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Influence of nutrient inputs from a wetland dominated by agriculture on the phytoplankton community in a shallow harbour at the Spanish Mediterranean coast

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Abstract

The Safor Wetland (Western Mediterranean) is a protected ecosystem declared Site of Community Importance under the Habitats Directive. Agricultural practices have been part of this ecosystem throughout history, and its hydrology is anthropogenically manipulated to satisfy cultivation needs. Freshwater from the wetland is discharged through surface channels to Gandia Harbour, a shallow water body with high water residence time. This study evaluated the linear eutrophication gradient downstream from the freshwater inflow locations. The role of the main nutrients in determining the phytoplankton community is discussed. The predominance of agricultural practices, 48% of the watershed soil, caused an excess of nitrogen and an imbalance in the nutrient ratios at all the sampling points. Phosphorus concentrations were particularly low, and did not exceed 1.0 µM. Chlorophyll-*a* concentration was of the order of that found in other eutrophic estuarine waters. In general, flagellates dominated over diatoms at all the harbour sampling points and depths. Potentially blooming species of both phytoplankton groups were detected. The correct implementation of the existing agricultural best management practices should continue to reduce nitrogen and phosphorus loading to the estuary. It seems reasonable that for effective control of the eutrophication effects in this area, strict control over wastewater point sources should be also exercised.

Keywords: Photosynthetic pigments; CHEMTAX; Eutrophication; Agricultural runoff; Estuary; Western Mediterranean

1. Introduction

It is well known that agriculture is the main source of nitrogen in many regions: less than a half of the total nitrogen input via fertilizers and animal manure in crop production is effectively used, while the remainder is dissipated into the wider environment, where it contributes to a range of ecological and human health effects (Galloway et al., 2008). One of the main ecological effects originates when nitrogen leaves fields in surface runoff and is discharged to coastal ecosystems. There, it induces enhanced primary phytoplankton production that can lead to severe eutrophication problems (Cloern, 2001; Glé et al., 2008).

However, the phenomenon of eutrophication does not only depend on nitrogen inputs, but also on the phosphorus and silica inputs and on the relative nutrient composition (Cloern, 2001; Ludwig et al., 2009). In addition to increasing primary production, the alteration of nutrient ratios, in particular the imbalance of nitrogen and phosphorus with respect to silica has inevitable effects on the taxonomic composition of phytoplankton communities, as it can provoke a shift in primary production from diatoms to non-siliceous algae, often harmful for the ecological equilibrium (Ludwig et al., 2009). Lastly, these changes in phytoplankton community often precede larger-scale, longer-term changes in ecosystem function, including shifts in nutrient cycles, food webs, and fisheries (Paerl et al., 2010).

The EU has already adopted several directives and policies intended to combat eutrophication with varying degrees of success. While phosphorus levels have been decreasing since the 1990s, a reduction in nitrogen emissions is more difficult to achieve. Phosphorus pollution is normally dominated by point sources which are easier to target, thanks mainly to the ban on phosphorus detergents and phosphate removal in sewage plants. But the relationship of nitrogen with farming and the diffuse nature of the sources makes nitrogen regulation more difficult (Artioli et al., 2008).

Eutrophication problems are especially relevant in wetlands. In recent decades, increased regulation of wetlands and more intensive farming have increased the nutrient loading to many coastal ecosystems world-wide. Proper functioning of wetlands depends on groundwater and surface water hydrology. However, the hydrology of these ecosystems has been traditionally manipulated to satisfy the different cultivation needs (Hook, 1993). The main environmental and socioeconomic impacts of water regulation in wetlands are discussed in López (1999).

The Safor Wetland (Western Mediterranean) is an example of wetland regulation. The main freshwater input to the Safor Wetland is groundwater discharge, and the second main input is due to precipitation and infiltration over the area. Climatically, autumn and spring are the rainy seasons while summer is the dry period in this Mediterranean area. Nowadays, the hydrology of the wetland is anthropogenically manipulated to satisfy cultivation needs (mainly citrus). To prevent crop root asphyxia, in the wet seasons, water is pumped through the irrigation channels to the sea to decrease the phreatic level. But, there are other factors that make this regulation necessary: the intense urbanization process experienced in recent decades also means pumping water in order to avoid flooding of urban areas, and the population increase in summer, as the Spanish coast is a popular tourist destination, makes it necessary to increase pumping to supply drinking water from the wells located in the detritic aquifer which nourishes the wetland.

Beklioglu et al. (2007) have highlighted the need for information on the role of hydrology and major nutrients (nitrogen and phosphorus) in the phytoplankton ecology of shallow Mediterranean lakes in order to develop criteria for water quality in this climatic zone. The phytoplanktonic community of the shallow water bodies of this area has been studied by Rodrigo et al. (2003) but no study has focused on the receiving waters. Studying the receiving waters is especially important given the oligotrophic character of Mediterranean waters, where discharges of freshwater and associated nutrients play a key role in marine productivity (Ludwig et al., 2009).

This study analyzes the role of nutrient and nutrient ratio variations in determining the phytoplankton community in Gandia Harbour in relation to freshwater inputs from the Safor Wetland. It analyzes these variations in terms of spatial and seasonal composition and abundance of phytoplankton groups, using diagnostic photopigment analysis.

2. Materials and methods

2.1. Study area

The Safor Wetland (on Spain’s Mediterranean coast) is a protected ecosystem declared a Site of Community Importance (SCI) under the Habitats Directive (92/43/EEC), as it is considered one of the best preserved wetlands in Spain. Agricultural practices have been part of this ecosystem with different crops throughout history (e.g. sugar cane in the 15th and 16th century; corn, wheat and the white mulberry tree in the 18th century; and rice in the 19th century). Nowadays, characteristic crops in this area are citrus and horticultural crops.

The Valencian Regional government (through Order 13/2000, DOGV nº 3677, 2000-01-31) declared the municipalities of the Safor Wetland a nitrate-vulnerable zone, in accordance with the Council Directive 91/676/EEC (hereafter referred to as the Nitrates Directive). The Good Agricultural Practices Code published by the regional government (through Order 7/2010, DOCV nº 6212/23.02.2010), also in accordance with the Nitrates Directive, establishes that the recommended nitrogen doses for citrus crops is 200-250 kg $ha^{-1}year^{-1}$ for surface irrigation and 180-220 kg ha^{-1} $year^{-1}$ for drip irrigation. For horticultural crops, doses are similar, depending on the crop. However, in this region agriculture has been more intensive than in other areas, due to the mild climate, and traditionally these doses have been exceeded outstandingly (MARM, 2010). In consequence, nitrogen excess has been lixiviated to the aquifers or poured into surface streams.

The area is drained by an orthogonal network of artificial channels (Fig. 1) and has several pumping stations. The Ahuir channel is the main collector and its flow can be pumped to two watercourses: northward to the Xeraco watercourse which outflows directly to the sea (the Mediterranean) or southward to the San Nicolas watercourse which outflows into the Gandia Harbour. San Nicolas is an ephemeral watercourse which drains an area of 50 km^2 and it is about 14 km long; it is generally inactive; it carries great quantities of water only when torrential rain falls. The flow is only continuous in the last 1.5 km due to the inputs of freshwater draining the Safor Wetland. The harbour has an average depth of 5 m, and a maximum depth of 10 m restricted to a small area where merchant ships tie up. Water residence time is above 30 days on average in the harbour, so eutrophication problems are more likely to be found here. The harbour can be considered a small stratified estuary with a shallow freshwater layer due to freshwater inputs from the wetland for most of the year. The last 1.5 km of the San Nicolas ephemeral watercourse and the Gandia Harbour were selected as the study area.

Gandia Harbour is a commercial, fishing and recreational harbour located in the southernmost sector of the Valencian Gulf (South-Western Mediterranean). Apart from the Ahuir channel, the harbour receives freshwater inputs from the Molí, Rei, and Nova channels (Fig. 1). The final few metres of the Rei, Nova and Ahuir channels are buried underground and flow into the harbour through two outlets. The Rei irrigation channel outlet is Point 3 (P3) in Fig. 1 (see detailed photo), while the Nova and Ahuir channels meet and flow out at Point 4 (P4).

Present land uses (Fig. 1) in the drainage area described include citrus (990.7 ha), horticultural crops (215.8 ha), forest (588.1 ha), wetland (224.8 ha) and urban use (409.6 ha). Anthropogenic land uses, including agricultural (48%) and urban (16%) use, represent 66% of the watershed, while forest and marsh account for 32% of the drainage area.

This area is located over the Plana de Gandia-Denia detritic unconfined aquifer, which provides the necessary water resources for crop irrigation; however its shallow phreatic level causes problems of root asphyxia. To prevent this problem, freshwater from the aquifer is pumped into Gandia Harbour through the irrigation channels described above. Due to continued agricultural practices, nitrate levels in the aquifer have exceeded the limit of 50 mg L^{-1} established by the Nitrates Directive, so freshwater discharges are characterized by high nitrogen loads.

Concerning the main phosphorus input of anthropogenic origin, municipal wastewater is treated in the sewage treatment plant of Gandia and discharged into the sea through a submarine outfall at an approximate distance of 1900 m from the harbour. However, there are some second homes on the non-urban soil of the Safor wetland (Fig. 1), which are not connected to the wastewater collection system. Many of them discharge wastewater directly into the surface channels (Nova, Ahuir and Rei channels), others have septic tanks. Even in the second case, wastewater ends in the irrigation channels, because wastewater infiltrates from the septic tanks to the shallow aquifer and water is continuously pumped from the aquifer into the surface channel as described above.

Regarding phosphorus input of agricultural origin, the recommended phosphorus doses for citrus crops is 70 kg ha^{-1} year^{-1} for surface irrigation and 80 kg ha^{-1} year^{-1} for drip irrigation (MARM, 2010). One unique dose of phosphorus fertilizers is surface-applied generally in March (Legaz and Primo-Millo, 1988). He et al. (2006) analysed the concentration and forms of phosphorus in the surface runoff from field-scale studies of an analogous study area: citrus and horticultural crops in a flat landscape with shallow water table, artificial drainage and similar phosphorus fertilization. They found that dissolved inorganic phosphorus (DIP) was the dominant form in the total dissolved phosphorus and its concentration varied widely from <0.01 to 9.85 mg L^{-1} in the runoff waters, but generally DIP concentration was above the 0.01 mg L^{-1} critical concentration for eutrophic shallow lakes recovery (Beklioglu et al., 2007).

2.2. Sampling strategy

Water samples were taken at the 8 sampling points shown in Fig.1. Sampling points were chosen to evaluate the nutrient input of the irrigation channels and its influence on Gandia Harbour phytoplankton structure. Sampling was designed with high spatial resolution as recommended in Zablotowicz et al. (2010) because phytoplankton can vary on a scale of meters.

Point 1 (P1) was situated in the first irrigation channel, the Molí channel, which flows into Gandia Harbour. Point 2 (P2) was located after the inflow of the Molí channel and before the inflow of the two other irrigation channels to the harbour. Point 3 (P3) and

Point 4 (P4) were situated in the Rei and Nova-Ahuir irrigation channels respectively. Points 5 (P5) to 8 (P8) were located on a longitudinal seaward transect, starting with P5 after the contribution of the Rei and Nova-Ahuir channels and finishing with P8 outside the harbour but under its direct influence.

Samples were taken in two hydrological periods: the wet one in spring, on 15 April 2009, and the dry one in summer, on 06 August 2009. Only one water sample was collected at 0.05 m depth in each irrigation channel because of their scarce depth and flow. At the other points, water samples were collected at different depths in the water column (0, 0.05, 0.10, 0.30, 0.50, 0.75 and 1 m) using a Superficial Water Sampler (Mösso et al., 2008) and one extra sample just above the bottom with a horizontal Van Dorn bottle. Water samples were kept in a cool box (4ºC) and transported to the laboratory.

Flow measurements were made with a calibrated current meter in the irrigation channels. At each measurement point, flow discharge was gauged by taking velocity within subsections (at 60% of the subsection's depth and averaged for 90 s) along the stream's cross section. A computer program integrated flows for the point.

Wind speed and direction were measured in the weather station located approximately 500 m from the harbour (Fig. 1).

2.3. Laboratory analysis

The following parameters were analyzed in all the samples: salinity, suspended solids (SS), nitrate, nitrite, and ammonium, dissolved inorganic phosphorus (DIP) and dissolved silicate (DSi). Dissolved inorganic nitrogen (DIN) was calculated as the sum of nitrate, nitrite and ammonium. Salinity was determined by means of a conductivity meter Multi 340i/SET WTW, using the Practical Salinity Scale. Nutrients were analyzed colorimetrically using the method of Aminot and Chaussepied (1983).

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 Targa et al. (2000). The system was calibrated with external standards obtained commercially from the DHI Water and Environment Institute (Hørsholm, Denmark). Once the concentration of important photosynthetic pigments was determined, the phytoplankton community was studied using the CHEMTAX program (Mackey et al., 1996). Diagnostic photopigment analyses are able to detect significant changes in phytoplankton community composition over a broad range of time scales and as such are well suited for monitoring programs designed to assess short- and long-term trends in water quality in response to nutrient enrichment (Niemi et al., 2004). Phytoplankton samples were fixed with formaldehyde, concentrated according to UNE EN 15204:2006, based on Utermohl (1958), and qualitatively examined under a LEICA DM IL inverted microscope. CHEMTAX was applied following the procedures described in Latasa (2007) using version 1.95 (S. Wright, pers. comm.) to obtain the contribution to chlorophyll-*a* (Chl-*a*) of the phytoplankton groups identified.

2.4. Statistical analysis

Sampling points were grouped according to similar salinity, DIN, DIP and DSi properties as determined by cluster analysis. Clustering dendograms were generated using STATGRAPHICS 5.1. City-block distances were calculated and samples clustered according to Ward’s method (Latasa et al., 2010). Pigment samples were separated into subsets following the results of the cluster analysis, and CHEMTAX was applied independently to each subset (Latasa et al., 2010) to obtain the contribution of 8 phytoplankton groups to the chlorophyll *a* stock: diatoms, dinoflagellates, euglenophytes, chlorophytes, cryptophytes, prymnesiophytes, prasinophytes and cyanobacteria.

A non parametric one-way analysis of variance (Kruskal-Wallis) was performed to statistically assess variations in the median fraction of Chl-*a* of each phytoplankton taxon within the identified clusters and sampling seasons. Variations in the nutrient concentration between clusters and seasons were also assessed.

Spearman rank correlation analyses were performed on environmental parameters (DIN, DIP, DSi, DIN/DIP, DSi/DIN, DSi/DIP, salinity and season) and phytoplankton groups in order to examine significant relationship.

3. Results

3.1. Physical and chemical parameters

Most of the physical and chemical parameters that were measured in this study showed a longitudinal gradient from the discharge points of the irrigation channels to the sea (Fig. 2). Salinity varied between 19.1 and 22.4 at the upper station (P2) surface and 37.0 and 37.5 at the lower station (P8) surface. The difference in temperature over the salinity gradient was rather small, generally less than 1ºC. In spring, temperatures ranged from 16.6ºC to 15.8ºC, surface and bottom respectively, at P2; and 15.8ºC and 14.9ºC at P8. In summer, temperatures ranged from 26ºC to 25.8ºC, surface and bottom respectively, and there were no significant changes along the longitudinal transect.

Predominant wind direction in the spring sampling was W-NW, while in summer it was E-SE. This caused greater marine water entry into the harbour in summer due to the orientation of its entrance channel (Fig. 1). Total flow measurements in the irrigation channels were 0.74 $m^3 s^{-1}$ in spring and 0.34 $m^3 s^{-1}$ in summer. More specifically, the Molí irrigation channel (sampling point P1) flow was 0.23 $m^3 s^{-1}$ in spring and 0.10 $m^3 s^{-1}$ in summer; Rei channel (P3) flow was 0.28 $m^3 s^{-1}$ and 0.04 $m^3 s^{-1}$ respectively; and the Nova-Ahuir channel (P4) was 0.23 $m^3 s^{-1}$ in spring and 0.20 $m^3 s^{-1}$ in summer. Nova-Ahuir flow was similar in both seasons, while Molí flow was reduced by half and Rei flow was practically non-existent in summer. Suspended solids were rather low, average 12 ± 5 mg L^{-1}, and so were not included in the statistical analysis.

The cluster analysis of salinity, DIN, DIP and DSi variables identified two major clusters, designated A and B. Samples included in cluster A were the samples from the irrigation channels (P1, P3 and P4) and the samples from P2 and P5 (from 0 to 0.75 m water column depth). All other samples were included in cluster B. Examination of the variables of the different clusters (Table 1) revealed that cluster A was characterized by significantly lower salinities and higher nutrient concentrations than cluster B. Nutrients showed an opposite longitudinal gradient to that of salinity and decreased from the landside to the seaside of the harbour. The spatial and seasonal variations in salinity and nutrients have been depicted in Fig. 2. No significant variation was observed for nutrient concentration between the two seasons, except for DIP concentration, which showed a significantly higher concentration in spring. Nitrate was the most dominant nitrogen form at all sampling points and the highest values were observed at the irrigation channel sampling points for both seasons with values around 200 µM. DIP concentrations were rather low and did not exceed 1 µM, for cluster A, and 0.2 µM, for cluster B. The highest DIP values were observed in the Rei (P3) and Nova-Ahuir (P4) irrigation channels and at sampling points P2 and P5 and were similar for both seasons, except in the Nova-Ahuir (P4) channel where DIP showed a summer increase (0.16 to 0.97 µM). Average DSi concentrations were 50.7 µM for Cluster A and 12.0 µM for Cluster B. In spring, the highest DSi values were found in the irrigation channels: all samples were around 100 µM DSi. In summer, DSi content in the irrigation channels decreased considerably, mainly in the Molí (P3) channel (9 µM).

In order to better define potential nutrient control, we compared nutrient ratios between DIN, DSi and DIP concentrations with Redfield ratios (Si:N:P = 16:16:1). In the DIN:DIP and DSi:DIP ratios, phosphorus was always the limiting nutrient, except for an isolated instance of DIN limitation in spring at the P8 near-bottom sample. The average DIN:DIP and DSi:DIP ratios were 1968 and 476 respectively in Cluster A and 379 and 300 respectively in Cluster B (Table 1), showing a seaward decreasing gradient (Fig. 3 a, b, e, f). Regarding the DSi:DIN ratio, conditions were Si-limited in Cluster A, where this ratio remained under 1 in both seasons. In the irrigation channels, due to the constant DIN levels and the silica decrease, the DSi:DIN ratio decreased from 0.5 in spring to less than 0.2 in summer. In cluster B, Si-limited conditions and N-limited conditions alternated (Fig. 3 c, d).

3.2. Phytoplankton abundance and composition

3.2.1. Total chlorophyll *a*

The spatial and seasonal variation in total chlorophyll *a* (Chl-*a*) is shown in Fig. 4.

Chl-*a* concentration showed significant spatial variation with the highest values observed in the harbour after the freshwater inputs and a decreasing seaward gradient. In spring, the highest Chl-*a* values were observed at P5, with a maximum of 8.8 µg L^{-1} at 1 m depth, and the lowest values were found at P8 with 1.4 µg L^{-1}. In summer, the highest values were measured at P2, with a maximum of 11.5 µg L^{-1} at 1 m depth, and the lowest values were found at P8 with 1.1 µg L^{-1} at surface. In the irrigation channels, Chl-*a* concentration did not show a significant seasonal variation in the Rei (P3) channel, while in the other channels it increased in summer. The Chl-*a* concentration varied from 2.4 to 10.2 µg L^{-1} in the Molí (P1) channel (spring and summer respectively); from 6.0 to 5.6 µg L^{-1} in the Rei (P3) channel; and from 1.9 to 4.5 µg L^{-1} in the Nova-Ahuir (P4) channel.

3.2.2. Irrigation channels: P1, P3 and P4 sampling points

In terms of the contribution of the different groups of algae to total chlorophyll *a*, in the irrigation channels diatoms were the most important group in spring (Table 2). The contribution of this group diminished in summer (Table 3), and even disappeared from the Molí (P1) channel, being replaced by an increase in flagellate organisms - mainly euglenophytes in the Molí and Nova-Ahuir (P4) channels and chlorophytes in the Rei (P3) and Nova-Ahuir (P4) channels. Although all three channels were under silica limiting conditions in both seasons, diatoms only disappeared from the Molí channel, which had the lowest DSi:DIN ratio (0.04).

The contribution of euglenophytes in spring to total Chl-*a* was small - around 10% in all three channels (Table 2) - while in summer they were the main group in the Molí channel (39%) and the second main group in the Nova-Ahuir (30%) (Table 3). Chlorophytes were the second main group in spring Chl-*a* contribution (Table 2), while in summer they were the main group in the Rei (61%) and Nova-Ahuir (36%) channels (Table 3). Abundance of dinoflagellates and prasinophytes was significantly higher in spring, though their contribution to total Chl-*a* was small, except for prasinophytes in the Rei channel (18%) (Table 2). Cryptophytes were an important group in the Molí channel in both seasons (18% spring and 23% summer) (Tables 2 and 3), while in the other two channels, they were more abundant in summer though their contribution to total Chl-*a* was less important (11% Rei channel and 7% Nova-Ahuir).

Prymnesiophytes were only found in the Molí channel with a 14% contribution to Chl-*a* in spring and 21% in summer (Tables 2 and 3). Cyanobacteria contribution to total Chl-*a* was only relevant in the Rei channel in summer where they accounted for 8% (Table 3).

3.2.3. The estuary: P2, P5; P6 and P7 sampling points

In the harbour, flagellates were the main contributing group to total Chl-*a* in both seasons, while the contribution of other groups such as diatoms and cyanobacteria was generally minor (Tables 2 and 3). In spring, euglenophytes were the most important group at P2, P5 and P6 down to a depth of 0.10 m, while in deeper waters (0.75 m), predominant groups were chlorophytes at P2, dinoflagellates at P5 and prasinophytes at P6 (Table 2). In summer, euglenophytes predominated in the whole water column at these same points (Table 3). At P7, the most important group in spring were prasinophytes (maximum of 68% at 0.10 m) and in summer, euglenophytes down to 0.10 m water depth (for reference see abundance at 0.05 m, Tables 2 and 3). The chlorophytes found were typical of freshwater, and as such they showed a negative correlation (Table 4) with salinity and their abundance was statistically higher in cluster A. They were also inversely correlated with DSi:DIN ratio and showed a positive correlation with DIN and DIP (Table 4). In the harbour, their maximum abundance was found at P2 in both seasons at 0.75 m depth (Tables 2 and 3). Dinoflagellate abundance decreased towards the coast and was only important at P2 and P5 just after the freshwater inputs. Freshwater prasinophytes were not found at P2 or P5 in spring or in summer (Tables 2 and 3). In spring, a prasinophytes population was found from P6 to P7, with its maximum abundance at 0.10 m depth accounting for 60% of total Chl-*a* at P6 and 68% at P7. The statistical analysis indicated that this group was more abundant in Cluster B (Table 2). Other flagellate contributions to total Chl-*a* were less important.

In spring, diatoms showed their maximum contribution to Chl-*a* at P2 and P5, after the freshwater inputs, where they were the third main group (Table 2). On the other hand, in summer, diatoms were the second main group at P5 and P6 in the whole water column, and at P7 they were the main group at 0.75 m depth, with a contribution even greater than that of euglenophytes (Table 3).

Cyanobacteria were more abundant in summer (statistical analysis), while their spring contribution to total Chl-*a* was reduced to less than 1% at all sampling points and depths (Tables 2 and 3). Two populations were detected: one population typical of freshwater and present in all irrigation channels in spring and only in the Rei channel in summer; and the other population, typical of marine waters. In the harbour, the freshwater population was observed in the surface samples from P2 and P5 (16.2% and 22.9% respectively in spring). The marine population showed an increase in abundance towards the sea; this population was directly correlated with salinity and inversely correlated with DIP concentration (Table 4).

3.2.4. Outside the estuary: P8 sampling point

Outside the harbour, at P8, prymnesiophytes were the most important group in spring, while an assemblage of prymnesiophytes (33.7%) and cyanobacteria (31.3%) predominated in summer (Tables 2 and 3). The prymnesiophyte marine population increased in abundance between P5 and P8 in spring and was present only at P8 in summer. Prymnesiophytes showed a negative correlation with DIN:DIP and DSi:DIP ratios (Table 4).

3.2.5. Blooming algal groups

It is important to highlight that in the microscope analysis, some potentially blooming species and genera (Moncheva et al., 2001) were detected: the dinoflagellates *Dinophysis caudata*, *Ceratium furca*, *Prorocentrum micans*, *Gymnodinium* spp., *Heterocapsa* sp., *Scrippsiella* spp.; diatoms of the genus *Amphora spp*. and *Pseudo-nitzschia* spp.; and euglenophytes of the genus *Eutreptiella* sp.

4. Discussion

Climatically, autumn and spring are the rainy seasons while summer is the dry period in this Mediterranean area. Thus, the phreatic level is higher in spring and to prevent crop root asphyxia, water is pumped through the irrigation channels to the sea. On the other hand, in summer, the population increase makes it necessary to increase pumping to supply drinking water from the wells located in the detritic aquifer. In addition, citrus crops need at least two waterings during summer. This causes the flow reduction found in the irrigation channels in summer, when irrigation return flows are the most important source of freshwater. Gandia harbour can be considered a small stratified estuary with a shallow freshwater layer for most of the year. However, in summer, due to the reduction in freshwater inputs and dominant wind direction, this freshwater layer nearly disappears.

In Gandia Harbour, the range of nitrate and silicate concentrations was of the order of those found in typical nutrient-enriched areas (Domingues et al., 2005). However, phosphorus concentrations were particularly low, in the same order of magnitude as those measured in non-polluted coastal areas (Aminot and Chaussepied, 1983; Glé et al., 2008).

It is broadly accepted that in marine systems, nitrogen is the limiting nutrient, whereas phosphorus limits freshwater systems. There is, however, evidence of seasonal and spatial variations of the limiting nutrient in estuarine systems (Domingues et al., 2005; García-Pintado et al., 2007). In the study area, the nutrient concentration in the irrigation channels and in the harbour is influenced by the soil use in their watershed. The clear predominance of agricultural practices (48% of watershed soil) and the discharge of treated urban wastewater through the submarine outfall, causes an excess of nitrogen and an imbalance in the DIN:DIP ratio. In addition, the entry of marine water into the harbour is also characterized by phosphorus limiting conditions, as shown by several studies in the Mediterranean, which identify phosphorus as the main limiting nutrient in phytoplankton productivity (Estrada, 1996; Olivos et al., 2002), in contrast with other marine systems.

Chemical weathering of silicates on land is the main process that supplies dissolved and particulate silicate to rivers. However, in this area, groundwater discharges from the Gandia-Denia detritic aquifer are also rich in silicates. The high silica levels found in both seasons also caused the imbalance in the DSi:DIP ratio. Thus, phosphorus was always the primary limiting nutrient for phytoplankton growth, while DIN and DSi spatially alternated as secondary limiting nutrients.

The reduction of DIP concentration to below 0.01 mg L^{-1} (approx. 0.32 μM) has been pointed out as the first necessary step in the recovery of eutrophic shallow lakes. However, for warm shallow lakes in the Mediterranean region there is experimental evidence that this threshold should be even lower (Villena and Romo, 2003). Despite the fact that the average DIP levels in the study area are generally below this limit, an isolated instance of DIP increase was observed in the Nova-Ahuir outflow in summer (0.97 µM). DIP concentrations in estuaries are often found to be highest during summer corresponding to a strong temperature-dependent release of phosphorus from sediments (García-Pintado et al., 2007). In Gandia Harbour, reduced water flow and the direction of dominant winds increase water residence time and this may provide longer contact with sediment that may also enhance internal release of phosphorus. However, DIP levels are significantly higher in spring, when phosphorus fertilizers are applied, which may point to a phosphorous origin from allochthonous sources (agricultural runoff) rather than from sediment release. DIP spring maximum was 0.30 µM, so the isolated DIP increase found in summer (see above) could not be explained by agricultural runoff. An alternative explanation for this increase can be found in the wastewater discharge from the second homes located in the study area (Fig. 1) which are only inhabited during summer.

Chlorophyll-*a* concentration was of the order of that found in other eutrophic estuarine waters (Rodríguez et al., 2003; Seoane et al., 2005) and coastal lagoons (Coelho et al., 2007) of moderate biomass but much lower than that found in very eutrophic estuaries (Ansotegui et al., 2001) and coastal lagoons (Villena and Romo, 2003).

Diatoms are recognised as the most opportunistic species as far as taking advantage of nutrient availability is concerned (Fogg, 1991). In this study, diatoms showed a significant positive correlation with DIN and DSi concentration, and with DIN:DIP and DSi:DIP ratios (Table 4), confirming a preference for eutrophic conditions. In the irrigation channels diatoms were the most important group in spring and their contribution diminished in summer, being replaced by an increase in flagellate contribution to total Chl-*a*. This numerical displacement of diatoms by flagellates, in geographically diverse regions experiencing decreased DSi:DIN and DSi:DIP ratios due to a decreased availability of silicate relative to nitrogen and phosphorus, has been well documented (Moncheva et al., 2001). In the harbour, high diatom contribution was also linked to high nutrient availability from freshwater discharges in spring (P2 and P5), while the highest contribution at P7 at 0.75 m depth in summer was attributed to the higher DSi:DIP ratios (Fig.3f) in this location due to lower phosphorus concentrations (Fig. 2f) in summer. DSi:DIN ratios, which showed a balanced value close to 1 at P7 (Fig. 3d), indicate diatom uptake (Glé et al., 2008). This high contribution of diatoms in summer has been reported and attributed to freshwater taxa that grow well in freshwaters as well as in brackish waters (Seoane et al., 2005). The diatom distribution can also be related with higher flushing rates as they are more abundant in the channels and in the sampling points located at their outflow (P2 and P5).

If we group the dinoflagellates, cryptophytes, prasinophytes, chlorophytes, and euglenophytes as flagellates, these dominated over diatoms at all the harbour sampling points and depths (P2, P5, P6 and P7), except for P7 at 0.75 m depth in summer. The dominance of euglenophytes has been observed in other eutrophic systems where it has been related with high nutrient levels and decreasing turbulence (Olli et al., 1996; Çelik and Ongun, 2007). The positive correlation of chlorophytes with DIN and DIP (Table 4) showed that this group was characteristic of the most eutrophic conditions. In contrast with diatoms, chlorophytes were inversely correlated with the DSi:DIN ratio (Table 4), so they outcompeted diatoms when this ratio value was lower. Their high contribution at P2 at 0.75 m depth in both seasons could be due to the sedimentation process of chlorophytes affected by the higher salinity. Dinoflagellates, cryptophytes and prasinophytes have been reported as eutrophic groups by Latasa et al. (2010) in the northwestern Mediterranean Sea. Higher abundance of prasinophytes at P6 and P7 could be attributed to euryhaline species (Seoane et al., 2005). The dominance of flagellates can explain the moderate chlorophyll-*a* levels observed. It is assumed that small phytoplankton (which includes flagellates) cannot reach high biomasses, since biomass elevation actuates a feedback mechanism favouring predators and which may finally enhance the rate of primary production rather than biomass accumulation (Fogg, 1991; Bel Hassen et al., 2008).

Outside the harbour, the dominance of prymnesiophytes in spring (Table 2) and the dominance of the prymnesiophyte and cyanobacteria assemblage in summer (Table 3) coincides with the observations of Latasa et al. (2010). These two groups prefer more oligotrophic waters, and prymnesiophytes are outcompeted by cyanobacteria when nutrient content decreases. In this case, prymnesiophytes were significantly less abundant in summer, when DIP and DSi values were lower at P8 (Fig. 2 e, f, g, h), and the cyanobacteria contribution to total Chl-*a* was higher. This preference for oligotrophic conditions is reflected in the prymnesiophytes' negative correlation with DIN:DIP and DSi:DIP ratios (Table 4). Higher contributions of smaller cells during summer are reported for several estuaries and it is well established that these organisms attain maximum growth rates at temperatures higher than those for diatoms and green algae (Domingues et al., 2005). In this study, cyanobacteria were positively and significantly correlated with season, while prasinophytes (green algae), prymnesiophytes and dinoflagellates were negatively correlated (Table 4). The dominance of these groups outside the harbour indicates that the eutrophication problems are mainly restricted to the harbour area.

Among the potentially blooming species identified, the dinoflagellate *Dinophysis caudata* is responsible for the synthesis of the toxin DSP okadaic acid and the diatom *Pseudo-nitzschia* spp. synthesises the ASP toxin (domoic acid). In humans, these toxins can cause the poisoning syndromes known as diarrheic and amnesic shellfish poisoning (DSP and ASP respectively) (Anderson, 2009).

5. Conclusions

To develop appropriate management strategies, it is necessary to fully understand how ecosystems function by first of all establishing the relationships between phytoplankton composition, abundance and nutrient patterns. Nutrient concentrations in the aquifers depend on anthropogenic land uses and agricultural practices. Intensive farming and wetland regulation have increased groundwater discharge characterized by high nitrate levels to coastal ecosystems, such as shallow estuaries. High phosphorus levels in receiving waters usually coincide with phosphorus fertilization period, but other sources such as uncontrolled wastewater discharges also cause phosphorus increases. In ecosystems with phosphorus limiting conditions and high nitrate and silicate levels, a continued input of phosphorus could trigger the undesired effects of phytoplankton species responsible for the generation of harmful blooms. The correct implementation of the existing agricultural best management practices is needed to reduce nitrogen and phosphorus loading. But for effective control of the eutrophication effects strict control over wastewater point sources should be also exercised. This management strategy has been proposed for similar eutrophicated systems such as La Albufera (Valencia, Spain) (Villena and Romo, 2003) and El Mar Menor (Murcia, Spain) (García-Pintado et al., 2007). Reducing phosphorus inputs would make phosphorus more limiting and result in nitrogen from anthropogenic sources being less useful for ecosystem productivity.

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Table 1 Descriptive statistics for salinity, nutrients (all expressed in µM units) and nutrient ratios for the identified clusters

	Salinity	**NH_4**	**NO_2**	**NO_3**	**DIN**	**DIP**	**DSi**	**DIN/DIP**	**DSi/DIP**	**DSi/DIN**
CLUSTER A										
Average	17.8	1.8	0.9	175.8	178.5	0.18	50.7	1968	476	0.3
Standard deviation	±13.5	±1.2	±0.3	±41.3	±41.3	±0.22	±36.6	±1435	±359	±0.2
Minimum	0.1	0.5	0.3	94.1	96.4	0.02	9.0	232	20	0.0
Maximum	34.8	4.6	1.4	243.2	245.5	0.97	106.0	5309	1367	0.6
CLUSTER B										
Average	36.3	0.8	0.2	12.0	13.0	0.06	12.0	399	316	1.0
Standard deviation	±1.2	±0.6	±0.1	±7.4	±8.0	±0.05	±8.7	±320	±200	±0.6
Minimum	34.2	0.1	0.0	2.9	3.4	0.01	2.8	53	46	0.4
Maximum	38.0	2.4	0.4	28.8	30.3	0.19	32.1	1143	622	2.5

DIN Dissolved Inorganic Nitrogen, DIP Dissolved Inorganic Phosphorus, DSi Dissolved Silicate

Cluster A includes samples from the irrigation channels (P1, P3 and P4) and samples from P2 to P5 (from 0 to 0.75 m water column depth)

Cluster B includes all other samples

Table 2 Contribution (%) of different phytoplankton groups to total chlorophyll *a* calculated using CHEMTAX for spring sampling. P1; P3 and P4 are the sampling points located in the irrigation channels which were only sampled at 0.05 m depth

Sampling point	Depth (m)	Diatoms	Dinoflagellates	Euglenophytes	Chlorophytes	Cryptophytes	Prasinophytes	Prymnesiophytes	Cyanobacteria
P1	0.05	23.3	1.6	11.4	21.4	17.8	9.2	14.1	1.3
P2	0.05	25.2	31.9	37.2	5.7	0.0	0.0	0.0	0.0
	0.75	0.0	34.3	0.0	62.7	0.6	2.5	0.0	0.0
P3	0.05	40.3	11.6	9.5	17.1	2.0	17.8	0.0	1.7
P4	0.05	52.7	6.3	12.5	20.5	0.0	6.9	0.0	1.1
P5	0.05	15.0	20.5	49.1	4.3	5.5	0.1	5.0	0.6
	0.75	11.8	45.6	14.7	14.1	5.6	1.0	7.2	0.0
P6	0.05	3.7	11.1	44.5	16.2	1.5	2.4	20.5	0.0
	0.75	6.8	14.6	17.6	5.7	3.0	42.6	9.7	0.0
P7	0.05	3.5	11.0	28.2	0.0	2.1	35.4	19.7	0.0
	0.75	2.7	9.7	18.5	0.0	2.9	37.0	29.2	0.0
P8	0.05	0.0	1.4	5.5	0.0	3.6	16.0	72.8	0.7
	0.75	0.0	0.0	0.0	0.0	3.2	32.1	64.7	0.0

Table 3 Contribution (%) of different phytoplankton groups to total chlorophyll *a* calculated using CHEMTAX for summer sampling. P1; P3 and P4 are the sampling points located in the irrigation channels which were only sampled at 0.05 m depth

Sampling point	Depth (m)	Diatoms	Dinoflagellates	Euglenophytes	Chlorophytes	Cryptophytes	Prasinophytes	Prymnesiophytes	Cyanobacteria
P1	0.05	0.0	0.0	39.0	16.8	22.7	0.0	21.5	0.0
P2	0.05	13.6	0.0	49.4	16.6	4.2	0.0	0.0	16.2
	0.75	4.3	0.4	58.3	18.7	13.4	0.0	0.0	4.9
P3	0.05	16.5	1.5	2.5	60.6	10.7	0.0	0.0	8.1
P4	0.05	22.7	0.0	30.0	35.7	7.1	4.5	0.0	0.0
P5	0.05	26.3	1.1	35.5	5.1	9.0	0.0	0.0	22.9
	0.75	28.9	7.7	29.2	3.8	9.8	0.0	0.0	20.6
P6	0.05	18.0	6.6	59.5	6.4	3.7	0.0	0.0	5.8
	0.75	21.8	5.2	59.3	0.0	4.0	0.0	0.0	9.7
P7	0.05	10.2	7.3	66.2	0.0	2.2	1.2	0.0	12.7
	0.75	44.6	0.0	0.0	0.0	20.9	0.0	0.0	34.5
P8	0.05	1.3	6.8	19.3	4.9	2.8	0.0	33.7	31.3
	0.75	0.0	6.7	11.3	8.2	1.7	0.0	33.2	38.9

Table 4 Rank correlation matrix (Spearman's) between phytoplankton groups and environmental variables

	Diatoms	**Dinoflagellates**	**Chlorophytes**	**Cryptophytes**	**Euglenophytes**	**Prasinophytes**	**Prymnesiophytes**	**Cyanobacteria**	**Marine cyanobacteria**
Season	0.07	-0.56[a]	0.00	0.16	0.25	-0.79[a]	-0.57[a]	0.74[a]	0.92[a]
Salinity	-0.31	0.02	-0.60[a]	-0.19	-0.05	0.03	0.18	0.18	0.55[b]
DIN	0.40[b]	-0.09	0.63[a]	0.20	0.20	-0.31	-0.40[b]	0.04	0.03
DIP	0.10	0.16	0.36[b]	0.01	-0.01	0.12	-0.05	-0.31	-0.61[b]
DSi	0.38[b]	0.20	0.40[b]	0.07	0.20	-0.15	-0.28	-0.01	-0.10
DIN/DIP	0.46[a]	-0.16	0.49[a]	0.24	0.30	-0.42[b]	-0.38[b]	0.21	0.40
DSi/DIN	0.32	0.16	-0.64[a]	-0.26	-0.14	0.35[b]	0.28	-0.12	-0.09
DSi/DIP	0.45[a]	0.09	0.04	0.08	0.23	-0.25	-0.35[b]	0.27	0.36

[a] $p < 0.01$

[b] $p < 0.05$

DIN - Dissolved Inorganic Nitrogen, DIP - Dissolved Inorganic Phosphorus, DSi - Dissolved Silicate

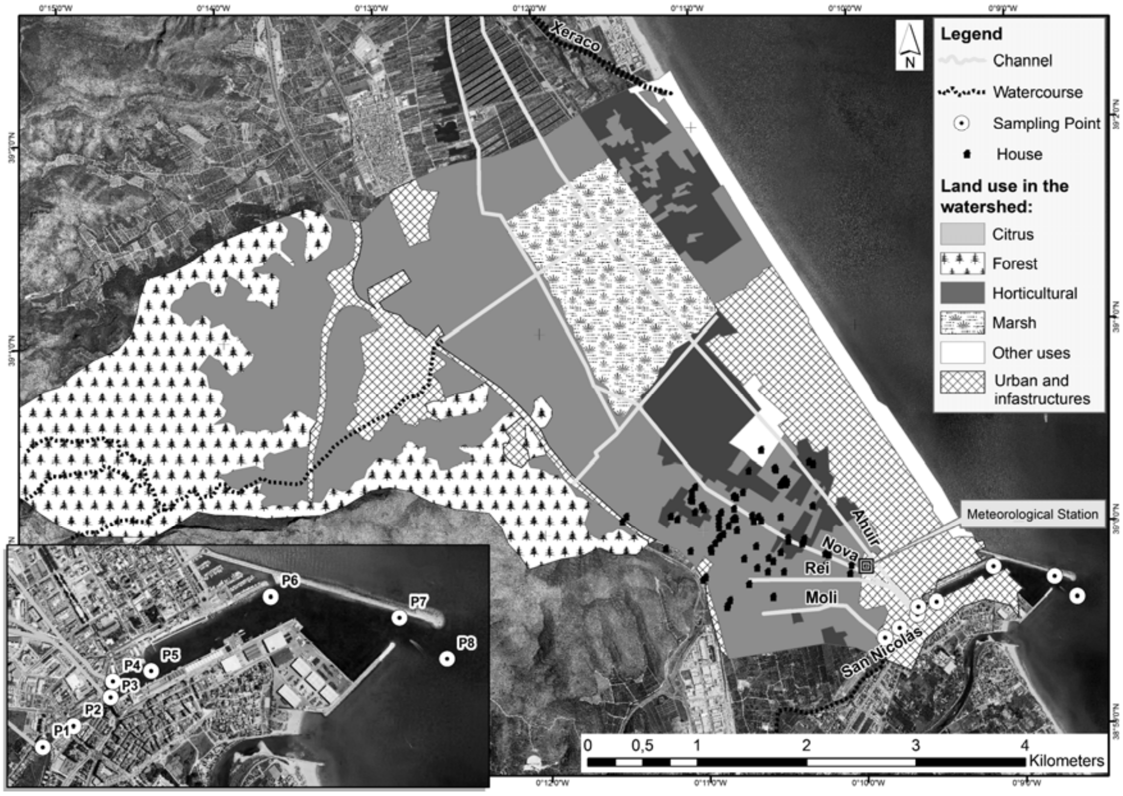

Fig. 1. The Gandia Harbour and land uses in its drainage area. Location of sampling stations: Point 1 (P1), Point 2 (P2), Point 3 (P3), Point 4 (P4), Point 5 (P5), Point 6 (P6), Point 7 (P7) and Point 8 (P8) and meteorological station. P1, P3 and P4 are located respectively in the channels of Molí, Rei and Ahuir-Nova

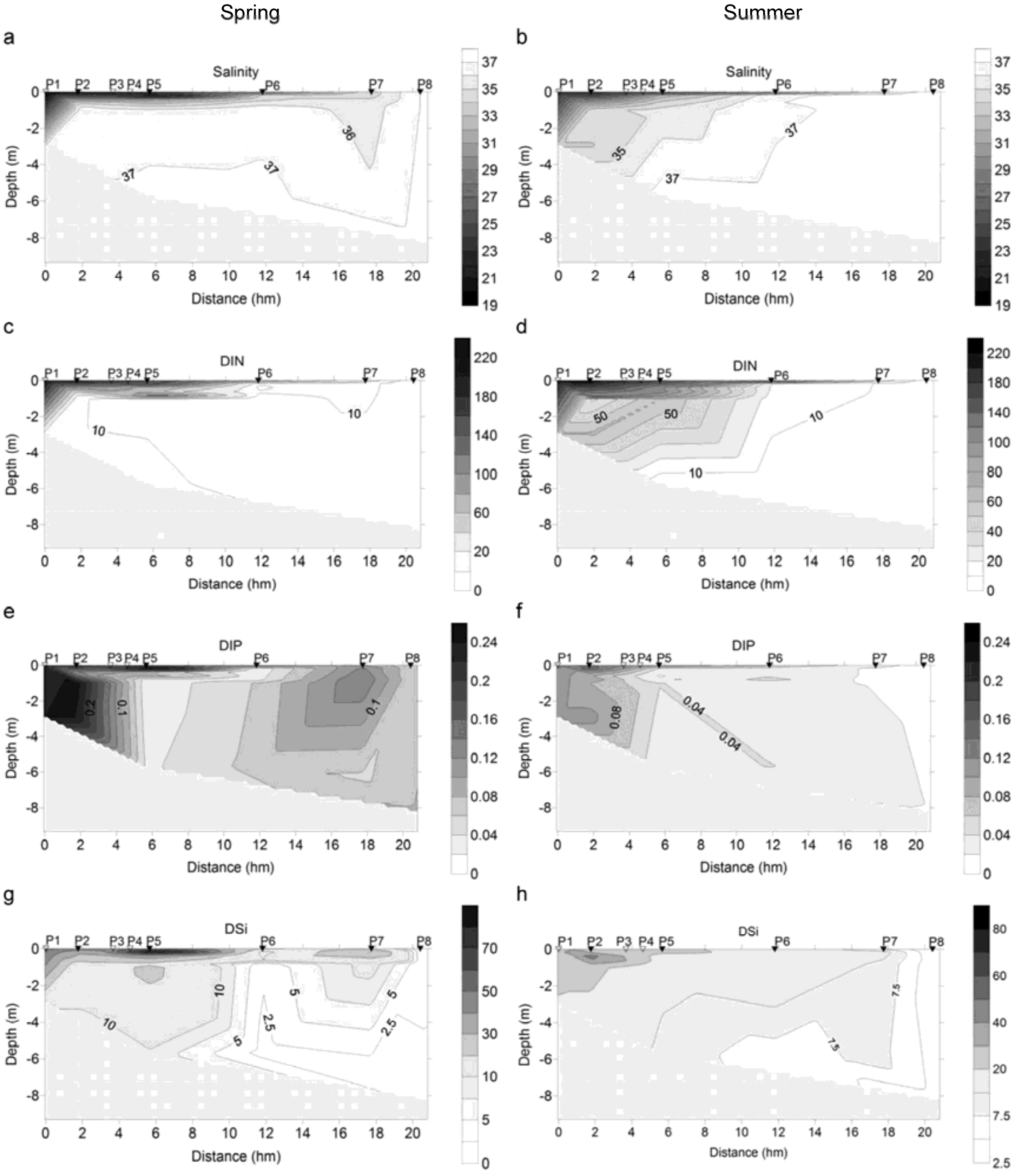

Fig. 2 Vertical profiles of salinity (PSU). DIN (μM). DIP (μM) and DSi (μM) according to a gradient of distance towards the coast. Two different periods have been distinguished: spring (left column) and summer (right column). Distance on the x-axis is scaled in hectometres from the starting point of the section: Point 1 (P1). The end point is located at the most distant station: Point 8 (P8). The black inverted triangles indicate the exact location of the sampling points and the white ones indicate the exact discharge point of irrigation channels

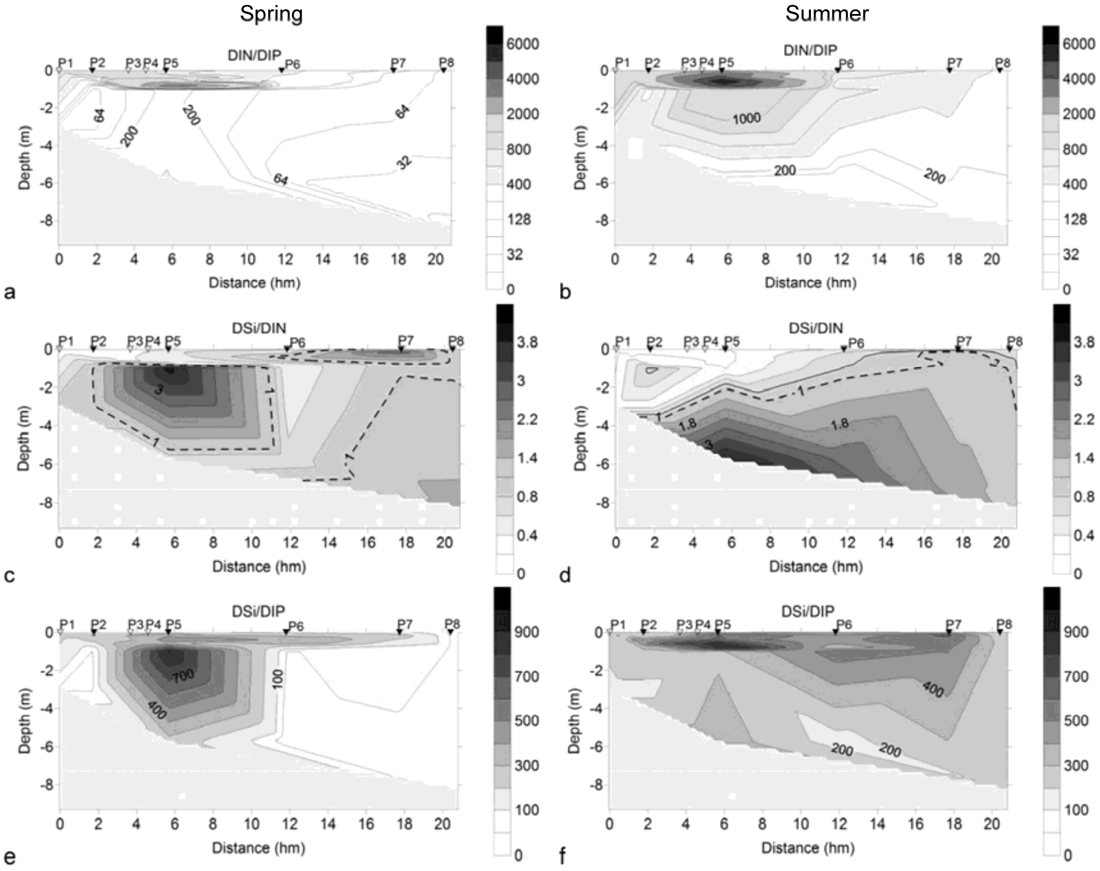

Fig. 3 Vertical profiles of DIN:DIP. DSi:DIN and DSi:DIP molar ratios. The section details are as for Fig. 2. The dashed line in figures c and d represents a 1:1 DSi:DIN molar ratio

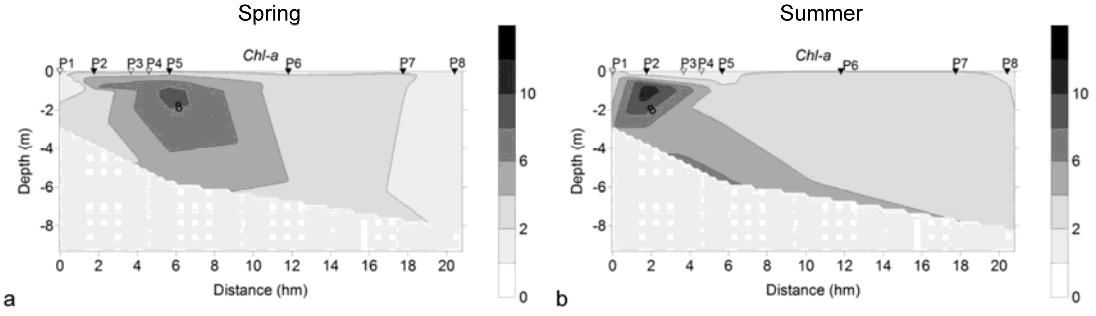

Fig. 4 Vertical profile of total Chl *a* (µg/L). The section details are as for Fig. 2