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ORIGINAL ARTICLE

Spatial and seasonal distribution of particulate phosphorus in coastal and offshore waters of the middle Adriatic Sea

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Abstract: The seasonal and spatial distributions of particulate phosphorus (PP) in the water column of the coastal area and open sea of the middle Adriatic Sea area were investigated for the first time. The relationship between PP and two other important forms of phosphorus (dissolved inorganic phosphorus (DIP), and organic phosphorus (P-ORG)), and the relationship between PP and other nutrients (nitrogen and silicon salts) and oceanographic parameters (temperature, salinity, oxygen saturation, chlorophyll *a*) from the water column were also examined. PP concentrations ranged from 0.007 µmol dm^{-3} in the open sea near Palagruža archipelago to 0.589 µmol dm^{-3} in the Krka River estuary. In the coastal area, PP concentrations were highest in autumn and lowest in winter. The vertical distribution of PP concentrations in the water column of the coastal area were highest in the surface layer during most of the year due to input of PP by freshwater inflows. In the open sea area, there were no significant seasonal or vertical differences in the distribution of PP concentrations in the water column. Seasonal analysis of the total phosphorus pool (PP, DIP and P-ORG) in the water column indicated that PP and P-ORG concentrations were higher in the coastal area compared to the open sea, while DIP concentrations were comparable. The results further demonstrate that PP can be an important fraction of the total phosphorus pool. A significant positive correlation was found between PP and chlorophyll *a*, indicating that PP was mainly of biological origin, and with nutrient salts of nitrogen and silicon, while a negative correlation was found with salinity.
Keywords: phosphorus; particulate phosphorus; nutrients; Adriatic Sea; particulate matter; water column

Sažetak: PROSTORNA I SEZONSKA RASPODJELA PARTIKULARNOG FOSFORA U OBALNIM VODAMA I OTVORENOM MORU SREDNJEG JADRANA. Sezonska i prostorna raspodjela partikularnog fosfora (PP) istraživana je u vodenom stupcu obalnog i otvorenog mora srednjeg Jadrana po prvi put. Istražen je i odnos PP-a s drugim važnim oblicima fosfora (otopljeni anorganski fosfor (DIP) i organski fosfor (P-ORG)), kao i odnos između PP-a i ostalih nutrijenata (soli dušika i silicija) i oceanografskih parametara (temperatura, salinitet, zasićenost kisikom, klorofil *a*) iz vodenog stupca. Koncentracije PP-a su bile u rasponu od 0.007 µmol dm^{-3} na otvorenom moru kod otočja Palagruže do 0.589 µmol dm^{-3} u estuariju rijeke Krke. U obalnom području srednjeg Jadrana najviše koncentracije PP izmjerene su tijekom jeseni, a najniže tijekom zime. Vertikalna raspodjela koncentracija PP-a u vodenom stupcu obalnog područja pokazala je najviše vrijednosti u površinskom sloju tijekom većeg dijela godine, što je posljedica unosa PP-a slatkovodnim dotocima. U području otvorenog mora nisu utvrđene značajne razlike u sezonskoj i vertikalnoj raspodjeli koncentracija PP u vodenom stupcu. Rezultati sezonskih istraživanja ukupnog fosfora u vodenom stupcu (PP, DIP i P-ORG) pokazala su da su koncentracije PP-a i P-ORG-a više u obalnom području u odnosu na koncentracije zabilježene u području otvorenog mora, dok su koncentracije DIP usporedive u oba područja. Rezultati također ukazuju da PP može imati važan udio u ukupnom fosforu. Statistička analiza rezultata pokazala je značajnu pozitivnu korelaciju PP-a s klorofilom *a*, što ukazuje da je PP uglavnom biološkog podrijetla, kao i s hranjivim solima dušika i silicija; dok je negativna korelacija utvrđena sa salinitetom.
Ključne riječi: fosfor; partikularni fosfor; hranjive soli; Jadransko more; partikularna tvar; vodeni stupac

INTRODUCTION

Phosphorus (P) and nitrogen (N) are essential nutrients for phytoplankton and microbial growth (Benitez-Nelson, 2000; Ruttenberg, 2014). A relatively large proportion of total phosphorus in seawater consists of the particulate phase, which is defined as solid particles in the water with a diameter larger than 0.45 µm. This phase is an essential factor in nutrient loading and can act as a potential source of dissolved nutrients in coastal waters through mineralisation and adsorption-desorption processes (Delaney, 1998; Paytan and McLaughin, 2007). It also has a significant role in the transport and cycling of energy and nutrients through the water column to sediment (Godet and Föllmi, 2021). In productive coastal areas, particles consist mainly of organic matter

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composed of live and dead phyto- and zooplankton and bacteria, along with excretion and decomposition products. In river deltas and estuaries, the inorganic component may predominate due to the terrestrial origin of the particles (Benitez-Nelson, 2000).

The allochthonous particulate phosphorus fraction (PP) is mainly introduced by rivers and atmospheric deposition. PP input from rivers into the marine environment is important for coastal areas (Ruttenberg, 2014); however, the majority of this phosphorus fraction is removed by the sedimentation process in the estuarine, lagoon or river mouth areas (Benitez-Nelson, 2000; Paytan and McLaughlin, 2007; Ruttenberg, 2014). Atmospheric deposition represents a less significant input of phosphorus to the marine ecosystem. In oligotrophic areas of the open sea, it can represent a significant additional source of phosphorus and promote the growth of autotrophic species of organisms (Pulido-Villena *et al*., 2008). The autochthonous organic PP fraction can be formed in the marine environment by various processes: photosynthesis and chemosynthesis of particulate organic matter; physical aggregation of particles (McCave, 1984; Kepkay, 1994); excretion of faecal pellets (Hernandez-Leon *et al.*, 2010; Yuan *et al.*, 2022); cellular lysis (Mine *et al.*, 2021), and leakage or exudation by phytoplankton and bacteria (Burd and Jackson, 2009; Thornton, 2014).

In addition to the particulate phase, phosphorus also occurs in seawater in dissolved form. Dissolved inorganic phosphorus (DIP) generally consists of orthophosphate, pyrophosphate and polyphosphate. Orthophosphate (predominantly in the form of HPO_4^{2-} (87%) and only 12% PO_4^{3-} and 1% H_2PO_4), is the most common compound and is preferentially utilised by phytoplankton, while some phytoplankton cannot directly assimilate pyro- and polyphosphate (Duan *et al.*, 2016). Similar to DIP, phytoplankton or microbial communities can also take up small organic molecules containing phosphorus when DIP is insufficient to meet the nutrient requirements of aquatic organisms (Yoshimura *et al*., 2014; Li *et al.*, 2017). The dissolved organic P (DOP) pool includes phosphate esters, phosphonates, and phosphorus associated with high molecular-weight organic matter, such as humic and fulvic acids. Most DOP compounds are not available for direct uptake by phytoplankton or bacteria, but can be rendered bioavailable by enzymatic hydrolytic production of orthophosphate (Ruttenberg, 2014). The issue of bioavailability is essential for assessing the coupled C–N–P cycle and related issues, such as nutrient limitation, coupled ocean–atmosphere CO_2 dynamics (Hopkinson and Vallino, 2005) and climate change through the nutrient–CO_2 connection (Tamburini *et al.*, 2003). Therefore, quantifying the organic phosphorus forms is relevant, especially when this fraction is more abundant and bioavailable compared to inorganic forms in natural waters (Björkman and Karl, 2003).

Phosphorus has been recognised as a limiting nutrient for certain marine ecosystems, including the Adriatic Sea (Vukadin and Stojanoski, 1976; Degobbis and Gilmartin, 1990; Viličić, 2014). Most studies on particulate phosphorus in Adriatic Sea have been focused on the shallow northern part of the basin (Gismondi *et al.*, 2002; Giani *et al*., 2003; Lipizer *et al*., 2011), which is under the influence of many rivers, particularly Po and Adige. Nutrients delivered by these northern rivers are quickly scavenged by primary producers, and thus their high concentrations are limited to the coastal and delta areas. A similar situation has been detected in the middle and southern parts of eastern Adriatic, where primary productivity is restricted to coastal areas around freshwater inputs (Ninčević Gladan *et al*., 2015). More recent research by Giani *et al*. (2018) on particulate matter in the northern Adriatic investigated the flux of this material to benthos and its effects on the carbon and biological pump. They found that the downward fluxes and elemental composition of the settling matter were characterised by high seasonal variations and linked these fluctuations to river discharge, primary production and the wind regime of the basin, while resuspension and advective transport in the shallow continental shelf area were the most important processes regulating the particle dynamics, concentrations, and sedimentation. PP studies have also been carried out for the southern part of the basin (Boldrin *et al.*, 2002), though information on the middle Adriatic is sparse.

The aim of this study was to obtain PP data for the eastern part of the middle Adriatic Sea for the first time, and to examine the availability, distribution and seasonal variability of the PP fraction in the water column. An additional aim was to investigate the relationship between different forms of phosphorus associated with the particulate matter or presented as organic or inorganic phosphorus in the dissolved phase. Furthermore, the relationships between PP and other nutrients and oceanographic parameters from the water column were analysed.

MATERIALS AND METHODS

Sampling and analytical methodology

Seawater samples were collected by the BIOS DVA research vessel in the middle Adriatic in January, April, August and November 2009 (corresponding to winter, spring, summer and autumn seasons) at a total of 11 stations (Fig. 1). Three stations were located in Kaštela Bay, a semi-enclosed basin under the anthropogenic influence of the surrounding mainland: station B1 is located in the central part of the bay, station B2 near the small town of Vranjic at the mouth of the river Jadro, a freshwater source with an annual mean inflow of 10 m^3 s^{-1}, and station B3 is located in the western part of the bay in the vicinity of the town of Trogir. One station (C) is located in the Split Channel extending between the islands of Čiovo and Šolta. The channel is characterised by relatively rapid ventilation, including the intrusion of open ocean water masses, therefore permitting

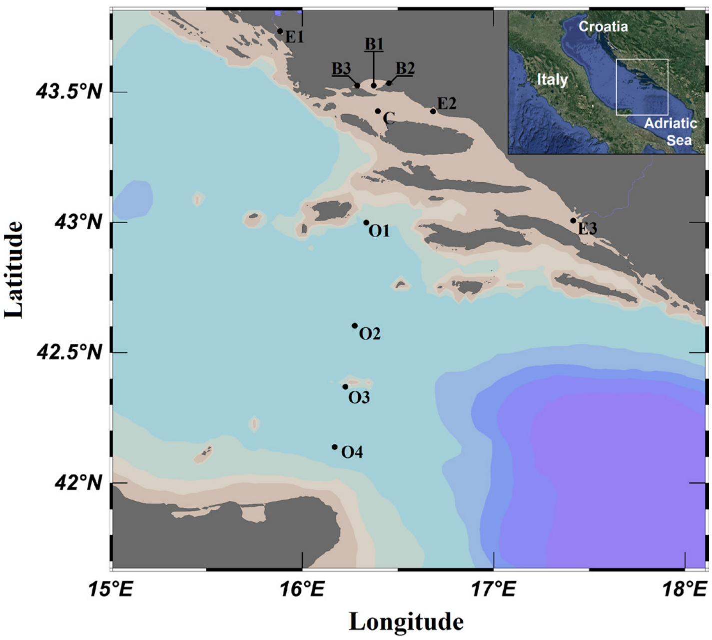

Fig. 1. Study area indicating sampling stations in the coastal (stations B1, B2, B3, E1, E2, E3 and C) and open sea area of the middle Adriatic (stations O1, O2, O3 and O4).

quick dispersion of loaded nutrients from coastal inputs (Bojanić *et al.*, 2012). As a result, it represents the area between the coastal and the open sea influence. Three stations were located in areas under the riverine influence: station E1 is located in the lower part of the Krka River estuary, which receives a substantial nutrient load coupled with sewage and domestic wastewater inputs from the city of Šibenik, making it one of the most productive areas in the eastern Adriatic (Kušpilić *et al.*, 2010); station E2 is located at the mouth of Cetina River near the town of Omiš, and station E3 is located in the Neretva Channel between the mainland and the Pelješac peninsula, a sparsely populated area where the Neretva River discharges a high nutrient load into the sea. Finally, four stations represent oligotrophic waters of the open Adriatic Sea, along a transect from Cape Stončica on the island of Vis (O1), along the Palagruža Sill (O2, O3) to Monte Gargano (O4).

Samples were obtained by Niskin bottles at standard oceanographic depths (0, 5, 10, 20, 30, 50, 75, 100, 150 and 2 m from the bottom). For PP analysis, 500 mL seawater subsamples were filtered through a precombusted (450ºC for 4 h) Whatman GF/F filters under vacuum at <0.01 MPa. Filters were stored at -20ºC until analysis in the on-shore laboratory. PP was recovered from a seawater sample on a membrane filter digested at 500°C for 1 hour and extracted with 1 N HCl. The released orthophosphate was analysed spectrophotometrically using the phosphomolybdate blue method described by Parsons *et al*. (1984).

Seawater samples for dissolved nutrient analysis were collected in polyethylene vials and immediately frozen at -20ºC. Determination of nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), orthophosphate (PO_4^{3-}) and silicate (SiO_4^{4-}) concentrations were done by AutoAnalyzer III colorimeter (Seal Analytical) using standard spectrophotometric methods (Grasshoff *et al.*, 1983). Dissolved inorganic N (DIN) represents the sum of NO_3^-, NO_2^- and NH_4^+. Total nitrogen (TN) and phosphorus (TP) were analysed in unfiltered samples after autoclaving in an acid potassium persulfate solution at 130ºC for 120 min (McKelvie *et al.*, 1995). In collaboration with Quasimeme Laboratory Performance Studies, the performance of the determination methods was tested with externally prepared sample material; the detection limit for orthophosphate was 0.005 μmol dm^{-3}. Organic bound P (P-ORG) was estimated as the difference between TP and DIP, while organic bound N (N-ORG) was calculated as the difference between TN and DIN. A Seabird-25 CTD probe was used to measure temperature and salinity, while oxygen was determined according to the standard Winkler method (Winkler, 1888). Chlorophyll *a* concentrations were determined fluorometrically according to Strickland and Parsons (1972).

Statistical data analysis

Principal component analysis (PCA) was performed to reveal the main pattern of spatial and seasonal variation between the forms of phosphorus (DIP, PP and P-ORG), nitrogen (NO_3^-, NO_2^-, NH_4^+ and N-ORG) and silicate (H_4SiO_4) as active variables and environmental variables as quantitative supplementary variables. These supplementary variables represented the spatial and temporal variability of seawater temperature, salinity, oxygen saturation and chlorophyll *a* concentration. In order to clarify the relationships within the data set and to facilitate the interpretation of the results, a varimax rotation of the average values of all variables (column average per station in each season) was performed. The cosine squares of the variables were used to estimate the best relationship between each variable and the extracted principal component. A triplot diagram was created to visualize the 2-D closeness between two groups of variables and observations in PCA space. The observations show the grouping of the data based on the combined factor "station-season". PCA was performed using the statistical package XLSTAT for Life Sciences (version 2023.1.5.1409, Addinsoft).

RESULTS AND DISCUSSION

The concentrations of the three forms of phosphorus investigated in the study area are shown in Fig. 2. The highest concentration range of the studied phosphorus forms was determined for P-ORG (below limit of detection (< LOD) to 0.955 μmol dm^{-3}), followed by PP (0.007 to 0.589 μmol dm^{-3}, while the range for DIP concentrations was <LOD to 0.359 μmol dm^{-3}). Median PP concentrations (Fig. 2) were higher than DIP con-

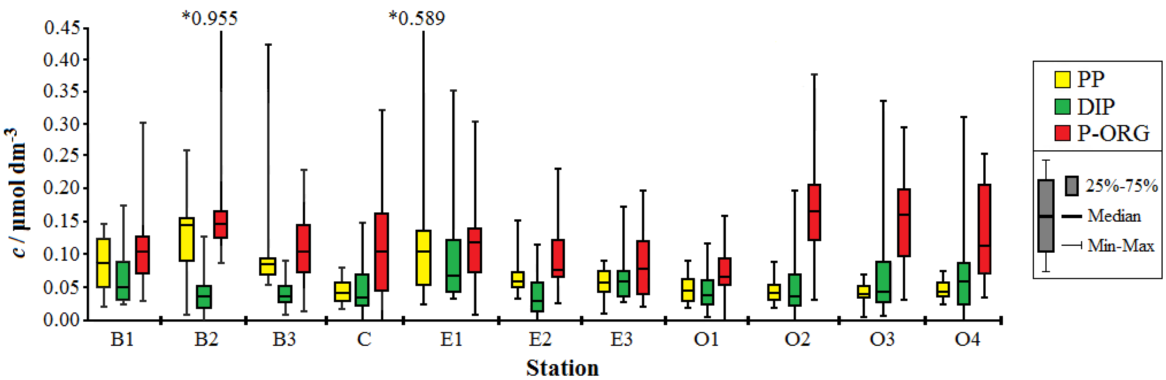

Fig 2. Box-whiskers diagram of particulate (PP), dissolved inorganic (DIP) and organic (P-ORG) phosphorus concentrations in the water column of the investigated stations in 2009. The lower and upper boundaries of the boxes mark the 25th and 75th percentiles of the data set, the centre line denotes the median value (50th percentile), while the lower and upper end lines represent the lowest and highest concentrations during the study period. Asterisk = maximum value out of range.

centrations at most stations (with the exception of E3 and O4), while median P-ORG concentrations were higher than PP and DIP at all stations. The comparison between the concentrations of the investigated P forms in the coastal zone and open sea showed differences in PP concentrations. The median values of PP concentrations in the coastal zone were higher than in the open sea, which can be attributed to the proximity of land, rivers and anthropogenic sources.

The annual median PP values (Fig. 2) in Kaštela Bay were similar at the western and central stations (B1 and B3), while the highest median PP concentration in this study (0.143 µmol dm^{-3}) was measured at station B2, likely due to the anthropogenic pressure of the surrounding, highly urbanised area and the proximity of the Jadro River (Marasović *et al.*, 2005). At three estuarine stations, the highest annual median PP concentration was observed at E1 in Šibenik Bay, where the highest PP concentration during this study (0.589 µmol dm^{-3}) was also recorded. The semi-enclosed nature of the bay into which the Krka River flows (connected to the coastal sea by the narrow Channel of St. Ante) and the proximity to the city of Šibenik are likely causes for the elevated nutrient concentrations in this area (Juračić and Crmarić, 2003; Liu *et al.,* 2019), which could also explain the high median PP value (0.121 µmol dm^{-3}). The median values of PP concentration at the mouths of the Neretva (E3) and Cetina rivers (E2) were the same (0.057 µmol dm^{-3}), slightly higher than at the offshore stations of the middle Adriatic (~0.047 µmol dm^{-3}). The annual median values of PP concentration at the open sea stations fluctuated around 0.045 µmol dm^{-3} at all stations (and in all seasons). From this we can conclude that the open waters of the middle Adriatic have a relatively stable PP pool throughout the year. Overall, the P-ORG fraction was the largest portion of the total phosphorus pool (range 24–74%; mean 47±12%), while DIP (7–72%; mean 29±17%) and PP (12–56%; mean 31±11%) were usually smaller fractions.

Coastal waters of the middle Adriatic

The concentration ranges and mean values of PP, DIP and P-ORG by season in the coastal area are shown in Table 1. The seasonal changes in PP concentrations along the middle Adriatic coast show that the lowest mean concentration was recorded in winter (0.068 µmol dm^{-3}). In spring and summer, the average PP concentrations increased and were highest in autumn (0.100 µmol dm^{-3}). This increase from winter to autumn differed significantly from the changes in mean seasonal concentrations of dissolved forms of phosphorus. Mean DIP concentrations showed an inverse seasonal distribution compared to PP, with the highest mean concentration (0.074 µmol dm^{-3}) in winter and the lowest (0.041 µmol dm^{-3}) in autumn. Mean P-ORG concentrations showed

Table 1. Concentration range and mean ± standard deviation (SD) (in µmol dm^{-3}) of particulate phosphorus (PP), dissolved inorganic (DIP) and organic (P-ORG) phosphorus in the coastal area of the middle Adriatic (stations B1, B2, B3, E1, E2, E3 and C) during 2009. LOD = limit of detection.

Season	PP		DIP		P-ORG	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Winter	0.021 - 0.153	0.068 ± 0.037	<LOD - 0.359	0.074 ± 0.064	0.028 - 0.192	0.105 ± 0.044
Spring	0.011 - 0.424	0.071 ± 0.070	<LOD - 0.232	0.059 ± 0.046	0.019 - 0.955	0.162 ± 0.188
Summer	0.044 - 0.222	0.091 ± 0.042	0.021 - 0.150	0.057 ± 0.033	0.007 - 0.323	0.143 ± 0.094
Autumn	0.018 - 0.589	0.100 ± 0.099	0.009 - 0.124	0.041 ± 0.032	0.036 - 0.200	0.096 ± 0.043

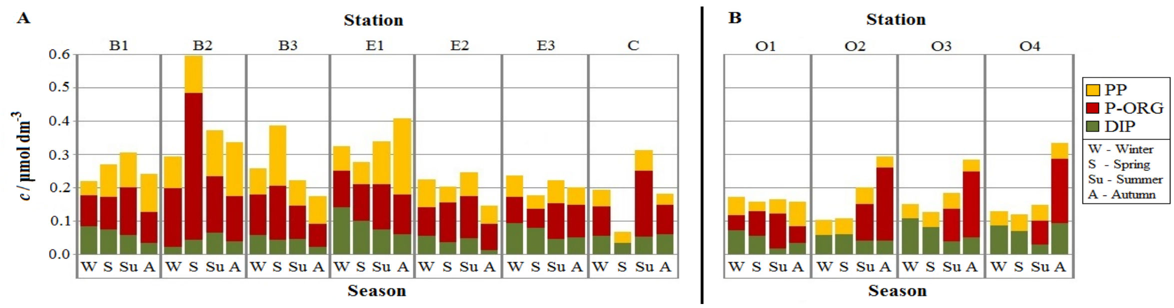

Fig 3. Seasonal distribution of mean particulate (PP), dissolved inorganic (DIP) and organic (P-ORG) phosphorus concentrations at the coastal (**A**) and offshore (**B**) stations in the middle Adriatic Sea.

no specific seasonal pattern, although higher concentrations were measured in warmer seasons (spring, summer). Gismondi *et al*. (2002) also recorded higher PP concentrations in June compared to February, which could be attributed to an increase in phytoplankton biomass in the warmer season. Similar seasonal changes in PP were also found in the Gulf of California (Lyons *et al*., 2011), where concentrations of PP and all its fractions (labile, oxy-hydroxide, authigenic, and detrital) were higher during the summer than in the winter.

The seasonal variability of PP, DIP and P-ORG concentrations at the coastal stations of the middle Adriatic are shown in Fig. 3A, with notable differences between the forms of phosphorus. The highest mean seasonal PP concentrations were observed at half of the stations (B1, B2, E1) during autumn, while others (E2, E3, C) showed a reasonably uniform pool throughout the year. The results also indicate that PP represents a significant fraction of the total P pool that has not been previously recognised, and could support further microbial and phytoplankton activity.

The PP concentrations determined at stations B1, B2, E2, E3 and C are consistent with the published data for other coastal regions. The ranges of PP concentrations for these stations are comparable to those found in the Gulf of Trieste (northeastern Adriatic Sea, Lipizer *et al.*, 2011), along the Mauritania coast (eastern Atlantic Ocean, Lomnitz, 2017) and in the Gulf of California (Lyons *et al.*, 2011). The PP values are also higher compared to the Egyptian coastal waters (eastern Mediterranean Sea, Abdel-Moati, 1990), Sagami Bay in the Japanese coastal area (northwestern Pacific Ocean, Suzumura and Ingall, 2004) and along the Australian coast near the Coral Sea (southwestern Pacific Ocean, Lønborg *et al.*, 2017). PP concentrations determined at stations B3 and E1 were higher compared than the above coastal stations, and are similar to concentrations in the River Po estuary (Giani *et al.*, 2001, 2003), in Chesapeake Bay (Li *et al*., 2017) and in the waters along the Florida coast (Murasko, 2009). In the northern Adriatic, the main anthropogenic influence originate from the Po River, which flushes large amounts of nutrient salts from intensive agriculture and urban centres along the river basin. In contrast, nutrient salts in the Gulf of Chesapeake (Li *et al*., 2017) and in Tampa Bay and Charlotte Harbor (Murasko, 2009) originate from municipal and industrial runoff and leachate from agricultural land. Stations B3 and E1 are located in the Kaštela and Šibenik Bays, respectively, both are under the influence of agriculture, freshwater inputs and increased anthropogenic impact of the surrounding urban area (Legović *et al*., 1994; Svensen *et al*. 2007; Bogner and Matijević, 2016; Blanco *et al*., 2018) and are comparable to these areas.

DIP concentrations at most stations (Fig. 3A) showed a seasonal trend, with the highest concentrations in winter, followed by a decrease during the spring phytoplankton bloom (Suppl. Fig. 1), that also marks a significant shift from DIP to the P-ORG pool. During summer, DIP values continued to decline and reached their minimum in autumn. The only exception was station B2 which displayed an inverse trend, although the reason for this remains unclear. The highest DIP concentration (0.359 μmol dm^{-3}) was measured in winter at E1 in the surface diluted layer, which also contained the highest amount of DIN (47.481 μmol dm^{-3}) found during this study. Although Šibenik Bay is significantly influenced by the Krka River, the river is characterised by low nutrient concentrations and an extremely low input of terrigenous material (Legović *et al*., 1994). The semi-enclosed nature of the Bay and the proximity of the city of Šibenik are likely sources of the increased nutrients. The P-ORG fraction remained stable over the seasons at most stations, although higher levels were usually observed during the warmer period.

The vertical distribution of PP in the coastal area was simplified by showing the average concentrations in three characteristic layers of the water column (surface - S, middle - M and bottom - B) (Fig. 4). The highest average PP concentrations were measured in the surface layer in all seasons except summer, when the highest PP concentration was measured in the bottom layer. Elevated PP concentrations in the surface layer were reported by Giani *et al*. (2001, 2003) and Gismondi *et al*. (2002) in the northern and middle Adriatic. PP concentrations in the middle layer of the water column were lower than in the surface and bottom layers at all stations throughout the year. In summer (Fig. 4C), the highest mean PP concentration in the water column was in the bottom layer. Increased abundance of phytoplankton in the bottom layer of coastal stations is common in summer, due to the increased concentrations of dissolved nutrient

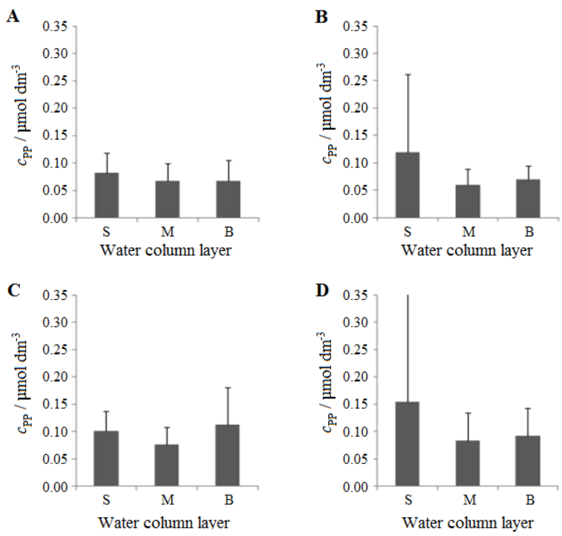

Fig. 4. Average concentration of particulate phosphorus (PP) in layers of the water column in the Adriatic coastal area (S - surface layer; M - middle layer; B - bottom layer) during winter (**A**), spring (**B**), summer (**C**) and autumn (**D**) of 2009.

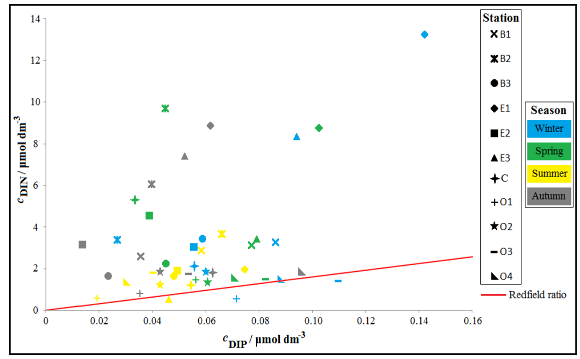

Fig 5. Relationship between seasonal mean dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) concentrations at coastal and offshore stations of the middle Adriatic Sea during 2009.

salts in this part of the water column (Ninčević *et al.*, 2002). In the studies by Li *et al.* (2013), a correlation was also found between the abundance of phytoplankton and the concentrations of PP and particulate nitrogen (PN) in the water column, which could explain the higher PP concentrations in the bottom layer.

The average seasonal DIN:DIP molar ratios for the coastal and offshore stations, derived from the linear regression slope (Fig. 5), were also calculated. According to Redfield *et al.* (1963), the optimum ratio of dissolved nitrogen to phosphorus is 16:1. A lower ratio indicates that primary production is limited by nitrogen, while a higher ratio indicates limitation by phosphorus. At all coastal and open sea stations, the DIN:DIP ratio was above 16, except at E3 in the coastal area during summer and at O1 and O3 in the open sea area in winter. The very low ratio at E3 (Fig. 5) during summer can be attributed to the very low DIN concentrations (0.540 µmol dm^{-3}) due to reduced inflow of the Neretva River during this part of the year (Vranješ *et al.*, 2013). These results are consistent with previous research on the ratio of inorganic N:P in the Adriatic Sea and clearly showed that phosphorus is a limiting factor for primary production (Giani *et al.*, 2012; Vilibić *et al.*, 2012; Viličić, 2014).

Pronounced seasonal fluctuations in the N:P ratio were observed at most coastal stations, with the highest ratios recorded in the spring (DIN:DIP = 41–218) and autumn (DIN:DIP = 73–231), while significantly lower ratios were determined in summer (DIN:DIP = 12–57). During the phytoplankton bloom in spring and autumn (Suppl. Fig. 1), DIN:DIP ratios increased due to the utilisation of DIP by the phytoplankton community, as is evident from increased chl *a* concentrations throughout the water column, high P-ORG concentrations, and increased O_2 saturation. The reasons for the seasonal changes in the DIN:DIP ratio were also confirmed by previous research in the middle Adriatic (Krstulović *et al.*, 1995; Šolić *et al.*, 2008), which found a temporal shift in phytoplanktonic and bacterial production of organic matter (phytoplanktonic production dominates over bacterial in spring, while the situation is reversed in summer).

Offshore waters of the middle Adriatic

The concentration ranges and mean values of PP, DIP and P-ORG by season in the offshore waters of the middle Adriatic are shown in Table 2. The mean seasonal PP concentrations in the offshore waters of the middle Adriatic Sea showed no significant fluctuations, which was expected since there are no additional sources of PP in the open sea, unlike near the coast (rivers, anthropogenic sources) (Benitez-Nelson, 2000; Karl, 2014; Ruttenberg, 2014). This is consistent with studies in the open part of the northern Adriatic Sea (Giani *et al.*, 2003) that found no significant seasonal variation of PP. DIP concentrations followed a similar trend as in the coastal region, with the exception that the lowest values were recorded in summer, while values were slightly higher in autumn. Krstulović *et al.* (1995) pointed out that bacterioplankton activity was highest in summer, when dissolved organic matter produced by phytoplankton accumulates and the amount of dead phytoplankton cells increases, which could lead to an increase in DIP concentrations. This process was further confirmed by Šolić *et al.* (2020) who reported a consistent short-term cyclic successions of the plankton food web types. According to these authors, another major effect of these types of food webs is that they accelerate mineralisation by restoring production in nutrient limited environments.

The spatial and seasonal distribution of PP, DIP and P-ORG concentrations by season in the open area of the middle Adriatic is shown in Fig. 6. During spring (Fig. 6B), PP distribution was uniform, without spatial and vertical concentration gradients, while an increase

Table 2. Concentration range and mean ± standard deviation (SD) (in µmol dm^{-3}) of particulate phosphorus (PP), dissolved inorganic (DIP) and organic (P-ORG) phosphorus in the offshore region of the middle Adriatic (stations O1, O2, O3 and O4) during 2009. (*missing data in January and April for O2, O3 and O4 stations). LOD = limit of detection.

Season	PP		DIP		P-ORG*	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Winter	0.022 - 0.082	0.045 ± 0.013	<LOD - 0.333	0.081 ± 0.083	0.008 - 0.103	0.047 ± 0.031
Spring	0.020 - 0.057	0.044 ± 0.011	0.006 - 0.189	0.067 ± 0.040	0.011 - 0.144	0.073 ± 0.039
Summer	0.021 - 0.089	0.046 ± 0.013	<LOD - 0.104	0.033 ± 0.024	0.030 - 0.161	0.096 ± 0.036
Autumn	0.007 - 0.091	0.047 ± 0.021	0.009 - 0.196	0.057 ± 0.047	<LOD - 0.379	0.168 ± 0.080

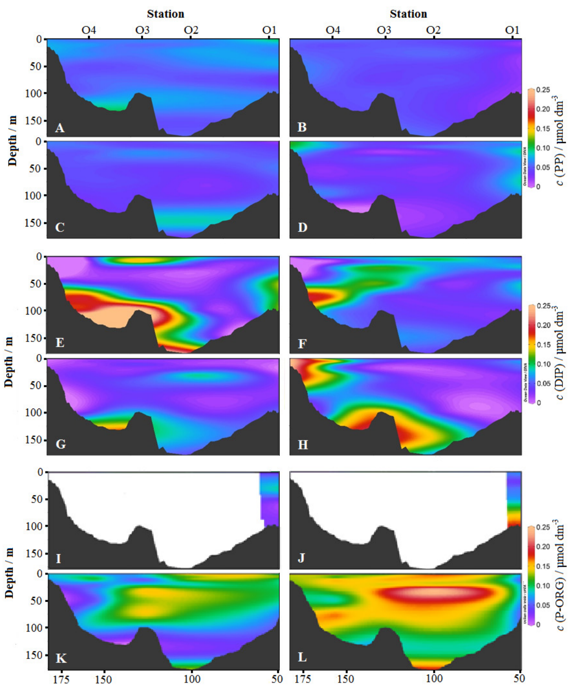

Fig. 6. Particulate (PP), dissolved inorganic (DIP) and organic (P-ORG) phosphorus concentrations along the offshore transect from the island of Vis (O1) to the Cape of Monte Gargano (O4) in the middle Adriatic during winter (**A**, **E**, **I**), spring (**B**. **F**, **J**), summer (**C**, **G**, **K**) and autumn (**D**, **H**, **L**) in 2009.

of PP was detected in some parts of the water column during winter, summer and autumn. In winter (Fig. 6A), elevated PP concentrations were measured in the bottom layer near the western Adriatic coast. Harris *et al*. (2008) recorded intense sediment resuspension near Cape Monte Gargano during winter, which could explain this increase in PP content in the bottom layer. According to these authors, the main causes of increased sediment resuspension in winter are sea currents and wind-induced waves. In summer (Fig. 6C), an increased PP concentration was recorded only in the bottom layer near the island of Sušac (O2). In the same area, Ninčević *et al*. (2002) reported a lack of larger phytoplankton cells in summer, which they linked to feeding by microzooplankton. It can be hypothesised that microzooplankton feeding may cause the increased PP concentrations during summer following enrichment of the water column with particulate matter (faecal pellets) through its metabolic processes (excretion), although further studies are needed to confirm this. The spatial distribution of PP during autumn (Fig. 6D) is characterised by elevated concentrations of PP at stations O1 and O4. At O4, elevated PP concentrations were found only in the surface layer, while at O1 most of the water column was enriched with PP. The increase in PP concentrations in the surface layer on the western side of the Adriatic is likely due to the outflow of seawater from the Adriatic Basin into the Otranto Sea, which contains increased amounts of nutrient salts (Orlić *et al*., 2006). Significant inflow of freshwater from the Po River (Pedro-Monzonis *et al*., 2016) and other rivers flowing into the northern part of the Adriatic basin are probably the cause of increased concentrations of PP and other nutrients (DIP, DIN, orthosilicates) in autumn (Degobbis, 1990; Cushman-Roisin *et al*., 2001). The reason for the elevated PP concentration in the entire water column at station O1 is unclear, as salinity values do not indicate the presence of freshwater, and chlorophyll *a* concentrations were even lower compared to the rest of the study.

The seasonal and spatial distribution of DIP on the offshore transect from stations O1 to O4 (Fig. 6E) showed that concentrations were increased during winter in the bottom layer of the western side of the Adriatic Sea (DIP> 0.25 µmol dm^{-3}) and in the bottom layer of O1 near the island of Vis. The flux of DIP from the sediments (Kušpilić, 2001) and the decomposition of organic matter in the deeper layers of the water column likely causes the elevated DIP levels (Loh and Bauer, 2000). In spring (Fig. 6F), DIP concentrations were generally below 0.1 µmol dm^{-3} in most of the study area, with an increase in DIP concentrations observed only in the middle layer along the western Adriatic coast, probably as a result of phytoplankton activity as suggested by the highest chlorophyll *a* values detected in this section of the transect (Suppl. Fig. 2B). In summer (Fig. 6G), the surface layer down to a depth of 20 meters was depleted in DIP due to the summer stratification of the water column. The distribution of DIP in the water column during autumn (Fig. 6H) showed higher concentrations in the surface layer of the water column in the western part of the Adriatic, similar to PP, and also due to the outflow of seawater from the Adriatic basin into the Otranto Sea.

The range of P-ORG concentrations (Table 2) determined in 2009 (from 0.068–0.389 μmol dm^{-3}) was similar to the range of DIP (< LOD–0.333 μmol dm^{-3}), and wider than the range of PP (0.007–0.091 μmol dm^{-3}). However, the data on nutrient concentrations at the offshore stations (O2, O3, O4) lack information on P-ORG in winter and spring (Fig. 6I, 6J), raising the possibility that some important features of the spatial distribution of P-ORG in the open sea area were not recorded. During summer (Fig. 6K), elevated P-ORG concentrations were measured mainly in areas with elevated chlorophyll *a* concentrations (Suppl. Fig. 2C). This phenomenon is referred to as the Deep Chlorophyll Maximum (DCM) and is characterised by intensive growth of phytoplankton in a layer with elevated concentrations of nutrient salts and sufficient light for photosynthesis. Summer distribution of P-ORG is probably due to the growth of cyanobacteria, as the dominant phytoplankton group in this area (Ninčević *et al.*, 2002). In autumn (Fig. 6L), the highest P-ORG concentrations were measured on the western side of the Adriatic with significant accumulation in 30–50 meter range, while the area near the island of Vis was relatively depleted.

The average seasonal DIN:DIP molar ratios for the offshore stations are shown in Fig. 5. The DIN:DIP molar ratios, derived from the linear regression slope, ranged from 8–46 and were higher than the Redfield ratio, indicating that the highest value usually occurs in summer due to orthophosphate depletion. The seasonal differences in the Redfield ratio were less pronounced at the open sea stations than in the coastal area. The differences in the ratio between stations in each area were also less pronounced in the offshore waters. Seasonal variations in the DIN:DIP ratios were also found in the northern Adriatic by Lipizer *et al.* (1997, 1999), who associated these fluctuations with general biological processes. After the phytoplankton bloom in spring, biological assimilation of inorganic forms of nutrient salts occurs, resulting in increased production of particulate organic matter (POM) and dissolved organic matter (DOM), which provide a suitable substrate for bacterial activity. The authors concluded that a classical food web develops in spring that utilises DIP and DIN, while in summer the "microbial loop" begins to mineralise the accumulated DOP and DON. Similar results were obtained by Karl *et al.* (1997), Cotner *et al.* (1997) and Ruttenberg and Dyhrman (2012) by investigating seasonal changes in the concentration of organic forms of phosphorus and primary production.

Statistical analysis

The patterns of spatial and seasonal variability of nutrient forms of phosphorus (DIP, PP and P-ORG), nitrogen (NO_3^-, NO_2^-, NH_4^+ and N-ORG) and silicate (SiO_4^{4-}), as well as environmental variables (seawater temperature, salinity, oxygen saturation and chlorophyll *a*) were analysed by principal component analysis (PCA) (Fig. 7). The PCA result summarised the pattern of data variability and linked sampling stations and nutrients with similar variances. Three PCA principal components (68.37% of total variability) were extracted in the analysis, which ranked all parameters (active and supplementary variables) according to their patterns of spatial and seasonal variability.

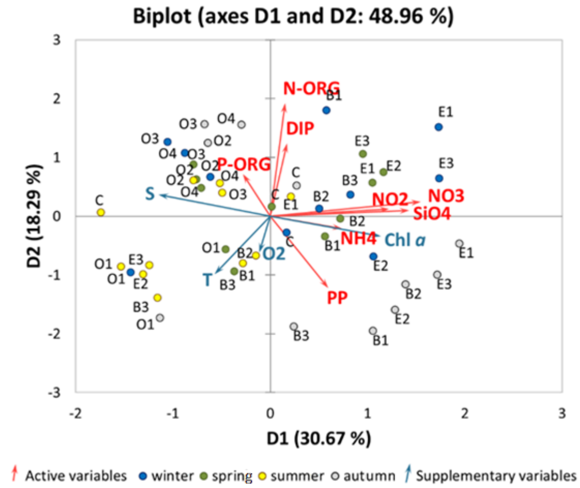

Fig. 7. Principal components analysis of the grouping of nutrient forms of phosphorus (DIP, PP and P-ORG), nitrogen (NO_3, NO_2, NH_4 and N-ORG) and silicate (SiO_4) and supplementary environmental parameters (T, temperature; S, salinity; O_2, oxygen saturation and Chl *a*, chlorophyll *a*), presented as a triplot diagram based on the average data of nutrient concentrations *per* station in each season. The length of each arrow represents the significance of the parameter, while the angles between the parameters show their correlation.

PC1 axis explained 30.67% of the total variability and mainly determines the variability of nitrogen salts NO_3^- and NO_2^- and silicate (Fig. 7, Table 3). This axis was strongly and positively related to chlorophyll *a* concentration and negatively related to salinity, indicating the strong influence of freshwater inflow on nutrient concentrations that support the development of the phytoplankton community. These conditions were observed at the stations in the Neretva (E3) and Krka (E1) estuaries during autumn and winter, when freshwater inflow and the supply of nutrients (especially nitrate and silicate) were the highest.

PC2 (18.29% of total variability) was positively associated with N-ORG and DIP and negatively with PP, seawater temperature, and oxygen saturation. High concentrations of organic nitrogen and dissolved inorganic phosphorus were observed at the open sea stations in autumn and in the central part of Kaštela Bay in winter. These correlations could be explained by the seasonal recycling of particles, which contributes to the enrichment of the water column with organic nitrogen and DIP (Pantoja *et al.*, 2002; Voss *et al.*, 2013; Li *et*

Table 3. Correlations between active variables [concentrations of nutrient forms of phosphorus (DIP, PP and P-ORG), nitrogen (NO_3, NO_2, NH_4 and N-ORG) and silicon (SiO_4)] and supplementary environmental variables (T, temperature; S, salinity; O_2, oxygen saturation and Chl *a*, chlorophyll *a*) and main factors after Varimax rotation (D). Values in bold correspond for each variable to the factor for which the squared cosine is the largest.

Parameter	D1	D2	D3
DIP	0.103	**0.553**	0.091
PP	0.353	**-0.544**	0.470
P-ORG	-0.166	0.319	**0.820**
NO_3^-	**0.920**	0.111	0.182
NO_2^-	**0.725**	0.053	0.319
NH_4^+	0.438	-0.093	**0.642**
N-ORG	0.089	**0.856**	0.071
SiO_4^{4-}	**0.849**	0.045	-0.316
T	-0.340	**-0.439**	0.095
S	**-0.684**	0.165	-0.187
O_2	-0.066	-0.277	0.195
Chl *a*	**0.675**	**-0.151**	0.393

al., 2017). In addition, anthropogenic pressure, vicinity to urban areas and the inflows of the Jadro River increase the PP concentrations in the Bay during autumn (Marasović *et al*., 2005). Due to the high anthropogenic input of nitrogen compounds *via* wastewater, the supply of nitrogen fertilisers *via* freshwater, and the burning of fossil fuels, coastal ecosystems are mainly phosphorus limited (Huang *et al.*, 2003).

PC3 (19.41% of total variability) highlighted the role of organic phosphorus and NH_4^+, both of which were positively correlated with this axis. Among the environmental variables, chlorophyll *a* concentration was positively correlated while salinity was negatively correlated, though these relationships were not statistically significant. These conditions were observed at most neritic stations during spring and summer, indicating the importance of freshwater nutrient supply and the role of phytoplankton in the processes of assimilation and remineralisation in the seawater column. As a semi-enclosed sea with a relatively limited exchange of water masses with neighbouring seas, especially the shallow coastal area, the Adriatic Sea is extremely sensitive to the input of nutrient salts, whether due to natural or anthropogenic influences.

CONCLUSION

The results of seasonal investigation of PP, DIP and P-ORG in the area of the middle Adriatic in 2009 indicated that PP and P-ORG concentrations were higher in the coastal area compared to concentrations determined in the open sea, while DIP concentrations were similar in both areas. In the coastal area of the middle Adriatic, PP concentrations were highest in autumn and lowest in winter. In the water column, the highest PP concentrations were found in the surface layer during most of the year (spring, autumn and winter) due to the introduction of PP by freshwater inflows. In the open sea area, no significant differences were detected in the spatial or temporal distribution of PP concentrations in the water column.

PP was mainly of biological origin, as there was a positive correlation between PP and chlorophyll *a* concentrations. It was also found that PP can be a significant fraction (12–56%) of the total phosphorus pool, which was not previously recognised. Microbial and phytoplankton communities could tap into this pool of available phosphorus to meet their growth and reproduction needs under less favourable conditions, such as during DIP depletion. This should ultimately provide information that would enable better prediction of primary production. In order to improve our understanding of the phosphorus cycle in the marine environment of the middle Adriatic, future studies should incorporate measurements of dissolved and particulate forms of carbon and nitrogen, and their organic and inorganic fractions, coupled with the use of sediment traps to estimate fluxes between the water column and sediment floor.

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