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Distribution of aerobic anoxygenic phototrophs in the Eastern Adriatic Sea

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ABSTRACT

The spatial patterns of aerobic anoxygenic phototrophs abundances were investigated, for the first time, in the Adriatic Sea. Also, the spatial patterns of the whole picoplankton community as well as the environmental factors that potentially influence these patterns were highlighted. AAP abundances was in average $66.9 \pm 66.8 \times 10^3$ cell mL^{-1}, and their proportion in total bacteria was $7.3 \pm 4.3\%$. These values are in the upper range of AAP abundances observed in marine environments. Multivariate analyses proved that environmental factors influenced the picoplankton community interdependently. Chl *a* was the main driving factor for the picoplankton community, accounting for 33.3% of picoplankton community variance, followed by NO_2^- (17.9% of variance explained) and temperature (14.2% of variance explained). Chl *a* showed stronger correlation with AAPs, non-pigmented bacteria and Picoeucaryotes than with cyanobacteria. Abundance of cyanobacteria was stronger correlated to salinity and the N:P ratio than to nutrient concentrations.

1. Introduction

Marine picoplankton encompass the smallest (<2 μm) marine organisms such as cyanobacteria, picoeukaryotes and non-pigmented bacteria. Marine picocyanobacteria are major contributors of biomass and primary production thus giving them an important role in food web dynamics and the carbon cycle in marine ecosystems (Li, 1994; Partensky et al., 1996; Grob et al., 2007). About 40 years ago, a novel bacterial functional group with small amounts of bacteriochlorophyll (BChl) *a* was discovered in Tokyo Bay, Japan (Harashima et al., 1978; Shiba et al., 1979). This group of bacteria, the aerobic anoxygenic phototrophs (AAPs), was found to account for a significant fraction of the microbial communities in marine environments (Kolber et al., 2001; Cottrell et al., 2006; Mašín et al., 2006; Sieracki et al., 2006; Lami et al., 2007; Michelou et al., 2007; Zhang and Jiao, 2007; Salka et al., 2008; Cottrell et al., 2010; Boeuf et al., 2013). In contrast to most anoxygenic phototrophs, AAPs photosynthesize and grow only in the presence of oxygen (Biebl and Wagner-Döbler, 2006; Yurkov and Csotonyi, 2009; Koblížek et al., 2010). Their primary metabolism is mostly heterotrophic (Harashima et al., 1987) but they can additionally satisfy their energy needs by utilizing the light harvesting pigment BChl *a* (Yurkov and van Gemerden, 1993; Fuchs et al., 2007; Hauruseu and Koblížek, 2012).

AAP has been a major topic in aquatic microbiology in the last two decades. Recent studies report on their unique role in ocean carbon cycling (Kolber et al., 2001; Jiao et al., 2003; Koblížek et al., 2007). AAPs questioned the classical view that marine bacteria are solely heterotrophic organisms fully dependant on recycled dissolved organic matter produced by photoautotrophic phytoplankton. Their ability to obtain energy by harvesting light and consuming organic substrates has led to considering their potential competitive advantage over strict heterotrophs in aquatic ecosystems (Ferrera et al., 2017).

A large number of ecological studies on AAPs have been carried out in open ocean waters, such as the Pacific (Cottrell et al., 2006; Lami et al., 2007; Ritchie and Johnson, 2012) and the Atlantic

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(Sieracki et al., 2006; Koblížek et al., 2007; Michelou et al., 2007). AAPs have also been investigated in enclosed seas, such as the Black Sea (Koblížek et al., 2006), the Baltic Sea (Koblížek et al., 2005; Salka et al., 2008) and the Mediterranean Sea (Lami et al., 2009; Ferrera et al., 2011; Hojerová et al., 2011; Lamy et al., 2011a, 2011b).

The picoplankton community structure in the Adriatic Sea has been studied intensively over the past few decades (Vilibić and Šantić, 2008; Šolić et al., 2008, 2009, 2010, 2015; Šilović et al., 2011; Šantić et al., 2011, 2012; Šestanović et al., 2015). However, the data on AAP presence and distribution patterns is scarce. So far, to our knowledge, only one study has explored the presence of AAP in the Adriatic Sea based on the concentration of BChl *a* (Celussi et al., 2015). Therefore, this survey is performed in order to determine AAPs abundances. We aimed to investigate the spatial patterns in their abundances in two different water types (coastal and transitional waters) along the eastern Adriatic coast. In addition, we highlighted the spatial patterns of the whole picoplankton community as well as the environmental factors that potentially influence these patterns. The results presented in this study constitute the first direct quantification of AAP abundances in the Adriatic Sea.

2. Materials and methods

2.1. Study area

Sampling was performed along the eastern Adriatic coast (Fig. 1) on board the RV Bios 2 in August 2015.

The samples were collected at 24 stations. Six were chosen in transitional waters (stations B1, B2, C1, C2, D1 and E1) and 18 were in coastal waters. Transitional and coastal water definitions correspond to the Water Framework Directive 2000/60/EC definition of waters. Transitional waters represent estuaries or mouths of Croatian rivers Krka, Jadro, Neretva and Ombla. They are characterized by shallow depths, high phosphate and low salinity values due to the substantial influence of freshwater flows. The largest variations in basic hydrographic parameters are found in the surface layer of a particular river (Šolić et al., 2015). Coastal waters are located further towards the open sea and have the typical biogeochemical features of marine waters.

2.2. Environmental parameters

Water samples were collected from the surface at all stations using 5 L Niskin bottles. A Seabird 25 CTD profiler recorded temperature and salinity data. Dissolved oxygen was determined by Winkler titration (Strickland and Parsons, 1972). Dissolved inorganic nutrient concentrations (nitrates, nitrites, ammonia and soluble reactive phosphorus) were determined colorimetrically using the auto-analyser modified method by Grasshof (1976). Chlorophyll *a* (Chl *a*) was determined in 500 mL samples that were filtered through Whatman GF/F glass-fibre filters and stored at −20 °C. These were homogenized and extracted in 90% acetone. Samples were analysed using a Turner TD-700 Laboratory Fluorometer calibrated with pure Chl *a* standards (Strickland and Parsons, 1972).

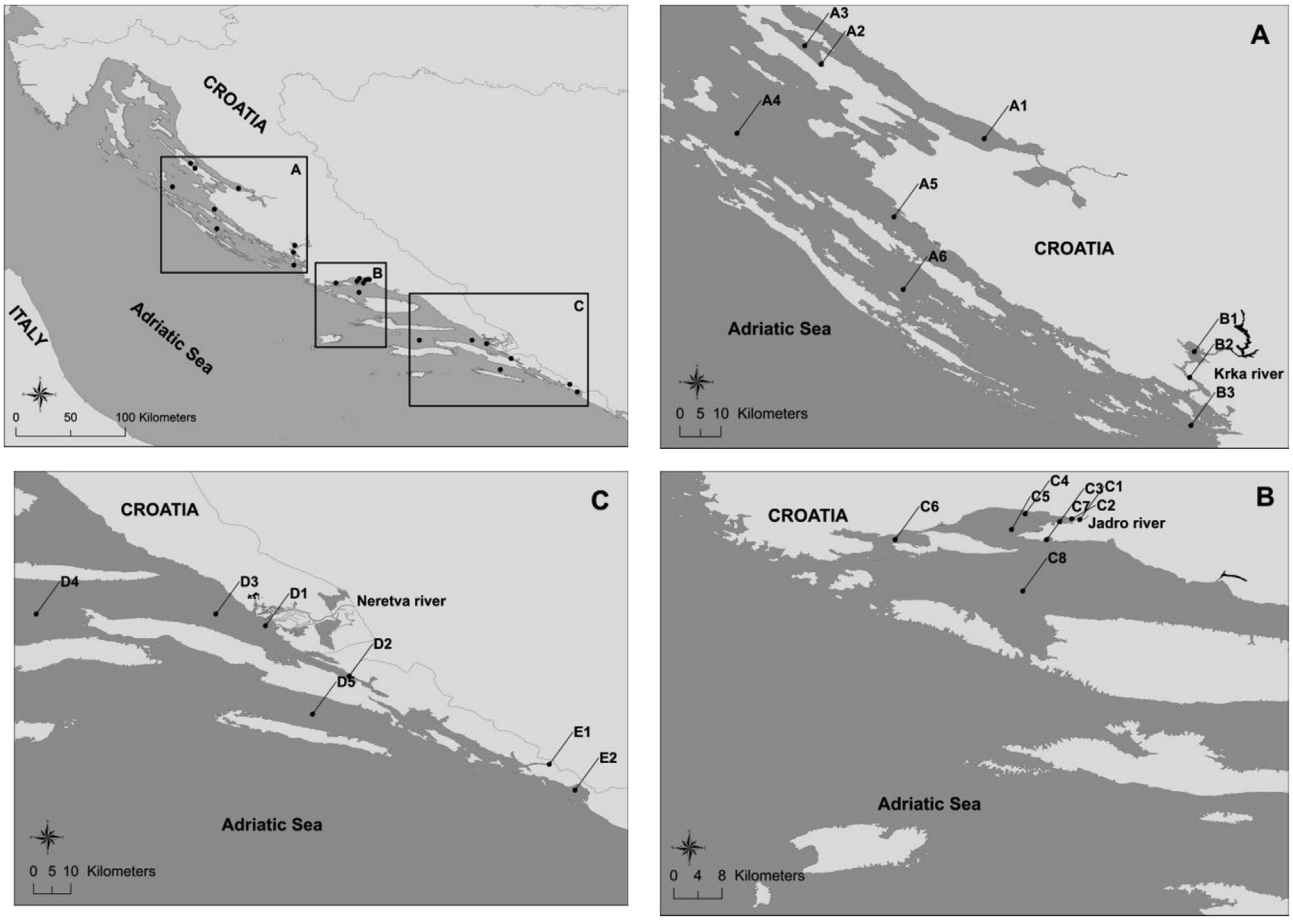

Fig. 1. Sampling stations in the Eastern Adriatic Sea.

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