Inter-annual recurrence of archaeal assemblages in the coastal NW Mediterranean Sea (Blanes Bay Microbial Observatory)

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Abstract

We report a long-term (i.e., 4.5 consecutive yr) monitoring of surface marine archaeal assemblages of the coastal Mediterranean Sea using quantitative polymerase chain reaction against specific phylogenetic and functional genes, and, for some specific samples, clone libraries of the 16S ribosomal ribonucleic acid gene. Archaea had a marked seasonal periodicity, with recurrent peaks of abundance in December and January and very low occurrence during summer, parallel to temporal changes in community composition. Group II.b *Euryarchaeota* sequences were mostly present during winter when water was nutrient-enriched, and phytoplankton were abundant. Group II.a sequences were, in turn, more abundant during summer when the water column is stratified, and nutrient concentrations and phytoplankton stocks were lower. Group I *Crenarchaeota* abundance was highest during winter and significantly correlated with that of archaeal ammonia monooxygenase (*amoA*) gene copies and nitrite concentrations, suggesting that Group I *Crenarchaeota* were ammonia oxidizers. The periodicity of archaeal assemblages matched the strong and predictable seasonality of the surface-water conditions in the northwestern Mediterranean Sea, and suggests a low degree of functional redundancy between archaeal groups. The distinct seasonal dynamics for Group II.a and II.b *Euryarchaeota*, and their close association with major ecosystem processes, indicate that they may play an important but as yet largely unknown role in the ocean.

Marine archaea are important in terms of metabolic activity and abundance (Karner et al. 2001; Wuchter et al. 2006). The most common phyla in the ocean are *Crenarchaeota* Group I and *Euryarchaeota* Group II (Massana et al. 2000), while *Euryarchaeota* Group III is rare and restricted to deep waters (Galand et al. 2009*a*). The physiology and ecology of Group II *Euryarchaeota* remain poorly understood. Nevertheless, the genomes of some bathypelagic Group II *Euryarchaeota* enriched in putative anaerobic respiration components, suggest that some may have anaerobic pathways in the deep sea (Martin-Cuadrado et al. 2008). And conversely, in surface waters some phylotypes of Group II *Euryarchaeota* (a group often retrieved in the photic zone [Massana et al. 2000]), possess a gene coding for light-capturing proteorhodopsins (Frigaard et al. 2006), which may indicate that some Group II *Euryarchaeota* could use light as an additional energy source.

In contrast to the poor understanding of *Euryarchaeota*'s role in the ocean, intense research on Group I *Crenarchaeota* has led to key discoveries linking the group to carbon and nitrogen biogeochemical cycling. Several lines of evidence indicate that they are fixing CO_2 and that chemoautotrophy is the dominant archaeal metabolism in deep waters (Ingalls et al. 2006). The cultivation of *Nitrosopumilus maritimus*, an ammonia-oxidizing autotrophic *Crenarchaeota* (Könneke et al. 2005), and the widespread occurrence of the archaeal ammonia monooxygenase gene (*amoA*) in the sea (Francis et al. 2005) have further highlighted their importance in the nitrogen cycle. Even though *Crenarchaeota* may be more important than marine bacteria driving the oxidation of ammonia to nitrite (Wuchter et al. 2006), new data indicate that not all *Crenarchaeota* are ammonia-oxidizers living autotrophically (Agogue et al. 2008; Pouliot et al. 2009). Evidence that *Crenarchaeota* take up organic compounds such as amino acids (Ouverney and Fuhrman 2000) suggests that some *Crenarchaeota* could be heterotrophs or mixotrophs. Several studies have shown that *Crenarchaeota* tend to be more abundant in surface waters during winter under many different oceanic regimes (Santa Barbara Channel in California, waters off Antarctic Peninsula, and the North Sea [Massana et al. 1997; Murray et al. 1998; Herfort et al. 2007]). In warmer oligotrophic marine waters such as those of the Mediterranean Sea, *Crenarchaeota* have been detected in both surface and deep waters (Garcia-Martinez and Rodriguez-Valera 2000). Their seasonal variability remains, however, poorly understood because they were sometimes more frequent in surface winter waters (Winter et al. 2009) but not always (Alonso-Sáez et al. 2007).

Attempts to unveil the role and diversity of marine microbes are challenged by the spatial and temporal intricacy of the marine environment. One solution to deal with such complexity is to investigate many oceanic regimes, representing different environmental conditions,

through both large-scale projects like the Global Ocean Survey, or international cooperative efforts such as the International Census of Marine Microbes. Those extensive global approaches can, however, miss the temporal component of microbial dynamics. A complementary strategy is to monitor microbes and ancillary environmental parameters at only one location, but over a long period of time, through the implementation of microbial observatories. Such marine microbial observatories have demonstrated reoccurring and predictable patterns in bacterial communities (Fuhrman et al. 2006) and have unveiled bacterial seasonal dynamics (Alonso-Sáez et al. 2007).

The long-term (> 2 yr) dynamics of marine archaea remain unexplored despite their important links with the oceanic carbon and nitrogen cycles. Here, we monitored archaeal communities in monthly intervals from January 2003 to May 2007 at the Blanes Bay Microbial Observatory, a site representative of the coastal northwest Mediterranean Sea, and characterized by a marked seasonality with high irradiance, and relatively warm, salty, and nutrient-poor waters (Duarte et al. 1999). We tested the hypothesis that archaeal groups show recurrent and predictable seasonal patterns by quantifying whole Archaea, Group II *Euryarchaeota*, Group I *Crenarchaeota*, and the functional *amoA* genes by quantitative polymerase chain reaction (qPCR). We also described phylotype dynamics through archaeal 16S ribosomal ribonucleic acid (rRNA) gene cloning and sequencing at selected periods.

Methods

Sampling and environmental parameters—Surface seawater samples (0.5 m) were collected monthly at the Blanes Bay Microbial Observatory (41°40′N, 2°48′E; 20 m depth) in the northwestern Mediterranean from January 2003 to May 2007 (53 samples). Water was filtered through a 200-μm-mesh net and kept in 25-liter polycarbonate carboys in the dark until being processed in the laboratory (within 1.5 h).

Methods for determination of surface-water temperature, chlorophyll *a* (Chl *a*) concentration, dissolved inorganic nutrient concentrations, and heterotrophic prokaryotes numbers were described earlier (Alonso-Sáez et al. 2008).

Deoxyribonucleic acid (DNA) extraction, PCR amplification, and cloning—Microbial biomass was collected by sequentially filtering 8 liters of seawater through a 3-μm-pore-size polycarbonate filter (Poretics) and a 0.2-μm Sterivex filter (Durapore, Millipore). Nucleic acids were extracted as described earlier (Massana et al. 1997). The concentration of DNA was measured by a NanoDrop. Archaeal 16S rRNA genes were amplified with primers 109F and 915R as previously described (Galand et al. 2009b). PCR products were purified with Qiaquick PCR Purification Kit (Qiagen) and cloned with TA cloning kit (Invitrogen). In total we constructed eight archaeal clone libraries covering year 2003. We chose to clone samples from year 2003 to allow comparison with bacterial

sequences published earlier (Alonso-Sáez et al. 2007). For each library, clones containing inserts were randomly chosen and directly sequenced using the vectors' T7p primer. Putative chimeras were checked by using basic local alignment search tool (BLAST) with sequence segments separately. The 16S rRNA sequence data have been archived in the GenBank database under accession Nos. GQ387680-GQ387925.

Quantitative PCR (qPCR)—The number of whole archaeal and Group I crenarchaeal-specific 16S rRNA genes, as well as archaeal *amoA* gene copy numbers were quantified for all samples in triplicate following conditions, reagents, and efficiencies described earlier (Galand et al. 2009b). For Group II euryarchaeal 16S rRNA gene copies we used primers GII-554-f (GTC GMT TTT ATT GGG CCT AA; Massana et al. 1997) and Eury806-r (CAC AGC GTT TAC ACC TAG; Teira et al. 2004) with similar qPCR conditions but with 59°C annealing temperature. All reactions were performed with 2 ng of template on a DNA Engine Thermal Cycler with Chromo 4 Real-Time Detector (Bio-Rad) using the fluorescent dye SYBRGreen I. Standards obtained from purified PCR products of clones containing the targeted gene were subjected to qPCR along with the samples (Galand et al. 2009b). Primer specificity was confirmed by melting curve analyses.

The specificity of the qPCR primers was also estimated with the OligoCheck function within the Primerose software package (Ashelford et al. 2002) against a database containing the Blanes Bay sequences obtained by cloning. The qPCR primers generally matched well the Blanes Bay sequences but there were a few exceptions. The forward qPCR primer for Group I *Crenarchaeota* had two mismatches with all sequences belonging to cluster B (*see* Web Appendix, www.aslo.org/lo/toc/vol_55/issue_5/2117a. pdf). The reverse primer for Group II *Euryarchaeota* had one mismatch to all sequences from the VIM2-36 clade of Group II.a Cluster L (*see* Web Appendix). Because we were interested in monitoring dynamics rather than quantifying absolute copy numbers, and because qPCR primers may miss some archaeal targets, we decided to present the quantification results as relative to a yearly averaged value, rather than absolute copy numbers, as in Hansman et al. (2009). qPCR results were normalized for each year separately to avoid possible quantification bias associated with different length of DNA storage. DNA levels decline substantially over time (Sozzi et al. 2005) and the samples we stored for > 5 yr may yield lower copy numbers than the ones stored for 1 yr only. Results are, thus, expressed as a relative abundance of gene copies per ng of total DNA normalized to yearly averaged gene abundances (normalized average = 1).

Phylogenetic analysis, diversity calculations, and statistic analysis—Sequences were compared with those in the GenBank database using the BLAST server at the National Center for Biotechnology Information. The ~ 800–base-pair sequences were aligned using the multiple-sequence comparison by log-expectation (MUSCLE) pack-
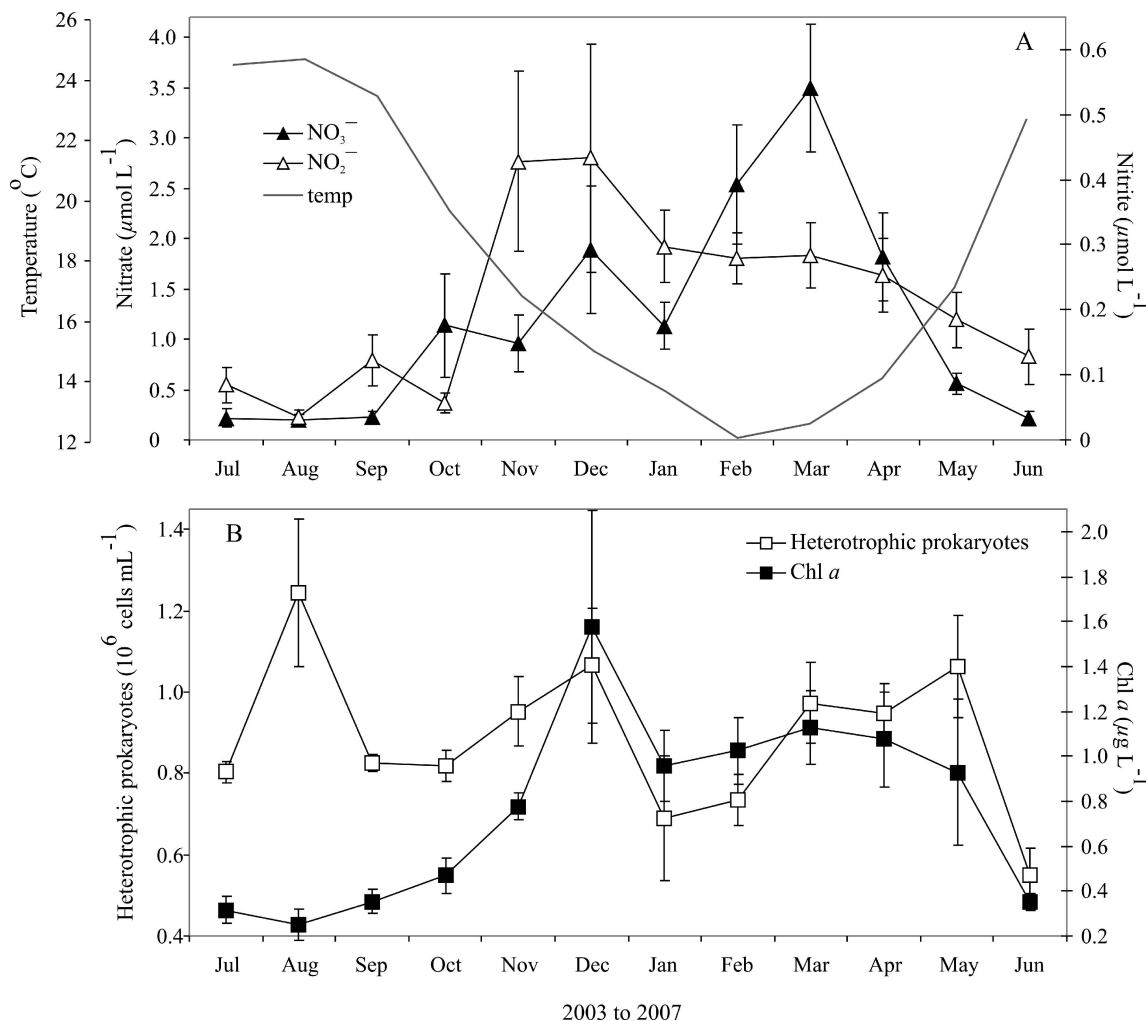
Fig. 1. Monthly average measurements ($\pm$ SE) of (A) Nitrate (NO_3^-), nitrite (NO_2^-), and temperature, and (B) bacteria and Chl *a* concentrations through the period January 2003–May 2007 at the Blanes Bay Microbial Observatory.

age (Edgar 2004) and manually checked. Phylogenetic analyses were completed with the program PHYLIP version 3.68 (Felsenstein 2008). DNADIST was used to calculate genetic distances with Kimura-2 model with 1000 data sets obtained by bootstrapping, and the distance tree was estimated with FITCH.

Sequences were grouped in operational taxonomic units (OTU) with the program DOTUR (Schloss and Handelsman 2005) through a Jukes–Cantor-corrected distance matrix obtained using the DNADIST program from PHYLIP, and rarefaction curves were calculated at a 97% identity cut off.

Correlations between the relative abundances of Archaea, Group II *Euryarchaeaota*, Group I *Crenarchaeota*, *amoA*, and environmental factors (temperature, salinity, Chl *a*, nitrate, nitrite, ammonium, phosphate, and silicate) were examined with the nonparametric Spearman's coefficients (*r*) because some variables did not comply with normality. Correlations were tested for significance at $\alpha = 0.05$ using a *t*-test as implemented in the program PAST, version 1.91 (Hammer et al. 2001).

Results

Physico-chemical and biological parameters—Average monthly values measured throughout the 4.5-yr survey showed that surface-water temperatures had clear seasonality, with values averaging 22–24°C in summer (Jun to Sep) and 12–14°C in winter (Dec to Mar; Fig. 1A). Nitrate and nitrite also showed seasonality, with higher concentrations in winter than in summer. The highest nitrite concentrations were found between November and January, whereas nitrate peaked along February–March (Fig. 1A). Ammonium concentrations did not show any seasonal trend (data not shown).

Chl *a* concentrations were on average five times higher in winter and early spring than in summer (Fig. 1B), and were strongly correlated to nitrate (Spearman correlation, $n = 53$, $r = 0.65$, $p < 0.001$) and nitrite ($n = 53$, $r = 0.49$, $p = 0.01$) concentrations. Heterotrophic prokaryotes concentrations ranged between 0.5×10^6 cells mL^{-1} and 1.5×10^6 cells mL^{-1} and followed the Chl *a* dynamic during winter and spring.
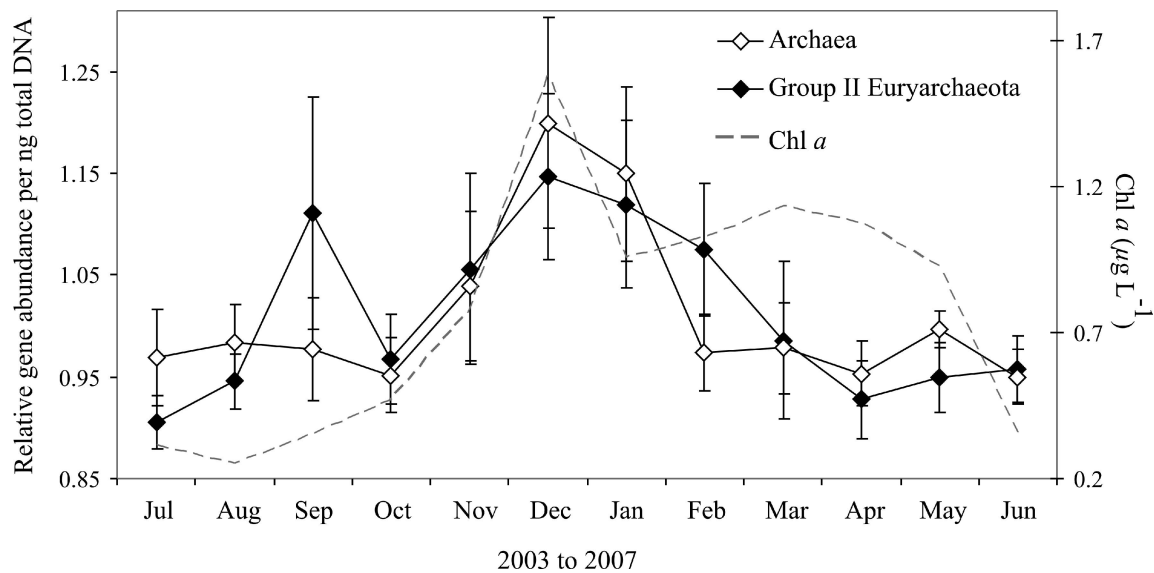
Fig. 2. Average seasonal abundance (± SE) of Archaeal and Euryarchaeal 16S rRNA gene. Gene copy numbers were quantified by qPCR through the period January 2003–May 2007 at the Blanes Bay Microbial Observatory. Data are normalized to yearly average values (= 1).

Long-term archaeal and amoA *gene dynamics*—The inter-annual qPCR quantification of the general archaeal 16S rRNA gene showed that archaeal relative abundance was higher in winter than in summer, with peaks detected in early winter (Dec to Jan; Fig. 2). Concentrations of Archaea were significantly correlated to silicate only (negative correlation, $n = 53$, $r = -0.30$, $p = 0.03$) among all the environmental parameters we tested (i.e., surface temperature, salinity, Chl *a*, nitrate, nitrite, ammonium, phosphate, silicate). The archaeal peak matched, however, the early winter Chl *a* abundance, but did not follow the later Chl *a* dynamics corresponding to the main late-winter phytoplankton bloom (Fig. 2).

Specific qPCR quantifications were done for Group II *Euryarchaeota* (Fig. 2) and Group I *Crenarchaeota* (Fig. 3). *Euryarchaeota* abundance was correlated to the abundance of total Archaea ($n = 53$, $r = 0.34$, $p = 0.01$) and followed a similar seasonal dynamic, with the highest abundance during winter (Nov to Mar) and a peak in December. The high abundance in September (Fig. 2) was due to an episodic peak of Group II *Euryarchaeota* in 2005 and was not considered as representative of the long-term euryarchaeal dynamics. Group I *Crenarchaeota* were more abundant during late autumn–early winter (Nov to Jan; Fig. 3) and were strongly correlated to nitrite concentrations ($n = 53$, $r = 0.37$, $p < 0.01$) but not to nitrate or ammonium.
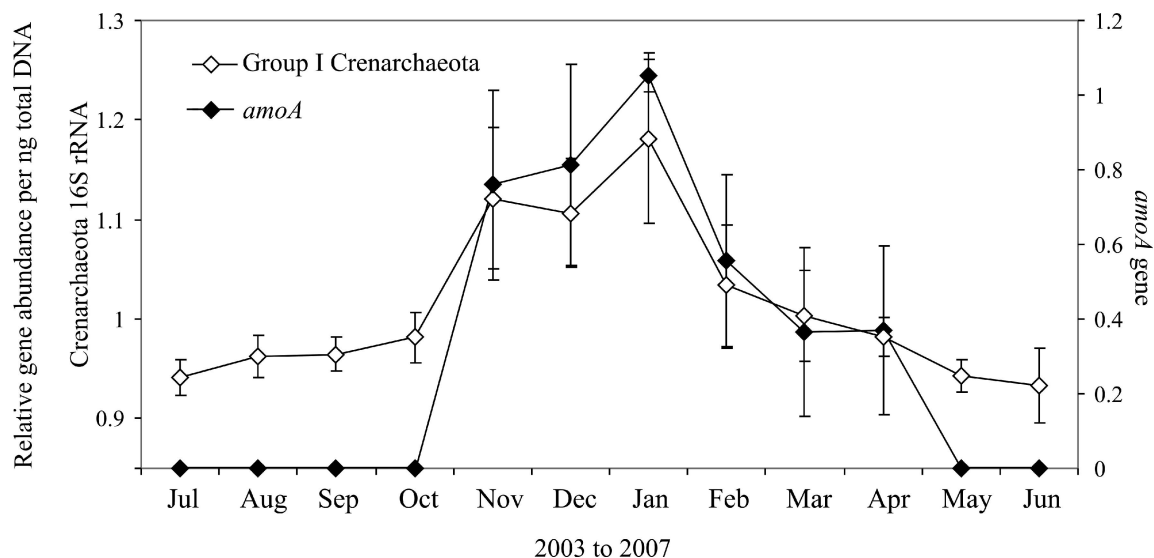

Fig. 3. Average seasonal abundance (± SE) of Group I *Crenarchaeota* 16S rRNA and archaeal *amoA* genes. Gene copy numbers were quantified by qPCR through the period January 2003–May 2007 at the Blanes Bay Microbial Observatory. Data are normalized to yearly average values (= 1).
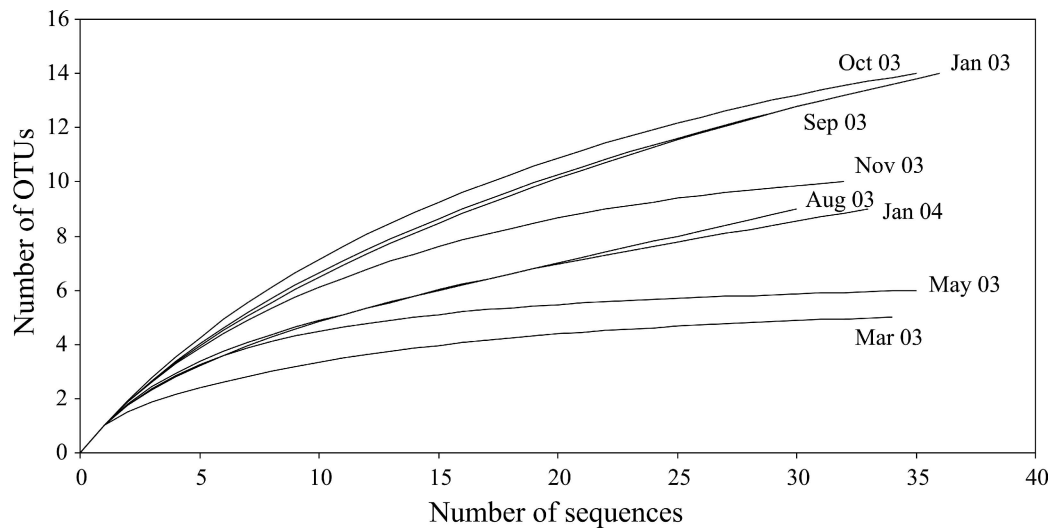
Fig. 4. Rarefaction curves for eight archaeal clone libraries from the Blanes Bay Microbial Observatory. OTUs are defined at a 3% difference cut off between 16S rRNA gene fragments.

Additionally, we surveyed the relative abundances of the Archaea-specific ammonia monooxygenase (*amoA*) gene by qPCR. The *amoA* gene was consistently detected between November and April throughout the complete 4.5-yr study. During the remaining months, it was always below detection limits. The seasonal dynamics of the *amoA* genes closely followed the abundance of Group I *Crenarchaeota* (Fig. 3; $n = 53$, $r = 0.47$, $p < 0.001$) and its relative abundance was positively correlated to nitrate concentrations ($n = 53$, $r = 0.38$, $p < 0.01$).

Seasonal dynamics of the archaeal phylotypes—We constructed eight clone libraries and obtained 246 16S

rRNA gene sequences covering an entire year (Jan 03 to Jan 04) to investigate the seasonal archaeal dynamics in detail. Rarefaction curves showed that some communities were still under-sampled but the abundant groups were probably well-covered at a 97% sequence identity cut off (Fig. 4). Most of the archaeal sequences belonged to Group II.a *Euryarchaeota* (43% of the sequences), II.b *Euryarchaeota* (31%), and Group I *Crenarchaeota* (18%). A few (< 10%) were from the *Euryarchaeota* Group III, the freshwater LDS and RC-V clusters (Grosskopf et al. 1998; Glissman et al. 2004), *Halobacteriaceae*, and *Methanospirillaceae* (Fig. 5). The phylogenetic trees are given in the Web Appendix.
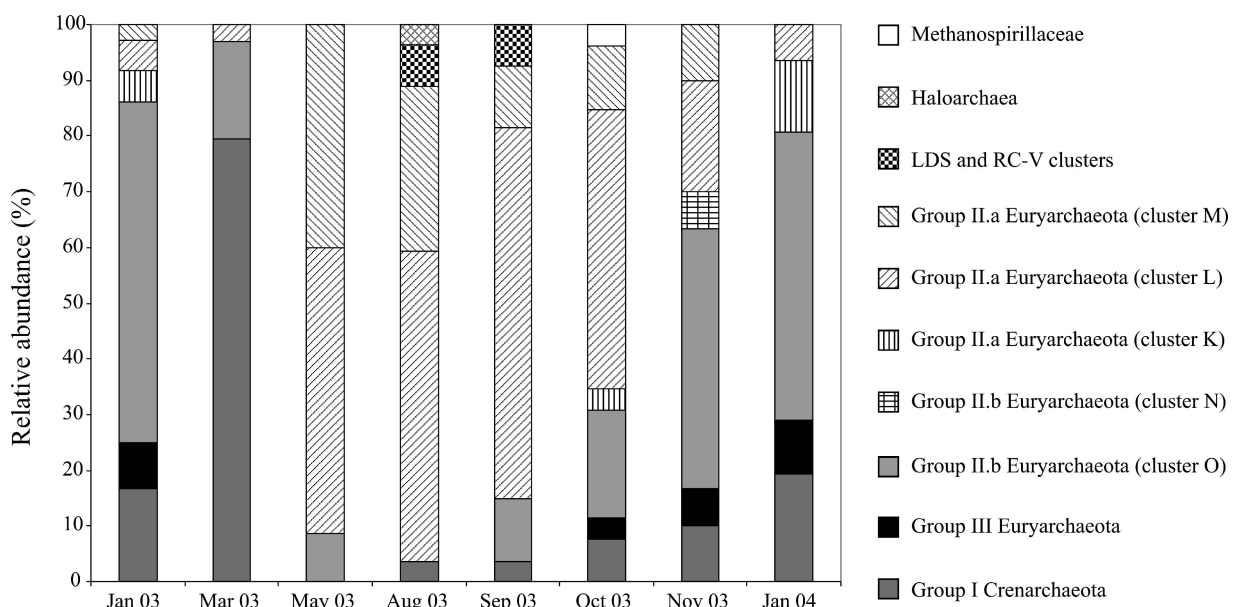

Fig. 5. Seasonal composition of eight archaeal clone libraries, covering an entire year at the Blanes Bay Microbial Observatory in the northwest coastal Mediterranean Sea. Naming of the archaeal clusters K to O follows our own definition given in the Web Appendix.

Group II euryarchaeal sequences were the most abundant all year round, except in March, and showed consistent seasonal changes in their relative abundances with marked differences between summer and winter (Fig. 5). Group II.b *Euryarchaeota* dominated the winter libraries, with the highest relative abundance in January (up to 60%; Fig. 5), and separated into two clusters (N and O) at a 90% similarity (*see* Web Appendix). Cluster O was predominant during late autumn and winter and contained mostly sequences belonging to the WHARN cluster (*see* Web Appendix). The WHARN cluster corresponds to phylotypes II-CC widely distributed in surface waters of various oceanic provinces (Massana et al. 2000). Cluster N only appeared anecdotally.

Group II.a was the most abundant archaeal type in the clone libraries from May to October and separated into three main clusters (K, L, M, with 89–93% similarity; *see* Web Appendix). Most sequences belonged to cluster L, followed by cluster M mostly detected in May–August (Fig. 5). The reverse *Euryarchaeota* qPCR primer had one mismatch to members of the cluster L (*see* Methods) and qPCR results may have underestimated the presence of cluster L and, thus, of *Euryarchaeota* generally during summer.

Crenarchaeotal sequences were more abundant during winter and all of them belonged to Group I, separated into two clusters at > 93% similarity (clusters A and B; *see* Web Appendix). Most of the sequences were closely related to *Nitrosopumilus maritimus* and there were no seasonal differences in the distributions of the two Group I clusters.

Less abundant groups included Group III *Euryarchaeota* (*see* Web Appendix), present during winter and not detected between March and September (Fig. 5), one sequence from *Methanospirillaceae*, one *Halobacteriaceae*, and four sequences from the euryarchaeal LDS and RC-V clusters (*see* Web Appendix), all found in summer and autumn (Fig. 5).

Discussion

Our long-term study of archaeal dynamics in the coastal northwest Mediterranean Sea revealed that archaea had a marked seasonal periodicity with recurrent peaks of relative abundance in December and January and lower occurrence during summer that matched changes in archaeal community composition. In winter, communities were dominated by Group II.b *Euryarchaeota*, whereas in summer Group II.a *Euryarchaeota* were more abundant. To the best of our knowledge, it is the first time that such a seasonal variation within Group II *Euryarchaeota* was observed. It suggests a probable niche partitioning between the two archaeal types, one developing in winter while the other is present during summer and in low numbers. There are currently no cultivated representatives of marine planktonic *Euryarchaeota* and their metabolic capabilities are not known. The discovery of proteorhodopsin in some genome fragments related to Group II.b suggests, however, that some may use light to gain a competitive advantage (Frigaard et al. 2006). It may explain why Group II.b *Euryarchaeota*, and particularly the ubiquitous WHARN

cluster, is more abundant in surface waters and declines below the photic zone in many oceanic areas (Massana et al. 1997). Group II.b in Blanes Bay followed patterns of abundance similar to the winter phytoplankton such as the cyanobacteria *Prochlorochoccus* (Alonso-Sáez et al. 2007), the flagellate *Micromonas* (Foulon et al. 2008), and diatoms (Agustí and Duarte 2000). We, therefore, hypothesize that members of Group II.b *Euryarchaeota*, and more specifically members of the WHARN cluster, may be able to use light as an additional energy source, and start growing as soon as nutrient concentrations increase after water column mixing, in correspondence to the first peak of chlorophyll. *Euryarchaeota* tend, however, to disappear when the main late-winter phytoplankton bloom occurs and may at that time be out-competed by true phototrophs. Conversely, the presence in summer of Group II.a *Euryarchaeota* matched high-summer phytoplankton lysis rates (Agustí and Duarte 2000). Together with the occurrence of Group II.a in particle-rich coastal waters (Galand et al. 2008), the available data make us speculate that members of the Group II.a, and especially those grouping under cluster L, could have a heterotrophic metabolism possibly closely associated with the summer phytoplankton lysis. Future population-specific studies will help us understand the physiology and ecology of Group II.a and II.b. The different seasonal cycles shown here and the association with major ecosystem processes, such as phytoplankton blooms and lysis, already suggest that they may play separate important roles in the ocean.

The cyclic seasonal dynamics of archaea were different from that of total heterotrophic prokaryotes. In Blanes Bay, heterotrophic prokaryotes were mostly bacteria (Alonso-Sáez et al. 2007), which suggests different responses of bacteria and archaea to environmental controls. During late winter, bacterial abundance increased according to Chl *a* concentrations, following phytoplankton growth. The late-winter phytoplankton bloom is triggered by the diffusion of nutrient-rich bottom water up to the surface when cold winter temperatures and strong northerly winds break the summer stratification (Duarte et al. 1999). In Blanes Bay, surface nutrient concentrations could also increase following strong rainy events associated with important run off (Guadayol et al. 2009). This late-winter peak of bacteria was characterized by the presence of the alphaproteobacterium *Roseobacter* (Alonso-Sáez et al. 2007). In turn, the second peak of bacterial abundance in August corresponded to a community dominated by SAR11 (Alonso-Sáez et al. 2007) that appeared at a time when the water column was stratified and nutrients depleted in the upper mixed layer (Duarte et al. 1999). This strong seasonality of both abiotic and biotic factors in the NW Mediterranean probably also influences archaeal dynamics. In particular, the time of summer stratification appears unfavorable for archaea in surface waters, as recently observed in the eastern Mediterranean Sea (Winter et al. 2009).

Euryarchaeota were always the dominant archaeal group in the clone libraries, even during the winter, with the exception of the month of March. This finding confirms preliminary results obtained by fluorescence in situ

hybridization showing that *Crenarchaeota* were always rare in Blanes Bay, whereas *Euryarchaeota* represented up to 6% of prokaryotes during winter (Alonso-Sáez et al. 2007). This winter predominance of *Euryarchaeota* contrasts with results showing *Crenarchaeota* dominating surface winter communities in the North Sea (Wuchter et al. 2006; Herfort et al. 2007) and the Southern Ocean (Murray et al. 1998).

Group I *Crenarchaeota* in Blanes Bay had, nevertheless, a seasonal periodicity illustrated by higher relative abundance during winter, with a peak in January. Group I is thought to play an important role in the ocean nitrogen cycle by oxidizing ammonia to nitrite (Francis et al. 2005; Wuchter et al. 2006). In Blanes Bay, winter Group I sequences were closely related to the only cultivated planktonic marine archaeum (i.e., *Nitrosopumilus maritimus*; Könneke et al. 2005). *N. maritimus* is an autotrophic ammonia-oxidizer that produces nitrite, suggesting that the Group I *Crenarchaeota* detected in the Blanes Bay also have the potential to oxidize ammonia. The strong relation between the dynamics of the *amoA* genes (involved in ammonia-oxidation) and Group I, as well as the correlation between the abundance of Group I and nitrite in the water, are additional clues indicating the presence of crenarchaeal nitrifiers in the coastal northwest Mediterranean. Even though *Crenarchaeota* were not abundant, their activity may be seasonally important. Microbes occurring in low numbers can be functionally important, as shown earlier for nitrogen-fixing bacteria (Montoya et al. 2004) or hydrocarbon-degrading organisms (Teira et al. 2007). The strong peak of nitrite appearing during early winter could, thus, indicate that the *Crenarchaeota* present at that time of the year are actively oxidizing ammonia to nitrite. However, because phytoplankton can also release nitrite (Lomas and Lipschultz 2006), the exact origin of the winter nitrite in Blanes Bay can not be conclusively determined. *Crenarchaeota* peaked in January when levels of Chl *a* decreased after the early winter December peak and before the late-winter March peak. Earlier studies have reported a negative correlation between Chl *a* and *Crenarchaeota* in marine and freshwater sites (Murray et al. 1998; Herfort et al. 2007; Auguet and Casamayor 2008), suggesting that organic material excreted by phototrophic primary producers may limit the abundance of *Crenarchaeota* in the environment (Könneke et al. 2005) or that competition for ammonium may be unfavorable to *Crenarchaeota*. The low abundance of *Crenarchaeota* in surface winter waters of the northwest Mediterranean could, thus, be explained by the recurrent late-winter phytoplankton bloom characterizing such regions (Duarte et al. 1999).

Group I *Crenarchaeota* showed recurrent seasonal patterns over the course of the study with higher relative abundance in winter as unveiled by the qPCR approach. Clone libraries also showed more Group I *Crenarchaeota* during winter and this good agreement between methods may support the fact that the seasonal patterns observed for the different groups of *Euryarchaeota* with clone libraries during 2003 are indeed reoccurring and predictable year after year. Such recurrent patterns have been observed earlier for bacteria and may reflect a low degree of functional redundancy in the community, where only a very limited repertoire of species would fill a particular niche (Fuhrman et al. 2006). Similarly, the predictable patterns we observed for *Crenarchaeota* and *Euryarchaeota* suggest that the physiology and ecology within each group were controlled by different abiotic or biotic constraints (Auguet et al. 2010).

In summary we showed that archaea had a marked seasonal dynamics in the coastal Mediterranean Sea and that the different components of the community were recurrent in a predictable way. Group II *Euryarchaeota* was the dominant phyla in the clone libraries but with distinct intra-group seasonal patterns. Group II.b was more abundant during winter, together with putative ammonia-oxidizing *Crenarchaeota*, while Group II.a was predominant in summer. Thus, the cyclic dynamics of archaea in surface waters of Blanes Bay appear to be closely associated with the strong and predictable seasonality of the Mediterranean Sea.

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Inter-annual recurrence of archaeal assemblages in the coastal NW Mediterranean Sea (Blanes Bay Microbial Observatory)

Pierre E. Galand, Carmen Gutiérrez-Provecho, Ramon Massana, Josep M. Gasol, and Emilio O. Casamayor

Web Appendix. Distance tree representing the position of archaeal 16S rRNA sequences from the Blanes Bay Microbial Observatory. (A) Group II.b *Euryarchaeota*, (B) Group II.a *Euryarchaeota*, (C) Group I *Crenarchaeota*, and (D) Group III *Euryarchaeota*. The four-digit number in the sequence name corresponds to the sampling date (month and yr). Reference sequences retrieved from GenBank are in boldface type. Bootstrap values > 50 are shown expressed as a percentage of 1000 replicates. Analyses are inferred from 800 base-pair-long 16S rRNA sequences using FITCH distance matrix analysis (from the program PHYLIP). Scale bar represents 10% sequence divergence.

59 B1003 G9B
B0103 B4C
sponge clone A13B3 (DQ299287)
B0104 G1C
B0104 H5C
70 B0104 F2C
B0103 A6A
B0104 H10C

K

100 B0303 B8B
coastal Beaufort Sea clone Ca303s.44 (EF014631)
99 B0503 C10A
B0503 C6C
B0503 C10C
B0503 D8A
B0503 C6A
B0503 D7A
B0503 D10A
94 B0503 C3C
99 B0103 B7A
B1103 G9A
B1103 E5C
B0503 D5A
B0903 D5B
B0903 E7B
B0903 E10B
B1103 H8A
72 B0503 C5A
B0503 C9A
B0803 E9A
B1003 G11B
54 B1103 H12A
B1003 H6B
B1003 H11B
B1103 E10C
B1003 A6C
B0503 D1A
antarctic clone OARB (U11042)
coral clone VIM2-36 (AY380646)
100 B0803 F3A
B0903 F11B
B0903 E3B
B0903 F1B
B0803 F9A
B0803 F5A
B0803 F10A
B0903 E4B
B1003 A10C
B0803 F8A
100 **North Pacific fosmid HF10_36B02 (DQ156391)**
B1103 E4C
B0503 D3A
B0903 D11B
B1003 G4B
coral clone VIDSA-25 (AY380705)
B0803 E6A
75 B0803 D11C
B0803 E8A
B1003 H5B
B0903 E9B
B0803 D4C
B0803 D12C
B0903 E11B
B0104 G10C
B1003 H1B
B0903 E6B
B0903 F10B
B1003 A2C
B1003 G5B
B0903 F5B
B0803 E1A
B0803 D1C
B0903 D7B
B0803 E7A
B1003 A8C
B0803 E11A
B0903 E5B
B0903 F7B
Santa Barbara channel clone SBAR16 (M88077)
94 B0503 C4A
B0503 C7A
B0503 C8A
B1003 A9C
B0104 G9C
B0503 C4C
B0103 A5A
B0903 D1B
B0503 D4A
B1003 A11C
B0903 D4B

L

69

66

69

99

48

55

53

B0103 A9A
B1003 A12C
B0903 D3B
100 **North Pacific fosmid HF10_90C09 (DQ156400)**
100 B0803 F1A
B0503 C2C
hydrothermal clone KAB187-13 (AB366594)
B0803 D2C
B0503 C7C
B0503 D12A
B0803 E2A
B0503 C1A
B1103 H9A
B0803 E5A
B1103 G2A
B0503 C1C
B0503 D2A
B0503 D6A
B0503 C12C
B0503 D9A
B0803 F4A
B1103 G10A
B0803 F6A
B0803 E4A
B0903 F4B
B1003 A3C
B0503 C11C
B0803 E10A
B0503 C8C
B0503 C12A
B0503 C11A
B1003 G10B
B0503 C2A
B0903 E8B
100

M

Group II.a Euryarchaeota

B

Continued.

B0303 A3B
B1103 G4A
B0104 F8C
B0303 A9B
B0303 C3B
B0103 B8A
B0103 B8C
B0303 A8B
B0104 F11C
B0104 G12C
B0303 C12B
B0303 C10B
B0303 B7B
B0303 B10B
B0303 C1B
B0303 B11B
B1003 H9B
B0303 B4B
B1003 G3B
B0303 B5B
B0303 C11B
B0303 C6B
B0104 G11C
B0303 A12B
B0303 C8B
B0303 C7B
B0103 A4A
B0303 C2B
B0303 B9B
B0104 F9C
B1103 E2C
B0303 A11B
B0303 B2B
B0303 B1B
B0303 B6B
Namibian upwelling system clone N67a_44 (EF645853)
B0803 D8C
B0903 E3B
Candidatus Nitrosopumilus maritimus (DQ085097)
B0303 C4B
tropical Atlantic Ocean clone AFRICA_1/18-1 (AY225425)
B0303 A1B
B0103 A12A
B0104 H7C
B1103 H1A
B0303 C5B
B0303 B3B
B0103 A8A
B0103 B1A

100

57

100

98
61

0.01

A

B

Group I Crenarchaeota

C

deep-sea water clone CTD005-2A (AY856360)
B0103 A7A
B0103 B12A
B1103 E1C
B0104 G7C
B1003 H2B
B1103 H10A
B0104 H11C
B0104 F12C
B0103 B6A
Surface North Pacific fosmid HF10_21C05 (DQ156479)

99
95
100
94
62
100

0.01

Group III Euryarchaeota

D

Continued.