Mediterranean Marine Science

Vol 15, No 2 (2014)

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doi: 10.12681/mms.565

Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
DOI: http://dx.doi.org/10.12681/mms.565

Dissolved organic matter cycling in eastern Mediterranean rivers experiencing multiple pressures. The case of the trans-boundary Evros River

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Handling Editor: Nikos Skoulikidis

Received: 31 June 2013; Accepted: 18 June 2014; Published on line: 30 June 2014

Abstract

Despite their high ecological value, transboundary Mediterranean rivers and their coastal wetlands are subject to increasing anthropogenic stressors (intensive agriculture, industrial activities, population density) and are predicted to be particularly vulnerable to future climate change. Improving understanding of elemental cycling in these systems is critical for understanding these ecosystems' functioning and response to future pressures. Here we studied dissolved organic matter (C, N and P) cycling in the Evros/Марица/Meriç River, one of the most heavily polluted and, simultaneously, most ecologically valuable transboundary river systems in the Eastern Mediterranean. Measurements were performed in different seasons over a year (April 2009, July 2009, September 2009, April 2010 and July 2010) and along transects extending more than 70 km from the freshwater end-member to 2-km offshore in the Aegean Sea. Dissolved organic carbon (DOC) in Evros waters (DOC: 119 – 496 μmol/L) was comparable to that of other large Mediterranean rivers (Rhone, Po). The organic component of N and P showed increased variability and ranged from 0.65 to 56 μmol/L for DON and from 0.03 to 4.15 μmol/L for DOP. In the lower parts of the river, where all point and non-point inputs converge, the high inorganic N inputs favored elevated assimilation rates by phytoplankton, and resulted in increased chl-a concentrations (up to 488μg/L) and autochthonous dissolved organic matter (DOM) production under dry conditions and low flushing rates. Moreover, the distribution of carbohydrates revealed a constant background of soil-derived mono-saccharides, and additional contributions of poly-saccharides during phytoplankton bloom events. During the dry season, inorganic nutrients and DOM from upstream sources remained restricted in the lower parts of the river (delta and coastal wetlands), while during high flow conditions upstream DOM was flushed to the sea and organic nitrogen forms became an important component of TDN (at least 40%) in the coastal and shelf waters. The co-existence of terrigenous material with autochthonous production and some anthropogenic contributions is supported by the large variability found in DOC:DON (4 - 96) and DOC:DOP (79 - >1000) ratios, the positive correlation of DOC *vs* chl-a (r^2= 0.76, p=0.01 for July 2009; r^2=0.77 p=0.01 for September 2009), the decoupling between DOC and DON, and the observed spatial distribution of inorganic N forms including ammonium. Our results illustrate the combined and complex influences of (i) seasonally dependent hydrological processes, (ii) consistent upstream anthropogenic pollution sources, and (iii) irregular water resource management practices on C, N, P dynamics along this heavily polluted trans-boundary river system, its delta and wetland ecosystem, and adjacent Mediterranean coastal waters.

Keywords: DOC, DON, DOP, carbohydrates, eutrophication, Evros River, eastern Mediterranean.

Introduction

Rivers are the major pathway of terrestrial organic matter to the coastal seas. Complex autochthonous processes within rivers, including estuaries and deltas, involve production and mineralization and may alter significantly both the quantity and composition of dissolved organic matter (DOM). Moreover, in modern times (1960 onwards) anthropogenic activities, such as dam construction and water resource management, extensive use of fertilizers on land crops, as well as wastewater discharges, have led to a 'reorganization' of the biogeochemical cycles of carbon (C), nitrogen (N) and phosphorus (P) (Rabouille *et al.,* 2001).

Traditionally most research dealing with eutrophication has focused on dissolved inorganic nutrients and research on organic forms of nutrients has lagged behind. Dissolved organic matter consists of a mixture of organic compounds and the concentration of DOC is considered a measure of DOM present in an aquatic system.

The importance of the dissolved organic carbon, nitrogen and phosphorus (DOC, DON, DOP) fluxes from rivers to coastal waters has been recognized during the last decades as a key factor regulating autotrophy *vs* heterotrophy (Kemp *et al.,* 1997; Caffrey, 2004). The bioavailability of DOM depends largely on the nature of the individual DOM compounds which in turn, is closely related to specific DOM sources (terrestrial plants, riverine phytoplankton, anthropogenic). In riverine DOM carbohydrates exhibit a high degree of variance accounting from 9% to 24 % of DOC (Senior & Chevolot, 1991; Hung *et al.*, 2001; Gueguen *et al.*, 2006). Carbohydrates (mostly PCHO) are excreted by both autotrophic and heterotrophic microorganisms and serve as energy units to heterotrophic microorganisms. In addition, substantial amounts of carbohydrates (as MCHO) in rivers originate from soil and leaf litter decomposition (Hedges *et al.*, 1994). Apart from microbial metabolism, photodegradation of DOM is another important process affecting mineralization rates (Vodacek *et al*., 1997; Hernes & Benner, 2003; Tzortziou *et al.,* 2007; Spencer *et al*., 2009). Moreover, removal processes taking place in estuaries involve flocculation and trapping of DOM in the sediments. In that sense, estuaries frequently act as permanent or occasional DOM sinks. The interactions between DOM source and sink processes however are complex and affected by multiple factors including hydrological conditions, catchment, soil and vegetation properties, biota and land uses. Previous studies on DOC transport and fate in river and estuarine systems showed that in many cases the apparent conservative character of DOC during estuarine mixing actually reflects the balance of simultaneous sources and sinks (Cifuentes & Eldridge, 1998; Moran *et al.,* 1999; Raymond & Bauer, 2000, Tzortziou *et al.,* 2011). The dynamics of dissolved organic nitrogen (DON), and to a lesser extent dissolved organic phosphorus (DOP), have been investigated in various world rivers (Aitkenhead-Peterson *et al.,* 2005; Amon & Beon, 2004; Duan *et al.,* 2008; Hopkinson *et al.,* 1998; Rinker & Powell, 2006; Seitzinger & Sanders, 1997; Townsend *et al.,* 2011; Wiegner *et al.,* 2006; Yao *et al.,* 2009). It have been shown that the organic species of N contribute substantially, from 30% up to 70%, to the total nitrogen pool in rivers, estuaries and coastal waters (Bronk, 2002; Dafner *et al.,* 2007; Badr *et al.,* 2008). Still, very limited information is available for C, N and P cycling in Mediterranean rivers, and particularly in trans-boundary systems that are subject to intensive and often conflicting uses. Although some large Mediterranean rivers (Rhone, Po) have been studied for their DOC dynamics (Pettine *et al.,* 1998; Cawvet, 1990) there are no systematic works on DON and DOP biogeochemistry. Only four recent works concerning small catchments in the Western and Central Mediterranean (Vazquez *et al.,* 2011; Bernal *et al.,* 2005; Mattsson *et al.,* 2009; Lorite-Herrera *et al.,* 2009) report DOC and DON data combined, while there is no published studies to our knowledge for river catchments in the Eastern Mediterranean.

This study provides new measurements on elemental and molecular DOM composition in the Evros River (*Evros* in Greek, *Maritsa* in Bulgarian, *Meriç Nehri* in Turkish), one of the most important natural resources in the Balkans with great impact on the economies of Bulgaria, Greece and Turkey. The Evros is a trans-boundary river, the second, in terms of water flux, in Eastern Europe after the Danube and the largest outflowing in the Aegean Sea. It is heavily polluted due to agricultural, urban and industrial activities. Moreover, the Evros is a river affected by floods, the frequency of which has increased dramatically during the last 13 years. The reason of this change is unclear; it could be due to climatic changes or to dam construction and water management practices (Angelidis *et al.,* 2010; Fotopoulos *et al.,* 2010). Here we studied the seasonal and spatial distribution of DOC, DON, DOP and carbohydrates along a > 70 km transect that extended from a freshwater end-member to 2-km offshore in the Aegean Sea. Our results illustrate the combined and complex influences of (i) seasonally dependent hydrological processes, (ii) consistent upstream anthropogenic pollution sources, and (iii) irregular water resource management practices on C, N, P dynamics along this heavily polluted trans-boundary river system, its delta and wetland ecosystem, and adjacent Mediterranean coastal waters.

Materials and Methods

Study Area

The Evros River drains the northeastern Balkan peninsula and outflows in the Aegean Sea (Fig. 1). It is the largest river (length 528 km) discharging in the Aegean Sea with a catchment area of 53,000 km^2 including parts in Bulgaria (66%), Turkey (28%) and Greece (6%). Its annual mean water flux fluctuates from 50 to 100 m^{3}s^{-1}. In the delta area, a number of wetlands are formed that are included in the Ramsar Convention. The Evros River catchment area is one of the most intensively cultivated areas in the Balkans and supports a population of 3.6 million people. During floods, water discharge exceeds 2,500 m^3 s^{-1}. In the Bulgarian part of the watershed a significant number of dams (>15) has been constructed after 1994 for irrigation and energy purposes. Thus, there is no longer a direct flow of waters from the Bulgarian watershed in the Greek-Turkish downstream part, especially during summer. Since 1997, the frequency of flood events has increased seven times compared to previous records from 1985 to 1995 (Angelidis *et al.,* 2010; Darmouth Flood Observatory, 2005). Major pollution pressures include mining industries in the Bulgarian part of the catchment area, industrial activities and domestic wastes around the town of Edirne, in Turkey, as well as agricultural areas in the Greek territory of the Evros catchment (Skoulikidis, 2009).

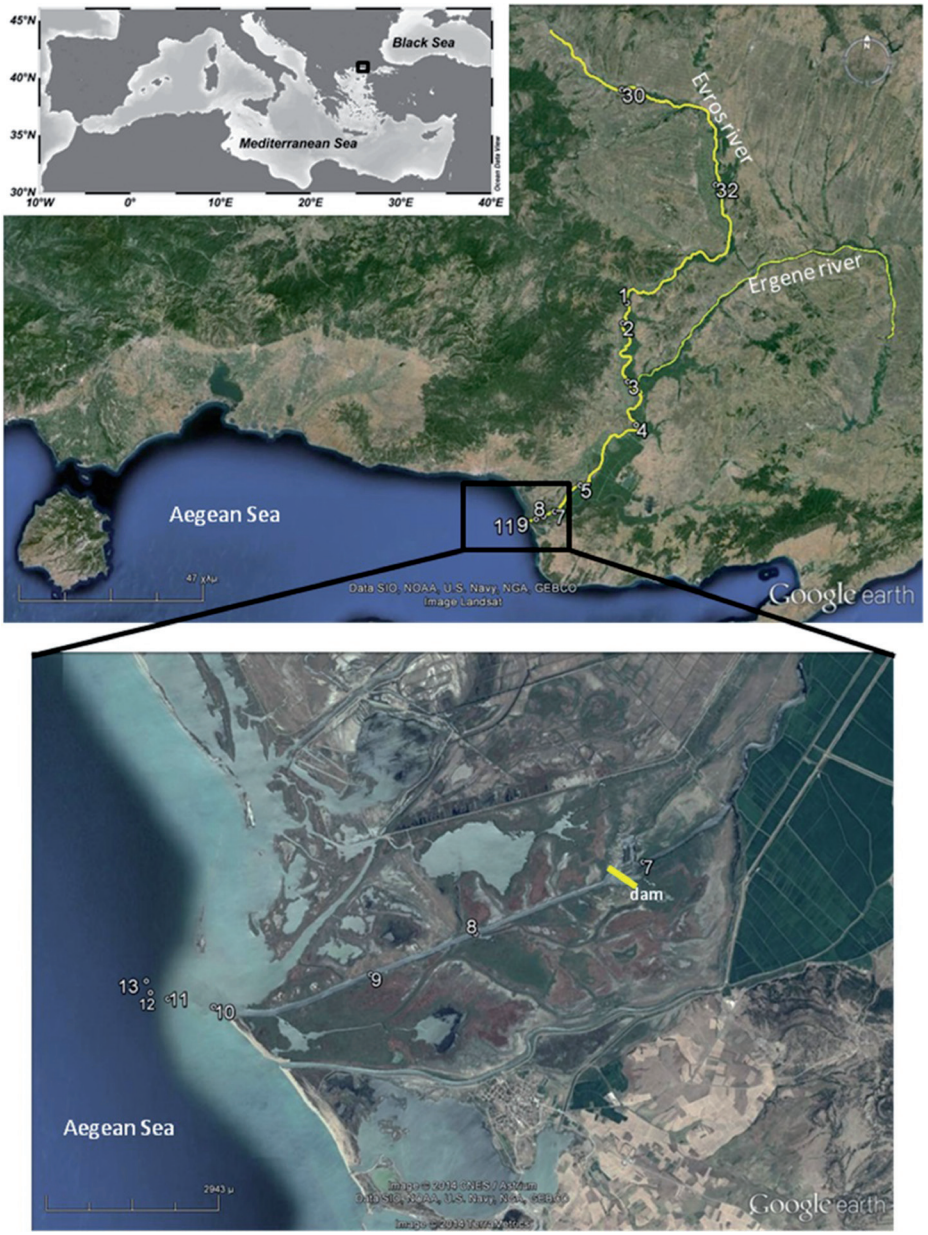

Fig. 1: The stations sampled along the Evros River (stns 30, 32 and stns 1 to 13). In the lower panel are shown in more detail, four downstream stations (7, 8, 9, 10), the marine stations (11, 12, 13) and the position of the dam.

Over 6,000 km^2 of cultivated land are located in the Greek part of the Evros River catchment which corresponds to one of the most important agricultural regions in northern Greece. Because of all these anthropogenic pressures, and in order to achieve the goals set by the WFD 2000/60/EC, various trans-boundary management schemes have been proposed (Dimitriou *et al.,* 2011; Nikolaou *et al.,* 2008).

Field sampling

Water sampling in the Evros River was conducted during five sampling periods: April 2009 (wet season), July 2009 (dry season), September 2009 (end of dry season), April 2010 (wet season, flood conditions) and July 2010 (dry season with increased water flow). Ten stations (st.1 to st.10) were sampled along the last 80 km of the main riverbed before the river reaches the sea. Three stations were sampled in marine waters (st.11, st.12, st.13) within a distance of ~ 2 km offshore. Additionally during April and July 2010 two more stations were sampled within a distance of 78 km further upstream (st.30, st.32). Overall, 3 groups of stations were considered: upstream stations 30, 32, 1, 2, 3 downstream stations 4, 5, 6, 7, 8, 9 10 and marine stations 11,12, 13 (Fig. 1). Between st. 3 and st.4, the Ergene tributary meets the Evros main river route. The Ergene River drains a catchment of approximately 14,000 km^2 in eastern Thrace (Turkey). Further downstream, and between st.7 and st.8, there is a small dam made out of soil material by the local authorities in summer 2008 in order to control the water flow and avoid sea intrusion. During our samplings, the dam was already

Table 1. Average values and range of measured hydrological parameters, inorganic nutrients and DOC, DON, DOP during each sampling.

	Precip.* (mm)	Q (m^3sec^{-1})	Q Ergene (m^3 sec^{-1})	Temp (°C)	Salinity	DO ($\mu molL^{-1}$)	D.O. sat.%	chl-a (μgL^{-1})	NO_3^- ($\mu molL^{-1}$)	NH_4^+ ($\mu molL^{-1}$)	PO_4^{-3} ($\mu molL^{-1}$)	DOC ($\mu molL^{-1}$)	DON ($\mu molL^{-1}$)	DOP ($\mu molL^{-1}$)
April 2009 *(n=10)*	129	114+	28	13.3 11.2- 15.0	0.5 0.0-28	307 275-359	95 86-118	5 2-8	70.82 9.07-90.39	9.06 0.93-18.35	3.22 0.63-4.31	219 132-259	23.68 5.54-55.94	1.12 0.03 -2.10
July 2009 *(n=11)*	20	-	8.3	26.4 24.6-29.8	9.9 0.0-35.7	328 222-538	133 89-180	132 18-488	9.52 0.43-20.72	24.60 1.44-62.32	3.73 0.10-9.80	314 206-496	18.69 6.10-33.77	1.27 0.23-4.15
Sept. 2009 *(n=11)*	54	32+	15.2	20.2 18.3-21.8	7.6 0.0-37.8	327 260-435	117 88-156	63 1-115	43.69 0.38-71.98	13.49 0.24-41.90	3.34 0.03-6.70	226 119-289	18.65 1.35-41.44	1.60 0.03-3.20
April 2010 *(n=8)*	67	198≠	38.8	15.1 13.4-15.9	0.3 0.1-0.9	290 278-306	93 88-99	4 1-8	148 103-203	6.87 1.62-15.49	3.80 3.34-4.11	217 181-257	12.65 0.65-44.31	0.36 0.03-0.63
July 2010 *(n=12)*	91	-	14.9	25.6 22.3-27.5	3.8 0.1-35.0	267 228-316	107 88-127	18 3-33	43.36 10.48-97.16	14.53 1.27-41.82	5.16 3.95-7.65	293 247-389	28.26 9.75-39.47	0.27 0.02-0.99

* Precipitation recorded at a coastal city (Alexandroupoli) ~20km distance from the delta. + Water flow measured at st.4 (70 km upstream). ≠ Water flow measured at st.30 (160 km upstream).

considerably eroded and freshwater escaped towards the Evros Delta even during summer months.

Water samples (500 mL) for chemical analyses were collected from surface waters using acid pre-cleaned Nalgene bottles. The samples were stored immediately at +4°C. For DOM analyses, samples were filtered through 0.22 μm Nucleopore filter, using a Nalgene filtration apparatus, within 24 h of collection. Five aliquots of the filtrate were stored for future chemical analyses.

Measurements of hydrological conditions, physico-chemical parameters and chlorophyll-a

Discharge measurements (in m^3 s^{-1}) were available at the monitoring station Kipoi (Q_K), located downstream of st.4 in Evros, during two of our samplings (April 2009, September 2009) and at the monitoring stations of Ormenio located at st.30 during July 2010. Discharge data were also available at station Yenicegörece along the Ergene river (Q_{ERG}), approx. 15 km upstream of the merging with Evros, during all sampling dates (data available from the Prefecture of Eastern Macedonia - Thrace, the Turkish General Directorate Of State Hydraulic Works, DSI 11th Regional Directorate - Edirne, and the Greek Ministry of Environment, Energy and Climate Change). Daily precipitation values (in mm/day) were measured consistently at the Alexandroupoli station during 2008-2010 (data available from the Hellenic National Meteorological Service- H.N.M.S., station WMO16627, 25°53′, 40°51′), from which we estimated the total precipitation accumulated over the two months preceding each of our samplings dates (P, in mm; Table 1).

At each station *in situ* measurements of temperature, pH, conductivity and dissolved oxygen, were conducted with a portable device (Hanna, HI-9828).

Water samples for chlorophyll-a determinations were filtered through a Whattman GF/F filter and chl-a was analyzed using a TURNER 00-AU-10 fluorescence meter according to the method described by Holm-Hansen *et al* (1965).

Analyses of inorganic and organic N, P

After filtration, samples for inorganic nutrients analyses were stored frozen in polyethylene bottles. Inorganic nutrients were analyzed using colorimetric techniques. Nitrates (including nitrites) were determined according to standard methods (Strickland & Parsons, 1977) using a Bran-Luebbe II autoanalyzer. Ammonia and phosphates were analyzed manually following the Nash reagent method (Solorzano, 1969) and the molybdenum phosphoric acid method (Murphy & Riley, 1962) respectively, with a Varian Cary-1E spectrophotometer. Detection limits of the analyses were 0.10 $\mu molL^{-1}$ for NO_3^-; 0.12 $\mu molL^{-1}$ for NH_4^+ and 0.04 $\mu molL^{-1}$ for PO_4^{3-}.

Filtered samples for the determination of DON and DOP were stored frozen until analyses in pre-digested bottles

(pyrex for DON and Teflon for DOP). For the measurement of DON and DOP, a conversion to inorganic forms was obtained by a persulfate wet-oxidation in low alkaline conditions (Valderrama, 1981; Raimbault *et al*., 1999). The concentrations of total dissolved nitrogen and phosphorus (TDN and TDP) in the sample were measured in the same manner as inorganic nutrients described above. The values obtained were corrected for the reagent blank. DON and DOP were calculated as the difference between total and mineral N, P species (DON=TDN-[NO_3^-+NH_4^+], DOP=TDP- [PO_4^{3-}]). The efficiency of the digestion for DON was tested with replicate analyses of EDTA standard solutions. DON recovery was 117% for the 5 μmol N L^{-1} , 107% for the 10 μmol N L^{-1} and 104% for the 15 μmol N L^{-1} solution.

Analysis of DOC

Duplicate samples (10 mL) for DOC determination were stored in pre-combusted (480ºC, 12 h) glass ampoules, acidified with 2.5 N HCl to pH ~2, flame – sealed immediately and kept at +4 ºC until analysis.

Analysis of DOC was carried out using a HTCO automatic analyzer (Shimadzu TOC-5000) following the method described by Sugimura & Suzuki (1988) and Cauwet (1994). The analytical precision and accuracy was tested on every analytical day against Deep Atlantic Seawater Reference Material provided by the DOC-CRM programme (University of Miami - D.A. Hansell); measured value: 44 ± 3 μmol C L^{-1} n=5 - certified value: 45 ± 1 μmol C L^{-1}.

Analysis of carbohydrates

Total carbohydrates (TCHO), monosaccharides (MCHO), and polysaccharides (PCHO) were analyzed using the 2,4,6-tripyridyl-s-triazine (TPTZ) colorimetric method (Myklestad *et al.,* 1997) with a Varian Cary-1E spectrophotometer.

The concentrations of TCHO were determined after hydrolysis of PCHO to MCHO. The MCHO concentration in samples was directly measured without hydrolysis. The reagent blank in Milli-Q was subtracted before calculating the concentration of monosaccharides. The PCHO concentration in samples was equal to the difference between TCHO and MCHO ([PCHO] = [TCHO] - [MCHO]). The standard deviation of the lowest TCHO concentration sample was 0.03 μmol C L^{-1} and the calculated limit of detection (LOD) was 3.00 μmol C L^{-1}.

Statistical treatment

Statistical treatment of data was carried out using the SPSS statistical package and in order to examine whether significant differences existed in the data sets during each season and between seasons. A Shapiro-Wilk test was used to determine whether the concentrations of a given parameter follow a normal distribution (for $p>0.05$). Most data sets did not follow a normal distribution, so a non-parametric test (Kruskal-Wailis) was used to compare the data between seasons. The differences between two groups of data were considered significant for $p<0.05$. The correlations between two sets of concentrations, following normal distribution, were calculated using the Pearson product-moment correlation (r^2). For those sets of concentrations that did not follow normal distribution the Spearman Rank correlation coefficient (r_s) was used.

Results

Interpretations of our data were made first by grouping our data in wet (April 2009, April 2010) and dry seasons (July 2009, September 2009, July 2010) and second taking into consideration specific patterns encountered within each season (e.g. flood events, phytoplankton blooms) (Table 1). In addition, we discuss spatial dynamics and the influences of various natural and anthropogenic point sources identified across the Evros River, delta and Aegean coastal waters. All physical and chemical data obtained during this study are presented in detail in the APPENDIX.

Hydrological conditions and influence on chlorophyll dynamics

Precipitation (mm), mean water discharge (m^3s^{-1}), temperature (ºC), salinity, D.O. (μmolL^{-1}) and chlorophyll-a (μgL^{-1}) are listed in Table 1 for all sampling periods. Climatic conditions and water management for irrigation purposes are reflected in the differences observed in water discharge volumes between the wet (April 2009, April 2010) and dry (July 2009, September 2009, July 2010) seasons. The maximum water discharge (198 m^3 s^{-1}) recorded in April 2010 was due to a flood episode in February-March 2010 (http://el.wikipedia.org/wiki/evros) that distinguishes this sampling as a post-flood situation. The variability in water discharge was reflected in the salinity distribution of the seaward stations (Fig. 2a). In April 2009, relative low salinities ($17<S<30$) were recorded at st.11, 12, 13, implying increased freshwater influence. Unfortunately, marine stations were not sampled in April 2010. A reversed pattern was observed during the dry season, when salt intrusion resulted in measurable salinities (S=2.1) as far as 14 km upstream of the river mouth (st.7) in mid-summer, and 2.7 km upstream of the river mouth (st.9) in late summer (September 2009). Water temperature values were low during the wet season (11.2 -15.9 ºC), intermediate in late-summer (18.3 -21.8 ºC) and reached their maximum in mid-summer months (24.6 – 29.8 ºC). Dissolved oxygen saturation was always >86% but never exceeded 100% during the wet season. An exception to this is the value of 118% in April 2009 that corresponds to the most seaward station (st.13). In summer however, river water was oversaturated in D.O., especially in the downstream parts; 132% in July 2009, 117% in September 2009 and 107% in July 2010. Average chlorophyll-a

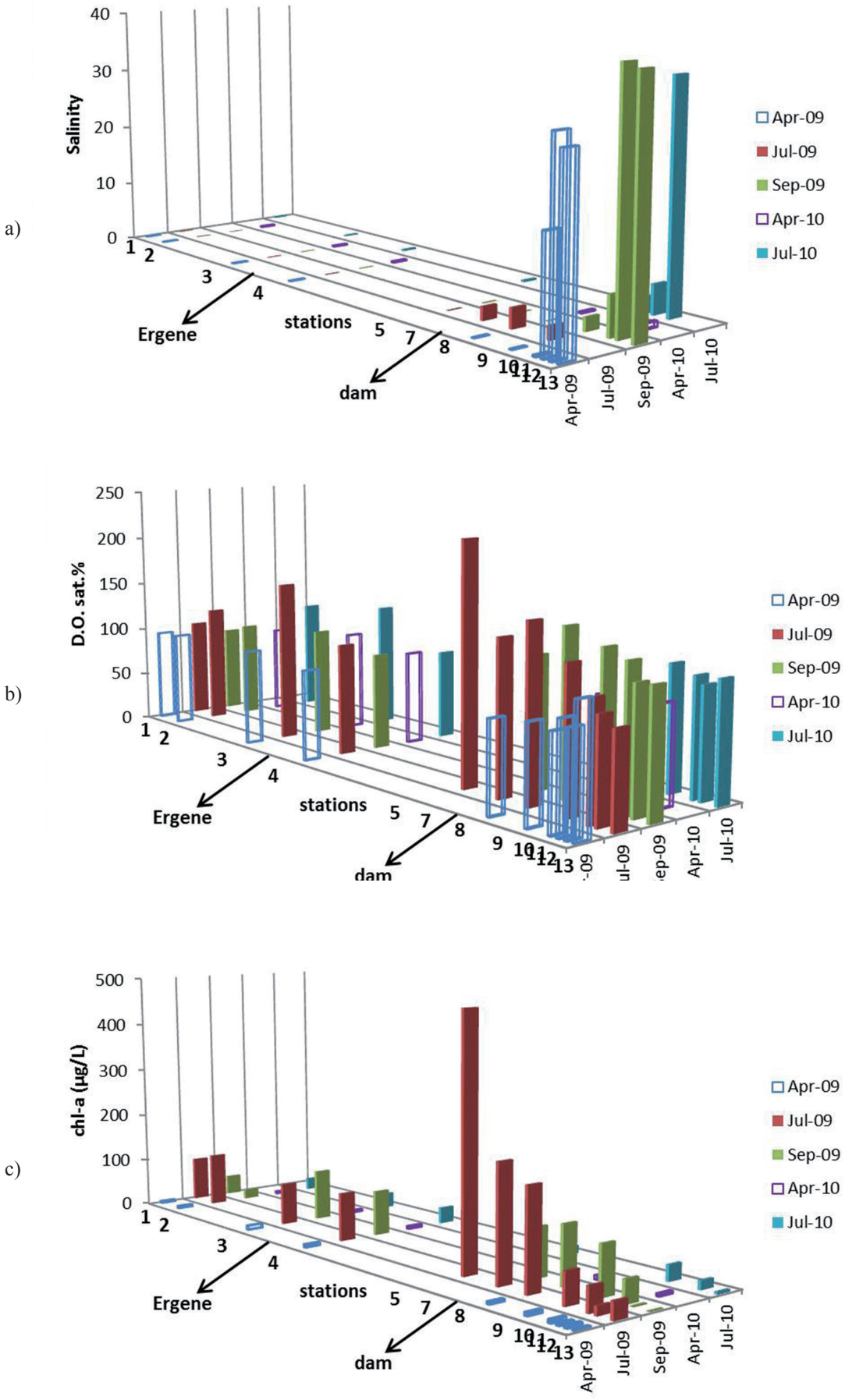

Fig. 2: Spatio-temporal distribution of (a) salinity; (b) O_2 saturation %; (c) chl-a (µg L^{-1}); along the Evros River during the 5 samplings (x-axis: station distances in km).

concentrations were as low as ~4-5 μgL^{-1} in the wet season and reached 132 μgL^{-1} in summer (dry season). More specifically during our summer samplings, chl-a showed similar variation to that observed for D.O. sat %, falling from 132 μgL^{-1} in July 2009 to 63 μgL^{-1} in September 2009 and 18 μgL^{-1} in July 2010. These results provide evidence that July 2009 coincided with a strong phytoplankton bloom and September 2009 either with a post bloom situation or with a second less intense bloom. The exceptionally high precipitation and subsequent water discharge that occurred in spring –summer 2010 hindered intense phytoplankton production in July 2010. The picture described, is clearly depicted in the D.O. and chl-a distribution shown in Figures 2b,c, where it is also shown that chl-a reaches its summer maximum values downstream the merging with Ergene and upstream of the dam (between st. 4 and st. 8)

Dissolved Organic Carbon

DOC in Evros waters ranged from 119 to 496 $\mu molL^{-1}$. The seasonal (wet vs dry) distribution of DOC showed significantly lower (p=0.02) concentrations during the wet season ranging from 132 to 259 µmol/L, while for the dry season the concentrations were in the range 206-496µmol/L. Spatial distribution of DOC (Fig. 3) was also variable and showed relatively low concentrations upstream (st. 1-3) followed by a consistent significant increase at station 4 (downstream the Ergene outflow) and an almost constant level at the downstream stations up to the delta. DOC concentrations in the delta and coastal waters (st. 10 to 13) were highly variable temporally, depending on the precipitation regime.

Inorganic and organic N, P

Nitrates showed a 1000-fold variation between seasons, with concentrations ranging from 0.43 to 203 $\mu molL^{-1}$ (Table 1). Significantly (p=0.00) higher nitrate concentrations were observed during the wet season, clearly shown in Figure 4a. No clear spatial trend was observed during each sampling month. Ammonium concentrations varied from 0.24 to 62.32 $\mu molL^{-1}$, with significantly (p=0.02) higher concentrations during the dry season (Table 1, Fig. 4b). The spatial distribution of ammonia concentrations was similar to that of DOC, showing a consistent sharp increase at station 4 downstream the merging with Ergene and maintaining high values down to the river mouth. DON concentrations varied in the range of 0.65 to 55.94 $\mu molL^{-1}$ and did not reveal any clear seasonal trends (Table 1, Fig. 4c). The spatial distribution of DON followed that of NH_4^+ and DOC, showing a consistent concentration increase downstream the merging with Ergene. In most northern European and American rivers, DON has been found to contribute to as much as 70% of TDN (Berman & Bronk, 2003). This was not the case for the Evros waters, where DON contribution was minor during the wet season (<10%) reaching ~40% during dry conditions. Particularly during the wet season, nitrates were the dominant TDN component with an average percentage contribution of 65% in April 2009 (70.82 μmoL^{-1}) and 89% in April 2010 (148 μmoL^{-1}) (wet and flood conditions, respectively). The dominant contribution of inorganic forms of N in Evros waters, especially during high flow conditions (Fig. 4a), was also reported by Skoulikidis (1993; 2009) and is most likely the result of draining large parts of agricultural land in the Evros watershed.

Large phosphorus loads have been previously recorded in the Evros River and according to Skoulikidis (2009), Evros has the highest total phosphorus concentrations among all rivers draining the Balkan Peninsula due to the presence of organophosphoric insecticides.

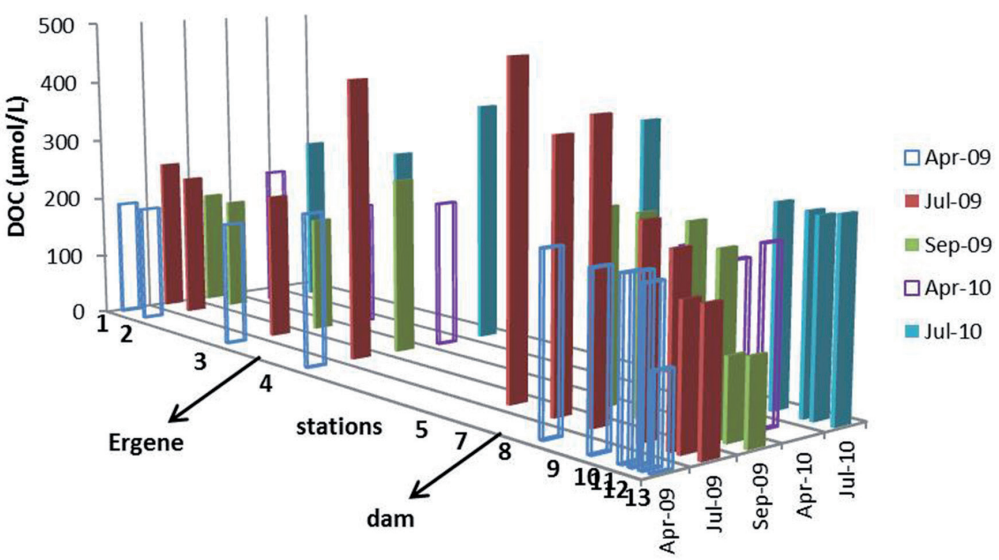

Fig. 3: Spatio-temporal distribution of DOC (µmol C L^{-1}) along the Evros River during the 5 samplings (x-axis: station distances in km).

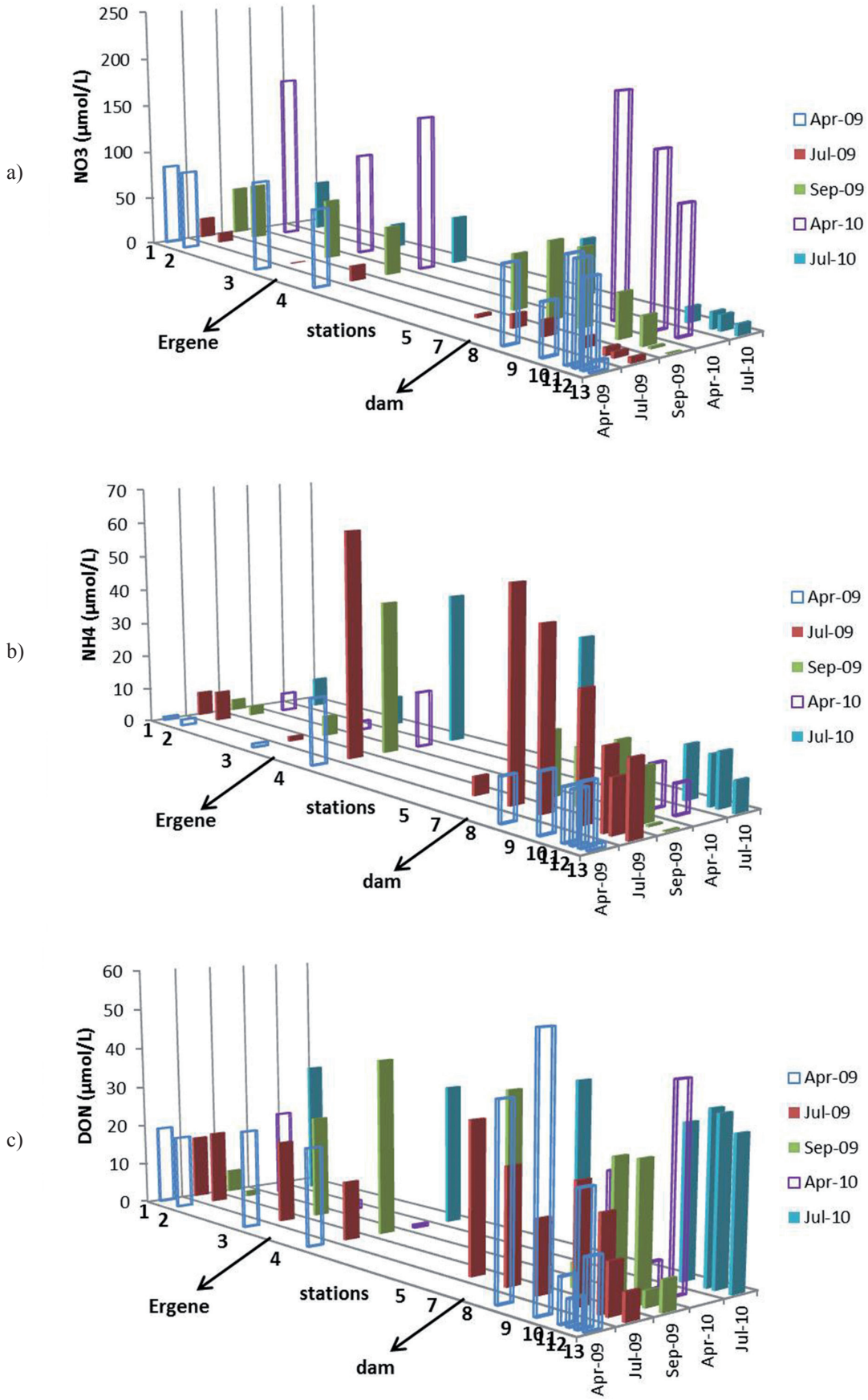

Fig. 4: Spatio-temporal distribution of (a) NO_3^- (µmol L^{-1}), (b) NH_4^+ (µmol L^{-1}), (c) DON (µmol N L^{-1}), along the Evros River during the 5 samplings (x-axis: station distances in km).

Phosphate concentrations during our study varied from <0.03 to 9.80 μmolL^{-1}. Higher concentrations were recorded during the dry period, but seasonal differences were only marginally statistically significant (p=0.06) (Table 1, Fig. 5a). DOP concentrations fluctuated from <0.03 μmolL^{-1} to 4.15 μmolL^{-1} and no significant seasonal (wet vs dry) differences were found. DOP concentrations were elevated during the dry months of July 2009 and September 2009, but not in July 2010 which followed an exceptionally high precipitation period. As shown in Figure 5, maximum concentrations for both P species were recorded downstream of st.3 to st.10.

DOC:DON and DOC:DOP ratios

DOC:DON ratios were highly variable in our samples and ranged from 4 to 96. Average DOC:DON however, (19±17), is comparable to DOC:DON ratios reported for various estuaries and world rivers (23.4±13.4) (Bronk, 2002). DOC:DOP ratios were also highly variable. In marine waters and occasionally at some upstream sites DOC:DOP was greater than 1000 whereas in all other cases DOC:DOP ratios ranged from ~120 to ~700. Mattsson *et al* (2009) reported DOC:DOP ratios ranging from 219 to 5590 in 34 European catchments and attributed this variation to differences in land uses. In Evros waters, the generally low DOC:DOP values (<1000) indicate the presence of organic phosphorus loads.

Carbohydrates

Total, poly- and mono-saccharides (TCHO, PCHO, MCHO) were measured during all sampling periods (Table 2). It should be noted here that the TPTZ method estimate of MCHO refers to the carbohydrate groups bound to humic substances. (Hung *et al.,* 2005; Buffle, 1990). Concentrations of MCHO ranged from 7.33 μmolCL^{-1} to 58.61 μmolCL^{-1} (Fig. 6a) and those of PCHO from as low as 3.00 μmolCL^{-1} to 86.75 μmolCL^{-1} (Fig. 6b). These large variations in the concentrations are due to spatial rather than temporal differences. Overall, MCHO exhibited considerably lower spatial variability (CV% ~30%) during both the wet and dry seasons compared to PCHO that had a more scattered spatial distribution especially during the dry season (CV%= 40% wet; CV%=91% dry). This picture is also reflected in the irregular contribution of PCHO/TCHO%, accounting for 9 - 76% of TCHO.

Table 2. Average values and range of measured concentrations of mono-, poly- and total polysaccharides during each sampling.

	MCHO (μmolCL^{-1})	PCHO (μmolCL^{-1})	TCHO (μmolCL^{-1})
April 2009	30.68	27.85	58.52
(n=9)	9.32-45.13	11.82-43.33	34.37-79.13
July 2009	27.72	27.67	55.39
(n=11)	12.99-40.96	3.00-128	25.78-170
Sept. 2009	24.49	21.66	46.16
(n=10)	7.33-41.63	8.86-49.29	16.19-90.92
April 2010	33.32	28.93	62.25
(n=8)	27.56-38.54	6.79-43.06	45.33-78.16
July 2010	40.19	34.90	75.09
(n=9)	31.14-58.61	7.96-86.75	39.76-130

Discussion

External sources of DOM

Because DOM in rivers originates from both natural and anthropogenic sources with variable elemental composition, a decoupling of DOC, DON, and DOP is expected. Previous studies found that urban areas contribute to all three constituents, whereas agricultural land contributes mostly to DON and DOP (Harrison *et al.,* 2005; Stedmon *et al.,* 2006). Waters draining forests and wetlands are typically enriched in DOC, and measurements of DOM along a European climatic gradient (Mattsson *et al*., 2009) showed a strong positive correlation between DOC concentrations/export and latitude. Lowest DOC levels were observed in the southern European catchment (Tech River, in France) due to warmer climate, steeper slopes, smaller accumulation of organic matter in soils and smaller proportion of wetlands. In Mediterranean rivers with warm climate, increased evaporation, soils poor in organic matter and steeper slopes, DOC concentrations are expected to be lower than those recorded in northern European rivers (208-675 μmoL^{-1}) (Abril *et al.,* 2002 and references therein; UNEP/MAP/MEDPOL, 2003). In Table 3 we have summarized the existing data on DOM for various Mediterranean rivers. It becomes clear that the lowest DOC concentrations have been recorded in the two undisturbed rivers; Krka in Croatia and Krathis in Southern Greece and the highest in two medium size rivers; the Eygoutier River (France) which drains an urban catchment with many anthropogenic activities and Furiosos River (Spain) which drains a forested catchment.

The Evros catchment (53,000 km^2) experiences many anthropogenic pressures. It has a population of 3.6 million with a density of 68 inhabitants/km^2. Most wastewater discharges are untreated and flow either directly into the river or through groundwaters. Additionally, the Evros catchment (especially the lower parts) is covered by 1,500 km^2 of agricultural land which is intensively cultivated with extensive use of N based fertilizers and organophosphoric insecticides (Dimitriou *et al.,* 2012, Vryzas *et al.,* 2009). Given this situation, it is expected that Evros waters will be enriched in DOC, DON, and DOP. Indeed, DOC concentrations measured in the Evros waters are comparable and even higher than those reported for other major Mediterranean rivers, including Rhone and Po, which also experience multiple pressures (Table 3). Also the observed enrichments in DOP and DON, relatively to the Tech River (forested catchment) (Table 3), are noteworthy, although these comparisons are based on

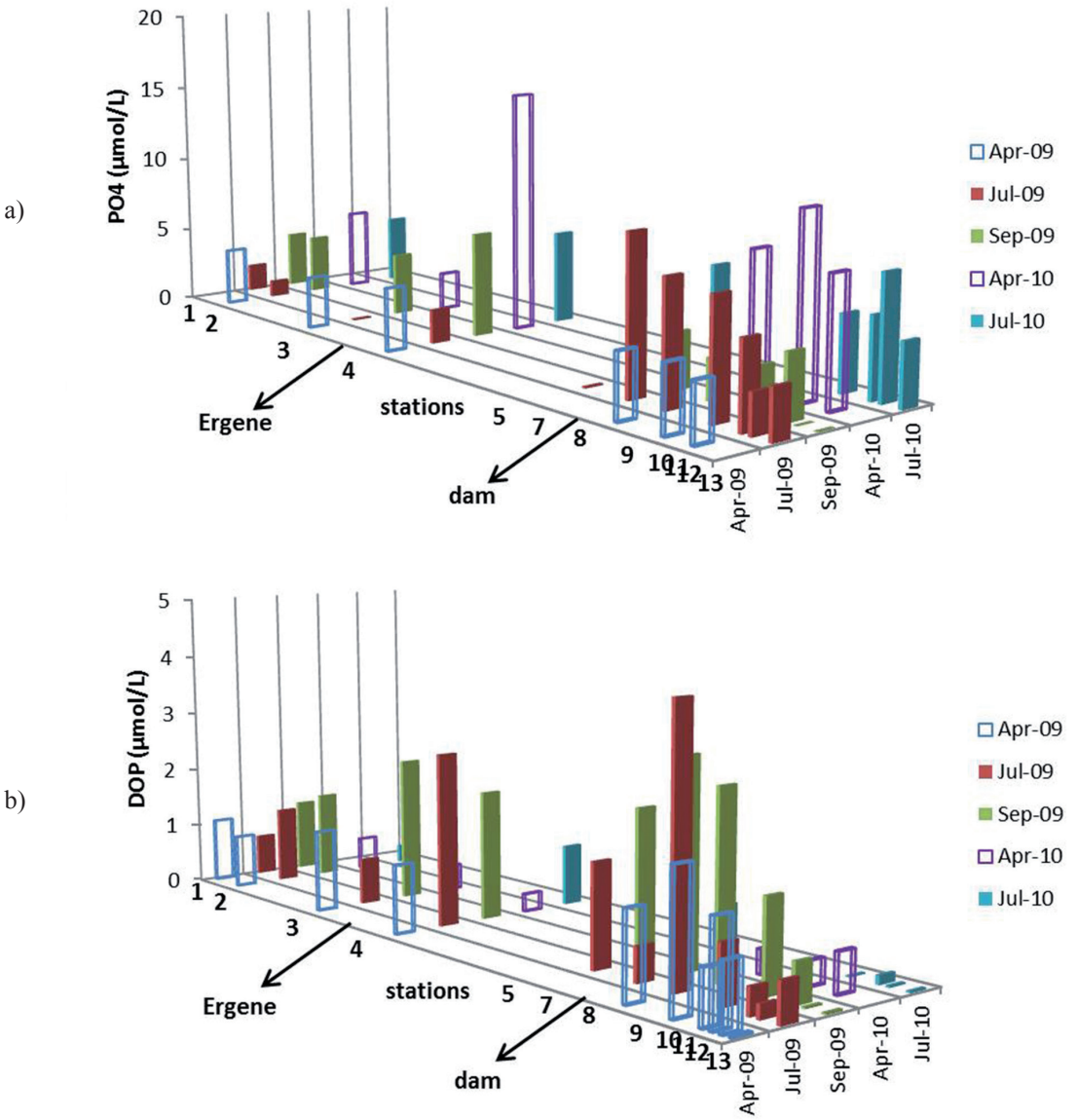

Fig. 5: Spatio-temporal distribution of (a) PO_4^{3-} (µmol L^{-1}), (b) DOP (µmol P L^{-1}), along the Evros River during the 5 samplings (x-axis: station distances in km).

limited data. The seasonal variability found for the various DOM components in the Evros waters, high for DOC but low for DON and DOP, implies that catchment draining is not the sole factor contributing to DOM but point sources and/or in situ processes are equally important.

The spatial distributions of DOC, DON, DOP along the river (Fig. 3, 4c, 5b), showed a consistent increase in DOC, DON, DOP downstream the Ergene outflow (st.4) during all samplings, highlighting the importance of Ergene as a source of allochtonous DOM for the Evros system. The coincident sharp increase in ammonium downstream st.4 (Fig. 4b) further indicates a considerable input of anthropogenic DOM from the Ergene tributary. Ammonium in freshwaters can be used as an indicator of microbial degradation processes or of the presence of wastewaters and agricultural run-off. High levels of ammonium have been recorded in Ergene waters and groundwaters of its catchment area in previous studies (Güneş *et al.* 2008; Ozler & Aydin, 2008) and a severe environmental deterioration of Ergene river has been reported (TWPCR, 2004). The high NH_4^+ concentrations observed at st. 4, after the Ergene merging point, during all seasons, correlate positively with DOC (r^2=0.82, y=4.35x+155.7), DON (r^2=0.77, y=1.07x-6.93) and DOP (r^2=0.76, y=0.04x-0.14) concentrations implying that all three DOM constituents in Evros waters can be associated with inputs from the Ergene river (Fig. 7). The fact that these positive correlations were observed only at st.4 and not at other stations downstream is most likely due to the combination of various DOM transformation processes in the Evros waters, discussed in more detail below.

In situ processes

Oxygen supersaturation coupled with the positive correlation of chl-a *vs* DOC (r^2= 0.76, p=0.01 for July 2009;

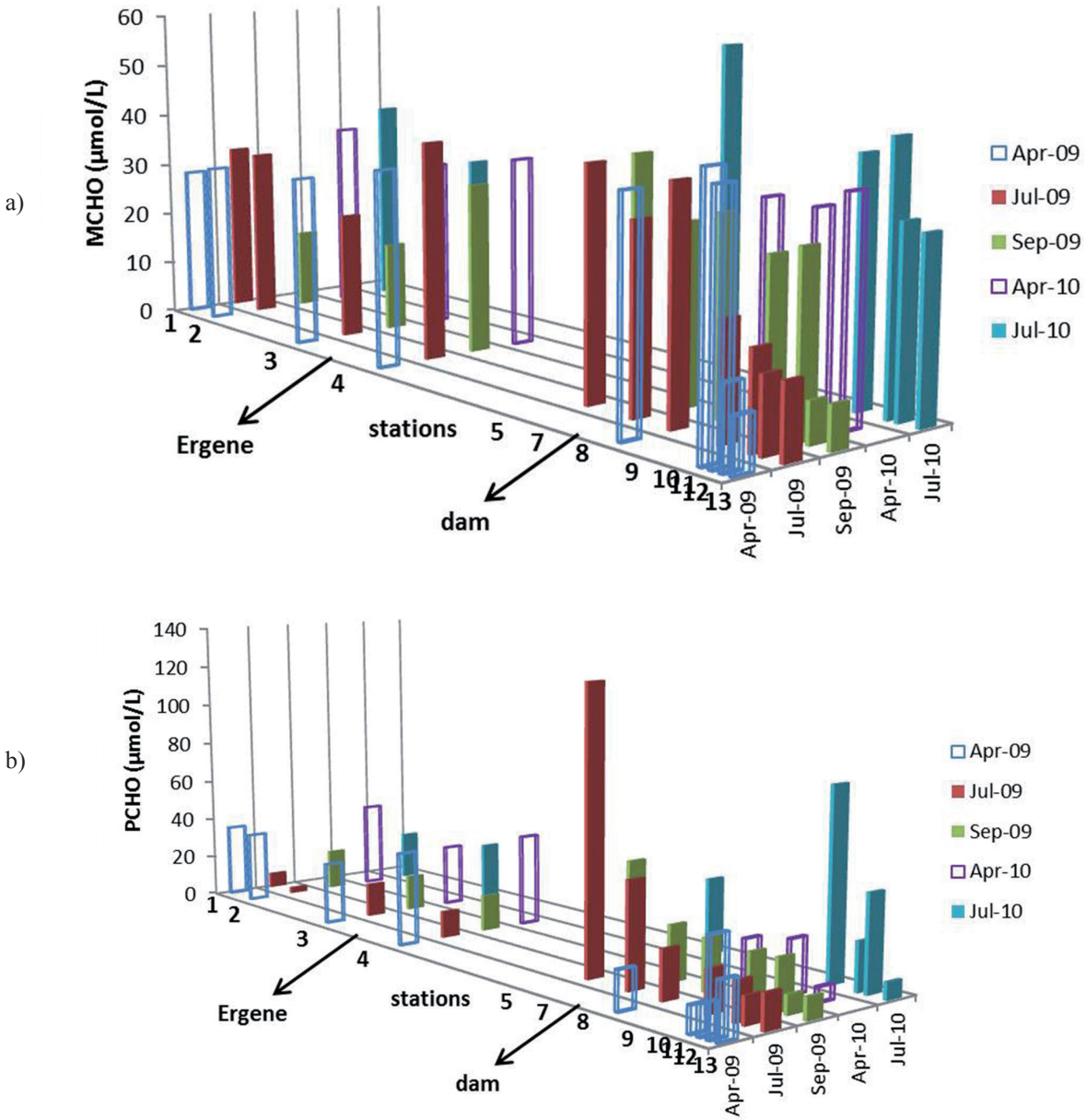

Fig. 6: Spatio-temporal distribution of (a) MCHO (µmol L^{-1}), (b) PCHO (µmol L^{-1}), along the Evros River during the 5 samplings (x-axis: station distances in km).

r^2=0.77 p=0.01 for September 2009), suggest that in addition to allochthonous sources, *in situ* production of DOM from phytoplanktonic cells is taking place. The latter is supported by the strong positive correlation of chl-a *vs* TCHO (r^2=0.970, p=0.00) and PCHO (r^2=0.872, p=0.00), during bloom conditions (July 2009). During post –bloom conditions (September 2009) the correlation of chl-a *vs* TCHO was still strong (r^2=0.617, p=0.01), but in this case it appears that MCHO was the critical factor (chl-a *vs* MCHO r^2=0.628, p=0.01). In July 2010 (post flood) no relationship was found between chl-a and carbohydrates. This is also true for the wet season samplings. The strong correlations we observed between carbohydrates and chl-a in July and September 2009 indicate that *in situ* carbohydrate production from phytoplanktonic cells is taking place during dry conditions, with PCHO being more important under bloom conditions and MCHO under post-bloom conditions. Estimates of the percentage contribution of mono- and poly-saccharides in the total DOC pool showed that average MCHO and PCHO concentrations corresponded to ~15% of DOC each during the wet season (TCHO/ DOC =30%), and decreased to 8-10% during the dry season (TCHO/DOC= 17-19%). However, this change was mainly due to changes in the absolute concentrations of DOC (concentrations of MCHO and PCHO did not change considerably among seasons) (Table 2), suggesting that TCHO is not a major contributor to the excess DOC observed during the dry months. Excess DOC should be attributed to a variety of organic molecules such as lipids, proteins, aromatic compounds, lignin, tannin, humics etc., from different sources (autochthonous, allochthonous or anthropogenic).

Table 3. DOM elemental composition in various Mediterranean rivers.

	DOC µmol/L	DON µmol/L	DOP µmol/L	*n*	Source
Rhone River France	150 -206				Cawvet *et al*., 1990
Rhone (Arles)	200				Eyrolle & Charmasson, 2001
Po River Italy	167 108 -308			*50*	Pettine *et al*., 1998
Po (estuarine waters)	134			*119*	Berto *et al*., 2010
Tech River France	150 83 -200	9 5-11	0.45 0.09 – 0.68	*35*	Mattsson *et al*., 2009
Eygoutier River France	417 83 - 2917			*58*	Nicolau *et al*., 2006
Furiosos River Spain	83 - 500	4-93			Vasquez *et al*., 2010
	83 - 1667	1 - 179		*115*	Bernal *et al*., 2005
Krka River Croatia	91 58 -292				Cawvet, 1991
Krathis River Greece	43				Skoulikidis & Amaxidis, 2009
Evros River Greece	231				Skoulikidis, 1993
Evros River Greece	257 119-496	21 1-56	0.94 0.03 - 4.15	*52*	Present work

Nitrogenous DOM is expected to follow complex dynamics. *In situ* processes such as phytoplankton uptake, mineralization and nitrification has been observed in other Mediterranean catchments (Lolite-Herera *et al.,* 2009; Aviles & Niell, 2007), whereas DON ammonification by bacteria and subsequent nitrification of the released ammonium in freshwater systems have been discussed in a number of previous studies using incubation experiments (Selmer, 1998; Kerner & Spitzy, 2001; Badr *et al*., 2008). The exact mechanisms responsible for the observed distribution of DON in our study system cannot be identified from these data alone and additional process-focused experiments are needed to further resolve relative contributions of different processes in this system. Nevertheless, the decoupling of DON *vs* chl-a (r_s=0.509, p=0.110 for July 2009; r_s=0.358, p=0.310 for September 2009) and

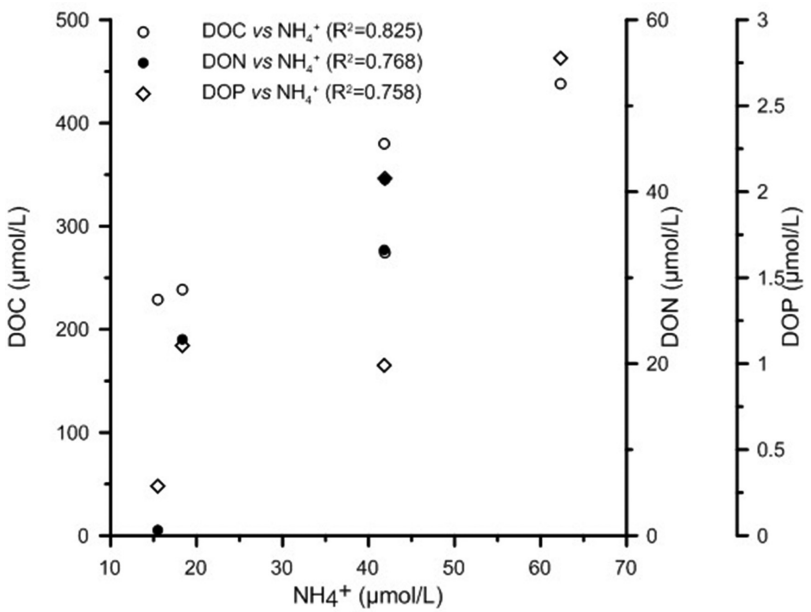

Fig. 7: The positive correlation of NH_4^+ with DOC, DON, DOP at station 4, shows the importance of Ergene as an external source of DOM.

DOC *vs* DON (r_s=0.536, p=0.089 for July 2009; r_s=0.220, p=0.515 for September 2009) implies the presence of allochthonous DON sources such as anthropogenic inputs, already discussed in the previous paragraph.

DOM availability to coastal waters

A major issue of concern, when dealing with DOM biogeochemistry in rivers is the availability of organic forms of N and P to marine bacterioplankton. Terrestrial DOM can become a source of nutrients in shelf waters during summer, when inorganic nutrients are limited. (Stepanauskas *et al.,* 1999; Leff & Meyer, 1991; Sun *et al.,* 1997). Previous work by Stepaunaskas *et al* (1999) showed an enhanced susceptibility of wetland-derived DON to bacterial mineralization during transport from freshwater to saline environments. The exact processes responsible for this have not been clarified yet, but it seems that factors such as changes in the availability of inorganic nutrients, adaptation of marine organisms to low DIN, and changes in the chemical structure of DOM when entering saline waters play a critical role. Some implications for DOM bioavailability in the mixing zone of the Evros River can be obtained from DOC:DON ratios. The coincidence of elevated DOC:DON ratios (18-34) at the marine stations (st.11 to st.13) with low DIN concentrations (Fig. 4a) in summer (July 2009, September 2009) shows that either DON serves as an alternative nitrogen source in coastal waters (Berg *et al.,* 2001; Anderson *et al.,* 2002), or that DON is already mineralized in the estuary and the DOM discharged to the sea is poor in nitrogen. The decrease in coastal DOC:DON ratios during the wet season (April 2009, July 2010), however, suggests that, under increased river flow conditions, freshwater nitrogenous DOM can be exported offshore to the Aegean waters, affecting coastal biological and biogeochemical processes.

Conclusions

Evros River is among the most polluted Eastern Mediterranean systems as is subjected to major anthropogenic pressures and at the same time its coastal wetland is an ecologically important Ramsar protected area. This study revealed that hydrological seasonal dynamics and anthropogenic interventions affect both the DOM pool and the inorganic nutrients. Our results indicate that DOM constituents should be included in monitoring schemes in order to predict in detail the impacts of future pressures to the system, such as increased coastal population, drier climate in Mediterranean and extreme flooding events.

We found that the Evros waters are enriched not only in inorganic nutrients but also in organic forms of C, N, P with concentrations comparable to those of larger Mediterranean rivers (Rhone, Po). DOM dynamics in this transboundary river are significantly affected by allochtonous sources and particularly by anthropogenic contributions from the Ergene tributary. Furthermore, the elevated DIN loads originating from intensive agriculture (mostly NO_3^-) and sewage (mostly NH_4^+) often give rise to production of autochthonous DOM, especially during summer months in the lower parts of the river when the river flow is significantly reduced. Occasionally, however, when this phase is interrupted by increased precipitation and discharge, the accumulated DOM from both allochtonous and autochonous sources is exported offshore, affecting biological and biogeochemical processes in the Aegean coastal waters.

Our results illustrate the combined and complex influences of (i) mostly seasonally dependent hydrologic conditions, (ii) consistent upstream anthropogenic pollution sources, and (iii) irregular water resource management practices on the organic and inorganic forms of C, N and P along this heavily polluted trans-boundary river system. During prolonged dryness and low flushing rates, or due to the existence of dams, increased residence times are expected often favoring extensive transformation of DOM most likely both through photochemical and microbial degradation processes. DOM cycling in rivers become therefore important and should be taken into consideration in management practices. Further studies on the residence time of C, N, P in the lower parts of the river, as well as on the exact processes that determine their fate (respiration, nitrification, flocculation, sedimentation), are needed to further understand DOM dynamics in transboundary Mediterranean rivers that are influenced by various natural and anthropogenic pressures. Detailed measurements of the photo-reactivity and bioavailability of the DOM exported from Evros would allow to fully understand the importance of anthropogenic influences on the coastal waters of eastern Mediterranean Sea.

Acknowledgements

This work was supported by the EU funded projects EcoDOM, MIRG-CT-2007-208841 and PreWEC, MEXT-CT-2006-038331.

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APPENDIX

Values of measured hydrological parameters, nutrients and DOC, DON, DOP at every station during each sampling.

Sampling	station	Temp (°C)	Salinity	DO		chl-a (µg/L)	DOC		NO_3		NH_4		DON		PO_4		DOP	
				mg/L	µmol/L		mg/L	µmol/L	mg/L	µmol/L	mg/L	µmol/L	mg/L	µmol/L	mg/L	µmol/L	mg/L	µmol/L
April 2009	1	11.2	0.01	10.3	322	4.15	2.26	188	5.15	83.07	0.02	0.93	0.27	19.14	0.25	2.62	0.03	1.06
	2	11.5	0.01	10.2	319	4.86	2.26	188	5.04	81.28	0.03	1.70	0.25	17.79	0.29	3.07	0.03	0.87
	3	12.1	0.01	10.2	319	8.43	2.33	194	5.50	88.72	0.02	1.02	0.33	23.28	0.30	3.18	0.04	1.32
	4	12.7	0.01	9.4	294	5.90	2.87	239	4.71	75.96	0.33	18.35	0.32	22.84	0.41	4.31	0.03	1.11
	8	13.6	0.01	8.8	275	4.31	3.11	259	4.34	70.05	0.20	11.38	0.58	41.22	0.35	3.71	0.04	1.36
	9	13.9	0.03	9.3	291	5.96	2.95	246	2.88	46.51	0.27	15.00	0.78	55.94	0.30	3.15	0.07	2.10
	10	13.4	0.2	9.2	288	6.13	2.98	248	5.60	90.39	0.23	12.97	0.13	9.42	0.40	4.21	0.03	0.83
	11	15	17	10	313	3.63	3.01	251	5.47	88.22	0.23	12.84	0.08	5.54	0.32	3.42	0.05	1.55
	12	14.6	30	9.4	294	4.57	2.89	241	4.64	74.91	0.27	15.06	0.38	27.13	0.37	3.87	0.03	1.00
	13	14.7	28	11.5	359	2.14	1.58	132	0.56	9.07	0.03	1.39	0.20	14.50	0.06	0.63	0.001	0.03
July 2009	1	26.2	0.01	8.11	253	89.49	3.01	251	1.28	20.72	0.13	7.09	0.22	15.62	0.17	1.80	0.02	0.68
	2	26.2	0.01	9.62	301	107.45	2.80	233	0.63	10.18	0.16	8.68	0.25	17.89	0.10	1.10	0.04	1.25
	3	26.6	0.01	12.73	398	84.12	2.77	231	0.03	0.43	0.03	1.44	0.27	19.60	0.01	0.10	0.02	0.75
	4	27.5	0.01	8.57	268	96.42	5.26	438	0.93	15.00	1.12	62.32	0.19	13.78	0.21	2.20	0.09	2.78
	5	29.8	0.01	17.22	538	488.11	5.95	496	0.20	3.17	0.09	4.98	0.47	33.77	0.01	0.10	0.05	1.63
	7	26.9	2.1	11.6	363	226.41	4.74	395	0.79	12.80	0.98	54.46	0.36	25.56	0.93	9.80	0.02	0.55
	8	26.8	3.1	13.1	409	195.76	5.15	429	0.93	15.04	0.83	45.89	0.23	16.11	0.73	7.70	0.13	4.15
	9	25.6	2.1	10.9	341	62.78	3.59	299	0.59	9.54	0.58	32.46	0.36	25.60	0.69	7.30	0.03	0.93
	10	25.4	32.2	8.79	275	48.79	3.23	269	0.45	7.21	0.37	20.44	0.29	20.36	0.50	5.30	0.01	0.43
	11	25.3	33.5	7.79	243	18.11	2.47	206	0.34	5.51	0.25	13.67	0.16	11.18	0.24	2.50	0.01	0.23
	13	24.6	35.7	7.1	222	34.49	2.48	207	0.31	5.06	0.34	19.16	0.09	6.10	0.29	3.10	0.02	0.63
September 2009	1	18.3		8.33	260	39.59	2.26	188	3.06	49.36	0.06	3.27	0.08	5.56	0.35	3.69	0.04	1.20
	2	18.7	0.01	8.98	281	19.43	2.20	183	3.56	57.49	0.05	2.77	0.02	1.35	0.36	3.84	0.04	1.43
	3	19.1	0.01	9.92	310	101.24	2.21	184	3.72	60.00	0.10	5.78	0.34	24.46	0.38	4.04	0.07	2.34
	4	19.3	0.02	8.82	276	89.59	3.30	275	2.98	48.00	0.75	41.90	0.58	41.44	0.64	6.70	0.06	2.08
	5	20.1	0.02	8.8	275	88.54	3.46	288	3.23	52.17	0.53	29.50	0.55	39.14	0.53	5.56	0.07	2.32
	7	20.4	0.02	11.4	356	96.35	3.46	288	4.45	71.77	0.31	17.00			0.33	3.50	0.10	3.20
	8	20.6	0.02	13.91	435	115.40	3.47	289	4.46	71.98	0.27	14.80	0.08	5.46	0.24	2.55	0.09	2.85
	9	20.8	2.1	12.77	399	96.92	3.46	288	2.54	41.01	0.34	18.75	0.41	29.13	0.27	2.81	0.04	1.44
	10	21.4	6.2	12.06	377	45.45	3.11	259	1.65	26.56	0.25	13.88	0.41	29.62	0.38	4.01	0.02	0.63
	11	21.8	37.8	10.01	313	2.20	1.43	119	0.12	1.91	0.01	0.49	0.05	3.59	0.003	0.03	0.001	0.03
	13	21.8	37.1	10.08	315	1.22	1.51	126	0.02	0.38	0.004	0.24	0.09	6.75	0.003	0.03	0.001	0.03

Sampling	station	Temp (°C)	Salinity	DO		chl-a (µg/L)	DOC		NO_3		NH_4		DON		PO_4		DOP	
				mg/L	µmol/L		mg/L	µmol/L	mg/L	µmol/L	mg/L	µmol/L	mg/L	µmol/L	mg/L	µmol/L	mg/L	µmol/L
April 2010	30	13.4	0.13	9.4	294	1.17	2.17	181	10.83	174.60	0.03	1.62	0.04	2.87	0.39	4.11	0.001	0.03
	31	14	0.11	10.3	322	0.85	2.00	167	2.82	45.56	0.02	1.37	0.06	4.30	0.05	0.48	0.002	0.06
	32	15	0.15	9.8	306	3.99	2.22	185	6.70	108.12	0.05	2.66	0.02	1.10	0.34	3.61	0.007	0.23
	2	14.7	0.16	8.9	278	4.42	2.75	229	10.59	170.78	0.09	5.23	0.29	20.97	0.35	3.70	0.016	0.53
	3	15.5	0.14	9.8	306	4.12	2.38	198	6.38	102.97	0.04	2.44	0.03	2.05	0.32	3.34	0.013	0.41
	4	15	0.18	9.3	291	5.32	2.75	229	9.49	153.09	0.28	15.49	0.01	0.65	0.38	4.04	0.009	0.29
	8	15.7	0.19	8.9	278	7.51	2.80	233	12.60	203.19	0.15	8.39	0.33	23.36	0.38	4.00	0.012	0.38
	9	15.6	0.23	8.9	278		2.71	226	9.78	157.74	0.20	11.21	0.08	5.87	0.39	4.08	0.012	0.39
	10	15.9	0.91	9.2	288	4.44	3.08	257	7.14	115.24	0.14	7.88	0.62	44.31	0.34	3.54	0.020	0.63
July 2010	32	22.3	0.1	8.2	256	5.01	3.01	251	5.67	91.52	0.08	4.38	0.24	17.48	0.49	5.13	0.001	0.03
	33	28.5	0.2	3.0	94	2.81	2.81	234	4.28	69.08	0.63	35.04	0.11	8.18	0.25	2.64	0.001	0.03
	2	25.3	0.2	9.3	291	24.57	3.30	275	3.23	52.02	0.15	8.55	0.46	32.65	0.43	4.48	0.009	0.30
	3	25.6	0.2	10.1	316	30.09	3.37	281	1.51	24.40	0.15	8.30	0.41	28.96	0.42	4.38	0.008	0.25
	4	25.2	0.3	7.3	228	32.60	4.56	380	2.92	47.16	0.75	41.82	0.47	33.25	0.57	6.00	0.031	0.99
	5	25.9	0.3	7.6	237	15.67	4.67	389	3.38	54.44	0.65	36.24	0.55	39.47	0.57	6.05	0.019	0.62
	9	27.1	1.4	9.5	297	31.84	3.58	298	0.90	14.51	0.26	14.23	0.47	33.89	0.45	4.78	0.001	0.02
	10	26.8	2.5	8.7	272		3.50	292	0.97	15.67	0.24	13.22	0.53	37.58	0.48	5.07	0.005	0.15
	11	27.3	4.7	8.2	256	17.55	3.46	288	0.92	14.81	0.26	14.31	0.52	36.80	0.73	7.65	0.001	0.03
	13	27.5	35.0	7.3	228	4.74	3.53	294	0.65	10.48	0.15	8.20	0.47	33.25	0.38	3.95	0.001	0.03