# Spatial and Temporal Patterns of Picoplankton Community in the Central and Southern Adriatic Sea

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## Contents

**Abstract** Laboratory of Microbiology at the Institute of Oceanography in Split, founded in 1947, covers numerous research in the field of marine microbial ecology. Marine microorganisms, heterotrophic bacteria, cyanobacteria *Prochlorococcus* and *Synechococcus*, heterotrophic nanoflagellates, aerobic anoxygenic phototrophs and viruses, are investigated in terms of structure, abundance, biomass, activity, regulation and production, as well as role of the microbial food web in biogeochemical processes in the sea. To assess the above-mentioned parameters, flow cytometry and infrared epifluorescent microscopy are used. Research is carried out in different marine environments, from coastal areas to open sea representing the trophic gradient, and also at estuarine areas, on different time scales. More recently, various

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grazing experiments are performed to study the bacterial carbon flux through the microbial food web, also in light of the global warming scenario using the experimental impact of temperature increase and phosphate addition on microbial community structure and carbon flux toward higher trophic levels. Understanding the factors driving the picoplankton group distribution and their relative contribution to total picoplankton biomass is essential for understanding the dynamics of the ecosystem. Thus, we present an overview of the results of many surveys on the microbial community in the Central and Southern Adriatic Sea.

**Keywords** Aerobic anoxygenic phototrophs, Autotrophic and heterotrophic picoplankton, Biomass, Flow cytometry, Heterotrophic nanoflagellates, Picoeukaryotes, The Adriatic Sea

## 1 Introduction

Extensive literature is available concerning picoplankton community distribution and dynamics in the Central Adriatic Sea [1–19]. In the last decade, the introduction of cytometry [20] expanded our knowledge with new members of the picoplankton community in water column research. *Prochlorococcus* (Prochl), *Synechococcus* (Syn), picoeukaryotes (PE), and heterotrophic bacteria represent the smallest size class of picoplankton (cells 0.2–<5 μm).

The autotrophic component of the picoplankton community includes cyanobacteria of the genera *Synechococcus* and *Prochlorococcus* and small eukaryotic cells of diverse taxa, picoeukaryotes. These tiny primary producers tend to dominate the photosynthetic biomass and primary production in oligotrophic waters like the Mediterranean Sea [21]. The eukaryotic component of picoplankton, picoeukaryotes, can contribute significantly to biomass and productivity in a wide variety of aquatic environments, even when present at lower abundances than cyanobacteria. This is due to their larger size and higher intracellular chlorophyll *a* (Chl *a*) and carbon content compared to cyanobacteria. Picoeukaryotes, as well as prokaryotic members, are consumed by grazers, thus forming a link to higher trophic levels, which has a variety of implications for the fate of their fixed carbon [22–24]. The importance of picoautotrophs as major contributors to biomass and primary production makes them an essential component for understanding the food web dynamics and the carbon cycle in marine ecosystems [22, 23, 25].

The heterotrophic component of the picoplankton community, heterotrophic bacteria, acts not only as decomposers of organic matter but also as important producers of new biomass. Heterotrophic bacteria often consume 10–50% of total primary production [26, 27], and through grazing by flagellates (mainly heterotrophic nanoflagellates – HNF) and ciliates, their biomass becomes available at higher trophic levels. Therefore, heterotrophic bacteria, as a part of the picoplankton community, undoubtedly play an important role in carbon flow through the marine ecosystem.

Aerobic anoxygenic phototrophs (AAP) are bacterial group recently recorded in the Adriatic Sea [28–31] using infrared epifluorescence microscopy (IREM). These photoheterotrophic microorganisms can harvest light energy using pigment bacteriochlorophyll *a* to supplement their primarily organotrophic metabolism only in the presence of oxygen [32]. AAP play a significant role in the aquatic food web and biogeochemical cycles [33–35]. Because of their metabolic flexibility, fast growth rates, and widespread occurrence, AAP challenge our view of carbon and energy budgets [36–39].

The role of the picoplankton community became more important, as mentioned before in oligotrophic and phosphorus-limited (P-limited) and nitrogen-limited (N-limited) areas, such as the investigated area of the Adriatic Sea [40, 41]. However, previous studies on picoplankton communities were mostly focused on investigating the epipelagic layer (i.e. depths less than 200 m). The deep sea is characterized by the absence of light, i.e. conditions that are unfavourable for the primary production. Tanaka and Rassoulzadegan [42] pointed out the importance of bacteria and their biomass in carbon flux in the deep sea. Moreover, Arístegui et al. [43] have highlighted that the deep ocean represents a key site for remineralisation of organic matter and long-term carbon storage. The discovery of cyanobacteria *Synechococcus* in the deep part of the Adriatic Sea revealed that they could be used to gain a better understanding of the effects of deep-ocean convection, such as ventilation and renewal of deep waters [44]. Hence, the vertical distribution of the picoplankton in the open Southern Adriatic Sea, below the euphotic zone, has recently started to be investigated more intensively [45–48].

This chapter aims to provide an overview of the recent results based on the picoplankton community in the open and coastal areas of the Central and Southern Adriatic Sea.

## 2 Investigated Area: The Adriatic Sea

The Adriatic Sea is an elongated semi-enclosed basin of the Eastern Mediterranean Sea. It can be divided into three sub-basins according to its morphology and bathymetry: the broad Northern Adriatic shelf with an average depth of 40 m, the Central Adriatic with depressions as deep as 280 m that is connected to the Southern Adriatic circular basin over the Palagruža Sill, with a depth reaching ~1,250 m. The Adriatic is characterized by a cyclonic basin-scale circulation. Through the Strait of Otranto at its southern end (~80 km wide, with a sill depth of ~800 m) [49, 50], the Adriatic exchanges water masses with the adjacent Ionian Sea. Waters from the Ionian Sea enrich the P-limited Adriatic Sea [51, 52] with nutrients and organic substances, causing changes in the food web [16] and the distribution of organisms [30, 44, 46, 48, 53]. Moreover, water masses flowing into the Adriatic along its eastern flank have important ecological implications.

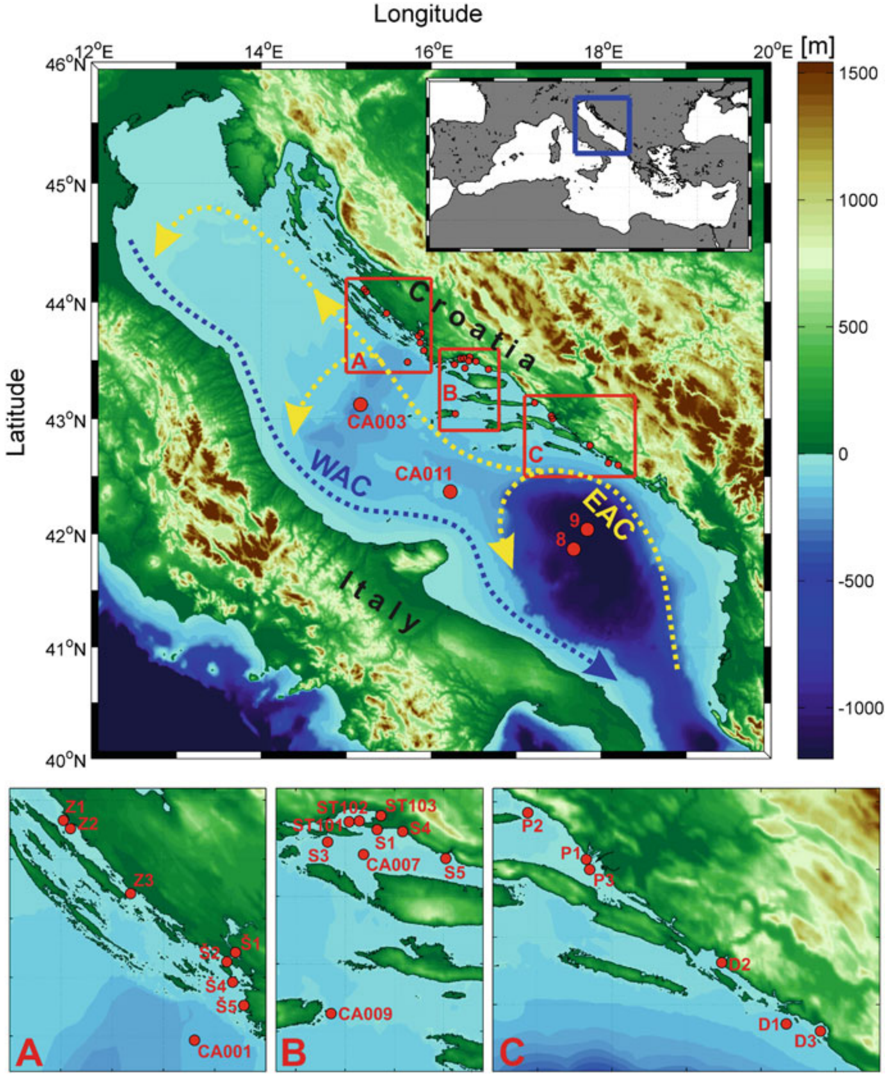

**Fig. 1** Map of Adriatic Sea illustrating water circulation and sampling stations. The yellow line represents the Eastern Adriatic Current (EAC), while blue line represents the Western Adriatic Current (WAC). The figure is made by Hrvoje Mihanović

The coastal area investigated is located in the Central and Southern Adriatic basin, covering the coastal zone from Zadar to Dubrovnik, and is partly under the influence of the karstic rivers: Krka, Jadro, Žrnovnica, Cetina, Neretva, and Trebišnjica (Fig. 1).

## 2.1 Autotrophic Picoplankton

Flow cytometry was used to determine the abundances of *Synechococcus, Prochlorococcus*, and picoeukaryotes [54, 55]. Autotrophic cells were divided into three groups: cyanobacteria (*Synechococcus* and *Prochlorococcus*) and picoeukaryotes, distinguished according to light scattering, cellular chlorophyll content and phycoerythrin-rich cells signals, respectively.

Both cyanobacterial genera were recorded in the water column during our surveys. The abundance of *Synechococcus* and *Prochlorococcus* were determined at 27 stations located along the eastern coast of the Central and Southern Adriatic and in the open area at the Jabuka Pit and the South Adriatic Pit [30, 41, 44, 56, 57]. *Synechococcus* abundance ranged from $10^2$ to $10^5$ cells $mL^{-1}$ in the coastal area and from $10^3$ to $10^4$ cells $mL^{-1}$ in the open sea. *Prochlorococcus* abundance in the coastal area ranged from 0 to $10^4$ cells $mL^{-1}$ and from $10^3$ to $10^4$ cells $mL^{-1}$ in the open sea. The seasonal distribution of both groups mostly showed an increase in abundance during the warmer period and a decrease during winter at the coastal stations but an increase of values during the winter on the open sea sites. Like *Synechococcus*, variations in the abundances of *Prochlorococcus* were more pronounced in the coastal sea areas compared to the open sea area [56]. The highest abundances of both cyanobacteria were found in the areas under the influence of karstic rivers. Generally, *Synechococcus* was found to be more abundant than *Prochlorococcus* in most cases [41, 57, 58], a phenomenon that has already been established for P-depleted environments [59, 60]. Due to the high affinity for inorganic P and higher phosphate uptake rates, *Synechococcus* hold the advantage over the genus *Prochlorococcus* and thrive in P-depleted environments, as reported recently [59, 61].

The study from the six estuaries along the coast, where P-limitation (not nitrogen) was common feature shows high values of autotrophic picoplankton, suggesting the potential importance of the picoplankton community in P-limited estuarine environments [41].

Distribution and abundance of two *Synechococcus* ecotypes, phycocyanin-rich cells (PC-SYN) and phycoerythrin-rich cells (PE-SYN), were studied in the surface layer of the Central Adriatic Sea during the 2015–2016 period [62]. The abundance of PC-SYN ranged from 0 to $\times 10^4$ cell $mL^{-1}$ and that of PE-SYN from $10^3$ to $10^4$ cell $mL^{-1}$. Both ecotypes coexisted in the studied waters with PC-SYN cells dominating during spring and PE-SYN during winter and autumn.

A first seasonal study of picoeukaryotes in the Adriatic (Fig. 2) showed that their biomass reached 21.85 $\mu gCL^{-1}$ with a clear trend of biomass decrease towards the open sea [58]. Furthermore, the general domination of picoeukaryotes within the picoautotrophic biomass along P-limited coastal estuaries was obtained [41, 57]. Picoeukaryotes also exhibited a strong seasonal pattern with noteworthy higher biomass values during the winter. During the investigated period, their biomass was notably higher than the biomass of heterotrophic bacteria, especially in the coastal area. The winter "bloom" of picoeukaryotes coincided with high nitrate

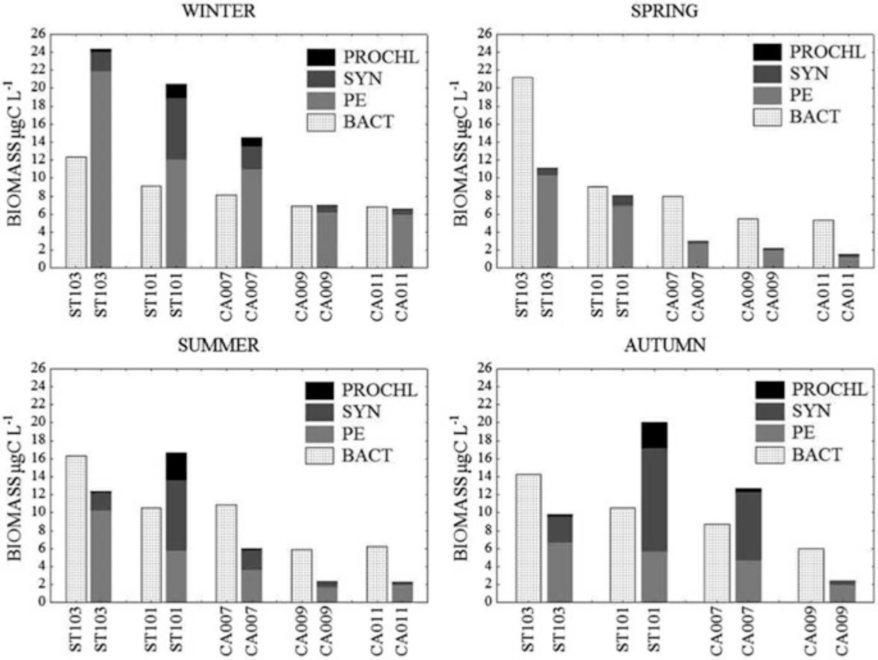

**Fig. 2** Biomass of different groups of picoplankton along the trophic gradient during four different seasons: total heterotrophic bacteria (BACT) and picoeukaryotes (PE); *Synechococcus* (SYN) and *Prochlorococcus* (PROCHL) [58]

concentration in the water column, especially in the coastal area. This finding is consistent with the fact that picoeukaryotes are highly successful in environments with elevated nitrate levels [63–65] since larger cells and autotrophs have a stronger response than heterotrophs to high nutrient availability [66].

## *2.2 Heterotrophic Bacteria and Aerobic Anoxygenic Phototrophs*

Bacterial abundance was determined in scatter plots of particle side scatter versus Sybr Green I fluorescence related to cellular nucleic acid content, to discriminate bacteria from other particles [54]. According to the cellular nucleic acid content, the bacterial population is divided into two sub-groups, HNA (high nucleic acid content) and LNA (low nucleic acid content) bacteria. The average monthly abundance of bacteria, obtained as the average value from the surface to the bottom layers of the investigated stations in the coastal area, ranged from $10^5$ to $10^6$ cells $mL^{-1}$, while at the open sea area ranged from $10^4$ to $10^5$ cells $mL^{-1}$ [30, 58, 67]. The seasonal distribution of bacteria in the coastal areas has shown an increased abundance during

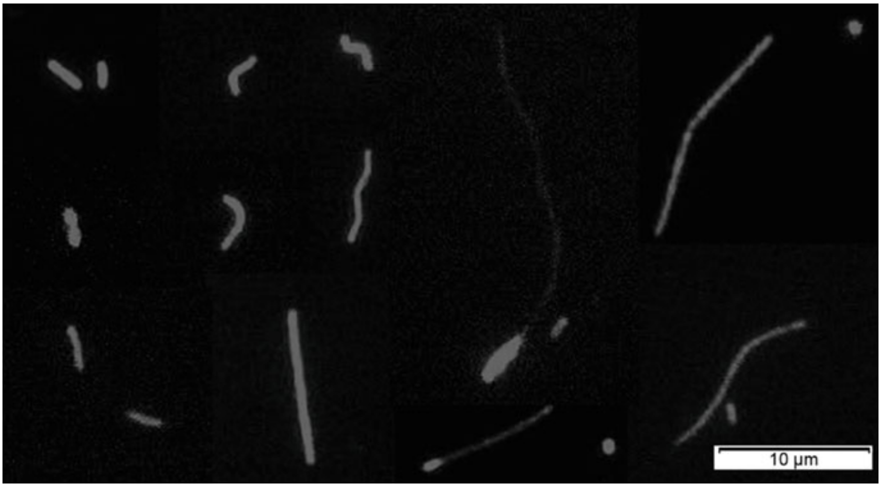

**Fig. 3** Various AAP morphotypes observed along the trophic gradient [31]

the warmer seasons and lower abundance during the colder seasons, with pronounced variations in bacterial densities concerning the open sea area [67]. Comparing all investigated areas, the highest numbers of bacteria were found in the area under the influence of the karstic river [67].

The average monthly percentage of HNA bacteria in the coastal areas ranged from 26 to 83% [57, 67]. Different temporal patterns were found for HNA and therefore for LNA bacterial groups. Seasonal distribution mostly showed the prevalence of the HNA group during the colder seasons and prevalence of LNA bacteria, or equal proportions of the LNA and HNA groups, in the bacterial community during spring and summer. However, a prevalence of the HNA group in the bacterial community during all four seasons was found in the coastal areas directly influenced by rivers [57, 67]. Comparing the investigated areas with the annual average, variations in the proportion of HNA bacteria were mostly more pronounced in the coastal sea areas compared with the open sea area. The average monthly percentage of HNA bacteria in the open sea ranged from 28 to 65% and showed the prevalence of LNA bacteria in the epipelagic layer and dominance of HNA in deep waters [30, 57, 67].

AAP were determined using the protocol described by Mašín et al. [68] (Fig. 3). Three epifluorescent filter sets were used: DAPI, IR, and chlorophyll to create the composite image. These images were subsequently used for distinguishing between organisms that contain bacteriochlorophyll *a* and Chl *a* but also for determining the number of heterotrophic bacteria, cyanobacteria and AAP bacteria in each sample. Chl *a* signal was subtracted from the IR image, due to its week emission tail in the IR area. The relative contribution of AAP to the total prokaryotic community was calculated. In addition, cell size was measured using image analysis from the corresponding DAPI-stained images to compare with total bacteria.

AAP counts along the Eastern Adriatic Sea ranged from $10^3$ to $10^5$ cells mL$^{-1}$ [29, 31] and from $10^2$ to $10^3$ cells mL$^{-1}$ in the deep waters of the Southern Adriatic Sea [30]. The proportion of AAP abundances in total prokaryotes ranged from 1.13 to 23.88% along the coastal areas of the Central and Southern Adriatic [29, 31], whereas in the deep Southern Adriatic proportions ranged from 0.65 to 2.48%, respectively [30]. The highest abundances were measured in the estuarine environments of the karstic rivers Krka and Jadro [29, 31]. The counts decreased from coastal eutrophic to the open sea oligotrophic areas and after 70 m depth [30, 31]. Counts from the Adriatic Sea are in the upper range of AAP abundances observed in marine environments, more comparable to the estuarine areas [69, 70] and lakes [71]. The abundance of AAP in the Mediterranean Sea has been recorded in several studies [35, 37, 72–75], with abundances ranging from $10^3$ to $10^5$ cells mL$^{-1}$ and portions in total prokaryotes from 1 to 11%. Seasonal changes with higher values in the warmer seasons were observed in the estuarine and open Adriatic Sea, and not in the coastal and channel station [31]. In the Mediterranean, AAP cell numbers were low in winter and high in spring and summer [73, 75].

To examine bacterial activity, our study [67] observed bacterial productivity concerning the distribution of the HNA and LNA bacterial groups. The analysis of these two bacterial groups concerning bacterial productivity in the coastal area showed a simultaneous increase in the percentage of the LNA group and bacterial production. However, exceptions with a prevalence of the HNA group in the bacterial community during the highest values of bacterial productivity were found in those areas influenced by the Krka and Jadro rivers. The analysis of HNA and LNA groups concerning bacterial productivity at the open sea stations showed a simultaneous increase in the percentage of the LNA group and bacterial production. Our result indicates the importance of both LNA and HNA bacterial groups in bacterial activity [67]. Higher values of bacterial production and domination of HNA bacteria were found in deep waters, suggesting that bacteria can have an active role in the deep-sea environment [30].

Microbial transformations of toxic monomethyl mercury and dissolved gaseous mercury at the lower levels of the marine food web are not well understood, especially in oligotrophic and P-limited seas. Recent research shows that the heterotrophic activity of LNA bacteria (abundant in oligotrophic regions) seems to be responsible for most of Hg methylation under P-limitation [76].

## 2.3 *Biomass Distribution from the Coast Towards the Open Sea*

The biomass of studied picoplankton groups was estimated using the following cell-to-carbon conversion factors: 20 fgC cell$^{-1}$ for heterotrophic bacteria [77, 78], 36 fgC cell$^{-1}$ for *Prochlorococcus* [79], 255 fgC cell$^{-1}$ for *Synechococcus* [79], 2,590 fgC cell$^{-1}$ for picoeukaryotes [79], and 2,659 fgC cell$^{-1}$ for AAP [31]. We observed the trend of biomass decrease from the coast towards the open sea for all members of the picoplankton community (Fig. 2). This pattern has already been described by many authors that conducted their research in this area [10, 11, 16, 17, 19, 57, 67] but also the Mediterranean [80] and the Pacific [25]. The same pattern of distribution (increasing from oligo- to eutrophic conditions) of both cyanobacterial groups is a feature not commonly found in marine environments. *Prochlorococcus* typically shows an opposite pattern compared to the distribution of *Synechococcus* along the trophic gradient and usually becomes a less important component of the picoplankton community from oligo- to eutrophic conditions [23, 81, 82]. In the Central Adriatic Sea, however, its contribution to picoplanktonic biomass is much larger in coastal eutrophic waters. This is a characteristic that is typical of the Central Adriatic [56, 57, 63] given that, in the Northern and Southern Adriatic, cyanobacteria are distributed uniformly along the trophic gradient [66]. The average annual picoeukaryotic biomass of 9.77 $\mu gCL^{-1}$ [58] is consistent with values for the North Temperate Zone [79], showing their greatest contribution to picoautotrophic biomass than either or both genera of cyanobacteria, especially in the coastal zone. The importance of picoeukaryotes has also been recorded for the Northern Adriatic [65] and other coastal areas [25, 79, 83, 84]. Their higher biomass in coastal waters than in the oligotrophic open sea is governed by their preference for the less stable water column and shallower nutricline, which allows the injection of nutrients into the surface, thus promoting their growth [23, 64].

Our result from coastal toward open sea survey showed that the annual biomass ratio of bacterial to autotrophic picoplankton was on average $>1$, which is consistent with the survey carried out in the Bay of Biscay [82] and in oligotrophic regions with low chlorophyll levels [85]. The ratio was higher during warmer seasons in oligotrophic waters stations, while values $<1$ were recorded during the winter and at coastal sites. This is since bacterial biomass tends to increase more slowly than phytoplankton biomass along the trophic gradient [86, 87]. These results show that within the picoplankton community, the autotrophic part makes a greater contribution to total picoplankton biomass in mesotrophic or relatively eutrophic areas, while heterotrophic bacteria become more important under oligotrophic conditions by contributing to the carbon cycle through the "microbial loop" [88]. Nevertheless, our recent research highlighted the domination biomass of picoautotrophs over heterotrophic at open sea area through the water column. The autotrophic biomass (an average of 13.6 $\mu gCL^{-1}$) was almost six times higher than heterotrophic (an average of 2.29 $\mu gCL^{-1}$), with the domination of *Synechococcus* (Fig. 4). Vertical distribution revealed the prevalence of autotrophic biomass over heterotrophic in the epipelagic layer but also deep waters [30].

AAP biomass was estimated from measured cells and the values ranged from 0.07 to 6.24 $\mu g\ C\ L^{-1}$ [31]. The proportion of AAP in picoplankton biomass ranged from 1.15 to 39.49% in the coastal areas and from 0.37 to 4.09% in the deep Adriatic Sea, respectively [30, 31]. AAP biomass displayed the highest values during summer in the estuarine areas and the lowest during winter in the open sea. We observed a trend of decreasing biomass of the AAP from eutrophic coastal to the oligotrophic open sea area [31]. The contribution of AAP to the total prokaryotic biomass was

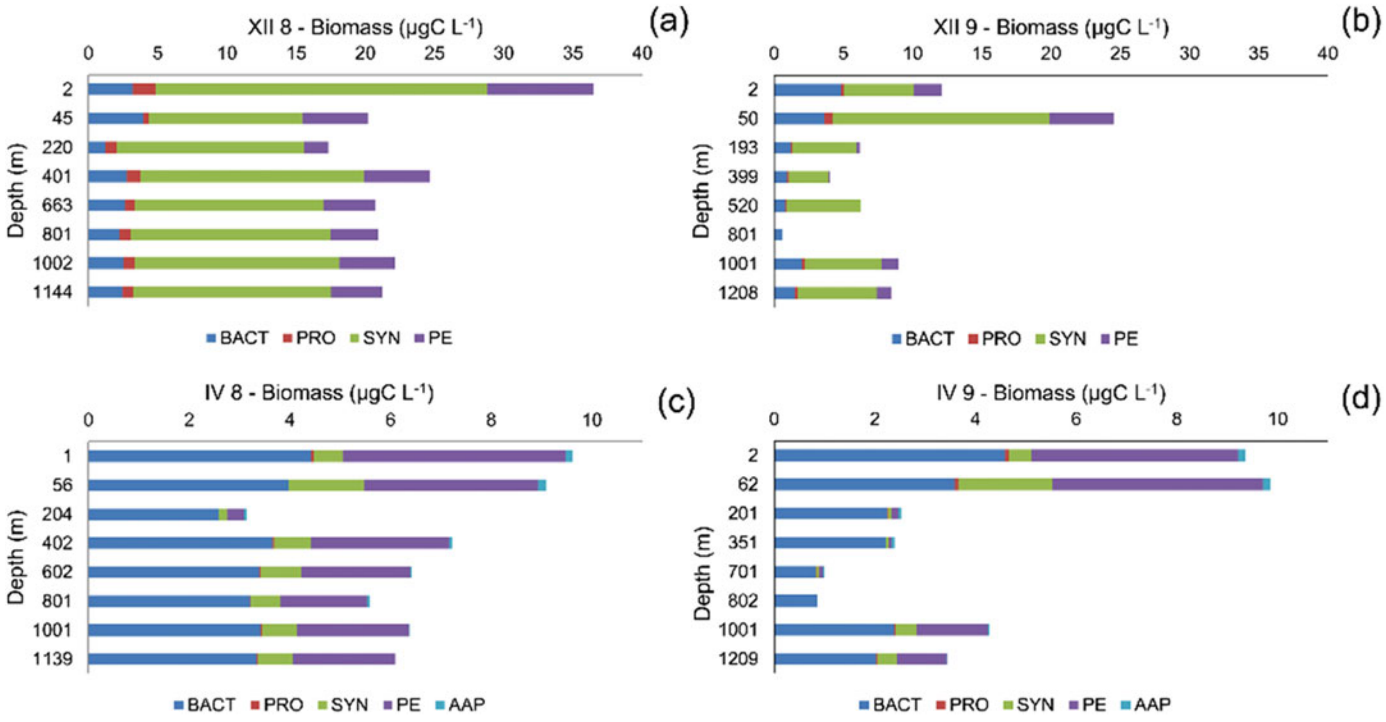

**Fig. 4** Vertical profiles of biomass for heterotrophic bacteria (BACT), *Prochlorococcus* (PRO), *Synechococcus* (SYN), picoeukaryotes (PE), aerobic anoxygenic phototrophs (AAP) at stations 8 (**a**) and 9 (**b**) in December 2015 and at stations 8 (**c**) and 9 (**d**) in April 2016 [30]

significantly higher than their contribution to the total abundance since an average biovolume of AAP cell is larger than of heterotrophic bacteria [31]. Accordingly, the role of AAP in the Adriatic Sea is reflected through their biomass.

# 3 Ecological Factors Affecting the Picoplankton Community

## *3.1 Salinity*

The only environmental factor that showed a significant correlation with all picoplanktonic groups during periods of thermohaline stratification and under the homogenous water column was salinity [57]. Negative relationships between biomass and salinity for all four picoplanktonic groups (Prochl, Syn, PE, HB), such as the ones found here, have already been observed along a marked salinity gradient for salinities higher than 23.5 [25, 89], although this is not always the case. The results show that salinity is an important parameter describing the habitat of the picoplanktonic community in the Central Adriatic, as well as for AAP [29, 31].

## *3.2 Nutrients*

Available nutrients (nitrates, nitrites, ammonium ion, soluble reactive phosphorus, silicates, organic-P, organic-N) are detected as important drivers of changes in the picoplankton community, both seasonally and spatially. Our results revealed that the highest values of all observed biological parameters exhibited the highest values in the coastal areas under the influence of karstic rivers that enriched the environment mostly with N-compounds. Detailed, the abundance of *Prochlorococcus* was influenced by nutrient availability and the movement of water masses more than by HNF [56]. PC-SYN showed a significant strong positive response to nitrogen nutrients, whereas PE-SYN positively responded to phosphate availability. The relative ratio of phosphorus availability and total inorganic nitrogen (N/P ratio) affects the spatial distribution of the two *Synechococcus* ecotypes [62].

Significant positive relationships between bacterial biomass and bacterial production as well as between bacterial parameters and Chl *a* during the stratified period [57] indicate that the ecosystem responds to higher substrate supply by accumulating bacterial biomass, which is consistent with [90]. The results indicate that the bacterial population could be bottom-up controlled during warm periods, which is in agreement with other seasonal studies (e.g. [90]), but contrary to previous investigations conducted in the Central Adriatic [17] where bottom-up control was dominant during the colder period. Two bacterial subpopulations responded differently to Chl *a* in different temperature regimes. HNA bacterial abundance was significantly correlated with Chl *a* during both periods with similar correlation coefficients. However, LNA yielded a stronger correlation with Chl *a* during the warm period and a rather weak one during cold months [57]. High values of HNA from the winter to the early spring and its stronger dependence on chlorophyll concentrations would reflect the direct dependence of HNA cells on dissolved primary production, as suggested by Scharek & Latasa [91] and Moran et al. [90]. LNA domination during the warmer period when dissolved nutrients are scarce as well as in the oligotrophic open sea reflects their successful adaptation to nutrient-poor conditions [92, 93] when the "microbial loop" in the marine ecosystem and regeneration processes become dominant.

AAP in the Adriatic were mainly influenced by Chl *a* and transparency of the water column [29, 31]. The strong correlation between AAP and Chl *a* is an already well-established feature [7, 94–96]. AAP thrive better in more eutrophic environments [37, 75], and in the Adriatic they prefer estuarine areas [29, 31]. Our data from the annual study highlighted P-limitation in the environment as an important factor for AAP growth [31].

## 3.3 Water Mass Movement

The movement of water masses is an extremely important environmental factor. Because, in addition to affecting the concentration of nutrients and salinity in the environment, it physically displaces the planktonic community by its motion and affects its horizontal and vertical distribution. Thus, autotrophic cells are found deep within the mesopelagic layer [30, 44]. So from time to time, water mass movement brings a high portion of available carbon in form of picoplankton biomass [30] and also influences which food web will be dominant in the environment [97].

## 3.4 Predation

Abundances of Sybr Green-I-stained HNF were also determined by cytometry [98]. An empirical model was used to examine the regulation of bacteria by predation [99]. Data close to the MAA line thus suggest a strong coupling between the bacteria and HNF abundance, likely interpreted as strong predation on the bacteria [99]. Data positioned below the MRA line instead suggest that bacterial abundance is not controlled by HNF grazing.

Our results showed that the increase in bacterial abundance (Fig. 5) and cell production supports the increase in the number of HNF, especially in the epipelagic layer [30]. It reveals that bacteria constitute a potential food resource for the nanoflagellate community and suggests a strong top-down control of bacteria. These results confirm previous findings showing [100, 101] that predators prefer

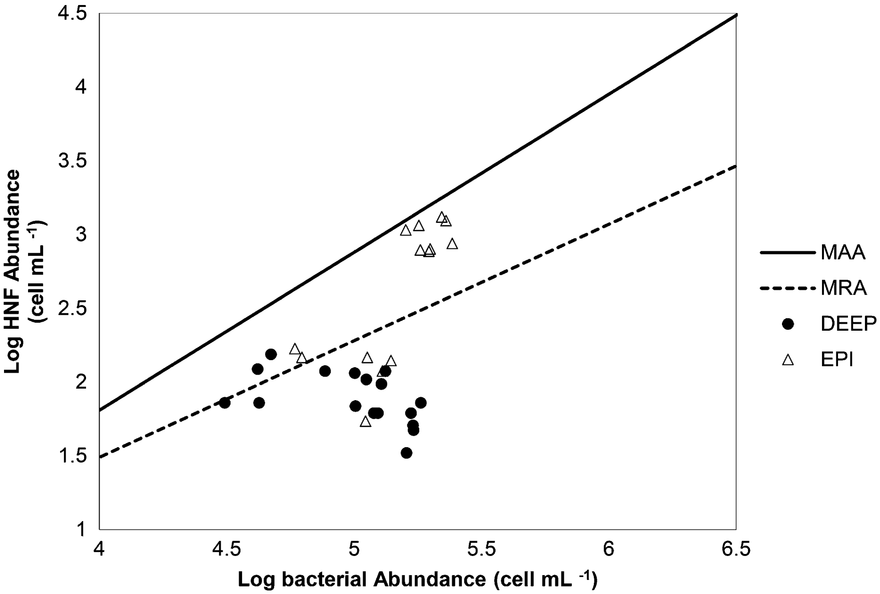

**Fig. 5** Relationship between bacterial and HNF abundance at study stations, plotted in a theoretical model [99] (*MAA* maximum attainable abundance, *MRA* mean realised abundance) in epipelagic (epi-) and deep (deep-) water layers [30]

active bacteria and remove bacterial production, and they also can control the abundance of the bacterial community in surface waters [99].

AAP are under intense "top-down" regulation [34, 35, 74, 102, 103]. As AAP cells in the Adriatic are 1.2 times larger than the average heterotrophic bacteria [31], they could represent an important prey for HNF, thus transferring their biomass to the higher trophic levels.

Our previous research showed that besides HNF viruses had a significant influence on bacterial mortality through high daily removal of the bacterial standing stock at the coastal and open-sea stations [104].

## *3.5 Temperature*

Besides the negative impact of salinity on all biological variables tested, the temperature is shown as a factor with a significant impact on investigated members. Generally, in our research all the observed picoplankton groups showed seasonality, the effect more pronounced in the coastal areas. The abundance of *Synechococcus* was influenced more by temperature than nutrient availability, especially during the isothermal period [56, 57]. Furthermore, PC-SYN showed a significant positive relationship with temperature [62]. The latest research sheds light on the role of

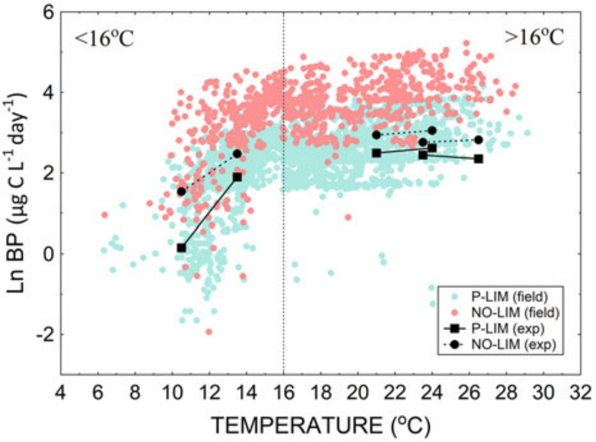

**Fig. 6** Bacterial production data obtained from manipulative laboratory experiments (black symbols) superimposed on field data divided concerning temperature (<16°C and >16°C) and concerning phosphorus limitation (limited – LIM and not limited – NOT-LIM) [106]

temperature rise in the global warming scenario (Fig. 6). The rise in temperature was associated with the increasing importance of microbial heterotrophic activities (increase bacterial growth and bacterial predator abundance, particularly HNF) and the increasing importance of autotrophic picoplankton in the microbial food web [100]. Detailed, an increase of the picoplankton carbon flux towards higher trophic levels can be expected in the Adriatic Sea, under the influence of temperature increase, particularly under phosphorus unlimited conditions [105, 106].

## 4 Anthropogenic Pollutants

Anthropogenic pollutants (e.g. trace metals and persistent organic pollutants) in the marine environment affect marine life from primary producers to fish. Contaminants such as trace metals and persistent organic pollutants reach the marine environment from numerous anthropogenic sources, e.g. industrial and urban wastewaters, agricultural runoff, mining wastewaters, shipping discharges, and oil spill accidents, are major sources of pollutants. Pollution may affect the abundance, growth, dominance, and succession patterns of phytoplankton forming the base of the aquatic food webs [107–112]. Moreover, certain contaminants may accumulate in phytoplankton and biomagnify from the bottom to the top of the food chain [111]. Cell size and high surface-to-volume ratio make picoplankton especially vulnerable to pollutants since the pollutant uptake is higher in smaller cells [113, 114].

The ecotoxicological effects of contaminants on picoplankton communities in the water column haven't been completely elucidated. A limited number of studies have explored the effects of exposure to a mixture of inorganic and organic contaminants in oligotrophic marine environments. These studies indicate that trace metals and organic contaminants in the marine environment impact primary production and entire picoplankton community structure (abundance, biomass, and composition) [107, 115, 116].

To our knowledge, no studies have been focusing on interactions between contaminants and picoplankton in the water column in the Eastern Adriatic. However, studies investigating complex interactions between contaminants and picoplankton in other oligotrophic regions of the Mediterranean and world's oceans indicate that the presence of multiple contaminants may exert additive, synergistic or antagonistic effects [107, 108, 112, 117].

Picoplankton responses induced by exposure to contaminants depend on contaminant bioavailability and species interactions, seawater chemistry, taxonomic differences, the cell size of picoplankton populations, grazing pressure, exposure to light, and contamination exposure history [116, 118–122]. Sensitivity and tolerance of picoplankton communities exposed to various contaminants are most likely dependent on several factors: nutrient and organic matter concentrations in seawater, the cell size of phytoplankton populations, and adapting capacity of plankton communities to contaminant exposure [116, 123]. Nutrients and DOM affect the bioavailability of certain contaminants, whereas cell size and surface-to-volume ratio affect the uptake rate of contaminants. Therefore, differences in bioavailability and contaminant uptake rates may explain the differences in sensitivity of picoplankton populations to contaminant exposure. Studies investigating the effects of chronic exposure to pollutants indicate that phytoplankton populations may develop adaptive mechanisms to cope with environmental pressures [115, 124]. Picoplankton communities may naturally evolve and acquire higher resistance to pollutants. Natural selection of more tolerant species increases the overall resistance of the population and ensures its survival in a polluted environment.

## 5 Conclusions

In the last decade, we significantly extended our research of the picoplankton community after introducing flow cytometry and IREM. We can conclude that autotrophic, heterotrophic, and aerobic anoxygenic phototrophic members are affected by abiotic (light, salinity, temperature, nutrients, physical forces) and biotic factors (grazing, viral lysis). We determined the distribution of *Synechococcus*, *Prochlorococcus*, picoeukaryotes, heterotrophic bacteria, and aerobic anoxygenic phototrophs and estimated their contribution to carbon budget from epipelagic to deep waters.

In conclusion, the picoplankton community is exposed to sudden physical-chemical changes in a dynamic environment of the Adriatic Sea, and their ability to physiologically acclimate to different environmental conditions determines their presence and activity throughout the water column.

Currently, in our laboratory, we started using molecular methods, 16S and *pufM* sequencing, a proxy for aerobic anoxygenic phototrophs, and CARD-FISH, to determine which taxa contribute most to the microbial community in the Adriatic Sea.

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