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Analysis of the effects of wet and dry seasons on a Mediterranean river basin: consequences for coastal waters and its quality management

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Abstract

Rivers play a major role in the delivery of nutrients to coastal ecosystems which are essential for ecosystem productivity. However, the increase of nutrients due to anthropogenic activities can cause eutrophication problems. This study analyzes the seasonal variation of phytoplankton communities in the coastal receiving waters of a Mediterranean river. Two scenarios are compared: the wet and the dry season with distinctive characteristics. During the wet season agricultural runoff and combined sewer overflows (CSO) were responsible for nutrient discharges, while during the dry season partially treated effluent from wastewater was the main nutrient source. In the receiving waters, diatoms typical seasonal cycle was modified by CSO discharges during rain episodes, while dinoflagellate abundance was higher in the dry season due to partially treated effluents discharges and low turbulence. We recommend that the design of the Water Framework Directive monitoring programs should take into account wastewater treatment plants and combined sewer systems located near the coast. Management decisions should take into account that only reductions in CSO and partially treated summer effluent are likely to be efficient in the short term. Analyzing the corrective measures cost through a cost-benefit analysis would help to determine whether the costs are excessive or not.

Keywords: CHEMTAX, pigments, phytoplankton, nutrients, Mediterranean river, combined sewer overflows

1. Introduction

Estuaries and coastal areas receive high loads of nutrients of terrestrial origin, either from point sources, which flow out at discrete and identifiable locations (e.g. rivers, submarine wastewater outfalls) or non-point sources, which are rather diffuse and highly variable from year to year depending on climate and rainfall (e.g. surface and groundwater runoff) (Paerl, 2006). Among nutrients, nitrogen and phosphorus are both required to support marine productivity and are the key limiting nutrients in most aquatic and terrestrial ecosystems. However, the increase in these nutrients due to anthropogenic activities requires their inputs into coastal marine ecosystems to be reduced in order to minimise eutrophication problems (Paerl et al., 2010). The influence of these terrestrial inputs is especially important in the marine oligotrophic areas, such as the Mediterranean Sea (Romero et al., 2007).

Several policies have been adopted to reduce nutrient inputs with varying degrees of success depending on the source (Artioli et al., 2008). While improvements in wastewater treatment have achieved reductions in inputs of phosphorus and nitrogen from point sources, the largest nutrient contribution for coastal marine environments is from diffuse sources, especially those from agricultural land runoff (fertilizers), and reducing this is a difficult and slow process (Carstersen et al., 2006). Then, there is a tendency to focus European Union (EU) policies on non-point sources rather than on point sources, which are supposed to have been successfully addressed in past legislation (Torrecilla et al., 2005).

Rivers play a major role in the delivery of nutrients to coastal ecosystems, both from natural (e.g. silicates weathering) and anthropogenic (urban and industrial wastewater, agriculture runoff) sources. Wastewater treatment plants (WWTPs) can discharge treated effluent to rivers or to coastal areas through submarine wastewater outfalls. The objective of the 91/271/EEC Council Directive, concerning urban wastewater treatment, was to protect the environment from the adverse effects of wastewater discharges. Different studies have demonstrated that the submarine outfall discharges of treated effluent which comply with the Directive, have either no significant effects or only minor effects on the quality of receiving waters (Juanes et al., 2005). However, wastewater discharge can cause water quality problems in rivers and this is especially relevant in Mediterranean rivers because of the hydrologic singularities of the Mediterranean climate and fluvial regime (Torrecilla et al., 2005).

In Mediterranean-type climate regions (areas surrounding the Mediterranean Sea, parts of western North America, parts of western and southern Australia, the south-west of South Africa, and parts of central Chile), characteristic precipitation is scarce and torrential and has an extremely high spatial and temporal variability. Natural river discharge, driven by precipitation variability, has two distinct seasons: wet and dry (Gasith and Resh, 1999; González-Hidalgo et al., 2005). Thus, wastewater discharges present different problems depending on the season.

During the wet season combined sewers overflows (CSO) can reach receiving waters without treatment (Clark et al., 2007). The importance of storm overflows for water quality have been recognized by the 91/271/EEC Directive: "the design, construction and maintenance of collecting systems shall be undertaken in accordance with the best technical knowledge not entailing excessive costs, notably regarding limitation of pollution of receiving waters due to storm water overflows" (Annex I, 91/271/EEC Directive). This importance is also recognized by the USA legislation in the CSO

Control Policy which was published on April 19, 1994 by the United States Environmental Protection Agency (USEPA).

During the dry season, urban sewage effluents are the main freshwater source in ephemeral streams in arid to semiarid areas of the world (Brooks et al., 2006; García-Pintado et al., 2007) and can account for up to 90% of the total river flow in some Mediterranean rivers such as the Serpis River (Molinos-Senante et al., 2011). In addition, fluctuating winter and summer populations in tourist areas, typical on Mediterranean coasts, provoke significantly variable wastewater loadings that can exceed the capacity of the treatment facilities (Aguilera et al., 2001; García-Pintado et al., 2007).

Several studies have addressed the quality problems of Mediterranean rivers receiving wastewater discharges, for instance, on the Mediterranean coast of the Iberian Peninsula, Torrecilla et al. (2005) studied the Ebro River (NE Spain) and Molinos-Senante et al. (2011) studied the Serpis River (Eastern Spain). On the other hand, studies on receiving coastal waters have mainly addressed the water quality in the submarine outfall areas (Aguilera et al., 2001) and not river mouths. Some studies of Californian recreational bathing beaches have addressed the effects of CSO discharges but have focused on fecal indicator bacteria (total coliforms, fecal coliforms and enterococci) and benthic invertebrates (Schiff et al., 2003; Clark et al., 2007).

Nutrient inputs into coastal ecosystems can induce eutrophication problems, among these, harmful algal blooms of species responsible for the synthesis of toxins and high-biomass producers that can cause hypoxia and anoxia and indiscriminant mortalities of marine life after reaching dense concentrations (Heisler et al., 2008). As regards marine recreational bathing beaches, these effects can cause beach closures that negatively impact the local economy of tourist areas. Thus, adequate management of nutrient sources is essential because clean water and healthy coastal habitats are clearly fundamental to successful coastal tourism which is characteristic of the Mediterranean climate regions (Hall, 2001). However, to develop appropriate management strategies, it is necessary to fully understand how ecosystems function by first of all establishing the relationships between phytoplankton and nutrient sources and patterns.

This study analyzes the seasonal variation of phytoplankton communities in the coastal receiving waters of the Serpis River input, which is a Mediterranean river basin in Spain. A comparison is established with marine waters to assess different patterns between high and low terrestrial influence areas. Two scenarios are analyzed: the wet and the dry season with distinctive characteristics. The aim is to provide information about ecosystem functioning that could help management decisions and could be extrapolated to other Mediterranean climate areas with similar characteristics.

2. Materials and methods

2.1. Study area

The study area (Fig. 1) is located in the East of the Iberian Peninsula, at the southernmost sector of the Valencian Gulf (Western-Mediterranean Sea) on the coast of the Safor County (Spain). The main annual rainfall is above 600-650 mm in the entire County, with values above 800 mm in the rainiest areas, with most precipitation concentrated into short-episodic storm events in autumn (Hermosilla, 2005). For a 10-year return period daily rainfall is expected to be higher than 200 mm (Hermosilla, 2005).

The main river draining this area is the Serpis River which has a basin of 753 km^2 and is 74.5 km in length (Hermosilla, 2005). The Serpis River receives freshwater from its tributary the Vernissa River (150 km^2). Both rivers have a Mediterranean regime characterized by a high seasonality, with a dry period during summer, and a wet period mainly in autumn (Hermosilla, 2005). These rivers are artificially regulated by a complex system of weirs and irrigation channels that provide freshwater for the irrigated crops of the Safor County. The Beniarrés reservoir (30 hm^3 maximum capacity) (Fig. 1) was built in the second half of the 20th century to increase the regularity of the Serpis River (Hermosilla, 2005).

In the Serpis basin, there are 30 WWTPs that receive wastewater from the main urban and industrial areas, treating around 31 Mm^3 per year (Molinos-Senante et al., 2011). In total, 24 of the 30 plants discharge treated water to the Serpis River, while the remaining six plants discharge treated water into the sea (Molinos-Senante et al., 2011). Downstream of the Beniarrés reservoir, the most important WWTP is the Gandia plant (Fig. 1) which serves a total of 133,300 inhabitants from 17 municipalities of the Safor County (EPSAR, 2013). This plant discharges 17 million m^3 of treated wastewater per year to the sea through a submarine outfall (approximately 1,900 m from the Serpis river mouth); however it also has two overflow channels that discharge directly to the river. One channel discharges besides the plant and the other near the river mouth.

The first overflow channel is used when the plant capacity is exceeded due to rain episodes. The plant treats an average flow of 46,350 m^3 day^{-1} and its design flow is 60,000 m^3 day^{-1} (EPSAR, 2013), which makes a difference of 13,650 m^3 day^{-1}. But the urban area without wastewater and pluvial waters separation amounts to nearly 15 million square meters (see areas in Fig. 1) with almost zero infiltration. So, even small rain episodes cause combined sewer overflows because the plant capacity is exceeded. The wastewater collector is parallel to the Serpis River and combined sewer is also discharged into the Serpis directly from the collector when its capacity is exceeded.

The second overflow channel is generally used in summer, when the plant capacity is exceeded due to the population increase and the effluent is partially evacuated without treatment. The average density in the coastal municipalities of the Safor county is 963 inhabitants km^{-2} in winter (1,314 inhabitants km^{-2} in Gandia city) (INE, 2009), but that figure triples in summer due to tourism activity which is mainly based on residential development (Mantecón and Huete, 2007). According to Ariza et al. (2008) urban beaches are much more likely to be closed, compared with natural beaches, mainly due to the presence of sewer systems. Beach closures adversely affect tourism, which is one of the main economic sectors in these coastal municipalities, based on the "sun & beach" product (Yepes and Medina, 2005). In August 2007, Venecia and Marenys de Rafalcaid beaches, which are located next to the mouth of the Serpis river, were closed due to fecal contamination and high bacteria levels (Santacreu, 2008). These two beaches only form a small part of the Gandia coastline. However tourism is mainly focused on the beaches on the northern side of Gandia Harbour. Unfortunately, national TV news reported the closure of the beach at Gandia, without specifying that only two small beach areas on the southern side were affected, and this caused many hotel booking cancellations. As Ariza et al. (2008) noticed to effectively manage such situations, it is necessary to carry an integral analysis of the drainage system in order to avoid prejudice to such an important activity for the local economy.

The Serpis River lower basin is characterised by dominant urban and agricultural uses. The alluvial plain was totally occupied by the Safor wetland and crops until the seventies. Nowadays, this area shares its agricultural activity, mainly citrus fruits and vegetables, with the tourism of the urban areas (Sebastiá et al., 2012). The recommended nitrogen doses for citrus and horticultural crops have been traditionally exceeded in this area (MARM, 2010). In consequence, both surface runoff and groundwater flow are characterized by high nitrate levels. In the detritic aquifer, nitrate levels have exceeded the limit of 50 mg L^{-1} established by the Nitrates Directive (Directive 91/676/EEC), and it has been declared a Nitrate Vulnerable Zone.

The Serpis River outflows on the southern side of Gandia Harbour creating an enclosed area with high residence time due to the barrier effect of the harbour (Fig. 1) and dominant currents (for a more detailed description see Sebastiá and Rodilla, 2012). The Venecia beach is located between the river mouth and the harbour, while the Marenys de Rafalcaid beach is located on the southern side of the river. High nutrient inputs to ecosystems with low water renewal rates are especially important for water quality deterioration as described in Sebastiá and Rodilla (2012).

The most important river near the Serpis is the Júcar River, located about 30 km north, and its discharge is significantly reduced for the same reasons than the Serpis (artificial regulation and intensive water use) (Mestres et al., 2007). The study by Mestres et al. (2007) demonstrated that the dynamics of the receiving waters of a small-scale river plume are mostly determined by local driving mechanisms, namely the prevailing wind and the freshwater discharge rate. So sampling was designed having into account that freshwater discharge in this area is mainly due to the Serpis River inputs (see Sebastiá et al., 2012 for a description of freshwater inputs into the Gandia Harbour and Sebastiá and Rodilla, 2012 for aquifer inputs).

2.2. Sampling design

Field work was done from May 2008 to August 2009, with an average interval of 15 days. Sampling frequency was higher in the warm seasons, while in January and February 2009 there was no sampling due to bad weather conditions. Sampling took place at two stations along a gradient of distance from the landside (Fig. 1). Point 1 (CW, coastal waters) was located near the coastline (7.5 m depth), south of Gandia Harbour and facing the Serpis river mouth. Point 2 (MW, marine waters) was located at approximately 9 km from the coastline (36.5 m depth) outside the imaginary line that would link the Cullera Cape and La Nao Cape and which separates coastal waters from marine waters. The sampling points were chosen to compare the nutrient concentrations and seasonal variations of phytoplankton in two areas characterized by high (CW) and low (MW) influence of terrestrial inputs. Two scenarios will be distinguished: 1) the dry season characterized by low discharge but with high nutrient input due to partial wastewater treatment; and, 2) the wet season characterized by high discharge of high nutrient CSO and agricultural runoff.

Temperature and salinity (expressed as practical salinity units, psu) were measured in the water column with a High Accuracy Conductivity, Temperature and Depth Meter (NXIC CTD). Samples were collected with Niskin bottles near the surface and with a Van Dorn bottle 50 cm above the bottom at each sampling point. Samples were separated into three subsamples for nutrient, pigment, and phytoplankton analysis. Water samples were kept in a cool box (4ºC) and transported to the laboratory.

Rainfall and river flow data for the studied period were available from the Vernissa and Font En Carrós meteorological and gauging stations of the Júcar Hydrographic Confederation (CHJ). The Vernissa gauging station measures the Vernissa River flow, while the Font En Carrós gauging station measures the Serpis River flow before receiving the Vernissa input (Fig. 1). Rainfall in this study is presented as daily data (Fig. 3). The cumulative values of the 5 days (120 h) prior to sampling were determined and used in further analysis.

2.3. Laboratory analysis

The following parameters were analysed in all the samples: salinity, suspended solids (SS), nitrate, nitrite, and ammonium, dissolved inorganic phosphorus (DIP), total phosphorus (TP) and dissolved silicate (DSi). Dissolved inorganic nitrogen (DIN) was calculated as the sum of nitrate, nitrite and ammonium. Salinity was determined by means of an induction conductivity meter Multi 340i/SET WTW, using the Practical Salinity Scale. Nutrients were analysed colorimetrically using the method of Aminot and Chaussepied (1983). A persulphate digestion (Valderrama, 1981) was made for TP and the phosphate was subsequently analyzed. Dissolved oxygen concentration was measured using the Winkler titration technique (Grasshoff, 1983).

Phytoplankton samples were fixed with formaldehyde, concentrated according to UNE EN 15204:2006, based on Utermohl (1985), and qualitatively examined under a LEICA DM IL inverted microscope. Only representative samples were screened according to the recommendations of Higgins et al. (2011).

Samples for phytoplankton pigment analysis were filtered on GF/F fiberglass filters (25 mm diameter). Pigments were extracted using acetone (100%, HPLC grade) and were measured using reverse-phase high-performance liquid chromatography (HPLC). The HPLC method employed was that proposed by Wright et al. (1991) slightly modified as per Hooker et al. (2000). The system was calibrated with external standards obtained commercially from the DHI Water and Environment Institute (Hørsholm, Denmark).

2.4. Phytoplankton composition and abundance

Once the concentration of important photosynthetic pigments was determined, the phytoplankton community was studied using the CHEMTAX program (Mackey et al., 1996) version 1.95 (S. Wright, pers. comm.) to obtain the contribution to chlorophyll-*a* (chl-*a*) of the 8 phytoplankton groups identified with microscopy (diatoms, dinoflagellates, euglenophytes, chlorophytes, cryptophytes, prymnesiophytes, prasinophytes and cyanobacteria).

To obtain reliable results, CHEMTAX was applied following the procedures described in Latasa et al. (2010) and using the same parameters (elements varied = 10). The average of the last 6 ratio estimations was incorporated into the final pigment ratio matrix when a clear convergence was observed. In the absence of a clear convergence, the average of each pigment ratio was incorporated. This final matrix (Supplemental Table 1) was then used to estimate the contribution of the different groups to chl-*a* stock.

2.5. Statistical analysis

In order to identify groups of samples with similar characteristics, a cluster analysis was performed using SPSS 16.0 to group samples according to salinity and nutrients (DIN, DIP and DSi). Squared Euclidean distances were calculated and samples clustered according to Ward’s method. CHEMTAX was applied independently to each identified subset to obtain the contribution of each phytoplankton group to the chl-*a* stock (Latasa et al., 2010).

A non parametric one-way analysis of variance (Kruskal-Wallis) was used to test differences in physico-chemical variables and phytoplankton composition and abundance between sampling points and seasons (wet and dry seasons).

Spearman rank correlation analyses were performed on environmental variables (Serpis flow, rain, salinity and dissolved oxygen) and nutrient concentrations (NH_4^+, DIN, DSi, DIP, TP) in order to examine significant relationship between environmental variables and nutrient concentrations in coastal waters (Supplemental Table 2). Significant relationship between physical-chemical variables (temperature, salinity, DO, NH_4^+, DIN, DSi, DIP, DIN:DIP, DSi:DIP, DSi:DIN) and phytoplankton groups was evaluated in coastal and marine waters (Supplemental Table 4).

3. Results

3.1 Environmental variables

The rainy season started in September 2008 and lasted until June 2009 (Fig. 3). Maximum rainfall was registered in October, 376 mm at Vernissa and 562 mm at Font En Carrós, with a daily maximum of 111 mm at Vernissa and 139 mm at Font En Carrós, where the 100 mm threshold was exceeded three times in October. Summer was the dry season, with 14 mm of average rainfall.

The general pattern of the Vernissa River flow rates recorded during the study period (Fig. 3) followed a seasonal cycle characterised by a 6.3 $m^3 s^{-1}$ average flow in the rainy season and 0 $m^3 s^{-1}$ values during summer. The Serpis River flow, recorded at the Font En Carrós station, was 1.1 $m^3 s^{-1}$ on average in the rainy season. During summer a water flow higher than 30.0 $m^3 s^{-1}$ (Fig. 3) was registered on 5 isolated days due to water releases from the Beniarrés reservoir to satisfy crop irrigation needs. Flow showed a significant correlation with rain which was weaker for the Font En Carrós station (r_s = 0.378, $p < 0.001$) while at Vernissa station, flow was r_s = 0.649, $p < 0.001$. No significant correlation was found between the flow measured at the Vernissa and the Font En Carrós gauging stations (Fig. 1) (r_s = 0.188, $p > 0.05$). At the coastal water station, NH_4^+, DIN and DSi concentrations were significantly correlated with rain and Serpis flow, calculated as the sum of the flow measured at the above mentioned gauging stations (Supplemental Table 2). DIP concentrations did not show any significant correlation with environmental variables or nutrient concentrations (Supplemental Table 2). TP was significantly correlated with rain but not with Serpis flow (Supplemental Table 2).

Water temperatures during the study period followed a seasonal evolution characterised by the lowest values in winter and maximum values in summer. Surface temperature was similar in CW (P1) and MW (P2) with a minimum value of 12.3ºC in winter and maximum value of 27.9ºC in summer. Bottom temperature was generally lower in MW (7.5 m depth CW and 36.5 m depth MW), where the lowest temperature, 11.8ºC, was observed in March. By the end of the summer, the temperature gradient between surface and bottom waters was 1ºC in CW and 10ºC in MW. Dissolved oxygen concentration ranged from 6.4 to 9.9 mg L^{-1} at CW, and from 6.7 to 10.5 mg L^{-1} at MW, with higher values from December to April. While water column temperature at the two sampling points was statistically different ($p < 0.05$), no significant differences ($p > 0.05$) were observed in the oxygen concentration, although it was slightly higher in marine waters.

Salinity values at CW were significantly lower than at MW ($p < 0.001$); mean values for the study period were 35.4 and 37.0 in CW and MW surface waters, and 36.8 and 37.4 respectively in bottom waters. At CW, salinity was significantly lower in surface waters ($p < 0.05$) and water column salinity was inversely correlated with rain (rain accumulated in the 5 days prior to sampling, Supplemental Table 2); however, no correlation was observed with the Serpis flow (Supplemental Table 2). At CW, salinity also showed a negative correlation with NH_4^+, DIN and TP concentrations (Supplemental Table 2). At MW, no significant differences were observed between surface and bottom waters ($p > 0.05$). The seasonal variation of salinity can be observed in Fig. 4. Salinity differences between the rainy and the dry season were more pronounced at CW: average surface salinity was 35.7 in the rainy season and 36.7 in the dry season, while minimum values were 24.3 and 33.8 respectively (MW average surface salinity 37.2 in both seasons). However, seasonal differences were not significant at either station ($p > 0.05$).

Suspended solids ranged from 6 to 19 mg L^{-1} at CW and from 2 to 15 mg L^{-1} at MW, and were significantly higher at CW ($p < 0.001$).

3.2. Nutrient concentrations and ratios

Analysis of variance showed that DIN ($p < 0.001$), DSi ($p < 0.05$) and TP ($p < 0.01$) concentrations were significantly higher at CW, as well as the DIN:DIP ratio ($p < 0.05$), while the DSi:DIN ratio was significantly lower ($p < 0.05$). No significant differences between sampling points were found in NH_4^+ and DIP concentrations ($p > 0.05$) or in the DSi:DIP ratio ($p > 0.05$). At CW, DIN and DSi concentrations ($p < 0.001$) and the DIN:DIP and DSi:DIP ratios ($p < 0.05$) were significantly higher in surface water samples. At MW, water column characteristics were more homogenous: there were no statistical differences between nutrient concentrations (NH_4^+, DIN, DIP, TP and DSi) ($p > 0.05$) and nutrient ratios ($p > 0.05$) of surface and bottom waters.

The cluster analysis identified 4 clusters numbered according to decreasing salinity and increasing DIN, DSi and DIP concentrations. The descriptive statistics are summarized in Supplemental Table 3. Cluster 1 included all samples from MW; cluster 2 included mainly bottom samples from CW; cluster 3 included mainly surface samples from CW; and cluster 4 included only 4 samples which were CW surface samples from 21 November 2008, 12 December 2008, 2 April 2009 and 22 April 2009. Bottom samples from CW which were included in cluster 3 where samples taken on 7 and 21 November 2008, 12 December 2008 and on April 2009 sampling campaigns (2, 8, 14 and 22 April). Bottom samples from CW included in cluster 3 and samples included in cluster 4 coincided with accumulated rain above 10 mm in the 5 days prior to sampling.

Dissolved inorganic nitrogen (DIN) concentrations during the study period ranged from 0.1 to 50.5 µM at CW and from 0.1 to 8.0 µM at MW (Fig. 4); the highest values were measured in the rainy season. DIN concentration was dominated by NO_3^- which registered maximum values of 49.4 µM at CW and 7.1 µM at MW. Though there were no significant differences in NH_4^+ concentrations at the sampling points ($p > 0.05$), concentrations below the method detection limit were more commonly found at MW than at CW. The seasonal tendency of NH_4^+ was similar to that of DIN (Fig. 4), with higher values after rain episodes, except for the maximum values attained on July 1 and 18 at CW bottom waters when no rain was registered.

Dissolved silicate (DSi) ranged from 0.5 to 16.3 µM at CW, and from 0.2 to 4.5 µM at MW (Fig. 4). The highest DSi concentrations were reached in the rainy season. Its maximum values were synchronous with rain events and with DIN maximum concentrations, except for April 2 (2009) when DSi did not peak. The lowest DSi concentrations were observed in June and early July.

Dissolved inorganic phosphorus (DIP) varied from < 0.01 µM to 0.43 µM at CW and from < 0.01 µM to 0.23 µM at MW (Fig. 3). The DIP seasonal pattern was not clear. Total phosphorus (TP) ranged from 0.04 to 1.14 at CW and from 0.01 to 1.20 at MW (Fig. 3) and showed a seasonal pattern similar to that of DIN and DSi (Fig. 3).

In order to better define potential nutrient control, nutrient ratios between DSi, DIN and DIP concentrations were compared with Redfield ratios (DSi:DIN:DIP = 16:16:1) as shown in Figure 4. The DIN:DIP ratio was always above 16 at CW surface waters (except an isolated ratio of 14 on August 5, 2008) (Fig. 4). The average ratio was 530, and the maximum ratio 3438, which was attained on April 22, 2009. At CW bottom waters and at MW (surface and bottom waters), DIN:DIP ratio values above 16 also prevailed. Values below the Redfield ratio were detected from mid-July to the beginning of November 2008 (only end of July at CW bottom waters) and in the second half of May 2009. The DSi:DIP ratios were high compared with Redfield ratios (average ratio was 179 at CW and 155 at MW), and only some points were below 16 with no clear seasonal tendency. As a consequence of these high DIN:DIP and DSi:DIP ratios, P was the potentially most deficient nutrient for phytoplankton growth during most of the study period, with DIN and DSi alternating as the secondary potentially deficient nutrient. In coastal waters, DSi secondary potentially limiting conditions were predominant all year round, except in May and from the second half of July to the beginning of September when DIN was the secondary potentially limiting nutrient. In contrast, in marine waters, DIN potentially limiting conditions prevailed. DSi was potentially limiting only in short periods: in April, from June to the beginning of July and from the second half of November to the end of December.

3.4. Phytoplankton abundance and composition

Comparison of both sampling points showed that coastal waters (CW) had the highest significant chl-*a* values ($p < 0.01$) which ranged from 0.31 to 4.32 mg m^{-3} and had an average value of 1.43 mg m^{-3}. In marine waters (MW) chl-*a* values varied from 0.05 to 1.43 mg m^{-3} and the average value was 0.47 mg m^{-3}. The maximum chl-*a* concentration attained in marine waters corresponded with the average value observed in coastal waters.

In both areas, high chl-*a* values were observed in March (early spring), followed by a chl-*a* decrease in April and a recovery in May. At CW, the highest chl-*a* values were attained on March 16, 2009, while at MW, maximum values were measured on May 26, 2009. From June to December chl-*a* concentration was lower in both coastal and marine waters (average 1.1 mg m^{-3} at CW and 0.36 mg m^{-3} at MW; maximum 2.05 mg m^{-3} at CW and 0.68 mg m^{-3} at MW).

Absolute phytoplankton abundance was significantly higher at CW for all groups ($p < 0.05$), except for euglenophytes and chlorophytes that did not show statistically significant differences ($p > 0.05$) between the two sampling points. Relative abundance of dinoflagellates, prasinophytes and prymnesiophytes was higher at CW ($p < 0.05$), while diatom, euglenophyte and cyanobacteria contribution to total Chl-*a* was higher at MW ($p < 0.05$). No significant differences were found in chlorophyte and cryptophyte relative abundance between the two sampling points ($p > 0.05$). In Fig. 5 the seasonal variation of each phytoplankton group is represented, as estimated with CHEMTAX, in terms of the contribution of the different groups of algae to total chlorophyll *a*.

In MW surface waters, diatom contribution to total chl-*a* was important all year round and ranged from 25 to 85% of chl-*a* (except for an isolated instance when diatoms were not detected on July 30, see Fig. 5). The diatom bloom started in early spring and lasted until the end of June. In this period the average diatom percentage of chl-*a* was 72%. No correlation was observed between diatoms and the variables included in the analysis (Supplemental Table 4). Amongst flagellates the main group was generally euglenophytes (Fig. 5). The maximum flagellate abundance was observed from November to December, when euglenophytes accounted for 30% of total chl-*a* on average. The prymnesiophyte contribution to chl-*a* was 12% on average, and their maximum abundance was observed on July 30, when they accounted for 42% of chl-*a*.

Cyanobacteria abundance was below 10% of chl-*a* from November to the end of May. An abundance increase was observed during the summer months with 34% of average contribution. Maximum cyanobacteria abundance reached 58% at the end of July 2008 and 47% at the beginning of August 2009 (Fig. 5). Cyanobacteria relative abundance was inversely correlated with DIN. The seasonal variation of phytoplankton groups in MW bottom waters followed the same tendency described for surface waters (Fig. 5). The main significant difference was a higher relative contribution of euglenophytes ($p < 0.01$) and a lower contribution of prymnesiophytes ($p < 0.05$).

In CW, several diatom peaks were observed throughout the study period and not only in spring (Fig. 5), and a maximum chl-*a* percentage of 77% was attained in June 2008. Diatom relative abundance was not correlated with any of the variables included in the analysis (Supplemental Table 4). Average flagellate abundance was 30% for the study period. Higher flagellate abundance was detected after the rain episodes due to an increase in euglenophyte, chlorophyte and cryptophyte abundance (Fig. 3 and 7). Chlorophyte abundance was inversely correlated with salinity (Supplemental Table 4). During the dry season, high flagellate abundance was linked with an increase in dinoflagellates (Fig. 5). Average abundance of prymnesiophytes was 30% for the study period although several peaks were observed, generally after rain periods, with a high of 90% of total chl-*a* on 1 March 2009 (Fig. 5). Cyanobacteria abundance was below 5% of chl-*a* from the end September to mid-May. An abundance increase was observed during the summer months with 20% of average contribution. Cyanobacteria relative abundance was inversely correlated with several variables: dissolved oxygen, DIN, DSi, DIN:DIP ratio and DSi:DIP ratio (Supplemental Table 4). The seasonal variation of phytoplankton groups in CW bottom waters was equal to that observed in surface waters. Only cryptophyte abundance, both absolute and relative, was significantly higher in surface waters ($p < 0.05$). This group was positively correlated with the following variables: NH_4^+, DIN, DSi, DIN:DIP ratio and DSi:DIP ratio (Supplemental Table 4). A negative correlation with the DSi:DIN ratio was also observed (Supplemental Table 4). None of the other phytoplankton groups produced any significant differences between surface and bottom waters.

The analysis of the seasonal variation revealed that in MW, diatom relative ($p < 0.05$) and absolute ($p < 0.001$) abundances were significantly higher in the wet season, while cyanobacteria abundances were higher in the dry season ($p < 0.001$). The other groups did not show any significant differences between seasons ($p > 0.05$). In CW, absolute abundance of all the phytoplankton groups was significantly higher in the wet season, except prasinophyte abundance which did not show any difference ($p > 0.05$). However, relative abundances of dinoflagellate ($p < 0.01$) and cyanobacteria ($p < 0.001$) were higher in the dry season and both showed a direct correlation with temperature (Supplemental Table 4).

4. Discussion

The Serpis River is a typical Mediterranean river subjected to the hydrologic singularities of the Mediterranean climate and fluvial regime which cause flow, physical and chemical conditions to change dramatically on a seasonal basis (Torrecilla et al., 2005). During summer, river average flow was 0 $m^3 s^{-1}$ and higher flows were linked to periodic releases from the Beniarrés reservoir to satisfy crop irrigation needs. This reservoir suffers severe eutrophication problems, especially during summer and it was declared a sensitive zone according to the 91/271/EEC Directive (Molinos-Senante et al., 2011).

Artificial regulation of Mediterranean rivers and intensive abstractions for agriculture cause moderate correlation between river flow and rain episodes (Struglia et al., 2004). Despite the river flow seasonality, salinity values in the receiving waters did not show the same seasonal variation (seasonal differences were not significant) and were not correlated with river flow. The absence of significant correlation is attributed to river regulation. However, seasonal salinity changes, though not significant, were observed during the dry season when values of CW and MW were more similar (Fig. 4) and indicated reduced river discharge. On the other hand, correlation between salinity and rain was significant. Measures of river flow were taken at the last gauging stations in the direction of flow and about 10 km from the river mouth (Vernissa and Font En Carrós). Rain events generated a significant river flow downstream of these stations, which also received combined sewer overflows (CSO) from the 15 million urban square meters located in the lower basin (Fig. 1). The seasonal variability of both rain and flow explained the seasonal variability in nutrient concentration patterns, which were correlated with either rain and flow (NH_4^+, DIN and DSi) or with rain only (TP) (Supplemental Table 2). It is important to highlight that other sources of freshwater did not contributed significantly to reduced salinity and nutrient concentration (DIN and DIP) as compared with the Serpis River (Sebastiá and Rodilla, 2012; Sebastiá et al., 2012).

The agricultural non-point sources generate the major part of DIN in the Mediterranean rivers basins (Torrecilla et al., 2005; Falco et al., 2010). In consequence, the DIN seasonal variation between the wet and the dry periods can be explained as agricultural mobilization by hydrological runoff from the catchment surface during rain events. This variation is also typical in other basins where agricultural land dominates the basin land uses (Cugier et al., 2005; Jarvie et al., 2008).

Chemical weathering of land silicates is the main process that supplies dissolved and particulate silicate to rivers, thus, higher DSi concentration in the wet period was also expected. In addition, groundwater discharges in the study area (wet period), are also characterized by high silica levels due to the lixiviation of biogenic silica from the wetland species of *Gramineae*, which are characterized by high silica content (typically 10-15% dry shoot weight), to the aquifer (Conley, 2002; Sebastiá and Rodilla, 2012). DSi levels were higher than those observed further north in the Balearic sea coastal area (NW Mediterranean) (Olivos et al., 2002; Sebastiá and Rodilla, 2012)

Phosphorus dynamics were more complex. DIP concentration was not correlated with river flow, did not present a clear seasonality and was not significantly different to that observed at MW. In the study area, higher phosphorus levels have been observed in surface irrigation channels during spring and have been attributed to diffuse sources, because they coincided with the period of phosphorus fertilizer application (Sebastiá et al., 2012). During the low-flow period, phosphorus contributions from agricultural diffuse sources (mobilized by hydrological runoff) are minimal and the major source is therefore likely to be derived from point sources (Jarvie et al., 2008). In the Serpis River the low-flow period coincided with summer, when the population increase due to tourist activity causes a higher wastewater flow, which exceeds the WWTP capacity. In this period, discharges of partially treated effluent through the overflow channels to the Serpis River have been observed (Sebastiá and Rodilla, 2012). However, phosphorus is the main potentially limiting nutrient, and any phosphorus discharge is rapidly consumed, which can cause the above described seasonal variations to go undetected (Falco et al., 2010).

On the other hand, total phosphorus correlated with rain, showed a seasonal variation similar to that of DIN and DSi, with higher values during the wet period, and was significantly higher in coastal waters. This highlighted the variability of phosphorus inputs, which were not detected when DIP levels were observed. As described above, DIP phosphorus inputs are rapidly consumed and this translates into biomass increase (total chlorophyll *a* concentration) which is correlated with TP increases ($p < 0.05$). This has been described for other coastal ecosystems with potential phosphorus limitation (Falco et al., 2010).

According to the 91/271/EEC Directive Annex II, the coastal waters of the study area meet the requisites of a sensitive area: coastal waters with poor water exchange and which receive large quantities of nutrients, though it has not been declared as such. In NE Spain the delta of the Ebro River was declared a sensitive zone (on 10 July 2006 Resolution, BOE nº 179, 2006-07-28). The Ebro River is the longest European river (928 km) that empties into the Mediterranean Sea, it has the second largest basin (88 835 km^2) and the third largest flow (mean annual discharge 384 m^3 s^{-1} (Falco et al., 2010). Despite the evident differences in river size and discharge, it presents some similarities with the Serpis River: it is a highly regulated Mediterranean river; low discharge occurs from July to October; the main activity in the basin is agriculture (mainly vineyards, orchards and corn crops); and it receives wastewater discharges near the mouth. These similarities allowed us to establish a comparison of the nutrient seasonal variability in the receiving waters of the two rivers.

In Falco et al. (2010), the mean and standard deviations for nutrients at sea (S) were presented for each season of the year. For comparison, autumn, winter and spring were grouped as the wet season, as in Supplemental Table 5. For the dry period, all nutrient concentrations were higher in the coastal waters of the Ebro River (Supplemental Table 5). However, for the wet period, the average nutrient values of coastal waters of the Serpis River were even higher than those observed for the Ebro River in some seasons (e.g. average DSi for the Serpis wet period was higher than the average DSi for the Ebro spring period, and DIP and TP were higher than the average for the Ebro winter period). The aim of this comparison is to illustrate the importance of rain episodes in Mediterranean rivers of scarce flow. Despite the different magnitude of average annual flow of these two rivers, mean annual discharge of 3 $m^3 s^{-1}$ for the Serpis (Pulido-Velázquez et al., 2011) and 384 $m^3 s^{-1}$ for the Ebro (Falco et al., 2010), the nutrient concentration in the receiving coastal waters is comparable.

On the other hand, all samples from MW were classified into one cluster which was characterized by the highest salinity and the lowest nutrient concentrations and variability. The range of nutrient concentrations (DIN, DSi and DIP) was of the order of those observed in previous studies further north in the Western Mediterranean Sea, 5 km from the coast (Olivos et al., 2002). As in CW, phosphorus was the main potentially limiting nutrient, but in MW the secondary potentially limiting nutrient was nitrogen, which agrees with the observations of Estrada (1996) for the Mediterranean Sea.

Coastal and marine waters also showed significant differences in phytoplankton biomass and relative group abundances. Total biomass (chl-*a*) and absolute abundance of phytoplankton groups was higher in coastal waters due to higher nutrient availability. The seasonal cycle showed maximum phytoplankton abundance in spring and minimum abundance in summer which is typical in coastal and marine waters (Garmendia et al., 2011).

In marine waters, abundance of diatoms was higher in spring and relative abundance of small cells (cyanobacteria and flagellates) increased in summer (Garmendia et al., 2011; Latasa et al., 2010). Cyanobacteria attain maximum growth rates at temperatures higher than those for diatoms and also prefer for more oligotrophic conditions (Latasa et al., 2010), in this study they were inversely correlated with DIN and DSi concentration. Maximum flagellate abundance was observed from November to December when silica was the potentially limiting nutrient after phosphorus.

In coastal waters, relative diatom abundance was lower than in marine waters and did not show the typical seasonal cycle of maximum abundance in spring and minimum abundance in summer. As the DSi:DIN and DIN:DIP ratios decreased diatom contribution was reduced and replaced by an increase in flagellates (Moncheva et al., 2001). In this case, minimum diatom abundance was observed after rain episodes as they were replaced by increases in relative flagellate (euglenophyte, chlorophyte and cryptophyte) and prymnesiophyte abundances. On the other hand, maximum relative diatom abundance was observed on 20 July 2008, with a 77% contribution to total chl-*a*. High relative diatom abundances during summer have been observed in other estuaries which receive wastewater discharges and are related to a continuous availability of silica (Garmendia et al., 2011). The negative correlation of chlorophytes with salinity (Supplemental Table 4) showed that this group was characteristic of freshwater. Cryptophytes are a group characteristic of eutrophic conditions (Latasa et al., 2010); as such they were positively correlated with NH_4^+, DIN and DSi. Cryptophytes were also inversely correlated with the DSi:DIN ratio (Supplemental Table 4), so they outcompeted diatoms when this ratio value was lower, mainly after rain episodes. Dinoflagellate, prasinophyte and prymnesiophyte relative abundances were higher in coastal waters than in marine waters. Dinoflagellates and prasinophytes have been identified as groups with preference for eutrophic conditions, but prymnesiophytes have been reported as a mesotrophic group (Latasa et al., 2010). Dinoflagellate blooms have been reported in estuaries and near-shore regions and are typically responsible for harmful algal blooms (Cugier et al., 2005; Latasa et al., 2010).

In the study area, higher relative dinoflagellate abundance was observed during the dry season and it showed a direct correlation with temperature. Dinoflagellate blooms have been observed in this season when nutrient levels are lower (lower DIN:DIP and DSi:DIP ratios) (Anderson et al., 2008). In addition, low silica concentrations reduce thcompetition with diatoms (Moncheva et al., 2001). In the microscope analysis two toxin producers were identified, *Alexandrium* sp. (paralytic shellfish-poisoning) and *D. caudata* (diarrheic shellfish-poisoning), these species were also detected in Sebastiá and Rodilla (2012). In the study area, prymnesiophytes were abundant throughout the year and did not show any significant difference between the wet and the dry period.

European policies have addressed point sources (PS) mainly through the improvement of wastewater treatment facilities (Artioli et al., 2008; Carstersen et al., 2006). However, many basins do not operate separated sanitary sewers but combined sewers that are designed to collect rainwater runoff, domestic sewage, and industrial wastewater in the same pipe. These systems are designed to overflow during periods of heavy rainfall and discharge directly to nearby rivers or coastal waters. The USEPA CSO Control Policy provides guidance on how communities with combined sewer systems can meet the Clean Water Act (CWA) goals and this includes monitoring to effectively characterize CSO impacts (USEPA, 1994). This Policy considers that characterization, monitoring, and modelling of the combined sewer system is an essential element of a long-term control plan (USEPA, 1994).

The goals and requirements of the US CWA are similar to those of the EU WFD (Ferreira et al., 2007), but in the EU there is no specific monitoring for CSO. Discharges of CSO may constitute a problem in relation to reaching "good status" in the quality of the receiving waters, which is one of the requirements of the WFD. According to Johansen et al. (2007) “recent surveys in the County of Aarhus, Denmark, have demonstrated that 17 % of the overflows lead to unacceptable conditions in the receiving waters". However, the classification of a water body strongly depends on the sampling design and if the combined sewer systems are not taken into consideration their impact may end up unnoticed. In Mediterranean areas, the impact of CSO is only important during the wet season because storm events are characteristic at this time of year. On the other hand, during the dry season, wastewater discharges constitute a problem because of the fluctuating population in tourist areas, which cause direct untreated effluent discharges into the receiving waters. So we recommend that the design of the WFD monitoring programs should take into account WWTP and combined sewer systems located near the coast.

To overcome the disturbances caused by CSO and partially treated effluent discharges our recommendation is to increase the capacity of the submarine outfall pipe and adapt it to the summer flow increase. We consider that increasing the WWTP capacity is not a priority as it serves the average population during most of the year.

5. Conclusions

In the Mediterranean region, the most common measures used to achieve the Water Framework Directive (WFD) quality targets for rivers and reservoirs involve increasing the quality of effluent produced from WWTPs in accordance with the 91/271/EEC Directive. Such measures have been recommended by the CHJ for the WWTPs discharging upstream the Beniarrés reservoir in the Serpis River Basin. However, in coastal WWTPs, which discharge effluent through submarine outfall, the quality of treated effluent is not the main problem. The main disturbances are localized near the coastline due to 1) CSO discharges, directly near the river mouths and subsequently into receiving coastal waters during rain episodes, and 2) partially treated effluent discharges due to the summer population increase. The importance of such discharges is such that despite the different magnitude of average annual flow of the Serpis River (3 $m^3 s^{-1}$) as compared with the Ebro River (384 $m^3 s^{-1}$) the nutrient concentration in the receiving coastal waters is comparable. The diatom seasonal cycle was modified by CSO discharges during rain episodes, while dinoflagellate abundance was higher in the dry season when partially treated effluents were the main source of freshwater and turbulence was low. The predicted increase in water residence time due to Gandia Harbour enlargement, as described in Sebastiá and Rodilla (2012) could be an additional factor that may trigger dinoflagellate blooms in this area.

The relevance of this problem is recognized in Annex I of the 91/271/EEC Directive, but the adoption of preventive measures is conditioned by economic considerations. Before implementing any management measures, the use of simulation models would be recommended to ensure that these measures have the anticipated effects on eutrophication problems. Taking into account that both agricultural land use and WWTP discharges are important sources of nutrients, different scenarios should be explored: e.g. different percentages of reductions in agricultural nutrient inputs and different percentages of reduction in CSO. In addition, different locations (depth and distance from the coast) should be simulated considering an increase in the submarine outfall flow; to make sure that the disturbances will not be relocated from the river mouth to the submarine outfall area. After selecting the best management option, analyzing the cost through a cost-benefit analysis would help to determine whether the costs are excessive or not. This may avoid that management measures will not be implemented due to economic considerations.

Management decisions should take into account that only reductions in CSO and partially treated summer effluent are likely to be efficient in the short term, while a reduction in diffuse agricultural sources, especially nitrogen, is only likely to give results in the long term due to the aquifer higher residence time.

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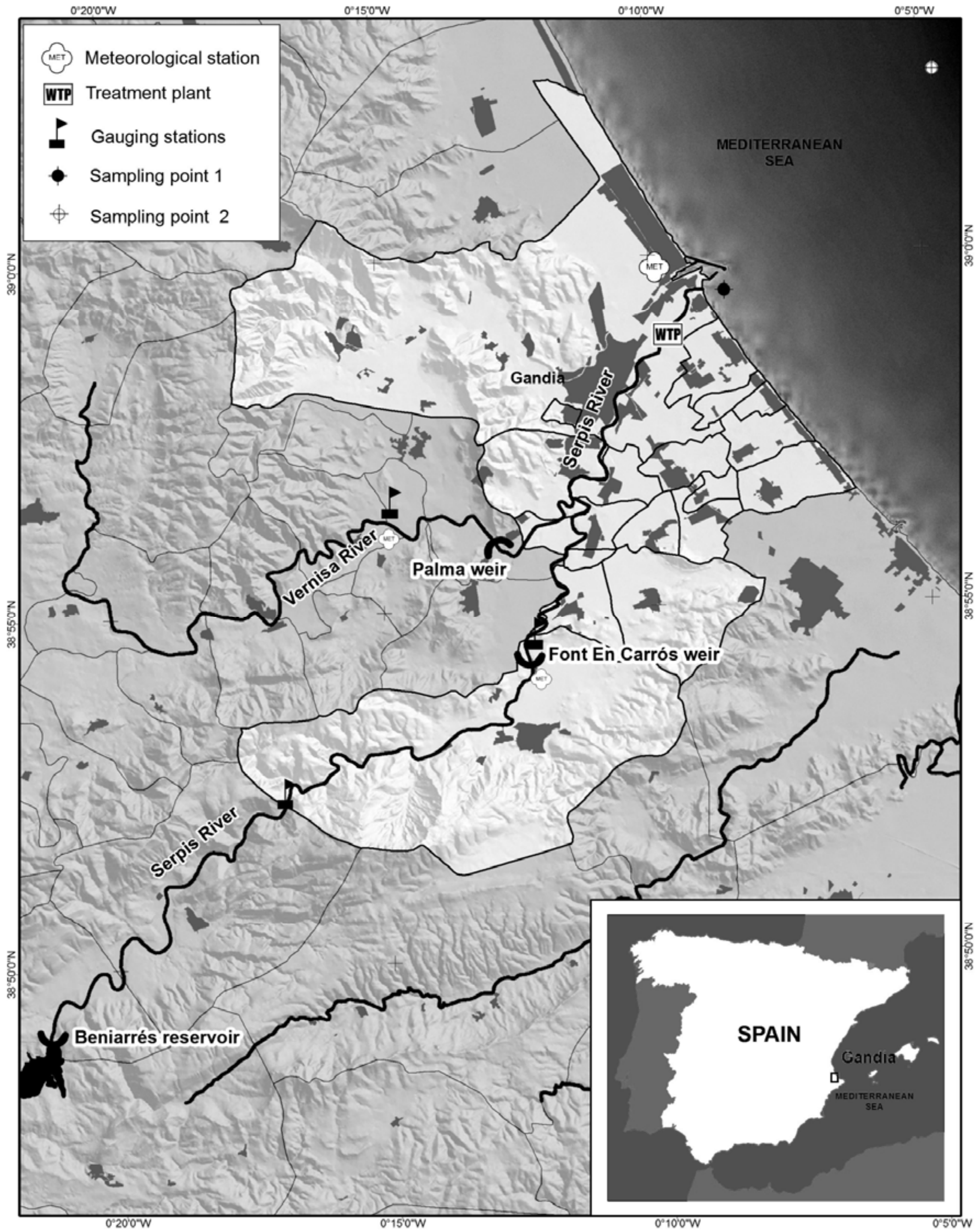

Figure 1. Study area and sampling points location

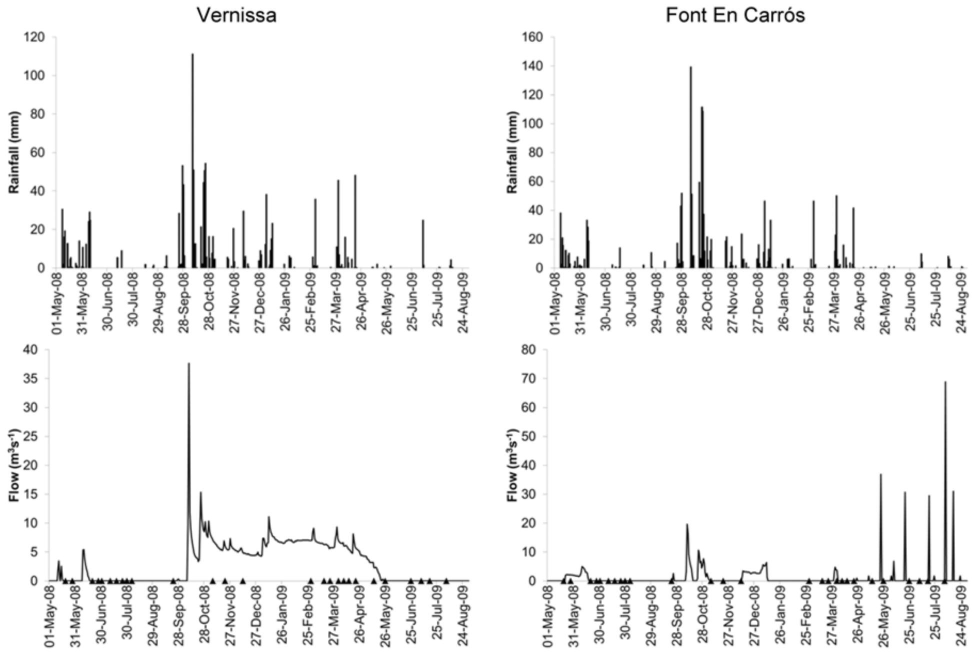

Figure 2. Daily rainfall and flow registered in Vernissa and Font En Carrós (CHJ) meteorological and gauging stations. The sampling dates are pointed out by triangles.

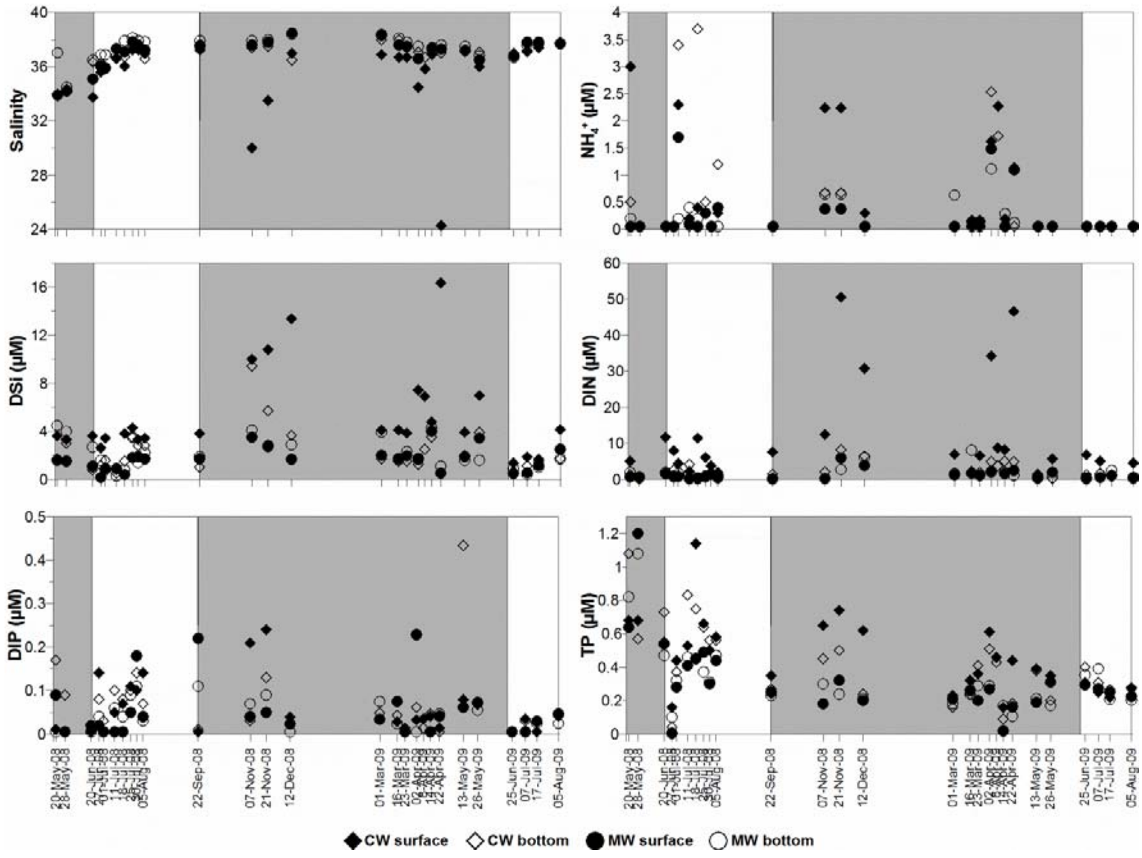

Figure 3. Temporal variation of salinity, dissolved inorganic nitrogen (DIN) (µM), ammonium (NH_4^+) (µM), dissolved silicate (DSi) (µM), dissolved inorganic phosphorus (DIP) (µM) and total phosphorus (TP) (µM)

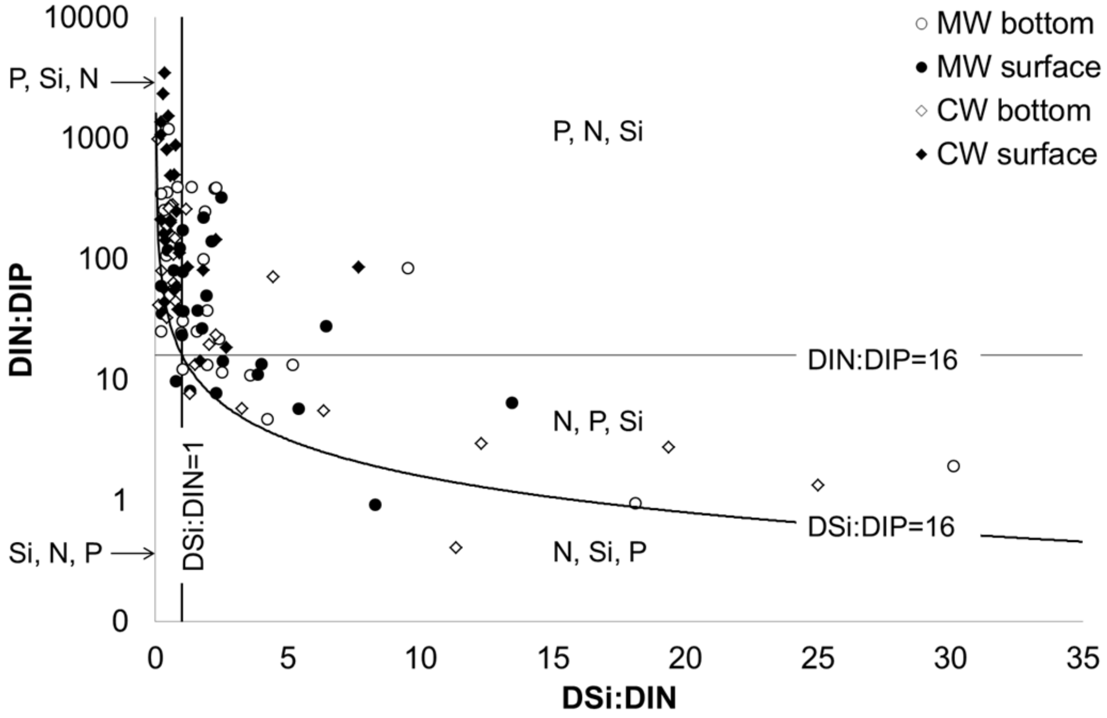

Figure 4. Synthetic graph of DSi:DIN:DIP molar ratios in coastal (CW) and marine waters (MW). In each area delimited Redfield ratios (DSi:DIN:DIP = 16:16:1), the potential limiting nutrients are reported in order of priority.

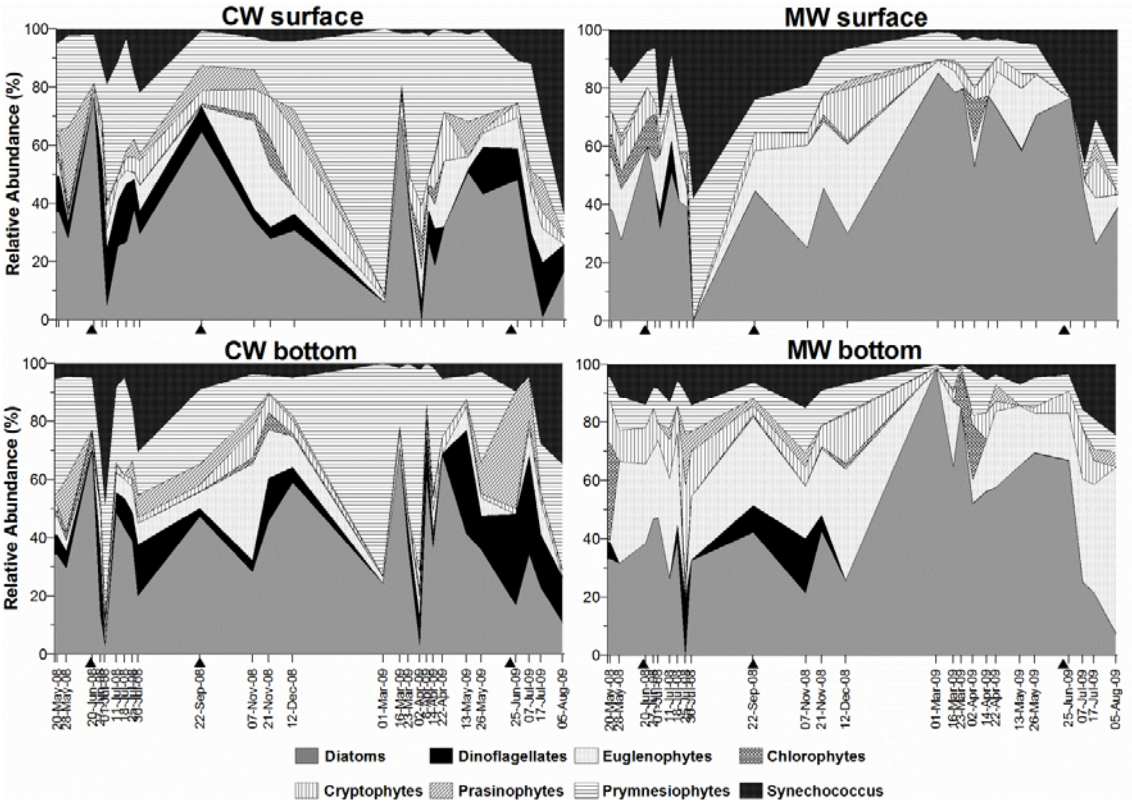

Figure 5. Temporal variation of relative abundance of phytoplankton groups as estimated with CHEMTAX

Table 1 Output matrices of pigment to chl-*a* ratios obtained from CHEMTAX for the samples of the clusters

	Per	19'But	Fuc	19'Hex	Neo	Pras	Viol	Allo	Lut	Zea	Chl_*b*
Diatoms											
Cluster 1	-	-	0.1753	-	0.0007	-	-	-	-	-	-
Cluster 2	-	-	0.2636	-	0.0000	-	-	-	-	-	-
Cluster 3	-	-	0.2634	-	0.0000	-	-	-	-	-	-
Dinoflagellates											
Cluster 1	0.5275	-	-	0.1095	-	-	-	-	-	-	-
Cluster 2	0.2221	-	-	0.1307	-	-	-	-	-	-	-
Cluster 3	0.2628	-	-	0.0933	-	-	-	-	-	-	-
Euglenophytes											
Cluster 1	-	-	-	-	0.0015	-	-	-	-	-	0.2965
Cluster 2	-	-	-	-	0.0052	-	-	-	-	-	0.4234
Cluster 3	-	-	-	-	0.0005	-	-	-	-	-	0.3575
Chlorophytes											
Cluster 1	-	-	-	-	0.1990	-	0.0310	-	0.0226	0.0503	0.0638
Cluster 2	-	-	-	-	0.3895	-	0.0162	-	0.0205	0.0411	0.0250
Cluster 3	-	-	-	-	0.5452	-	0.0001	-	0.0569	0.0042	0.0064
Cryptophytes											
Cluster 1	-	-	-	-	-	-	-	0.3136	-	-	-
Cluster 2	-	-	-	-	-	-	-	0.3561	-	-	-
Cluster 3	-	-	-	-	-	-	-	0.3600	-	-	-
Prasinophytes											
Cluster 1	-	-	-	-	0.0535	0.1721	0.1057	-	0.0823	0.0973	0.1631
Cluster 2	-	-	-	-	0.0068	0.0428	0.2202	-	0.0250	0.0474	0.0271
Cluster 3	-	-	-	-	0.0093	0.1205	0.1751	-	0.0592	0.0804	0.1070
Prymnesiophytes											

	Per	19’But	Fuc	19’Hex	Neo	Pras	Viol	Allo	Lut	Zea	Chl_*b*
Cluster 1	-	0.0447	0.0056	0.4331	-	-	-	-	-	-	-
Cluster 2	-	0.0267	0.0844	0.1833	-	-	-	-	-	-	-
Cluster 3	-	0.0265	0.0860	0.1818	-	-	-	-	-	-	-
Cyanobacteria											
Cluster 1	-	-	-	-	-	-	-	-	-	0.4605	-
Cluster 2	-	-	-	-	-	-	-	-	-	0.4033	-
Cluster 3	-	-	-	-	-	-	-	-	-	0.4551	-

Per: peridinin; 19’But: 19’-butanoyloxyfucoxanthin; Fuco: fucoxanthin; 19’Hex: 19’-hexanoyloxyfucoxanthin; Neo: neoxanthin; Pras: prasinoxanthin; Viol: violaxanthin; Allo: alloxanthin; Lut: lutein; Zea: zeaxanthin; Chl-*b:* chlorophyll *b*. –: pigment not present in phytoplankton group

The output matrix for Cluster 4 was the same that Cluster 1 in Sebastiá et al. (2012), both groups of samples were calculated together because samples have similar characteristics, and sample number for Cluster 4 (n = 4) was not enough to be calculated alone by CHEMTAX

Table 2. Rank correlation matrix (Spearman's r_s) of main physical and chemical parameters measured in Gandia coastal waters (n=54) from May 2008 to August 2009. ***p < 0.001, **p < 0.01, *p < 0.05

	Salinity	**DO**	**NH_4^+**	**DIN**	**DSi**	**DIP**	**TP**
Serpis flow	-0.04	0.53***	0.28*	0.35*	0.39**	0.00	-0.04
Rain	-0.32*	0.25	0.50***	0.41**	0.30*	0.09	0.30*
Salinity		0.04	-0.32*	-0.37**	-0.24	0.02	-0.39**
DO			0.12	0.27*	0.27*	-0.14	-0.12
NH_4^+				0.23	0.27*	0.14	0.52***
DIN					0.56***	-0.05	0.05
DSi						0.19	0.16
DIP							0.29*

Table 3. Descriptive statistics for salinity and nutrients (expressed in µM units) for the identified clusters

	Salinity	**DIN**	**DSi**	**DIP**
CLUSTER 1				
Average	37.2	1.47	1.90	0.04
Standard deviation	1.0	1.53	1.14	0.05
Maximum	38.5	8.00	4.50	0.23
Minimum	33.9	0.10	0.20	<0.01
CLUSTER 2				
Average	36.8	1.38	2.11	0.07
Standard deviation	1.2	1.02	1.14	0.09
Maximum	38.0	4.56	4.10	0.43
Minimum	34.0	0.12	0.70	<0.01
CLUSTER 3				
Average	36.2	6.56	3.96	0.05
Standard deviation	1.7	2.59	2.44	0.05
Maximum	37.5	12.34	10.00	0.21
Minimum	30.0	2.12	0.50	<0.01
CLUSTER 4				
Average	32.3	40.54	12.00	0.08
Standard deviation	5.5	9.43	3.75	0.11
Maximum	37.0	50.48	16.30	0.24
Minimum	24.3	30.87	7.50	0.01

Table 4. Rank correlation matrix (Spearman's r_s) of main physical and chemical parameters with relative abundance of phytoplankton groups, in coastal waters (P1, n=52) and marine waters (P2, n=50) of Gandia coast from May 2008 to August 2009. ***p < 0.001, **p < 0.01, *p < 0.05

	Diatoms		**Dinoflagellates**		**Euglenophytes**		**Chlorophytes**		**Cryptophytes**		**Prasinophytes**		**Prymnesiophytes**		**Cyanobacteria**	
	CW	MW	CW	MW	CW	MW	CW	MW	CW	MW	CW	MW	CW	MW	CW	MW
Temperature	-0.16	-0.32*	0.45**	0.09	-0.17	-0.19	-0.35*	-0.13	-0.28*	-0.16	0.22	0.00	-0.39**	0.27	0.60***	0.72***
Salinity	0.03	-0.11	0.13	0.00	-0.06	0.05	-0.31*	-0.03	-0.18	0.02	0.10	0.39**	-0.14	-0.21	0.16	-0.05
DO	-0.16	0.21	-0.19	-0.14	-0.14	-0.13	0.05	0.05	-0.03	-0.24	-0.25	0.08	0.35*	-0.04	-0.41**	-0.27
NH_4^+	-0.13	0.00	-0.04	0.34*	0.20	-0.05	0.50***	0.09	0.44**	0.06	-0.15	-0.02	0.23	0.12	-0.11	-0.17
DIN	0.03	0.13	-0.15	-0.26	0.23	0.01	0.24	0.18	0.62***	0.28	-0.34*	0.03	0.02	0.04	-0.34**	-0.37**
DSi	-0.05	-0.01	-0.18	0.13	0.21	-0.12	0.22	0.15	0.40***	0.08	0.06	0.06	0.03	0.14	-0.38**	-0.19
DIP	-0.05	-0.09	0.16	0.13	0.27	0.00	0.07	0.03	-0.04	-0.08	0.07	0.15	0.03	0.17	0.12	0.01
PT	0.11	-0.32	-0.03	-0.10	-0.06	-0.02	0.46**	0.21	0.09	0.25	0.08	0.03	0.02	0.18	0.04	0.32
DIN:DIP	0.08	0.14	-0.14	-0.25	-0.03	0.00	0.13	0.10	0.50***	0.23	-0.32*	-0.06	-0.05	-0.13	-0.27*	-0.20
DSi:DIP	0.05	0.06	-0.21	-0.07	-0.12	-0.05	0.09	0.01	0.30*	0.09	0.00	0.09	-0.03	-0.07	-0.31*	-0.09
DSi:DIN	-0.07	-0.06	0.07	0.31*	-0.09	-0.12	-0.10	-0.10	-0.43**	-0.15	0.43**	0.00	0.01	0.00	0.11	0.14

Table 5. Means (M) and standard deviation (SD) nutrients in receiving waters of the Serpis River (SR) and the Ebro River (ER) for wet and dry periods

		NH_4^+		**DIN**		**DSi**		**DIP**		**TP**	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dry period	SR	0.5	1.0	3.5	3.2	2.2	1.2	0.05	0.05	0.47	0.25
	ER	5.5	4.9	9.7	9.0	11.9	9.6	0.23	0.24	0.83	0.25
Wet period	SR	0.7	0.9	9.0	13.3	4.9	3.8	0.07	0.09	0.42	0.21
	ER (autumn)	7.1		9.0		9.2		0.38		0.91	
	ER (winter)	1.8	0.8	15.6	9.1	6.2	4.3	0.03	0.01	0.21	0.04
	ER (spring)	1.2	0.5	15.2	18.2	1.4	1.6	0.11	0.14	0.92	1.06