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Abundance of virus-like particles (VLPs) and microbial plankton community composition in a Mediterranean Sea coastal area

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ABSTRACT: In the northern coastal area of the Alboran Sea (the most western basin of the Mediterranean Sea), the distribution of plankton at the surface follows complex patterns corresponding to the variability of abiotic (environmental) and biotic factors, including virus–host interactions. To assess these interactions, we measured the abundances of virus-like particles (VLPs) and different microbial virus hosts of the plankton community, including heterotrophic bacteria (HB), cyanobacteria and autotrophic eukaryote pico-, nano- and microplankton (APP, ANP and AMP, respectively). Samplings were carried out in the euphotic zone during 2 contrasting seasonal periods (autumn and spring), in 3 coastal areas of the Alboran basin. Virioplankton abundance varied, ranging from 8.5×10^5 to 2.2×10^7 VLPs ml^{-1}, covering almost the whole range of variability described for the Mediterranean Sea. The mean VLP to microbial ratio (VMR) was 13.6 and the median was 10.5, although the VMR varied substantially from 1.3 to 44. In autumn, the abundance of VLPs did not correlate with HB, although it correlated positively with *Prochlorococcus* and *Synechococcus* and negatively with APP and AMP. In contrast, in spring, VLPs correlated positively with HB and APP and negatively with *Synechococcus*. These abundance data suggest that not only bacteria but also cyanobacteria could play a relevant role as virus hosts in the Alboran Sea, at least in autumn. The changes in abiotic factors that favor the growth of cyanobacteria vs. HB or vice versa and their possible role in regulating cyanophage to bacteriophage dynamics are further discussed.

KEY WORDS: Virus-like particles · Bacteria · Phytoplankton · Pelagic ecosystem · Mediterranean Sea · Phosphate

INTRODUCTION

Viruses are the most abundant predators of the marine pelagic ecosystem, forming the second largest biomass in the oceans after prokaryotes (Suttle 2005). There is growing evidence that viruses play a role in the shaping and functioning of marine microbial communities. For instance, Suttle (2007) reported that viral lysis could eliminate between 20 and 40% of the prokaryote stock per day in surface seawater, equaling grazing as a source of microbial mortality. Virus infections have been proposed as one of the mechanisms causing the decline in phytoplankton blooms (Brussaard 2004a), as well as a driving force in the population dynamics of phytoplankton (Larsen et al. 2001). Furthermore, viral lysis contributes to the carbon, nitrogen and phosphorus biogeochemical cycles by releasing dissolved and particulate organic matter from host organisms that is usable by the microbial loop (Suttle 2007, Haaber & Middelboe 2009). In spite of the ecological importance of viruses, the factors that determine their spatio-temporal vari-

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ability patterns in the ocean remain poorly understood (Wigington et al. 2016). The abundance of virus-like particles (VLPs) varies by 4 orders of magnitude, with a high abundance (typically about 10^7 VLPs ml^{-1}) and variability in the euphotic layer of productive and nutrient-rich waters (Wommack & Colwell 2000), and a reduced and relatively stable abundance below the surface layer (below ca. 200 m depth). Many studies have assumed that viruses display abundances about 1 order of magnitude higher than microbial cells, and consequently that both viruses and microbial cells follow similar distribution patterns. However, Wigington et al. (2016, 2017) recently compiled the results of multiple studies which allowed them to conclude that the virus to microbial ratio (VMR) in the surface layer of the ocean varies between 2.6 and 160, and that contrary to what has been previously postulated, the VMR tends to decrease with increasing microbial abundance. This high variability in VMR across different regions would be a consequence of the variability not only of the abiotic factors (temperature, salinity) but also of the endogenous features of the microbial community (i.e. related to the traits of both viruses and microbial hosts) which affect viral abundance differently depending on the aquatic environment (Clasen et al. 2008, Finke et al. 2017). In marine ecosystems, the influence of environmental variables on virus dynamics and virus–host interactions has been reviewed by Mojica & Brussaard (2014). Biotic factors modulate the process of infection beyond the encounter rates, which largely depend on the abundance of both populations. Indeed, different studies show temporal uncoupling between viruses and prokaryote populations (Ory et al. 2011, Bouvy et al. 2012, Karuza et al. 2012). However, high microbial densities drive a shift from lytic to temperate viral communities, supporting a model in which the viral abundance decreases with increased microbial abundance (Knowles et al. 2016).

The objective of this study was to assess virus–host interactions by analyzing the abundances of VLPs and different microbial virus hosts in the plankton community. The covariance of viruses with biotic and abiotic variables was researched in different areas in the northern Alboran Sea, where plankton communities present distribution patterns that are shaped by the influence of the Atlantic water jet penetrating into the Alboran Sea through the Strait of Gibraltar. Consequently, strong gradients of nutrients and chlorophyll, decreasing from west to east, are normally found (Rodríguez 1994, Ramírez et al. 2005, Mercado et al. 2007, 2012). Trophic interactions could also play an important role in determining the structure of the plankton community (Amorim et al. 2016). Concerning virus–host interactions, studies reporting data on virus abundance in the Alboran Sea are scarce (Alonso et al. 2001, Magagnini et al. 2007). Alonso et al. (2001) concluded that bacteriophages were dominant based on a research survey carried out in summer during which both coastal and open-ocean stations were sampled. Our data indicate that the viral abundance in the Alboran Sea co-varied with autotrophic prokaryotes in autumn and heterotrophic bacteria in spring, suggesting that cyanobacteria played an important role in determining the virus distribution in autumn. This hypothesis should be tested with molecular data in the future.

MATERIALS AND METHODS

Sampling and characterization of biotic and abiotic variables

Two oceanographic cruises were carried out in November 2014 and April 2015 on board the RV 'Francisco de Paula Navarro.' During the surveys, samples were taken from 4 stations located 0.5, 1, 3 and 10 km off the coast in the bays of Algeciras, Malaga and Almeria (AG, MA and AL, respectively; Fig. 1). At each station, temperature and salinity profiles were obtained with a CTD Seabird 25. Water samples were taken with 5 l Niskin bottles at 2 or 3 depths depending on the water column depth. The samples were collected at 0, 10 and 25 m depth at stations deeper than 25 m. At the other stations (6 in all), samples were collected at the surface and close to the bottom. Additionally, 10 ml sub-samples were collected at each depth, and immediately frozen at

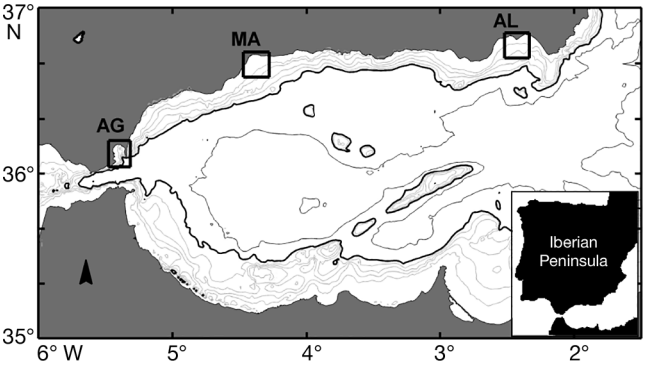

Fig. 1. Sampling areas. Two oceanographic cruises were carried out in November 2014 and April 2015. During the surveys, samples were taken from 4 stations located 0.5, 1, 3 and 10 km off the coast in the bays of Algeciras (AG), Malaga (MA) and Almeria (AL)

–20°C. These non-filtered samples were used for the analysis of nutrient concentrations (nitrate, phosphate and silicate) by means of a segmented-flow Bran-Luebbe AA3 autoanalyzer as described by Ramírez et al. (2005). In addition, 0.5–1 l of seawater was filtered through Whatman GF/F filters and frozen aboard for analysis of the chlorophyll *a* (chl *a*) concentration by spectrophotometry after extraction in 90% acetone at 4–5°C overnight.

The abundances of VLPs, heterotrophic bacteria (HB), *Synechococcus* (Syn), *Prochlorococcus* (Prochl), autotrophic eukaryote picoplankton (APP) and autotrophic eukaryote nanoplankton (ANP) were determined by flow cytometry in samples of 5 ml of seawater preserved with glutaraldehyde (1% final concentration) and immediately frozen in liquid nitrogen (Vaulot et al. 1989). The samples were analyzed individually without being integrated using a Becton Dickinson FACScan flow cytometer; an example of representative cytograms is shown in Fig. 2. The analysis was performed based on orange and red fluorescence signals together with side-light scatter (SSC). This analysis allowed the identification of cyanobacteria (Prochl and Syn), APP (1–2 µm cell size) and ANP (2–5 µm cell size). HB and VLPs were enumerated according to particle light dispersion and content in nucleic acids by staining with SYBR Green I (Sigma) (Gasol & del Giorgio 2000). Samples for VLP enumeration were diluted in sterilized filtered TE buffer (pH 8) before staining (Brussaard 2004b, Brussaard et al. 2010) and were analyzed after thorough cleaning of the flow cytometer using ultrapure grade II water as sheath fluid. This way the levels of colloids or other particles that would skew VLP counts were minimized in our fluid system. Data were acquired and analyzed using CELL QUEST Software (BD Biosciences). TruCOUNT Tubes (BD Biosciences) were used to determine absolute counts. The precision (variation coefficient) of the abundance measurements for VLPs, HB, Syn, Prochl, APP and ANP were 5, 3, 3, 10, 7 and 8%, respectively.

The abundance of large phytoplankton (i.e. >5 µm cell size; photoautotrophic microplankton, AMP) including diatoms, dinoflagellates and flagellates, was determined by microscopy in the samples taken at the surface and at 25 m depth (or close to the bottom when the station was shallower). These samples were fixed in dark glass bottles with Lugol's solution (2% f.c.). In the laboratory, 100 ml of each fixed sample were allowed to settle in a composite chamber for 24 h, following the technique developed by Utermöhl (1958). Cells were counted at 200× and 600× magnification with a Leica DMIL inverted microscope.

The abundance of photoautotrophs was estimated as the sum of cyanobacteria, APP, ANP and AMP.

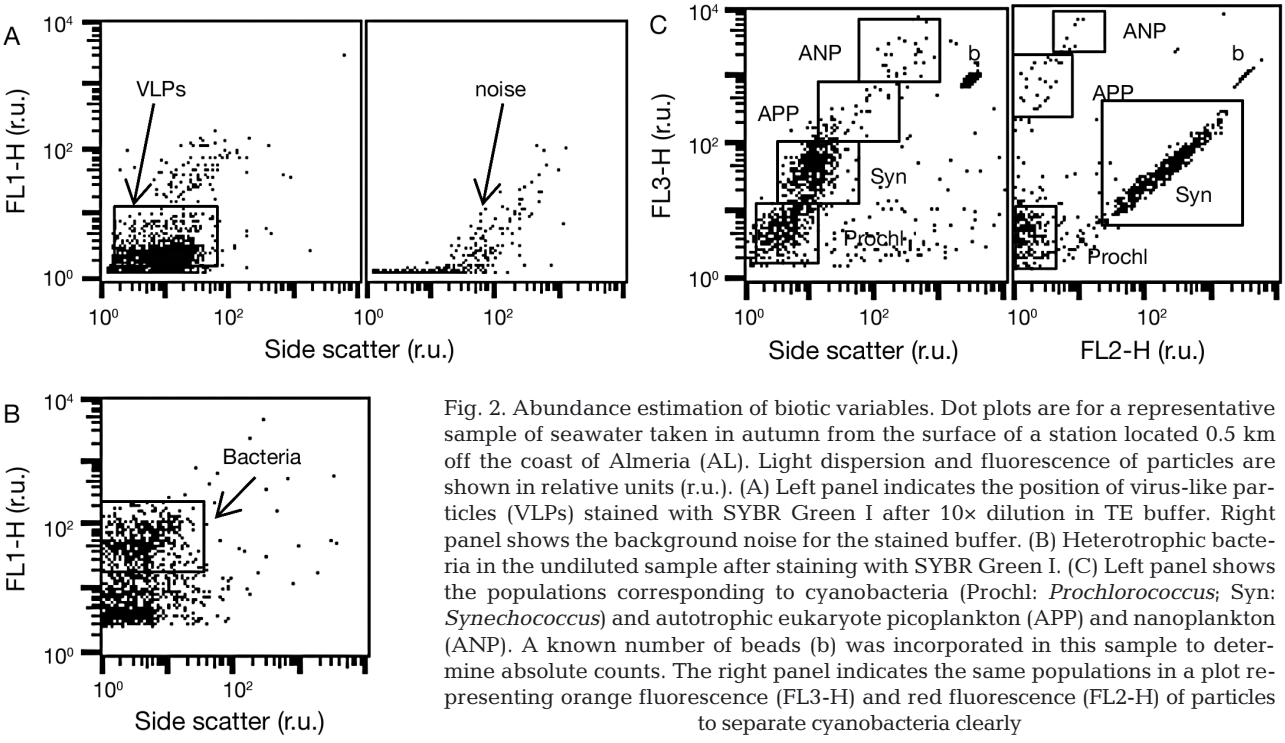

Fig. 2. Abundance estimation of biotic variables. Dot plots are for a representative sample of seawater taken in autumn from the surface of a station located 0.5 km off the coast of Almeria (AL). Light dispersion and fluorescence of particles are shown in relative units (r.u.). (A) Left panel indicates the position of virus-like particles (VLPs) stained with SYBR Green I after 10× dilution in TE buffer. Right panel shows the background noise for the stained buffer. (B) Heterotrophic bacteria in the undiluted sample after staining with SYBR Green I. (C) Left panel shows the populations corresponding to cyanobacteria (Prochl: *Prochlorococcus*; Syn: *Synechococcus*) and autotrophic eukaryote picoplankton (APP) and nanoplankton (ANP). A known number of beads (b) was incorporated in this sample to determine absolute counts. The right panel indicates the same populations in a plot representing orange fluorescence (FL3-H) and red fluorescence (FL2-H) of particles to separate cyanobacteria clearly

The VMR was calculated by dividing VLPs by the sum of photoautotrophs plus the HB abundance.

Statistical analysis

The microbial community was analyzed in 60 samples (30 in each season: 11 from AG, 9 from MA and 10 from AL). This dataset was used to test the statistical significance of the differences in the variables analyzed between AG, MA and AL. Kolmogorov-Smirnov and Shapiro-Wilk tests and Levene's test were used to research which variables fulfilled the criteria of normality and homoscedasticity, respectively. Temperature, salinity, chl *a*, nitrate, silicate, Syn, APP and ANP did not satisfy these criteria. Consequently, we used the non-parametrical Kruskal-Wallis test. Similarly, non-parametric Spearman rank correlation analyses were carried out to examine relationships between variables. For all of these analyses, the significance level was established at 0.05.

An analysis of similarity (ANOSIM) with the abundances of the different plankton groups was carried out in order to test for differences in composition between the 2 sampling periods as well as among AG, MA and AL during each period. This analysis used a matrix composed of Bray Curtis similarity coefficient generated with the abundance data. Analysis of similarity percentage (SIMPER) was used to identify which group primarily counted for the differences obtained between the 2 sampling periods. Abundance data were transformed logarithmically, and the R package 'vegan 2.4-5' was used (Oksanen et al. 2016).

Additionally, a principal component analysis (PCA) was performed to identify the main variation patterns among the abiotic and biotic variables (Savenkoff et al. 1995). The variables included in the analysis were temperature, salinity, phosphate concentration, chl *a*, VLP, Prochl, Syn, APP and ANP. Note that AMP was not included in this analysis as this plankton fraction was analyzed in a comparatively reduced number of samples. Nitrate and silicate were also excluded, because we obtained no clear temporal or spatial variation patterns for either nutrient (see Fig. 3). Furthermore, nitrate was below the detection limit in the same samples, implying that certain concentrations were not available for all samples. Each variable was normalized to 0 mean by first subtracting the mean value for the whole data set and then dividing it by the standard deviation. PCA was performed separately for the 2 surveys. STATISTICA software version 7.1. (StatSoft) was used for this statistical analysis.

RESULTS

Hydrology and nutrients

Two oceanographic cruises were carried out in November 2014 and April 2015 sampling the surface layer (up to 25 m depth in the euphotic zone) at 4 stations located 0.5, 1, 3 and 10 km off the coast in the bays of AG, MA and AL (Fig. 1). See Figs. 3–5 for the means of the different analyzed variables calculated for each season and bay from the data obtained at the 4 stations and depths sampled. The average surface temperature (ST) in autumn ranged from 18.3°C in AG to 21.2°C in AL (Fig. 3). In spring, the ST decreased in all 3 areas by ca. 3–5°C. Significant differences in salinity were found between autumn and spring in AG and MA (ranging from 36.6 to 37.3), but not in AL, where the salinity was relatively elevated in autumn (37.5; Fig. 3). Nitrate concentrations averaged 0.5 µM during the 2 surveys in AG. In contrast, in MA and AL the concentration means were lower than 0.5 µM, and multiple samples had concentrations below the detection limit (0.05 µM). Phosphate and silicate concentrations were always above the detection limits (0.04 and 0.10 µM, respectively). In AG and MA, phosphate concentrations were significantly lower in autumn (0.19 and 0.12 µM, respectively) compared to spring (0.30 and 0.28 µM). However, no seasonal differences were found for AL, where the phosphate concentration averaged 0.14 µM. In contrast, although silicate was higher in spring compared to autumn in AG and MA, the differences were not significant. The nitrate and phosphate concentrations were significantly higher in AG during the 2 surveys, unlike the silicate, which showed similar values in the 3 areas. The low nitrate concentrations resulted in nitrate:phosphate ratios departing significantly from the Redfield ratio (16:1) in the 3 areas and during the 2 surveys. This result suggests that phytoplankton growth under the different sampling conditions was probably limited by nitrate. Concordantly with this hypothesis, the chl *a* concentration was relatively low and only exceeded 1 µg l^{-1} in some samples collected in AG. For each area, there were no significant differences in chl *a* between the 2 surveys, although chl *a* in AG was higher than in MA during autumn, and higher than in AL during autumn and spring (Fig. 3). Furthermore, there were significant positive correlations between chl *a* and concentrations of nitrate ($R = 0.74$, $n = 60$, $p < 0.01$), phosphate ($R = 0.52$, $n = 60$, $p < 0.01$) and silicate ($R = 0.67$, $n = 60$, $p < 0.01$).

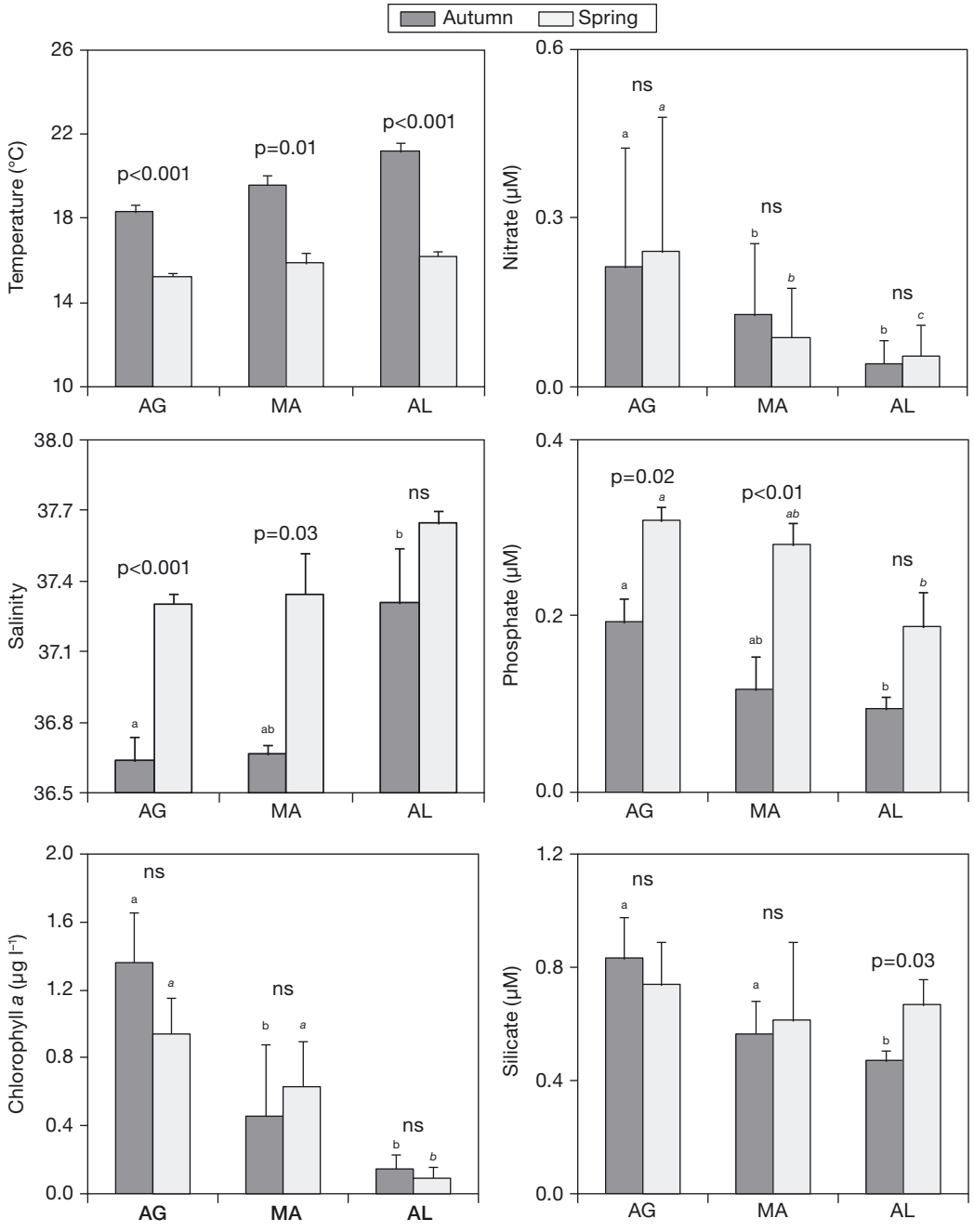

Fig. 3. Temperature, salinity (PSU), and concentrations of chl *a* and 3 nutrients in the 3 areas (see Fig. 1) sampled in autumn and spring. Means ± SD for each area were calculated from data obtained at 3 sampling stations within the 25 m depth layer. For each area, the significance level of the differences between autumn and spring is indicated according to the results of the Kruskal-Wallis analysis (ns: non-significant). For each survey, significant differences between the 3 areas at p = 0.05 are shown by superscript letters

Composition of the virus host phytoplankton and bacterioplankton community

Prochl was the most abundant autotrophic cell group in 25% of the samples (data not shown). Its numbers were higher in April, in particular in AL where the highest abundances were registered (Fig. 4). However, variability among samples was extensive, and the statistical test failed to detect significant differences between surveys or areas. In terms of abundance, Syn dominated in 35% of the samples. Most of these samples (18 out of 21) were collected in MA and AL during autumn (data not shown), surprisingly, the period with the lowest

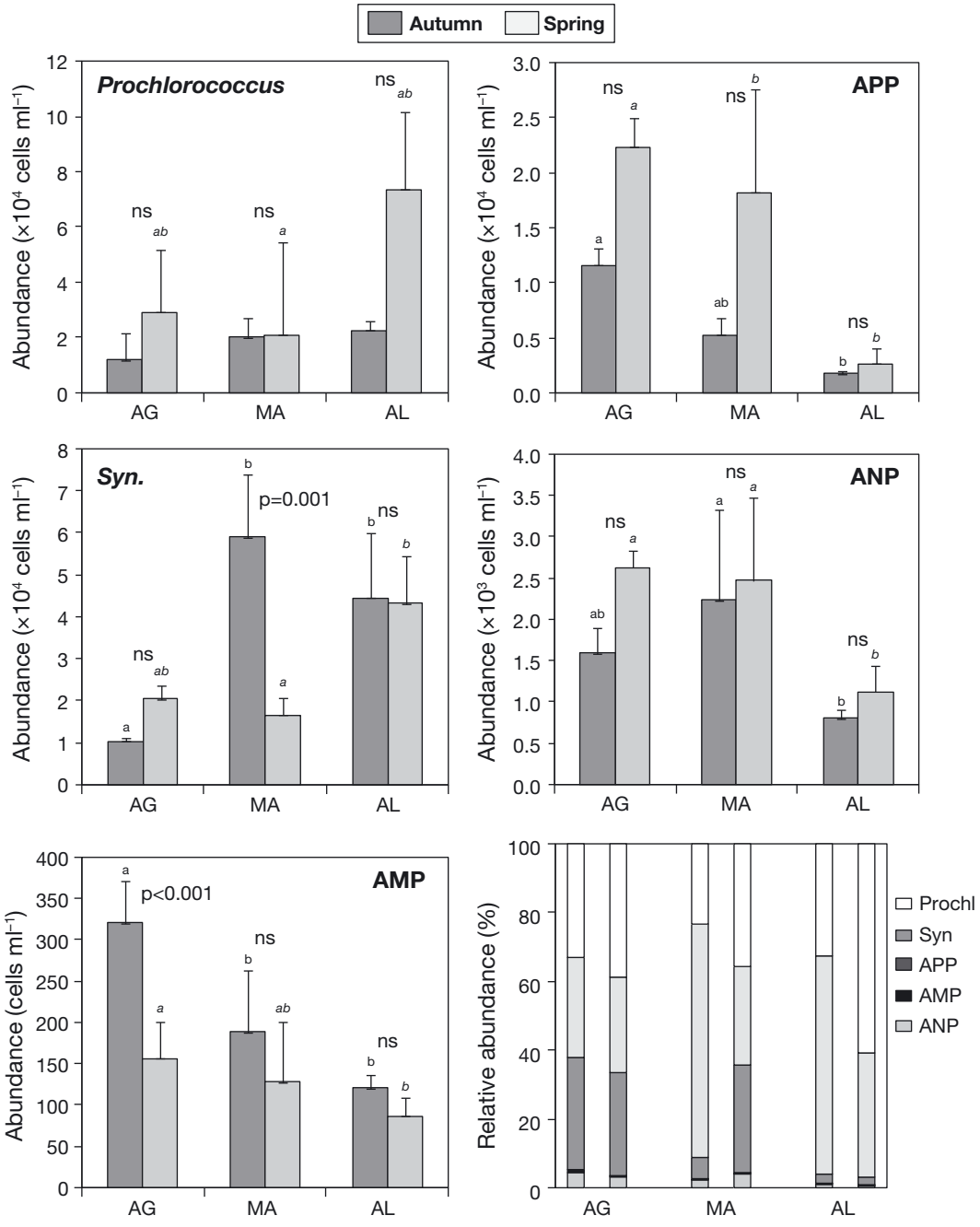

Fig. 4. Abundance of different autotrophic plankton fractions analyzed (abbreviations as in Fig. 2) in the 3 areas (see Fig. 1) sampled in autumn and spring. Note the different scales of the *y*-axes used for each group. Other details as in Fig. 3. The bottom right panel shows the relative abundance of the different autotrophic plankton fractions analyzed at each of the 3 sampling areas in autumn (left bars) and spring (right bars)

abundance of Syn in AG (Fig. 4). Syn abundance decreased sharply in MA from autumn to spring and did not vary significantly in AL. Twenty percent of the samples featured APP abundances higher than Prochl and Syn (data not shown). Most of these samples were collected in AG and none in AL. In fact, the APP abundance in AL was significantly lower than in AG during autumn and spring as well as in MA during spring (Fig. 4). In AG and MA, the abundance of this cell group was higher in spring, although the high variability impeded the detection of any significant differences. The ANP abundance was 10 times lower than the abundance of smaller autotrophic cells. ANP was less abundant in AL compared to AG

and MA, and did not vary significantly between the 2 surveys (Fig. 4). AMP represented less than 0.5% of the total autotrophic cells in terms of abundance, apart from the samples collected in AG during autumn, where it accounted for about 1% of autotrophic cells (data not shown). The AMP abundance was significantly higher in AG compared to AL during the 2 surveys (Fig. 4).

These data reveal that there were differences in the composition of the phytoplankton community between the 3 areas during the 2 surveys (Fig. 4). Thus, in autumn, a higher contribution of APP, ANP and AMP occurred in AG compared to MA and AL. In spring, the main differences in the phytoplankton composition were obtained in AL, where the most important group contributing to shape the community in terms of abundance was Prochl.

The microbial planktonic community was further studied by enumerating the abundance of HB which was 10 times higher than autotrophs (Fig. 5). The HB abundance did not differ between the 2 surveys in the 3 areas. The only remarkable variation pattern was a significant reduction in AL with respect to AG and MA. It is worth noting that this spatial variation pattern of HB was not apparently related to variation in the abundance of autotrophic cells, as the 2 variables were not significantly correlated.

The results of ANOSIM carried out with the abundance data of the different plankton groups revealed significant differences in community composition between the 2 surveys ($R = 0.20$, $p = 0.001$). According to the results of SIMPER, APP and Prochl were the groups with higher contribution to this dissimilarity (22%). Syn also showed a high contribution (21%), whereas the contribution of NNP and HB was comparatively lower (11%). Additionally, the differences in the community composition among the 3 sampling areas were significant for both time periods, i.e. during autumn ($R = 0.76$, $p < 0.001$) and spring ($R = 0.55$, $p < 0.001$).

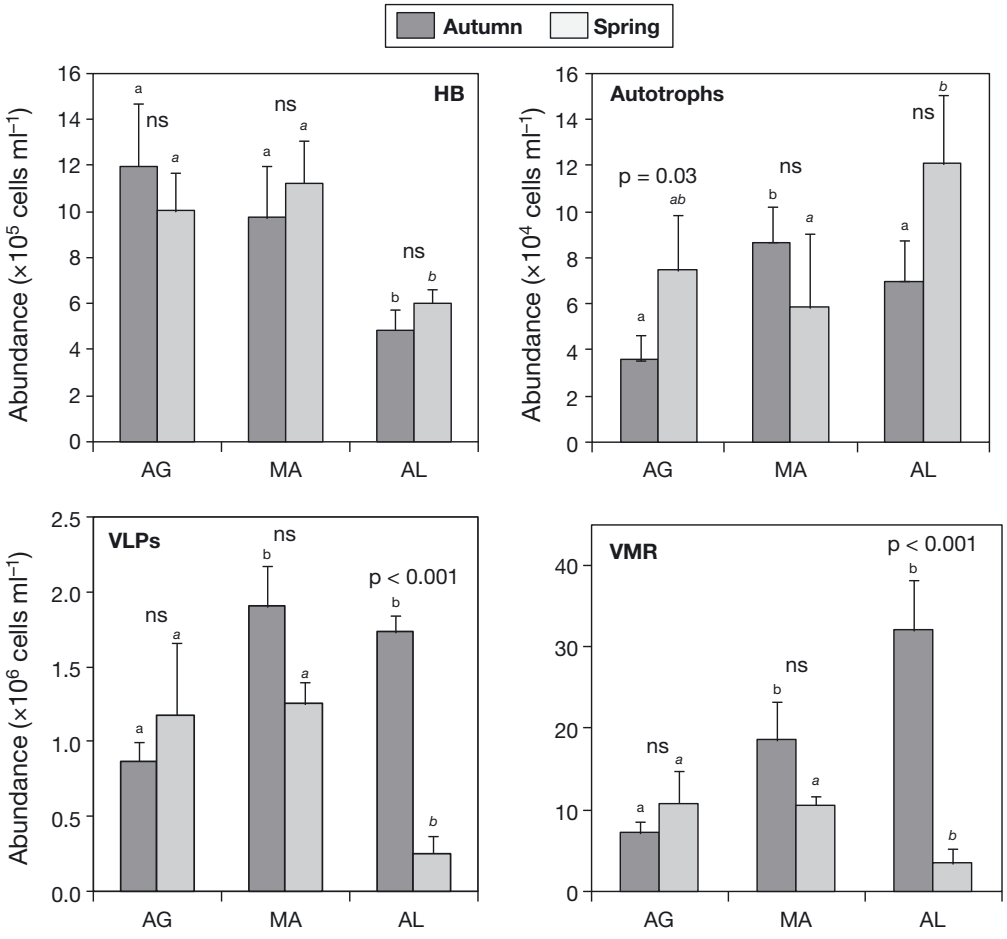

Fig. 5. Abundance of heterotrophic bacteria (HB), autotrophs and virus-like particles (VLPs) in autumn and spring. Other details as in Fig. 2. Lower right panel shows values for VMR, i.e. the ratio between VLPs and microbial abundance (i.e. the sum of pico-, nano- and microplankton)

Abundance of VLPs and covariation with biotic and abiotic variables

Abundance of VLPs varied from 8.5×10^5 to 2.2×10^7 VLPs ml^{-1}. No significant differences in the distribution of VLPs according to depth or distance to the coast were found (data not shown). Only in AL were significant differences in VLP abundance found between the 2 surveys (Fig. 5). In autumn, the VLP abundance was significantly lower in AG compared to MA and AL, as occurred with autotrophic cell abundance, but not with HB. In contrast, the lowest abundance of VLPs during spring was found in AL. The mean VMR was 13.6 and the median 10.5, although VMR showed a substantial variability ranging from 1.3 to 44 (Fig. 5). The lowest VMR value was obtained during spring in AL. The VMR followed a distribution pattern roughly similar to VLPs ($R = 0.91$, $n = 60$, $p < 0.01$), indicating that the variability of this ratio was mainly due to variability in the VLPs. In fact, VMR and microbial abundance (autotrophs plus HB) were not significantly correlated.

Correlation analyses between VLPs and the abiotic and biotic variables were carried out (Table 1). When the results of both surveys were pooled, the VLPs correlated positively and strongly with temperature ($R = 0.50$, $p < 0.001$). Correlations with salinity and phosphate were also significant although comparatively weaker ($R = 0.39$, $p < 0.01$; and $R = 0.23$, $p < 0.05$, respectively). Among the biotic variables, there was a negative correlation with the abundance of Syn ($R = 0.35$, $p < 0.01$). When the data of the 2 surveys were considered separately, the factors correlating (and the signs of the correlations) with VLPs were almost the opposite. Thus, in autumn VLPs correlated positively with temperature ($R = 0.53$, $p < 0.01$) and abundance of Prochl ($R = 0.52$, $p > 0.01$) and Syn ($R = 0.69$, $p < 0.001$). For this time period, VLPs correlated negatively with AMP ($R = -0.76$, $p < 0.001$) and more weakly with chl *a*, phosphate, and APP ($R = -0.57$, -0.56 and -0.55, $p < 0.01$, respectively). In contrast, in spring, strong positive correlations were obtained between VLP abundance and phosphate ($R = 0.73$, $p < 0.001$) and HB ($R = 0.88$, $p < 0.001$). VLP was also significantly correlated with chl *a*, APP and ANP and negatively with salinity and Syn.

PCAs were carried out to visualize covariance of the abiotic and biotic variables listed in Table 1, with the exception of AMP since data for this variable were not available for all the samples. In autumn, the first 2 factors extracted from the PCA explained 82% of the overall variance (Fig. 6A). APP and ANP presented negative correlations with the first factor, while abundance of cyanobacteria (Prochl and Syn) had positive correlations (Table 2). ANP, Syn and HB were the main variables contributing to the second factor. Consequently, the results of PCA indicate that higher abundance of HB appeared to occur in samples with relatively higher abundance of eukaryotic phytoplankton. Interestingly, VLP was strongly related to Prochl and Syn (Fig. 6A). PCA clearly discriminated the samples coming from the 3 sampling sites (Fig. 6B). Thus, all samples collected in AG had negative scores for the first factor. In spring, the first 2 factors explained more than 80% of the total variability (Fig. 6C). As for autumn, in spring APP, ANP and HB contributed negatively to the first factor, and temperature, salinity, Syn and Prochl contributed

Table 1. Values of Spearman rank R obtained from the correlation analyses between virus-like particles (VLPs) and the abiotic and biotic variables researched in this work (variables that did not correlate with VLPs are not listed). Sample numbers (n) are shown. APP: autotrophic eukaryote picoplankton; ANP: autotrophic eukaryote nanoplankton; AMP: autotrophic eukaryote microplankton. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

Variable	Total (n = 60)	Autumn (n = 30)	Spring (n = 30)
Temperature	0.50***	0.53**	−0.15
Salinity	−0.39**	0.27	−0.79***
Chlorophyll *a*	−0.07	−0.57***	0.56**
Phosphate	−0.32*	−0.56**	0.73***
Heterotrophic bacteria	0.04	−0.31	0.88***
Prochlorococcus	−0.06	0.52**	−0.35
Synechococcus	0.35**	0.69***	−0.57**
APP	−0.05	−0.55**	0.68***
ANP	0.09	0.02	0.67**
AMP	0.01	−0.76***	0.29

Table 2. Correlations between the 2 first principal components obtained from the principal component analysis (PCA) and the abiotic and biotic variables. Abbreviations as in Table 1

Variable	Autumn		Spring	
	PC1	PC2	PC1	PC2
Temperature	0.65	−0.61	0.65	−0.61
Salinity	0.77	0.43	0.77	0.43
Phosphate	−0.90	−0.02	−0.90	−0.02
Chlorophyll *a*	−0.90	0.31	−0.90	0.31
Virus-like particles	−0.81	−0.45	−0.81	−0.44
Heterotrophic bacteria	−0.83	−0.43	−0.83	−0.43
Prochlorococcus	0.70	−0.47	0.70	−0.47
Synechococcus	0.85	−0.05	0.85	−0.05
APP	−0.94	0.04	−0.94	0.04
ANP	−0.89	−0.02	−0.89	−0.02

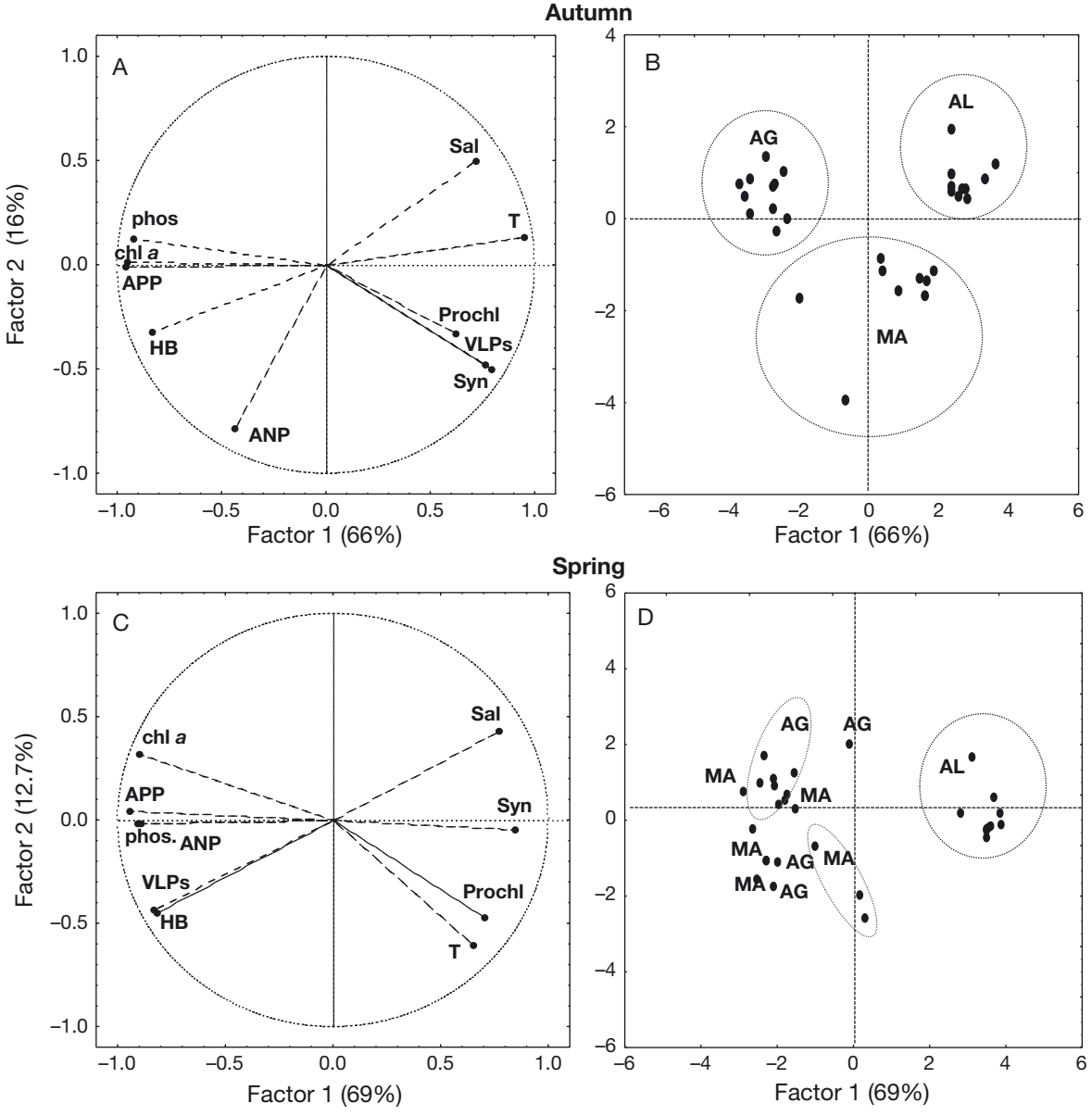

Fig. 6. Vector plots of (A,C) the variable factor coordinates and (B,D) scores obtained for each sample extracted from the principal component analysis (PCA) carried out for (A,B) autumn and (C,D) spring. Sal: salinity; T: temperature; phos.: phosphate concentration; chl *a*: chlorophyll *a* concentration; VLPs: virus-like particles; HB: heterotrophic bacteria; Prochl: *Prochlorococcus*; Syn: *Synechococcus*; APP: autotrophic eukaryote picoplankton; ANP: autotrophic eukaryote nanoplankton

positively. However, VLP was strongly correlated to HB in spring. As occurred in autumn, this first factor clearly discriminated the samples coming from AL from the other sites (Fig. 6D), which were characterized by significantly lower VLPs.

DISCUSSION

The variability of VLP abundance in the Alboran Sea in our study ranged from 8.5×10^5 to 2.2×10^7 VLPs ml^{-1}. This covers almost the whole variability range described for the Mediterranean Sea. The available information about virus distribution in the Mediterranean Sea is limited and cannot reliably be used to research spatial and temporal variability patterns within a given sub-region, with the exception of the Adriatic Sea (eastern Mediterranean Sea), where the temporal dynamics of viruses and bacterioplankton have been described in more detail (Karuza et al. 2012). Nonetheless, the large-scale distribution patterns of VLPs in the Mediterranean Sea have been analyzed by some authors (Magagnini et al. 2007, Christaki et al. 2011). Based on these studies (and

other available data), Siokou-Frangou et al. (2010) and Estrada & Vaqué (2014) concluded that the VLP abundance in the upper 200 m depth layer of the Mediterranean Sea generally decreases from coast to open sea and from west to east following the plankton productivity gradients. According to these studies, VLPs in the surface layer of the Mediterranean Sea range from 2.3×10^5 to 2.7×10^7 VLPs ml^{-1}. Interestingly, this lower limit was reported by Magagnini et al. (2007) in an open sea station located in the Alboran Sea. In addition, Alonso et al. (2001) reported virus abundance for the Alboran Sea ranging from 2.6×10^5 to 1.8×10^6 VLPs ml^{-1}, i.e. the maximum abundances were 10 times lower than those generally reported for the Mediterranean Sea. In contrast, the VLP abundance in our work falls within the range of abundances reported by Wigington et al. (2016), where data from multiple regions were reviewed and compared (i.e. 3.7×10^5 to 6.4×10^7 VLPs ml^{-1}). Therefore, our study demonstrates that variability in VLP distribution in the Alboran Sea could be as significant as inter-regional variability. Interestingly, the range of variability obtained in the abundance of microbial cells in our study (from 3.9×10^5 to 1.6×10^6 cells ml^{-1}) is considerably lower than the range in microbial abundance variability described by Wigington et al. (2016), even though the span for virus variation is similar. This means that most of the VMR variability in the Alboran Sea can be attributed to the variability in virus abundance.

According to Siokou-Frangou et al. (2010) and Estrada & Vaqué (2014), VLPs are significantly correlated to HB abundance and only weakly to chl *a* in the Mediterranean Sea, indicating that in this basin, bacteria are more probable viral hosts than phytoplankton cells. Likewise, Alonso et al. (2001) reported that most of the viruses analyzed in the Alboran Sea during summer were bacteriophages. Coincidently, in our study, a significant correlation between VLPs and HB was obtained in spring. However, when the whole data set was considered, the correlation between VLPs and HB was not significant. This lack of correlation can be due to differences in viral burst size (BS) of HB since it appears to be fairly variable in the marine environment (see Parada et al. 2006 for a review). BS is influenced by a number of factors, including the size of the host cell and the viruses and the metabolic activity of the bacterial community that in turn is controlled by abiotic factors. However, in our study, VLPs were significantly correlated not only with HB but also with other plankton fractions. Thus, in autumn VLPs correlated positively with Prochl and Syn and negatively with APP and AMP, while in spring VLPs correlated positively with HB, APP and ANP and negatively with Syn. Altogether, these results suggest that photoautotrophs would also play a role as viral hosts.

To our knowledge, this is the first report presenting evidence in support of the importance of photoautotrophs as viral hosts in a particular area in the Mediterranean Sea. A high abundance of cyanophages has been described for other marine ecosystems, with abundances up to 10^6 cells ml^{-1} at some stations (Waterbury & Valois 1993, Garza & Suttle 1998, Matteson et al. 2013). Parsons et al. (2012) described a significant positive correlation between VLPs and Prochl abundance in the Sargasso Sea, implying that cyanophages infecting Prochl were abundant. In contrast, the same study found a negative correlation between VLPs and Syn. Similarly, Matteson et al. (2013) described VLP increases with decreases in Syn and Prochl abundance. According to these authors, the differences in the sign of the correlations would reflect the fact that different phases of the lysis process (host lysis or virus production) were captured during the samplings. This hypothesis would explain the opposite correlations obtained in our study between VLPs and Syn in autumn compared to spring, assuming that by chance the samples for each season represent a snapshot of different phases of the virus production cycle that occurs within hours. However, other factors could also contribute to this finding. For instance, the cyanobacteria contributed positively to the first factor extracted from the PCA while HB did so negatively (Fig. 6) during the 2 surveys, indicating that both populations presented opposite distribution patterns (in fact, there was a negative correlation between cyanobacteria and HB; $R = -0.47$, $n = 60$, $p < 0.001$). Furthermore, HB was positively correlated to phosphate concentration ($R = 0.50$, $n = 60$, $p < 0.001$) while the correlation between cyanobacteria and phosphate was negative ($R = -0.29$, $n = 60$, $p < 0.05$). Published data regarding the phosphate uptake kinetics in marine cyanobacteria and HB indicate that cyanobacteria are superior for phosphate uptake (see, e.g., Tanaka et al. 2003). Therefore, our results suggest that higher phosphate availability favored the growth of HB vs. cyanobacteria in spring. The opposite situation (i.e. improved competitive advantage of cyanobacteria compared to HB) is found in autumn. APP and AMP abundances correlated positively with HB ($R = 0.70$, $n = 60$, $p < 0.001$ and $R = 0.38$, $n = 32$, $p < 0.05$; respectively), probably indicating that eukaryotic phytoplankton growth was partially dependent on the release of nutrients by the heterotrophic bacterial community (note that the

nitrate concentration was low at all stations during the 2 surveys). Consequently, the positive correlation found between VLPs and APP and ANP in spring could be the result of covariation with HB more than a direct link to VLPs.

If the 'kill-the-winner' theory is assumed (Thingstad 2000), HB growing more actively will favor the proliferation of bacteriophages in spring. In contrast, more favorable conditions for cyanobacterial growth in autumn would favor the proliferation of cyanophages. Additionally, the fact that BS increases in environments that favor host growth (Parada et al. 2006) would contribute to the relationship obtained between cyanobacterial abundance and VLPs in autumn despite HB abundance being comparatively higher (as occurred in spring). Consequently, our results allow us to hypothesize that, in our sampling areas, cyanobacteria were the more likely viral hosts during the autumn survey. Therefore, virus dynamics in the 3 areas analyzed appeared to be regulated by changes in the abundance of cyanobacteria and HB, which in turn are related to variability in abiotic factors that directly affect virus–host interactions (reviewed by Mojica & Brussaard 2014) by improving the competitive advantage of either one population or the other. Further molecular information on virus dynamics in the Alboran Sea obtained from controlled virus–host systems is necessary in order to test this hypothesis and interpret virus to host ratios obtained in our study area (Parikka et al. 2017).

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LITERATURE CITED

Alonso MC, Jiménez-Gómez F, Rodríguez J, Borrego JJ (2001) Distribution of virus-like particles in an oligotrophic marine environment (Alboran Sea, Western Mediterranean). Microb Ecol 42:407–415

Amorim AL, León P, Mercado JM, Cortés D and others (2016) Controls of picophytoplankton abundance and composition in a highly dynamic marine system, the Northern Alboran Sea (Western Mediterranean). J Sea Res 112:13–22

Bouvy M, Combe M, Bettarel Y, Dupuy C, Rochelle-Newall E, Charpy L (2012) Uncoupled viral and bacterial distributions in coral reef waters of Tuamotu Archipelago (French Polynesia). Mar Pollut Bull 65:506–515

Brussaard CPD (2004a) Viral control of phytoplankton populations — a review. J Eukaryot Microbiol 51: 125–138

Brussaard CPD (2004b) Optimization of procedures for counting viruses by flow cytometry. Appl Environ Microbiol 70:1506–1513

Brussaard CPD, Payet JP, Winter C, Weinbauer MG (2010) Quantification of aquatic viruses by flow cytometry. In: Wilhelm S, Weinbauer M, Suttle C (eds) Manual of aquatic viral ecology. American Society of Limnology and Oceanography, Waco, TX, p 102–109

Christaki U, Van Wambeke F, Lefevre D, Lagaria A and others (2011) Microbial food webs and metabolic state across oligotrophic waters of the Mediterranean Sea during summer. Biogeosciences 8:1839–1852

Clasen JL, Brigden SM, Payet JP, Suttle CA (2008) Evidence that viral abundance across oceans and lakes is driven by different biological factors. Freshw Biol 53:1090–1100

Estrada M, Vaqué D (2014) Microbial components. In: Goffredo S, Dubinsky Z (eds) The Mediterranean Sea: its history and present challenges. Springer, Dordrecht, p 87–111

Finke JF, Hunt BP, Winter C, Carmack EC, Suttle CA (2017) Nutrients and other environmental factors influence virus abundances across oxic and hypoxic marine environments. Viruses 9:152–166

Garza DR, Suttle CA (1998) The effect of cyanophages on the mortality of *Synechococcus* spp. and selection for UV resistant viral communities. Microb Ecol 36:281–292

Gasol JM, del Giorgio PA (2000) Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Sci Mar 64:197–224

Haaber J, Middelboe M (2009) Viral lysis of *Phaeocystis pouchetii*: implications for algal population dynamics and heterotrophic C, N and P cycling. ISME J 3:430–441

Karuza A, Umani SF, Del Negro P (2012) The un(coupling) between viruses and prokaryotes in the Gulf of Trieste. Estuar Coast Shelf Sci 115:87–97

Knowles B, Silveira CB, Bailey BA, Barott K and others (2016) Lytic to temperate switching of viral communities. Nature 531:466–470

Larsen A, Castberg T, Sandaa RA, Brussaard CPD and others (2001) Population dynamics and diversity of phytoplankton, bacteria and viruses in a seawater enclosure. Mar Ecol Prog Ser 221:47–57

Magagnini M, Corinaldesi C, Monticelli LS, De Domenico E, Danovaro R (2007) Viral abundance and distribution in mesopelagic and bathypelagic waters of the Mediterranean Sea. Deep-Sea Res I 54:1209–1220

Matteson AR, Rowe JM, Ponsero AJ, Pimentel TM, Boyd PW, Wilhelm SW (2013) High abundances of cyanomyoviruses in marine ecosystems demonstrate ecological relevance. FEMS Microbiol Ecol 84:223–234

Mercado JM, Cortés D, García A, Ramírez T (2007) Seasonal and inter-annual changes in the planktonic communities of the northwest Alboran Sea (Mediterranean Sea). Prog Oceanogr 74:273–293

Mercado JM, Cortés D, Ramírez T, Gómez F (2012) Hydrological forcing masks the potential impact of nutrient release from diffuse sources in the NW coast of the Alboran Sea. Hydrobiologia 680:91–107

Mojica KDA, Brussaard CPD (2014) Factors affecting virus dynamics and microbial host–virus interactions in marine environments. FEMS Microbiol Ecol 89:495–515

Oksanen J, Blanchet FG, Friendly M, Kindt R and others (2016) vegan: Community ecology package version 2.4-0. https://cran.r-project.org/package=vegan

Ory P, Palesse S, Delmas D, Montanié H (2011) *In situ* structuring of virioplankton through bacterial exoenzymatic activity: interaction with phytoplankton. Aquat Microb Ecol 64:233–252

Parada V, Herndl GJ, Weinbauer MG (2006) Viral burst size of heterotrophic prokaryotes in aquatic system. J Mar Biol Assoc UK 86:613–621

Parikka KJ, Le Romancer M, Wauters N, Jacquet S (2017) Deciphering the virus-to-prokaryote ratio (VPR): insights into virus-host relationships in a variety of ecosystems. Biol Rev Camb Philos Soc 92:1081–1100

Parsons RJ, Breitbart M, Lomas MW, Carlson CA (2012) Ocean time-series reveals recurring seasonal patterns of virioplankton dynamics in the northwestern Sargasso Sea. ISME J 6:273–284

Ramírez T, Cortés D, Mercado JM, Vargas-Yañez M, Sebastián M, Liger E (2005) Seasonal dynamics of inorganic nutrients and phytoplankton biomass in the NW Alboran Sea. Estuar Coast Shelf Sci 65:654–670

Rodríguez J (1994) Some comments on the size-based structural analysis of the pelagic ecosystem. Sci Mar 58:1–10

Savenkoff C, Chanut JP, Vezina AF, Gratton Y (1995) Distribution of biological activity in the lower St. Lawrence estuary as determined by multivariate analysis. Estuar Coast Shelf Sci 40:647–664

Siokou-Frangou I, Christaki U, Mazzocchi MG, Montresor M, Ribera d'Alcalá M, Vaqué D, Zingone A (2010) Plankton in the open Mediterranean Sea: a review. Biogeosciences 7:1543–1586

Suttle CA (2005) Viruses in the sea. Nature 437:356–361

Suttle CA (2007) Marine viruses — major players in the global ecosystem. Nat Rev Microbiol 5:801–812

Tanaka T, Rassoulzadegan F, Thingstad TF (2003) Measurements of phosphate affinity constants and phosphorus release rates from the microbial food web in Villefranche Bay, northwestern Mediterranean. Limnol Oceanogr 48: 1150–1160

Thingstad TF (2000) Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. Limnol Oceanogr 45:1320–1328

Utermöhl H (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Verh Int Verein Limnol 9: 1–38

Vaulot D, Courties C, Partensky F (1989) A simple method to preserve oceanic phytoplankton for flow cytometric analyses. Cytometry 10:629–635

Waterbury JB, Valois FW (1993) Resistance to co-occurring phages enables marine *Synechococcus* communities to coexist with cyanophages abundant in seawater. Appl Environ Microbiol 59:3393–3399

Wigington CH, Sonderegger D, Brussaard CPD, Buchan A and others (2016) Re-examination of the relationship between marine virus and microbial cell abundances. Nat Microbiol 1:15024

Wigington CH, Sonderegger D, Brussaard CPD, Buchan A and others (2017) Author correction: Re-examination of the relationship between marine virus and microbial cell abundances. Nat Microbiol 2:1571

Wommack KE, Colwell RR (2000) Virioplankton: viruses in aquatic ecosystems. Microbiol Mol Biol Rev 64:69–114

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