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Aerobic anoxygenic phototrophs of the Adriatic Sea

Ana Vrdoljak Tomaš[1], Danijela Šantić[1], Iva Stojan[1,2*] and Mladen Šolić[1]

[1] *Institute of Oceanography and Fisheries, Šetalište Ivana Meštrovića 63, 21000 Split, Croatia*
[2] *Doctoral study of Biophysics, Faculty of Science, University of Split, Ruđera Boškovića 37, 21000 Split, Croatia*

Abstract: Aerobic anoxygenic phototrophs are a newly discovered member of the bacterial community in the Adriatic Sea. During the last seven years, when we started to study these organisms, we have collected a considerable number of samples from different environments, from the coast, the estuary, and the open sea. Here we have compiled data from 34 georeferenced study sites from four studies that summarize all that is known about aerobic anoxygenic phototrophs and examine the spatial and vertical distribution and environmental factors affecting this community in the Adriatic Sea. We found horizontal and vertical influences on AAP distribution, mainly salinity, nitrates, chlorophyll *a*, ammonium, temperature, and soluble reactive phosphorus. Much is known about their ecology in the Adriatic, and with a new survey underway, we will expand our knowledge of their community composition and their role in carbon flux to higher trophic levels.

Keywords: picoplankton; vertical distribution; multivariate analysis; Adriatic Sea; spatial distribution; bacteria biomass

Sažetak: AEROBNI ANOKSIGENI FOTOTROFI JADRANSKOG MORA. Aerobni anoksigeni fototrofi relativno su novi član bakterijske zajednice u Jadranskom moru. U posljednjih sedam godina, od kada smo započeli proučavati ove organizme, prikupili smo znatan broj uzoraka iz različitih okoliša, obalnog, estuarnog i područja otvorenog mora. U ovom radu objedinili smo podatke s 34 georeferencirane lokacije istraživanja iz četiri studije koje sažimaju sva saznanja o aerobnim anoksigenim fototrofima te istražili prostornu i vertikalnu raspodjelu, kao i okolišne čimbenike koji utječu na ovu zajednicu u Jadranskom moru. Utvrdili smo čimbenike koji utječu na horizontalnu i vertikalnu raspodjelu AAP, uglavnom salinitet, nitrate, klorofil a, amonijeve ione, temperaturu te topljivi reaktivni fosfor. Iako se dosta zna o njihovoj ekologiji u Jadranu, aktualnim istraživanjem proširit ćemo naše znanje o sastavu ove zajednice i njihovoj ulozi u protoku ugljika prema višim trofičkim razinama.

Ključne riječi: pikoplankton; vertikalna raspodjela; multivarijatna analiza; Jadran; prostorna rasprostranjenost; bakterijska biomasa

INTRODUCTION

It is now well known that microbes are the drivers of ecosystem function. In the Adriatic, there is extensive literature on the distribution, dynamics, and regulation of the picoplankton community both in the central and southern and in the open and nearshore areas. Phototrophs (cyanobacteria *Prochlorococcus* and *Synechococcus* and picoeukaryotes) and heterotrophs together are extensively studied by flow cytometry, epifluorescence microscopy and heterotrophic cell production (Šolić and Krstulović, 2016; Šolić *et al*., 2022; Šantić *et al*., 2021). While most biogeochemical studies use the dichotomy of dividing microorganisms into photo(auto)trophs and organo(hetero)trophs to define their role in the carbon cycle, there are different types of metabolism, and photoheterotrophy is one of them. Photoheterotrophic organisms are fascinating because they can use both light and dissolved organic matter as energy sources (EILER, 2006) and are an important part of the microbial communities that inhabit the euphotic zone of the world's oceans (Béjà *et al*., 2000; Kolber *et al*., 2000, 2001).

In the 1970s, a new bacterial functional group, aerobic anoxygenic phototrophs (AAPs), containing small amounts of bacteriochlorophyll *a* (BChl *a*) was discovered near Tokyo (Harashima *et al*., 1989). These photoheterotrophic microorganisms can harvest light energy using pigment BChl *a* to supplement their primarily organotrophic metabolism (Yurkov and van Gemerden, 1993; Hauruseu and Koblížek, 2012) only in the presence of oxygen (Biebl and Wagner-Döbler, 2006; Yurkov and Csotonyi, 2009; Koblížek *et al*., 2010). Besides cyanobacteria and proteorhodopsin-containing bacteria, they are the third most abundant group of photo(hetero)trophic prokaryotes in the ocean and have been an important topic in aquatic microbiology over the last two decades (Kolber *et al*., 2000; Koblížek, 2015). AAPs are known to constitute about 10% of all prokaryotes in various marine environments (Koblížek, 2015). Because of their metabolic flexibility, fast growth rates, and widespread occurrence, AAPs play a significant role in the aquatic food web and biogeochemical cycles (Kolber *et al*., 2001; Koblížek *et al*., 2007; Ferrera *et al*., 2011, 2017), *ergo* challenge our

**Corresponding author:* e-mail: stojan@izor.hr
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view of carbon and energy budgets (Eiler 2006; Moran and Miller, 2007; Mašín *et al.*, 2008; Lamy *et al.*, 2011a; Sánchez *et al.*, 2020). Here, we summarize all data on AAPs collected in the Adriatic Sea in coastal, estuarine, and open areas, and examine their spatio-temporal distribution as well as environmental factors affecting this important bacterial community.

MATERIAL AND METHODS

Study area and sampling

The Adriatic Sea is the northernmost basin of the Mediterranean, 800 km long and 200-250 km wide, which exchanges waters with the rest of the Mediterranean Sea (i.e., the Ionian Basin) through the Strait of Otranto (Kovačević *et al.*, 1999; Yari *et al.*, 2012). Bathymetry divides the basin into three parts: the wide shelf of the northern Adriatic with an average depth of 40 m, the central Adriatic with depressions up to 280 m deep, which is, with the Palagruža sill, connected to the circular basin of the southern Adriatic, whose maximum depth exceeds 1200 m. The estuaries can be characterized as salt wedges with typical halo- and nutriclines, with the largest variations in hydrographic parameters occurring in the surface layer (Šolić *et al.*, 2015). A typical feature of the studied estuaries is their limitation by phosphorus, but not by nitrogen, which is due to the fact that the karst rivers of the eastern Adriatic carry small amounts of phosphorus (UNEP/MAP, 2003). They are also characterized by shallow depths and low salinity, which is due to the strong influence of freshwater flows. The offshore area of the eastern Adriatic coast is characterized as oligotrophic and phosphorus-limited (P-limited) (Buljan and Zore-Armanda, 1976; Vukadin and Stojanoski, 2001). The studied stations are located in the central and southern Adriatic Sea and cover the trophic gradient from karstic estuaries through the coastal sea to the open sea (Fig. 1). Samples were collected aboard the R/V BIOS DVA on several cruises in 2015, 2016, 2017, and 2019, during which we collected and observed more than 300 samples. Samples were collected at 34 stations; 6 are in estuarine waters of the Croatian rivers Krka, Jadro, Neretva and Ombla, 18 in coastal sea, and 10 in the open sea.

Environmental parameters

The ecological parameters used in this study have been described in previous publications (Šantić *et al.*, 2017; 2019; 2021; Vrdoljak Tomaš *et al.*, 2019).

Epifluorescence microscopy

AAPs were counted using an Olympus BX51 microscope equipped with an Olympus UPlanSApo 100×/1.40 OIL, IR objective, and image analysis software (CellSens). The microscope was equipped with a U-RFL-T Hg lamp for excitation, and fluorescence images were

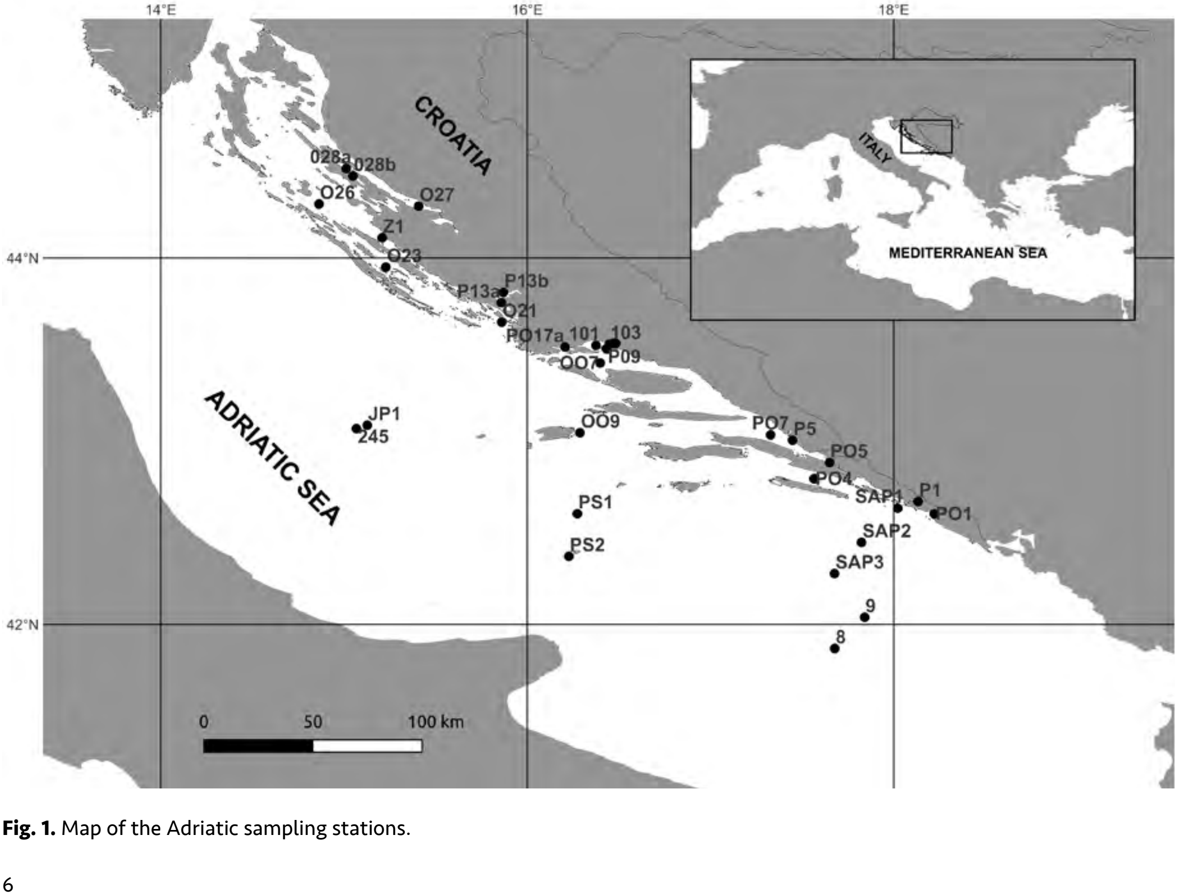

Fig. 1. Map of the Adriatic sampling stations.

acquired using an XM10- IR camera, Olympus. AAPs were determined according to the protocol described by Mašín et al. (2006). Subsamples were fixed with 2% formaldehyde, incubated for 30-60 min at room temperature in the dark, and stored at -80°C. Briefly, triplicate samples were thawed and mixed gently. Cells were collected by filtration on 0.2-µm PC filters and after drying were stained with 4',6-diamidino-2-phenylindole (DAPI) using a 3:1 mixture of Citifluor™ AF1 and Vectashield®. Three epifluorescent filter sets were used: DAPI, IR, and chlorophyll, to create the composite image. These images were then used to distinguish between organisms with bacteriochlorophyll *a* and chlorophyll *a* (Chl *a*), but also to determine the absolute abundance of AAPs in each sample. The chlorophyll signal was subtracted from the IR image because Chl *a* emits weakly in the infrared region. The relative proportion of AAPs in the total prokaryotic community was also calculated. In addition to abundance, cell size in the samples along the trophic gradient was measured by image analysis of the corresponding DAPI-stained images. AAP cell biovolumes were calculated by approximating the most similar geometric shapes, as recently described (Vrdoljak Tomaš *et al.*, 2019).

Statistical analyzes

Statistical operations were performed using STATISTICA 9.0 and PRIMER 7 (Clarke and Gorley, 2015). A Spearman's rank order correlation analysis was performed to assess the influence of environmental variables on the AAP community. Non-metric Multi-Dimensional Scaling (nMDS) based on Euclidean distance matrices was used to examine differences in AAP abundances along trophic and vertical gradients. nMDS ordination plots were created to represent the samples as points in low dimensional space (2D in the present study) such that the relative distances apart of all points were in the same rank order as the relative dissimilarities of the samples. Thus, points close to each other represented very similar samples. The stress value indicated how accurately the multivariate relationships are represented in the 2D ordination plot; the lower the stress value, the more representative the plot (Clarke and Gorley, 2015).

Having established the partitioning of the entire microbial community, including cytometric populations and AAPs counts, along the trophic gradient, we aim to examine which differences in environmental variables influenced the most these communities in the coastal area and the open sea. To this end, we have used the PERMANOVA+ add-on package (PRIMER 7 software) for CAP (canonical analysis of principal coordinates) based on Euclidean distances of transformed microbial abundance data (Clarke and Gorley, 2006; Anderson *et al.*, 2008). Community abundance matrix is log(X+1)-transformed and Euclidean distances are calculated on log-transformed data. Ecological data are also log(X+1)-transformed except for salinity, which is exponentially transformed due to the left-skewed distribution. Following transformation, normalization and application of missing data tool (algorithm EM, maximum iterations: 1000) is performed. Prior to CAP, we have used the BIOENV procedure (Clarke and Warwick, 2001) to identify the environmental variables that best explain variation in biological communities. BIOENV is a non-parametric method that calculates Spearman's rank correlation coefficients between the matrices of microbial abundances and those of environmental variables based on Euclidean distances. This method then selects the environmental variables that best explain the biological patterns by maximizing the Spearman's rank correlation between the biotic and ecological matrices. On the other hand, CAP is a constrained ordination method that finds an axis through a multivariate community data cloud which maximizes the relationship with a predictor variable, in this case the environmental data. CAP is run on a subset of 4 principal coordinate axes that explain 99.64% of the total variation in communities. The *a priori* hypothesis that different environmental factors shape the coastal and open-sea microbial communities was determined using Spearman's rank correlations.

RESULTS AND DISCUSSION

Spatio-temporal distribution of aerobic anoxygenic phototrophs

The first study of AAPs in the Adriatic Sea referred to their pigment BChl *a* along a latitudinal transect covering the entire Adriatic Sea. By converting pigment concentration to abundance, the authors assumed that AAPs could represent up to 10% of the total prokaryotes (Celussi *et al.*, 2015). Based on the abundance of cells using infrared epifluorescence microscopy, AAPs were described for the first time on a spatial scale in coastal and estuarine (transitional) waters along the eastern Adriatic coast in summer (Šantić *et al.*, 2017). Among karstic estuaries, the highest numbers were measured in the Jadro River, with absolute abundances of 23.59×10^4 cells mL^{-1} and relative abundances of 21.63% of total prokaryotes. Later, their distribution was studied along the trophic gradient from the coast to the open sea and vertically over a year-round period (Vrdoljak Tomaš *et al.*, 2019). Through this study, we gained important insights into their role in the microbial community structure and dynamics. AAPs' relative abundances were higher in April at all stations; nevertheless, these abundances were highest in the estuaries during the period studied, compared to the bay and open ocean areas. In the Krka River estuary, the abundance detected (23.45×10^4 cells mL^{-1}, 23.88% of total prokaryotes) was slightly lower than in the Jadro River (Šantić *et al.*, 2017). In previous studies in the Mediterranean, AAPs accounted for up to 11% of the total prokaryotes (Lami *et al.*, 2009; Lamy *et al.*, 2011a, b; Hojerová *et al.*, 2011; Ferrera *et al*., 2011; 2014), similar to other marine areas (Sieracki *et al*., 2006; Jiao *et al*., 2007). AAPs accounted for up to 34% of prokaryotes in the Delaware estuary (Waidner and Kirchman, 2007), a higher percentage than in the Krka River estuary. Moreover, AAPs were often more abundant in the sea surface microlayer, a boundary layer of ≤1 mm thickness between the ocean and the atmosphere, than in the subsurface, where clearly the highest abundance was recently found in the Adriatic Sea, reaching 55.86×10^4 cells mL^{-1} and the resulting proportion of 29.78% (Vrdoljak Tomaš *et al*., 2022). The vertical distribution of AAPs in the open deep areas of the Adriatic was determined in April (Šantić *et al*., 2019; 2021). They were detected up to 1209 m with abundances of 400 cells mL^{-1}.

In general, AAPs' abundances considerably decrease with increasing depth and towards the open sea (Fig. 2). When all data from the Adriatic are combined, the nMDS ordination plot consistently shows a decrease in abundance across the vertical gradient (Fig. 2A). Due to their phototrophic nature, the AAPs inhabit the euphotic zone. AAP cells are most abundant up to 20 m depth, then decrease, with a minimum at depths greater than 200 m. Consistent with the nutricline and minimum fluorescence layer, the abundance of AAPs began to decline steeply below 200 m depth (Šantić *et al*., 2019). The nMDS plot also shows a clear separation of AAPs along the trophic gradient from the coast to the open Adriatic (Fig. 2B). This observation supports the hypothesis that AAPs prefer coastal, more eutrophic environments, which is in agreement with previous studies (Schwalbach and Fuhrman, 2005; Cottrell *et al*., 2006; Sieracki *et al*., 2006; Lami *et al*., 2007; Hojerová *et al*., 2011; Lamy *et al*., 2011a, b; Ferrera *et al*., 2014). The influx of freshwater from the Adriatic karst rivers and underground springs enriches coastal waters with nutrients and particles (UNEP/MAP, 2003), which drives the picoplankton community (Šolić *et al*., 2015).

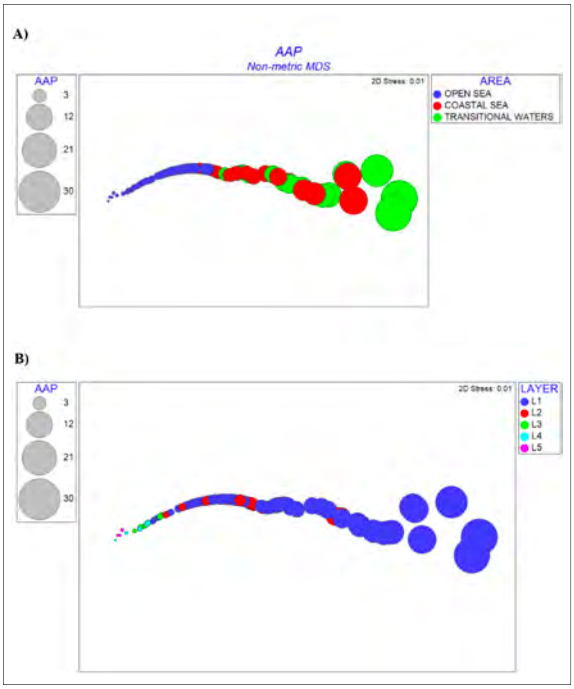

Fig. 2. Two-dimensional nMDS ordination plot comparing abundances of aerobic anoxygenic phototrophs based on Euclidean distances along the vertical gradient (layer (L) 1 0-20m, layer 2 21-50 m, layer 3 51-100 m, layer 4 101-200 m, layer 5 > 200 m) (**A**) and the trophic gradient (**B**).

We have not observed clear seasonality regarding abundances of AAPs in the Adriatic Sea, nonetheless we have noticed higher abundances in the warmer season compared to lower abundances in the colder season, which is consistent with a study in the Mediterranean Sea (Ferrera *et al*., 2014). The most striking seasonal changes were observed in the estuaries and open sea, while no clear seasonality of AAPs was detected in coastal areas, compared to the open sea (Vrdoljak Tomaš *et al*., 2019). A study in the northwestern Mediterranean coast shows that AAPs are highly seasonal and recurrent, but contain ecotypes that exhibit different temporal niche partitioning (Auladell *et al*., 2019). If extreme events due to high nutrient levels are excluded, the values in the Adriatic Sea are within the range observed in other marine environments.

Morphology and biovolume of AAP cells

The morphotypes of AAPs from the Adriatic Sea are shown in Fig. 3. In the studied area, AAPs are mainly represented by short rods and arc-shaped cells at all stations. Filaments were observed mainly at the estuary of the Krka River. Arc-shaped cells, spirilla and short rods were detected at the open sea station, while rods and filaments were found mainly at the stations in the estuary. The AAP cell types are consistent with the study by Sieracki *et al.* (2006). The mean biovolume of AAP cells ranged from 0.001 to 14.73, median 0.09 μm^3 (Vrdoljak Tomaš *et al*., 2019). It is known that the cell size of AAPs is larger than that of average heterotrophic bacteria (Sieracki *et al*., 2006; Lamy *et al*., 2011a; Kirchman *et al*., 2014; Stegman *et al*., 2014; Fauteux

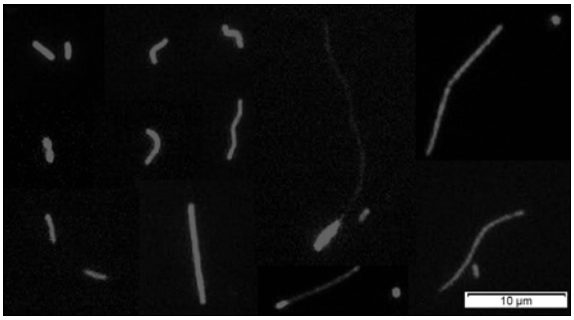

Fig. 3. Various AAP morphotypes observed along the trophic gradient (This figure was published in Deep Sea Research Part II: Topical Studies in Oceanography, 164, Vrdoljak Tomaš et al., Dynamics of Aerobic Anoxygenic Phototrophs along the trophic gradient in the central Adriatic Sea. 112-121, Copyright Elsevier, 2019).

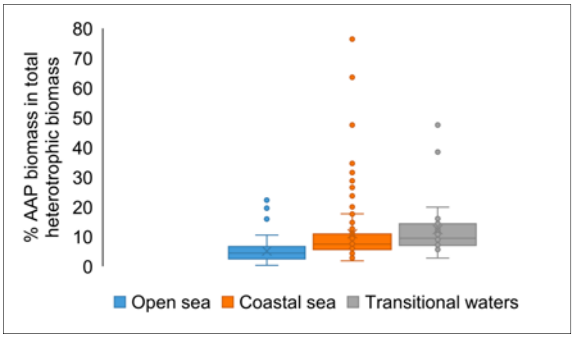

Fig. 4. The portion of aerobic anoxygenic phototrophs in the total heterotrophic biomass along the trophic gradient. The upper and lower quartiles of the data define the top and the bottom of a box plot; the line inside the box represents the median value and the size of the box represents the spread of the central value; dots indicate outliers.

et al., 2015). Our measurements of AAP biovolume showed that they were on average 1.2 times larger than the average heterotrophic bacteria from the Adriatic Sea (Krstulović, 1992). This suggests that AAP bacteria may be more susceptible to top-down control than other bacteria and may be crucial contributors to the recycling of organic matter in marine environments (Koblížek *et al.*, 2007). Ferrera *et al.* (2011, 2017) confirmed the hypothesis that AAPs actively grow under predator free conditions.

We have also calculated the biomass of AAP cells and it ranged from 0.01 to 14.85 $\mu g\ C\ L^{-1}$ (6.27 when excluding maximum value), median 0.49 μgCL^{-1}. If we exclude the maximum value of AAP and HB in the coastal area due to favorable weather conditions, the values agree with previous studies related to different areas of the Adriatic Sea (Šantić *et al.*, 2019; Vrdoljak Tomaš *et al.*, 2019). The highest portion of AAP biomass to total heterotrophic biomass was generally measured in transitional waters, with the highest value in the coastal area of Kaštela Bay (76.38%, median 6.55), decreasing towards the open sea (Fig. 4). The contribution of AAPs to total prokaryotic biomass was significantly higher than their contribution to total abundance because the average biovolume of AAP cells is significantly larger than the biovolume of heterotrophic bacteria.

Influence of environmental factors on aerobic anoxygenic phototrophs

AAP studies have shown that temperature, salinity, day length, chlorophyll concentration, carbon and nitrogen components influence AAP population dynamics and distribution (Mašín *et al.* 2006; Hojerová *et al.*, 2011; Lamy *et al.*, 2011a; Jiao *et al.*, 2007; Zhang and Jiao 2007; Ferrera *et al.*, 2014; Auladell *et al.*, 2019). However, untangling this complex interplay is difficult as it is not clear whether the relationship between AAP and Chl *a* reflects direct association with primary producers for DOC they primarily release or the same dependence on limiting nutrients such as phosphate or nitrogen have a similar dependence on light (Zhang and Jiao, 2007; Ferrera *et al.*, 2011; Koblížek, 2015). By measuring environmental parameters simultaneously with biotic ones, we were able to reveal which environmental variables explain the temporal and spatial variations in the abundance of AAPs in the Adriatic Sea. It seems that AAPs are regulated by several factors, the most important of which in the Adriatic are salinity, Chl *a*, nitrates, ammonium ions, temperature, and soluble reactive phosphorus (SRP) ($r = 0.60$, $r = 0.22$, $r = 0.20$, $r = 0.14$, respectively, $p < 0.05$). Chl *a* followed by nitrates and temperature are the main driving factors in the coastal and transitional waters (Šantić *et al.*, 2017), on the trophic gradient Chl *a*, silicate and *vice versa* are salinity, water column transparency, nitrates, nitrites (Vrdoljak Tomaš *et al.*, 2019) and in the open and deep sea are Chl *a*, nitrates, nitrites, SRP and total dissolved phosphates (Šantić *et al.*, 2019).

AAPs are known to covary with heterotrophic bacteria and correlate with HNF on trophic bases. We have found a correlation between AAP and picoeukaryotes ($r = 0.69$, $p < 0.05$), which are known to prefer areas not limited by nutrients. Other surveys also documented these relationships (Šantić *et al.*, 2019; Vrdoljak Tomaš *et al.*, 2019). In a year-round study in the Adriatic Sea, the seawater column was P-limited during the studied period, except in April, when a more favorable N/P ratio likely resulted in higher AAP numbers, especially in the Krka River estuary (Vrdoljak Tomaš *et al.*, 2019). Recently, the markedly highest abundance in the Adriatic Sea was detected in the sea surface microlayer, following the fire event that enriched this unique niche with ammonium ions ($DIN/PO_4^{3-} = 169$), an optimal food source for direct consumption, and nitrates (Vrdoljak Tomaš *et al.*, 2022). At this time, photoheterotrophic AAPs comprised nearly 30% of the prokaryotic population. In previous investigations of AAPs in mountain lakes and Mediterranean coastal lagoon it was speculated that the capacity to harvest light energy is beneficial in N- and P-depleted environments where it could be utilized in carbon metabolism and potentially also in phosphorus acquisition (Mašín *et al.*, 2008; Lamy *et al.*, 2011b).

To determine association, distinctions were plotted using a canonical analysis of principal coordinates (CAP) from entire microbial community and environmental data (Fig. 5). The results showed a clear spatial separation between the coast and the open sea and we wanted to determine environmental factors influencing the total picoplankton community, particularly AAPs. Thus, the community was significantly shaped by several key physical and chemical variables, which, according to the BIO-ENV procedure, were the subset of salinity, nitrates, and Chl *a* that explained most of the variation in biological communities (q=0.595). CAP1 and CAP2 explained 78.89% of the total variance in the biological dataset in the coastal and open sea. CAP1 is determined by Chl *a* (0.642) and inversely with salin-

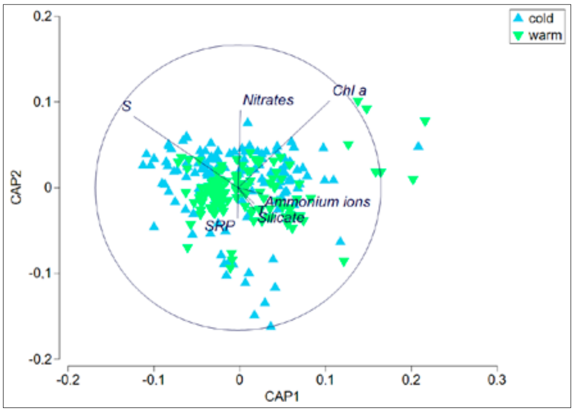

Fig. 5. Canonical analysis of principal coordinates (CAP) of microbial community and environmental parameters in the open and coastal Adriatic Sea using Euclidean distances calculated on log-transformed data.

ity (-0.729), indicating coastal environment. CAP 2 is determined by nitrates (0.542) and inversely by SRP (-0.215).

The frequently observed correlation between AAPs and HB (r = 0.66, or $p < 0.05$) may indicate similar environmental preferences or constraints on the same environmental factors. They were also associated with PE (r = 0.69, respectively, $p < 0.05$) that are known to prefer areas not limited by nutrients, but to a lesser extent with cyanobacteria *Synechococcus* and *Prochlorococcus* (r = 0.44 and r = 0.33, respectively, $p < 0.05$). The high correlation with HNF (r = 0.59, respectively, $p < 0.05$) indicates that AAPs are ideal prey, suggesting that these organisms play an important role in carbon flow to higher trophic levels. A study suggests that by identifying ecologically cohesive clades for AAP bacteria based on marine habitat characteristics, predictions about AAP bacterial communities are possible (Lehours *et al.*, 2018).

Recently, molecular methods were used for the first time to determine bacterial diversity in open areas of the central and southern Adriatic Sea, and the use of Neural Gas showed a heterogeneous response to different environmental factors (Šantić *et al.*, 2021). Moreover, in Kaštela Bay, qualitative annotation by amplicon reads with normalization by the standard internal method was applied for the first time to determine the growth curves of different phylotypes of bacteria and AAPs using the 16S rRNA and *pufM* genes (Fecskeová *et al.*, 2021) and compared them with counts by microscopy and catalyzed reporter deposition - fluorescence in situ hybridization (CARD-FISH).

The new project ADRISAAF focuses on conducting eDNA metabarcoding surveys in our laboratory at spatial and temporal scales based on *pufM* amplicon sequencing to estimate AAP diversity in the central Adriatic Sea. Phylogenetically, AAPs belong to the *Alphaproteobacteria, Betaproteobacteria* and *Gammaproteobacteria*. *PufM*, a marker gene associated with anoxygenic phototrophy, encodes the M subunit of the bacterial type 2-reaction center and is routinely used in studies of AAP diversity (Koblížek, 2015). Preliminary amplicon sequencing results for samples collected in Kaštela Bay in 2020 show the number of AAP species observed (mean 108, SD 2.83) with an average Shannon index of 3.36 (SD 0.075) and an average Pielou evenness of 0.718 (SD 0.024). A manually curated taxonomic database was used for taxonomic assignment (Villena-Alemany *et al.*, 2022). Regarding the relative abundances of a given taxon obtained from Illumina NGS, preliminary results have shown the prevalence of the class *Gammaproteobacteri*a (genus *Group K*) followed by *Alphaproteobacteri*a (family *Rhodobacteraceae*) in the composition of the AAP community in Kaštela Bay. In addition, the application of two combined epifluorescence-based methods, FISH and IR microscopy (FISH-IR), provides quantitative estimates of specific AAP groups representing either *Gammaproteobacteria*, *Alphaproteobacteria*, or *Roseobacter* clades (Kasalický *et al*., 2018). In parallel, we are conducting research on 16S rRNA, which qualitatively annotates prokaryotes, and CARD-FISH, which qualitatively and quantitatively annotates various bacterial groups of interest. ADRISAAF will also contribute to new insights into the flux of AAP biomass through marine food webs which will include controlled experimental studies.

CONCLUSIONS

The overall data set of AAPs from the Adriatic Sea is gathered here to determine spatio-temporal distribution and their environmental drivers. Results support the notion that AAP abundances considerably decrease with increasing depth and toward the open sea. Explaining the temporal dynamics of marine microorganisms is critical for predicting their changing patterns in the light of environmental perturbations. The subset of salinity, Chl *a*, nitrates ammonium, temperature, and soluble reactive phosphorus influenced the entire microbial community as well as the AAPS. This study has contributed to extending the current knowledge of the microbial ecology of aerobic anoxygenic phototrophs in the Adriatic Sea.

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