversity imported in the Mediterranean basin. Exogenous species are spatially distributed along the Mediterranean Sea coasts, displaying a higher abundance in correspondence of the Levantine basin (Coll et al., 2010). Possibly a similar distribution model is followed by planktonic microorganisms, which would diversely impact on the surface water of the eastern and western Mediterranean Sea thus concurring in the results of the present study. The influence of latitude on the microbiome inhabiting surface waters was already reported by Fuhrman et al. (2008) investigating the microbial community assemblages by ARISA fingerprinting in 57 different locations around the world. Salinity values were higher in the eastern Mediterranean surface water analyzed in this study, compared to those collected in the western basin (Table 1). Salinity, together with temperature and oxygen content is a signature of specific water masses. Its different value in the two sub-basins of the Mediterranean Sea is typically mirroring the influence of distinct water masses in the surface layers of the Mediterranean basin, due for example to the intrusion of cold and less saline seawater from the Gibraltar Strait into the Alboran Sea and the intense evaporation phenomena in the Levantine basin. The detection of significant relationship between salinity and oxygen content values and planktonic bacterial assemblages in surface waters collected from the eastern and western most areas of the Mediterranean Sea (Fig. 1) suggested that specific water masses in the surface seawater layers influence the impact of certain bacterial taxa in the total planktonic

OSD
10, 291–319, 2013

community structure. These data supported the concept that specific water masses host a peculiar microbial community, as demonstrated in the North Atlantic (Varela et al., 2008a, b) and the high Arctic (Galand et al., 2009; Hamilton et al., 2008).

3.5 Bacterial populations are stratified across the water column according to environmental variables

The vertical distribution of planktonic bacterial communities throughout the water column was assessed on eight stations, sited both in the eastern and western Mediterranean basins. Water samples were collected at different depths covering the subsurface (0–100 m), upper (100–500 m) and lower (500–1000 m) meso-, and bathypelagic (1000–3500 m) realms. Similarly to what we observed for prokaryotic abundance in subsurface, diversity assessment by ARISA fingerprinting in the meso- and bathypelagic depths revealed the occurrence of a vertical profile of bacterial communities across the water column (Fig. 5). As illustrated by NMDS analysis (Fig. 5a), the pattern of microbiome composition is less distinct than previously observed taking into account only the edge of the water profile (Fig. 3a), nevertheless surface samples clustered differently from meso- and bathypelagic depths. The microbial community structure of upper meso- and bathypelagic layers were sharply defined while the samples collected at the lower mesopelagic depths presented a higher variability (Fig. 5a). A certain degree of variability of sample distribution was detected within the categories also according to physico-chemical parameters (Fig. 5b). In spite of the reported unevenness, DistLM analysis showed that four significant variables, represented by longitude ($p = 0.0166$), pressure ($p = 0.0014$), salinity ($p = 0.024$) and temperature ($p = 0.0007$) explained only the 32.79 % of the total variation of the bacterial community composition along the investigated vertical profile (Table 4). Distance-based redundancy analysis (db-RDA) displayed 24.20 % of the total variation (Fig. 5c), showing that microbial communities are shaped by longitude, pressure, salinity and temperature.

The investigation of bacterial β-diversity along the depth gradient by ARISA fingerprinting and NMDS analysis (stress value: 0.13) was consistent with the results of the

OSD
10, 291–319, 2013

PERMANOVA statistical test. The bacterial species composition in the subsurface samples was significantly diverse from those collected in the upper (p = 0.0085) and lower (p = 0.033) meso- and bathypelagic layers (p = 0.0005). The upper meso- and bathypelagic strata were also different (p = 0.0017). As inferable by NMDS and DistLM analyses, where the distribution of lower mesopelagic samples was partially overlapped to upper mesopelagic and bathypelagic depths, PERMANOVA test showed that bacterial community structure of the lower mesopelagic samples was not significantly diverse from upper mesopelagic (p = 0.1553) and bathypelagic (p = 0.1296) microbiomes.

Several recent studies provided hints of correlation between prokaryotic diversity and water masses, defined as "bio-oceanographic islands" (Agogué et al., 2011; Galand et al., 2009) which carry their own specific microbiome. Fingerprinting methods applied to seawater collected along a north-south transect in the Aegean Sea demonstrated that both free-living and attached bacterial communities clustered according to the region and depth of sampling, highlighting the existence of horizontal and vertical profiling of bacteria in the Aegean Sea (Moesender et al., 2001). The samples analyzed in the present study were collected on a larger scale, along a wider transect covering both the eastern and western Mediterranean Sea, making more difficult the interpretation of bacteria distribution data according to water masses circulation. The absence of a sharp profiling of bacterial communities according to depth in the intermediate layers of the water column can be possibly influenced by the extremely hydrographically complex nature of the system, where several water masses at different spatial and temporal scale concur to the water composition of meso- and bathypelagic layers (Gačić et al., 2010; Rubino et al., 2012).

The observed spatial pattern of bacterial communities throughout the water column were related to different variables comprising the site of sampling (longitude), the depth of collection (pressure) and the water salinity and temperature values. Our results on bacterial stratification across the water column are in agreement with previous studies realized on different oceanic regions (De Corte et al., 2009; DeLong et al., 2006; Winter et al., 2008). Further studies will be necessary to correlate bacterial zonation

OSD
10, 291–319, 2013

according to water masses composition, also in the light of the high variability of microbiome composition in samples characterized by the same *T*-*S* values in the eastern Mediterranean Sea (Yokokawa et al., 2010). These findings are consistent with the notion that physical conditions of the deep environments are more stable than those in surface ocean, while concentration and composition of organic constituents could display relatively high variability (Nagata et al., 2010 and references therein).

4 Conclusions

Our study provides a comprehensive depiction of planktonic bacterial community structure across different layers of the water column over a longitudinal transect covering the whole Mediterranean Sea. As recently emerged from the study of specific bacterial taxa (Feingersch et al., 2010; Lamy et al., 2011; Mella-Flores et al., 2011), our investigation demonstrated that also the overall planktonic bacterial community inhabiting surface seawater was spatially patterned in the Mediterranean Sea, exhibiting different populations in the eastern and western basins. The bacterial taxa distribution shifted along the sub-surface, upper and lower meso- and bathypelagic realms of the Mediterranean Sea according to physico-chemical parameters including longitude, pressure, temperature and salinity. Bacterial zonation according to depth was also demonstrated. Partial evidences were hence provided, concerning the relation of bacterial community structure and water masses in the different water column layers. Distribution patchiness of the water column intermediate levels and water masses interleaving phenomena (Rubino et al., 2012) makes the correlation between planktonic bacterial communities and water masses a hypothesis to be further confirmed by studies to be realized on large scale covering the whole Mediterranean Sea. Besides, future measurements on quantity and quality of dissolved organic matter might enhance the insight on the explaining variables that determine microbiome composition along the eastern and western Mediterranean Sea. The global warming is estimated to have an impact on oceanic circulation and exotic species entrance in the Mediterranean Sea, factors that

OSD
10, 291–319, 2013

are putatively involved in shifts of the biogeochemical cycles, which are lastly triggered by microbial activity. A deeper knowledge on the mechanisms driving bacterial diversity in distinct water masses might hence be crucial in the climate change perspective.

Supplementary material related to this article is available online at: http://www.ocean-sci-discuss.net/10/291/2013/osd-10-291-2013-supplement.pdf.

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OSD
10, 291–319, 2013

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OSD
10, 291–319, 2013

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10, 291–319, 2013

Table 1. Region name, layer, physical parameters (salinity and temperature) and chemical parameters (phosphate, nitrate and nitrite) of the stations sampled during the M84/3 METEOR cruise. Mean ± SD are given for the subsurface, meso- and bathypelagic waters. Numbers between brackets indicate the number of stations.

Region	Layer (m)	Salinity	Temperature (°C)	Dissolved Oxygen (µmol L^{-1})	PO_4 (µmol L^{-1})	NO_3 (µmol L^{-1})	NO_2 (µmol L^{-1})
Eastern	< 100	38.80 ± 0.29 (26)	16.58 ± 0.99 (26)	220.46 ± 1.25 (26)	0.01 ± 0.002 (15)	0.18 ± 0.04 (25)	0.02 ± 0.01 (20)
	100–1000	38.93 ± 0.03 (28)	14.93 ± 0.13 (28)	189.48 ± 1.97 (25)	0.10 ± 0.007 (25)	3.27 ± 0.18 (25)	0.02 ± 0.005 (22)
	> 1000	38.76 ± 0.01 (23)	13.89 ± 0.04 (23)	175.02 ± 1.24 (23)	0.18 ± 0.008 (21)	4.69 ± 0.08 (21)	0.006 ± 0.001 (15)
Western	< 100	37.15 ± 0.12 (22)	15.77 ± 0.14 (22)	218.77 ± 1.90 (22)	0.08 ± 0.01 (21)	1.79 ± 0.37 (22)	0.04 ± 0.006 (22)
	100–1000	38.10 ± 0.17 (26)	13.42 ± 0.07 (26)	172.25 ± 1.58 (26)	0.40 ± 0.03 (19)	8.02 ± 0.45 (22)	0.01 ± 0.001 (21)
	> 1000	38.36 ± 0.12 (20)	13.05 ± 0.20 (20)	174.94 ± 0.88 (19)	0.41 ± 0.03 (17)	8.57 ± 0.55 (17)	0.008 ± 0.003 (13)

OSD
10, 291–319, 2013

Table 2. Results of distance-based multivariate linear model (DistLM) for bacterial community composition in subsurface and deep water layers showing the % variation explained by individual axes.

Axis	% Explained variation (fitted model)		% Explained variation (total variation)	
	Individual	Cumulative	Individual	Cumulative
dbRDA1	71.44	71.44	48.85	48.85
dbRDA2	11.03	82.47	7.54	56.39
dbRDA3	9.29	91.76	6.35	62.74
dbRDA4	4.65	96.41	3.18	65.92
dbRDA5	2.54	98.95	1.74	67.65
dbRDA6	1.05	100.00	0.72	68.37

OSD
10, 291–319, 2013

Table 3. Results of distance-based multivariate linear model (DistLM) for bacterial community composition in subsurface waters showing the % variation explained by individual axes.

Axis	% Explained variation (fitted model)		% Explained variation (total variation)	
	Individual	Cumulative	Individual	Cumulative
dbRDA1	57.33	57.33	43.65	43.65
dbRDA2	19.25	76.59	14.65	58.30
dbRDA3	13.44	90.03	10.23	68.53
dbRDA4	6.54	96.57	4.98	73.51
dbRDA5	3.43	100.00	2.61	76.12

OSD
10, 291–319, 2013

Table 4. Results of distance-based multivariate linear model (DistLM) for bacterial community composition in subsurface, meso- and bathypelagic seawater layers showing the % variation explained by individual axes.

Axis	% Explained variation (fitted model)		% Explained variation (total variation)	
	Individual	Cumulative	Individual	Cumulative
dbRDA1	55.96	55.96	18.35	18.35
dbRDA2	17.84	73.81	5.85	24.20
dbRDA3	11.58	85.39	3.8	28.00
dbRDA4	7.87	93.27	2.58	30.58
dbRDA5	4.55	97.82	1.49	32.07
dbRDA6	2.18	100.00	0.72	32.79

OSD
10, 291–319, 2013

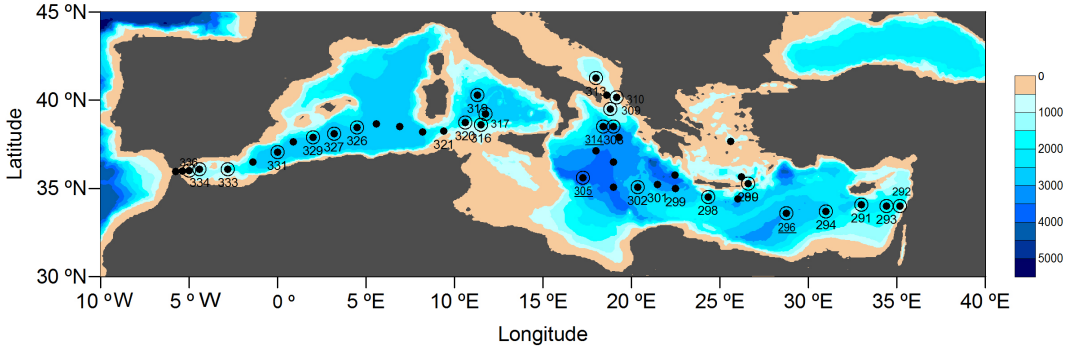

Fig. 1. Location of sampling stations during the M84/3 cruise. Map of the study area of the Mediterranean Sea with the stations occupied during the METEOR cruise indicated by dots. Stations where both bacterial abundance and community composition (ARISA fingerprinting) were determined are indicated by encircle dots and station numbers. Underlined stations numbers indicate those stations were only the bacterial abundance was measured.

OSD
10, 291–319, 2013

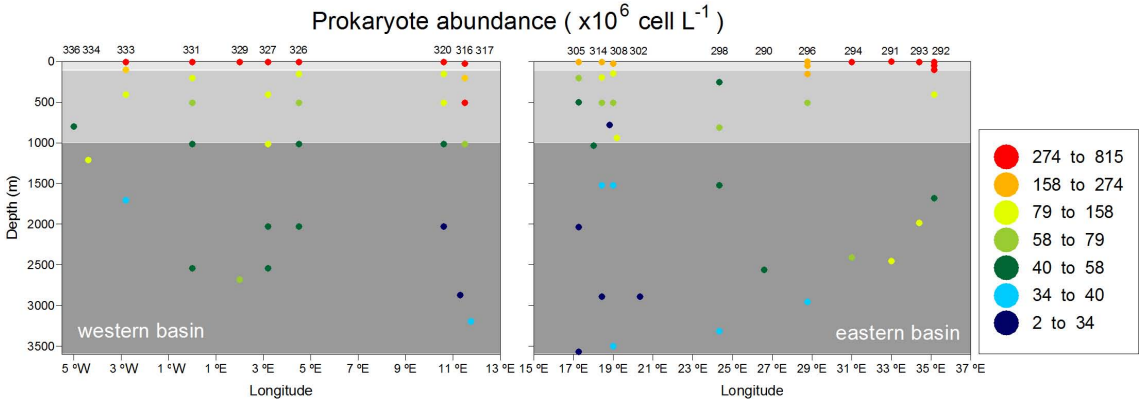

Fig. 2. Prokaryotic abundance across the water column in the eastern and western Mediterranean Sea. Prokaryotic abundance in each of the three water layers (subsurface: ≤ 100 m; meso-: 100–1000 m and bathypelagic: > 1000 m) at the eastern and western basin of the Mediterranean Sea during the METEOR cruise. Station name label are reported only for those stations where prokaryotic abundance was evaluated at two or more depths.

OSD
10, 291–319, 2013

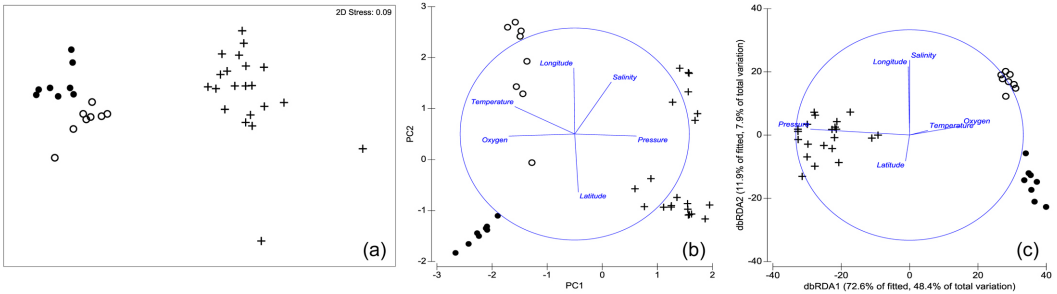

Fig. 3. Diversity of surface and bathypelagic waters according to biotic and environmental data. **(a)** Non-metric multidimensional scaling (NMDS) results based on qualitative ARISA fingerprinting showing a sharp clustering of surface (open and filled circles corresponding respectively to eastern and western surface samples) and bathypelagic (crosses) seawater samples. **(b)** Principal Component Analysis performed on the environmental data reported in Table 1. **(c)** dbRDA ordinations of the presence/absence ARISA dataset overlaid with the partial correlations of the tested environmental variables explaining the clustering of surface (open and filled circles) and bathypelagic (crosses) seawater samples.

OSD
10, 291–319, 2013

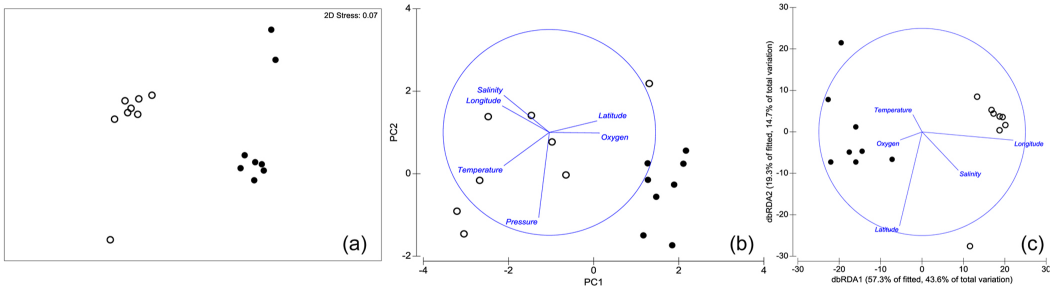

Fig. 4. Diversity of Mediterranean Sea surface waters according to biotic and environmental data. **(a)** Non-metric multidimensional scaling (NMDS) results based on qualitative ARISA fingerprinting illustrating a clear clustering of eastern (open circles) and western (filled circles) Mediterranean Sea surface water. **(b)** Principal Component Analysis performed on the environmental data reported in Table 1. **(c)** dbRDA ordinations of the presence/absence ARISA dataset overlaid with the partial correlations of the tested environmental variables explaining the clustering of eastern (open circles) and western (filled circles) Mediterranean Sea surface water.

OSD
10, 291–319, 2013

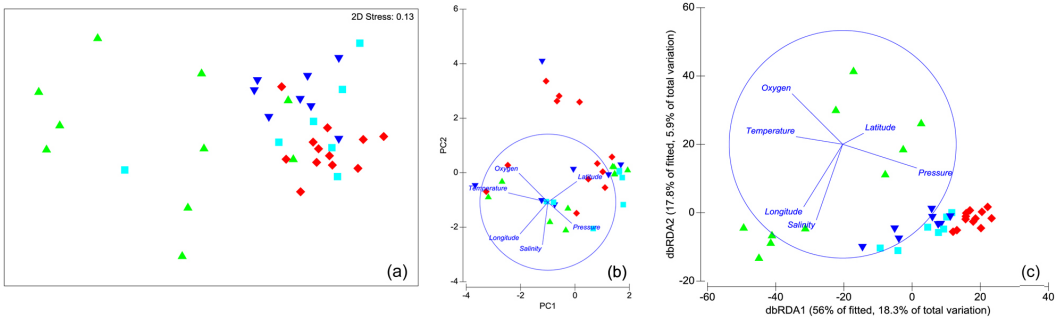

Fig. 5. Sample diversity throughout the water column according to biotic and environmental data. **(a)** Non-metric multidimensional scaling (NMDS) results based on qualitative ARISA fingerprinting of the samples across the water column, showing the distribution of surface (green triangles), upper mesopelagic (blue triangles), lower mesopelagic (light blue squares) and bathypelagic (red diamonds) samples. **(b)** Principal Component Analysis performed on the environmental data reported in Table 1. **(c)** dbRDA ordinations of the presence/absence ARISA dataset overlaid with the partial correlations of the tested environmental variables.

OSD
10, 291–319, 2013