SEASONAL DYNAMICS OF THE PHYTOPLANKTON COMMUNITY IN THE GANDIA COASTAL AREA, SOUTHERN GULF OF VALENCIA

I. GADEA[1], M. RODILLA[1], J. SOSPEDRA[1], S. FALCO[1], T. MORATA[1]

(1) Institut d'Investigació per a la Gestió Integrada de Zones Costaneres (IGIC),
Universitat Politècnica de València, C/Paranimf 1, 46730 Grau de Gandia, Spain

ABSTRACT

Seasonal variability in the phytoplankton community of the coastal area of Gandia in the south of the Gulf of Valencia (Western Mediterranean Sea) was examined in relation to physical and chemical surface water variables (i.e. salinity, nutrients, dissolved oxygen and temperature). This small area presents most of the point and non-point nutrients inputs that affect coastal areas as wastewater discharges through submarine outfall, river discharges and groundwater discharges from a detritic aquifer. Furthermore, surface channels that drain the Safor wetland, which is used mainly for agricultural crops, outflow into the confined harbour. The main objective of the study was to observe the variations in phytoplankton groups as a response to environmental variables during different seasons and understand which species could be used as indicators of anthropogenic pressure. For this purpose, the taxonomic composition of the micro-phytoplankton communities at 32 fixed stations was determined in four sampling campaigns from summer 2010 to spring 2011. The results indicate that nutrient inputs mainly from the Serpis river and channels that drain the Safor Wetland determine the composition and abundance of the phytoplankton community, and that several key environmental factors such as water temperature, radiation, nutrients, and the molar ratios of nutrients influence seasonal phytoplankton assemblages. However, the discharge of effluent from a sewage treatment plant through the submarine outfall did not appear to have a significant impact on the phytoplankton community. The phytoplankton community comprised two main groups: diatoms and dinoflagellates and a total of 108 taxa were identified. The diatom population primarily flourished in autumn and winter whereas in spring, dinoflagellate bloom occurred with high radiation, very low DIP and high DIN:DIP and low DSi:DIN molar ratios. In this paper we discuss the possible rationale for these nutrient changes. Furthermore, potentially blooming species were detected in the Gandia harbour and in the mouth of the Serpis river at Venecia Beach.

Key words: Phytoplankton, nutrients, eutrophication, seasonality, Western Mediterranean.

RESUMEN (Variabilidad estacional de la comunidad de fitoplancton de las aguas superficiales de la zona costera de Gandía en el sur del Golfo de Valencia)

La variabilidad estacional de la comunidad de fitoplancton de las aguas superficiales de la zona costera de Gandía en el sur del Golfo de Valencia (Mediterráneo occidental) fue examinada, teniendo en cuenta su relación con diferentes variables físicas y químicas (p.e. salinidad, nutrientes, oxígeno disuelto, temperatura, etc.). En esta pequeña área se dan la mayoría de las entradas de nutrientes, tanto puntuales como difusas, que afectan las áreas costeras: descargas de aguas residuales mediante emisarios submarinos, aportes fluviales y descargas de aguas subterráneas procedentes de acuíferos costeros. Además, los canales superficiales que drenan el humedal de La Safor, cuyo uso fundamental es la agricultura, vierten en las aguas confinadas del puerto. El objetivo principal de este estudio fue observar las variaciones de los grupos de fitoplancton como respuesta a las variaciones ambientales en diferentes estaciones del año, y determinar que especies pueden ser utilizadas como indicadores de presión antrópica. Para ello, se determinó la composición taxonómica de la comunidad de micro-fitoplancton en 32 puntos de muestreo fijos en cuatro campañas de muestreo, desde verano de 2010 hasta primavera de 2011. Los resultados muestran que la entrada de nutrientes, principalmente a través del Río Serpis y los canales que drenan el humedal, determinan la composición y abundancia de la comunidad de fitoplancton. Además diversos factores ambientales clave como temperatura del agua, radiación, nutrientes, así como las relaciones entre nutrientes influyen en las diferentes asociaciones de fitoplancton observadas en cada estación. Por otro lado, la descarga del efluente procedente de la depuradora de aguas residuales no tuvo un impacto significativo sobre la comunidad de fitoplancton. La comunidad de fitoplancton estuvo formada principalmente por dos grupos: diatomeas y dinoflagelados. Se identificaron un total de 108 taxones. Las diatomeas proliferaron principalmente en otoño e invierno. Los dinoflagelados proliferaron en primavera, bajo condiciones de elevada radiación, baja concentración de DIP, razones DIN:DIP elevadas y razones DSi:DIN bajas. En este documento se discute la justificación de los cambios en la concentración de nutrientes. Además, se detectaron especies potencialmente nocivas en el Puerto de Gandía y en la desembocadura del río Serpis, en la playa de Venecia.

Palabras clave: Fitoplancton, nutrientes, eutrofización, estacionalidad, Mediterráneo Occidental
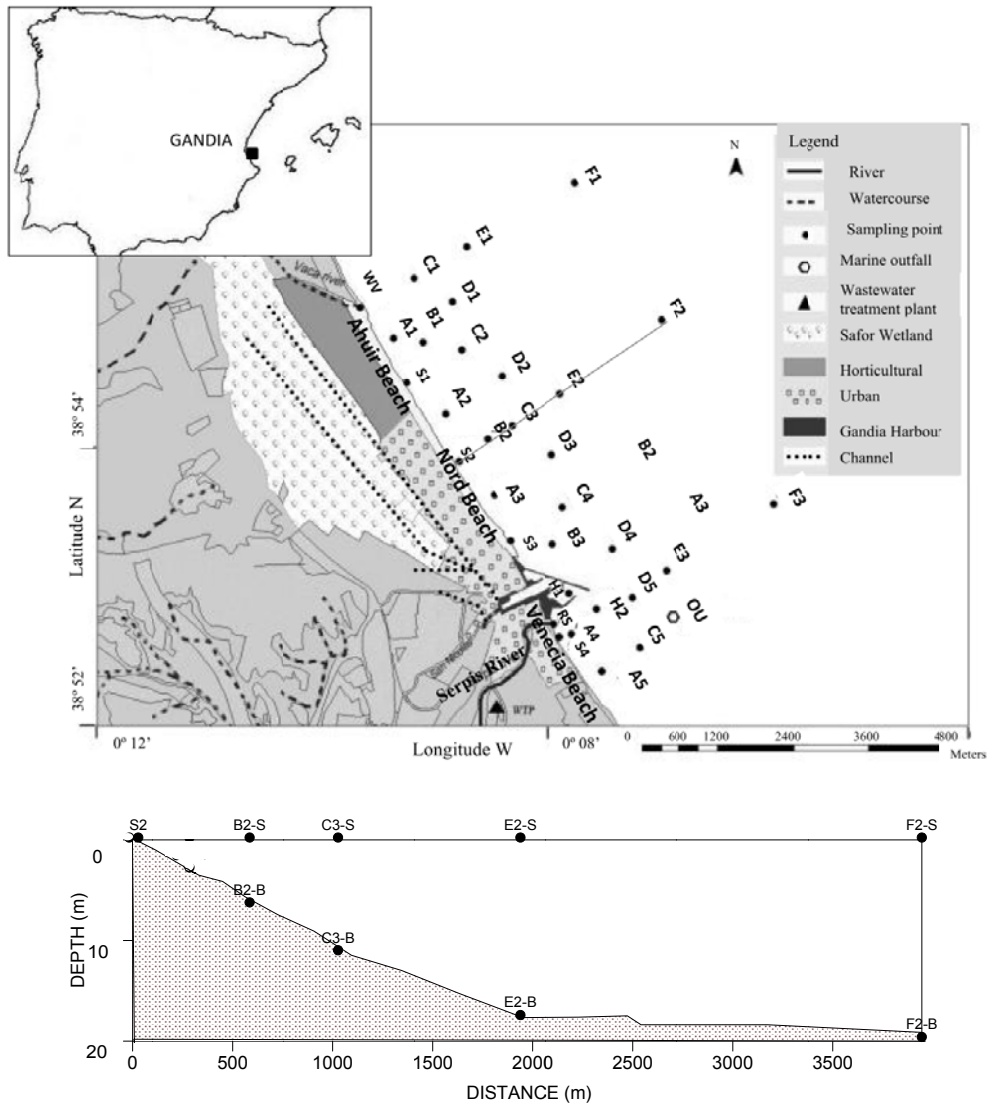
Figure 1:
Sampling site in the south of the Gulf of Valencia (Western Mediterranean Sea). Sample sites distribution through the transect.

INTRODUCTION

Phytoplankton biomass remains relatively low throughout most of the Mediterranean coastal areas (Yilmaz *et al*., 1992; Duarte *et al*., 2000; Drira *et al*., 2010). The quality of coastal waters in many regions of the world has deteriorated in recent years as human population and activities have increased along coastal regions (Newton *et al*., 2003). On the Mediterranean Sea, this human pressure is responsible for major changes in coastal ecosystems (Duarte *et al*., 1999, 2000), particularly in areas with excessive nutrient loading and/or restricted water exchange. Elevated inputs of nutrients can produce eutrophication (Newton *et al*., 2003) with its associated problems, such as harmful algal blooms and deterioration of water quality (Domingues *et al*., 2011). Phytoplankton

dynamics are the result of a complex interplay of physical, chemical and biological processes (Choudhury and Pal, 2010). In coastal and shelf waters particularly, plankton abundance and species composition are characterized by a very high degree of spatial and temporal variability (Ribera d'Alcalá *et al*., 2004), and these reflect the environmental conditions of the ecosystem, among which nutrient availability plays a significant role (Beman *et al*., 2005; Puigserver *et al*., 2010). The role of nutrients, especially nitrogen and phosphorus as limiting factors of phytoplankton, is an important aspect of any attempt to mitigate and manage eutrophication (Conley, 2000; Conley et al., 2009; Paerl, 2009). A comprehensive understanding of how nutrients affect phytoplankton growth, diversity, and production, is therefore needed to properly assess the impact of nutrient enrichment and
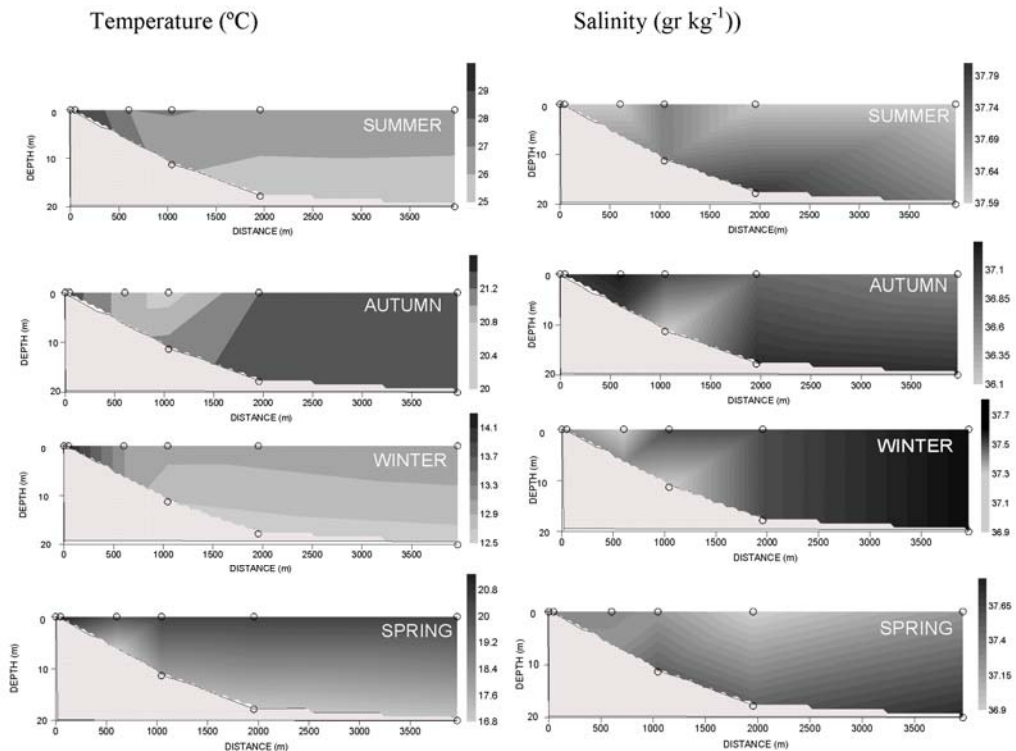
Figure 2:
Vertical profiles of temperature and salinity.

the efficiency of subsequent nutrient reduction strategies (Gobler *et al*., 2006; Domingues *et al*., 2011).

The North Western Mediterranean has a very dense human population (100 habitants/m of coastline in some regions); therefore, near-shore waters are very susceptible to anthropogenic alterations (Olivos *et al*., 2002). Siokou-Frangou *et al*., (2010) consider that the Catalan front is an area of higher nutrient availability and intense biological activity. In this area,cyanobacteria and picoeukaryotes often coexist or alternate with diatoms, dinoflagellates and other flagellates belonging to different algal groups. In the Catalan Sea, the strong seasonality creates optimum conditions for changes in the alternance of dominant groups in the phytoplankton community (Siokou-Frangou *et al.,* 2010).Spatial and seasonal variability of primary production values can be high (Granata *et al*., 2004, Siokou-Frangou *et al*., 2010).

The Gulf of Valencia, south of the Catalan Sea, has suffered from progressive eutrophication process that has been accelerated in recent decades due to the strong industrial and demographic development of coastal cities (Soler and del Río, 1995). The coast of Gandia, located in the south of Gulf of Valencia, has different freshwater inputs: pumped water from the Safor Wetland is discharged through surface channels to Gandia Harbour and to the

Vaca intermittent watercourse which outflows, directly to the sea, on the Ahuir beach (Sebastiá *et al*., 2012). The hydrology of this wetland is manipulated to satisfy the different cultivation needs and these discharges are characterized by high nitrogen loads due to intensive agriculture practices (mainly citriculture). The nitrogen in these fertilisers seeps into ground water, rivers, and streams, gradually making its way into coastal waters (Newton *et al*., 2003). An important source of nitrogen and phosphorus is the sewage plant that treats the municipal wastewater of Gandia which discharges treated wastewater into the sea through a submarine out fall at an approximate distance of 1700 m from the coastline.

An other freshwater input is the Serpis river which has a marked Mediterranean regime characterized by a dry period in summer and humid period with torrential rains, mainly at the beginning of autumn (Garófano *et al*., 2009).This river is associated with important point and diffuse pollution sources due to certain land uses and fires, respectively (CHJ, 2005; Garófano *et al*., 2011). The sewage plant of Gandia has two overflow channels that discharge directly to the river near the mouth, which happens mainly during torrential rain episodes because wastewater and pluvial waters are not separate and in summer as consequence of the increase in the population. While the Vaca watercourse outflows in a more open
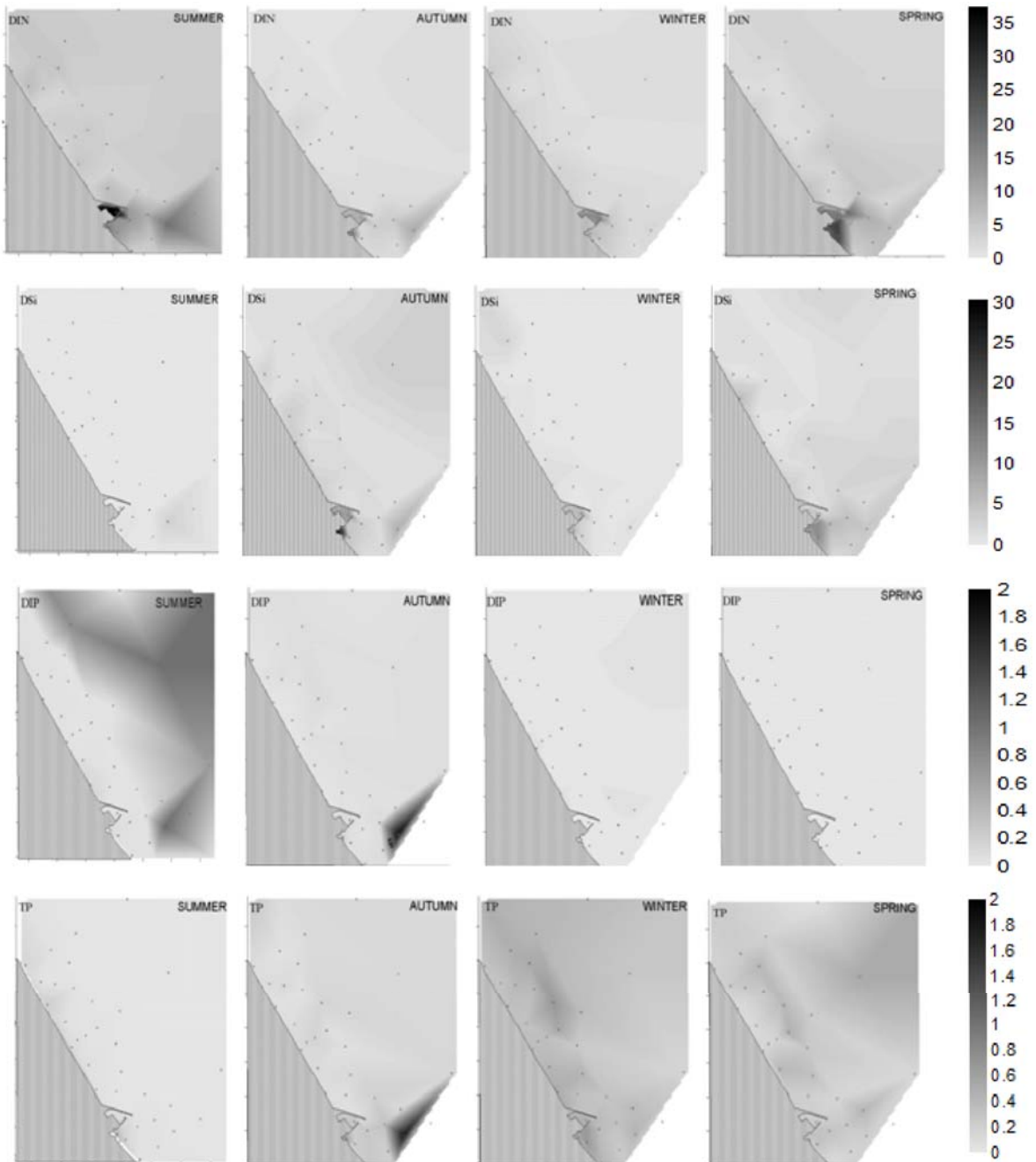
Figure 3:
Spatial and temporal variation of DIN (µmol l⁻¹), DSi (µmol l⁻¹), DIP (µmol l⁻¹) and TP (µmol l⁻¹).

area, the Serpis River outflows on Venecia Beach, on the southern side of Gandia Harbour and it is a semi-enclosed bay (enclosed by the harbour and the river mouth). Furthermore, this coastal area receives a diffuse input of freshwater and nutrients from the Plana Gandia-Denia detritic aquifer through groundwater discharges that are rich in silicates (Sebastiá *et al*., 2012).

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). Threat it faces from increased nutrient loading from anthropogenic activities may be a cause for concern. This may imply a shift from oligotrophic to eutrophic

status whose influence on the phytoplankton community is interesting to evaluate. In the south of the Gulf of Valencia (Western Mediterranean) and its coastal zone few studies have been carried out in relation to this topic: Rodrigo *et al*., (2003) studied the phytoplankton community of the Safor Wetland and Sebastiá *et al*., (2012) studied the abundance of phytoplankton groups using diagnostic photopigment analysis,the study was carried out specifically in Gandia Harbour and only in spring and summer. However, the phytoplankton community structure and dynamics in relation to the physicochemistry of the Gandia coastal area is still subject to investigations.

The main objectives of this study was to analyze the variations in the phytoplankton community as a response to physical and chemical water variables during different seasons in a coastal area with different inputs of nutrients and to understand which species could be used as indicators of anthropogenic pressure. To achieve this objective, it was necessary to study phytoplankton composition, seasonal variations of environmental variables and examine the possible relationship between the phytoplankton successional patterns and the changes in nutrient and environmental regimen. The spatial patterns of the phytoplankton community were studied using a cluster analysis and a canonical correspondence analysis (CCA) was performed to understand if there was any environmental factor that triggers the development of HABs.

MATERIAL AND METHODS

Study site, sampling and laboratory procedures

Coastal area of Gandia is located in the Western Mediterranean Sea, in the south of the Gulf of Valencia (Spain). In this study, samples were taken from 33 sampling sites (fig.1) placed in parallel lines at different distances from the shoreline: surf zone (SZ), 200 m (A); 600 m (B), 1000 m (C), 1300 m (D), 2000 m (E) and 4000 m (F).The sampling sites formed a grid whose north and south limits were the mouths of the Vaca watercourse in the Ahuir Beach (station WV) and the Serpis river in Venecia Beach (station RS), respectively. This grid included the submarine outfall of the Gandia sewage treatment plant (station OU) and Gandia Harbour (stations H1, H2), where the ephemeral San Nicolás watercourse and surface channels that drain the Safor Wetland outflow. The depth of the study area varied from 1.3 m to 20 m.

Four samplings were carried out from summer 2010 to spring 2011. Specifically, 26 August, 21 October, 23 February and 16 May, these samplings were designated as: Summer, Autumn, Winter and Spring monitoring, respectively. Samples for the physical, chemical and chlorophyll

a analysis and for the phytoplankton counts were taken at the surface layer (0.3 m depth) using a Van Dorn bottle. In order to study the vertical structure of the water column, temperature and salinity were measured in five extra samples above the bottom. These samples were taken in an orthogonal transect (fig.1).

Temperature and dissolved oxygen were measured in situ with a YSI proODO sensor and light penetration was measured with Secchi disc. Water samples were kept in a cool box (4ºC) and transported to the laboratory.

Phytoplankton quantitative samples were placed in 0.125 1 jars and fixed in situ with 20% formaldehyde solution neutralized with hexametilenentramina (Throndsen, 1978). Sub-samples (50 ml) were allowed to settle for 24h in HydroBios chambers and then counted and identified at x400 magnifications with a Leica DMIL inverted microscope (Utermohl, 1958). Counts were made following the methodology of Andersen and Throndsen (2003). This did not include the small size fraction of phytoplankton (picoplankton and some portion of the nanoplankton). Qualitative phytoplankton samples were collected by plankton nets (mesh size 50 µm). The phytoplankton community was classified to the lowest taxonomic level possible in accordance with Tomas (1997).

The following parameters were analysed in all the samples: salinity, chlorophyll-a (Chl-*a*) suspended solids (SS), nitrate, nitrite and ammonium, dissolved inorganic phosphorus (DIP), total phosphate (TP) and dissolved silicate (DSi). Dissolved inorganic nitrogen (DIN) was calculated as the sum of nitrate, nitrite and ammonium. Nutrients were analysed using the method of Aminot and Chaussepied (1983). Chl-*a* and SS were analyzed following the methodology described in APHA (2005). Salinity was determined by means of a conductivity meter Multi 340i/SET WTW.

Precipitation, environmental temperature, wind speed and direction, as well as radiation, were measured at the weather station situated in the Royal Sailing Club of Gandia located in Gandia Harbour.

Data processing and statistical analysis.

The phytoplankton community was analysed in terms of species richness and abundance, diversity (Shannon-Weaver diversity index) and evenness (Pielou) (Zar, 1984) using the PC-ORD v.4 statistical package. The cluster analysis allowed the grouping of study sites according to their similarity in phytoplankton composition. Bray-Curtis distance along with group average was used as a linkage
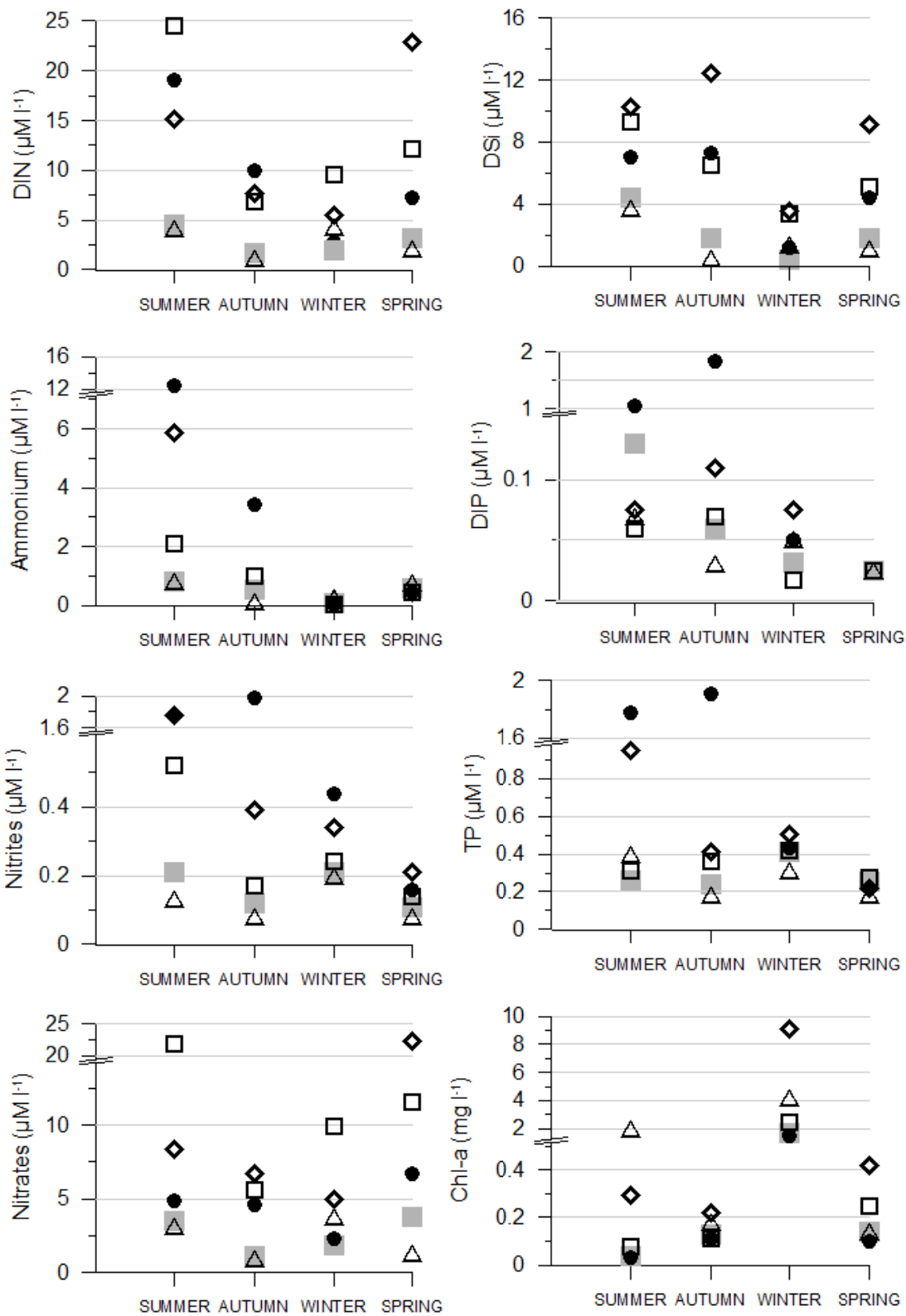
Figure 4:

Seasonal changes in the mean concentrations of nutrients, chl-a and main phytoplankton groups from two stations in the entrance of the harbour (□ H1, H2), Ahuir beach (∆ WV), submarine outfall (●OU), river Serpis and Venecia Beach (◊ RS, S4, A4) and from rest of stations (■).

method. A ranked triangular matrix of similarities was calculated for the stations using Bray-Curtis similarity matrices following log (x+1) transformations.

A non parametric one-way analysis of variance (Kruskal-Wallis) was used to test differences in physic-chemical variables and phytoplankton composition between sampling areas and seasons. This analysis was performed using STATGRAPHICS 5.1.

Phytoplankton and nutrient maps were made using the Surfer v. 8 program. The method of interpolation used was triangulation with linear interpolation.

Spearman Rank correlation (Rs) analyses were performed on environmental parameters (nutrients, molar ratios, radiation, dissolved oxygen, water transparency, and salinity) and phytoplankton groups in order to examine significant relationship. This analysis was performed using SPSS v.16.

The relationship between potentially harmful and bloom forming species and environmental parameters was determined with the multivariate ordination methods Canonical Correspondence Analysis (CCA) using the PC.ORD v4 statistical package. Bloom forming species were included as dependent variables and physicochemical variables were included as independent variables. The statistical significance of the relationships was evaluated using Monte Carlo permutation tests.

Spearman Rank correlation (Rs) analyses were performed on environmental parameters (nutrients, molar ratios, radiation, dissolved oxygen, water transparency, and salinity) and phytoplankton groups in order to examine significant relationship. This analysis was performed using SPSS v.16.

Parameters shown in the tables have been calculated using Microsoft Office Excel.

RESULTS

Physicochemical variables

1. *Temperature, dissolved oxygen, salinity and light penetration.*

Data collected during the four seasons are shown in table 1. The overall means of environmental parameters show that water temperatures followed the expected annual dynamics with winter minima (13.16 ± 0.40 ºC) and summer maxima (27.30 ± 0.87 ºC). The surf zone, in all seasons, registered significantly higher temperatures

($p < 0.05$) than the other areas. In summer a thermal stratification of the water column was observed (fig. 2), in surface samples the temperature was about 28 ºC while at the bottom,the temperature was 25 ºC. Salinity exhibited seasonal fluctuations and reached maximum values (37.37 ± 0.69 g kg^{-1}; 37.29 ± 0.33 g kg^{-1}) in the dry seasons (summer and winter, respectively) whereas the lowest values (36.66 ± 1.63g kg^{-1}; 36.75 ± 1.36 g kg^{-1}) were measured in the rainy seasons (autumn and spring). Minimum values were observed at the mouth of the Serpis river, the lowest values being registered in the rainy seasons. In the submarine outfall plume, the lowest values were observed in summer and autumn (36.6 g kg^{-1}, 35.9 g kg^{-1}, respectively). Freshwater discharges from the aquifer were observed mainly in autumn (36.1 g kg^{-1} in the swash area) (fig.2).Oxygen concentration reached maximum values in winter (9.65 ± 0.22 mg l^{-1}) and minimum values is summer (7.00 ± 0.14 mg l^{-1}). In all monitoring campaigns there were significant differences ($p < 0.05$) between the surf zone and the rest of the sample points. In summer, winter and spring, the surf zone registered lower concentrations but in autumn, oxygen in this zone was higher. The average light penetration (7.58 ± 5.22 m) in summer was significantly higher ($p < 0.05$) than in the other seasons. In all seasons light penetration increased from the shoreline to stations located 4000 m away. Minimum pH was (7.91 ± 0.06) in summer, while the highest value of pH (8.20 ± 0.03) was measured in spring.

2. *Nutrients concentrations and nutrient molar ratios*

Seasonal and spatial variation of nutrient concentrations is shown in figures 3 and 4. Nitrate was the most dominant nitrogen form in all seasons. The highest values of ammonium were observed at the submarine outfall area and at the mouth of the river Serpis in summer. In this season maximum values of nitrites were registered at the submarine outfall and the mouth of the river too. The highest values of DIN were observed in summer (6.05 ± 6.59 µmol l^{-1}) and spring (5.79 ± 6.24 µmol l^{-1}), and were, in fact, significantly higher ($p < 0.05$) than the values registered in autumn and winter. In the study area, three zones presented peaks of DIN: Gandia Harbour showed 36.52 µmol l^{-1} in summer, the submarine outfall plume reached 19.07 µmol l^{-1} in summer too, and the mouth of the river Serpis presented 17.02 µmol l^{-1} , 17.26 µmol l^{-1} and 21.54 µmol l^{-1} in summer, autumn and spring, respectively. In the same areas, the highest values of DSi were measured during the study period. Maximum values were observe dat the mouth of the Serpis river in autumn and spring (28.11 µmol l^{-1} and 12.08 µmol l^{-1}, respectively) and in the submarine outfall plume in autumn (7.34 µmol l^{-1}). In general, low DIP concentrations were observed throughout whole study, in fact, concentrations in the spring samples were undetectable.

Summer

0.2%

99%

Autumn

0.3%   1%
4.5%
2%   0.05%

93%

Winter

0.1%   1.5%
5.5 %
0.1%
3.5%

89%

Spring

2.8%   2.2%
0%   0.45%

48%   46%

▨ Diatoms   ■ Dinoflagellates   ▣ Raphydophytes
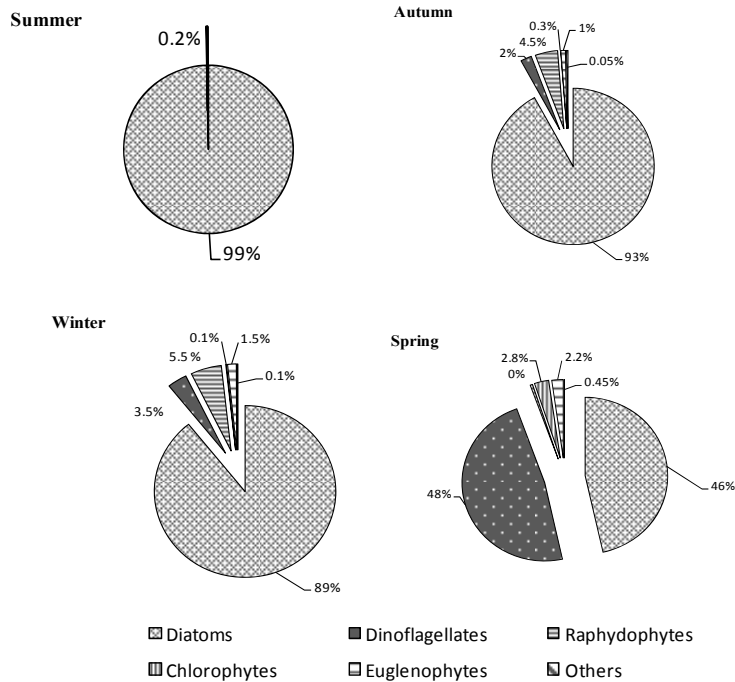
▥ Chlorophytes   ▢ Euglenophytes   ◩ Others

Figure 5:
Contribution of different phytoplankton groups to abundance in each season.

The highest DIP concentrations were measured in summer (0.16 ± 0.28 μmol l^{-1}). The submarine outfall area reached maximum values, 1.06 μmol l^{-1}, in summer and 1.84 μmol l^{-1},in autumn. Winter showed significantly higher ($p<0.05$) TP values, however maximum concentrations were registered at the submarine outfall plume in summer and autumn (1.78 μmol l^{-1} and 1.91 μmol l^{-1}, respectively).

The criteria for stoichiometric nutrient limitation were the same as those used by Dotch and Whitledge (1992), Justic *et al*., (1995) and Olivos *et al*., (2002) to identify a nutrient-limited environment for elements based on nutrient uptake kinetics: P is limiting if DSi: PO$_4$>22 and DIN: PO$_4$>22; N is limiting if DSi: DIN>1 and DIN: PO$_4$<10; and Si is limiting if DSi: PO$_4$<10 and DSi: DIN<1. In general, phosphorus acted to a great extent as the potential limiting nutrient (table 2) and silica acted as the limiting nutrient on the submarine outfall plume in summer and autumn and in more than 30% of the cases in winter. Nitrogen as a limiting nutrient acted only in two samples located 4000m from the coast, in summer. In autumn,33.3 % of cases did not present limiting nutrient (table 2).

3. *Total chlorophyll a*

Chl-*a* concentration showed significantly higher values ($p<0.05$) in winter (2.55 ± 2.54 μg l^{-1}) and lower in summer (0.03 ± 0.08 μg l^{-1}) In winter, the highest values

were observed mainly at the surf zone and around the first 100 meters from the coastline; the maximum concentration measured was 11.71 μg l^{-1} in the Serpis estuary. In this season, the lowest values were register at the plume of the outfall. In the other seasons, the highest values of Chl-*a* were observed on the mouth of the Serpis river too, but the concentrations were always lower than those found in the winter monitoring.

Phytoplankton community structure

A total of 108 different taxa were identified in this study in different seasons (table 3a;3b), Dinophyceae (45%), Diatoms (39%), Dictyochophytes (3%), Chloro-phytes (3%), Euglenophytes (2%), Haptophytes (2%), Raphydophytes (2%), Cryptophytes (2%) and Chrysophytes (2%). In terms of species richness, dinoflagellates and diatoms were the most important groups. Dinoflagellates were the largest group with respect to the total number of taxa identified (49 species were identified) which belonged to 24 different genera. The most representative dinoflagellate genera were *Protoperidinium* and *Ceratium* with 11 and 10 species respectively. The diatom species identified belonged to 36 genera. The most representative diatom genus was *Chaetoceros* with 7 species. Only 32 taxa had a frequency higher than 10% of the samples. Most of these taxa were diatoms, followed by dinoflagellates and raphydophytes.
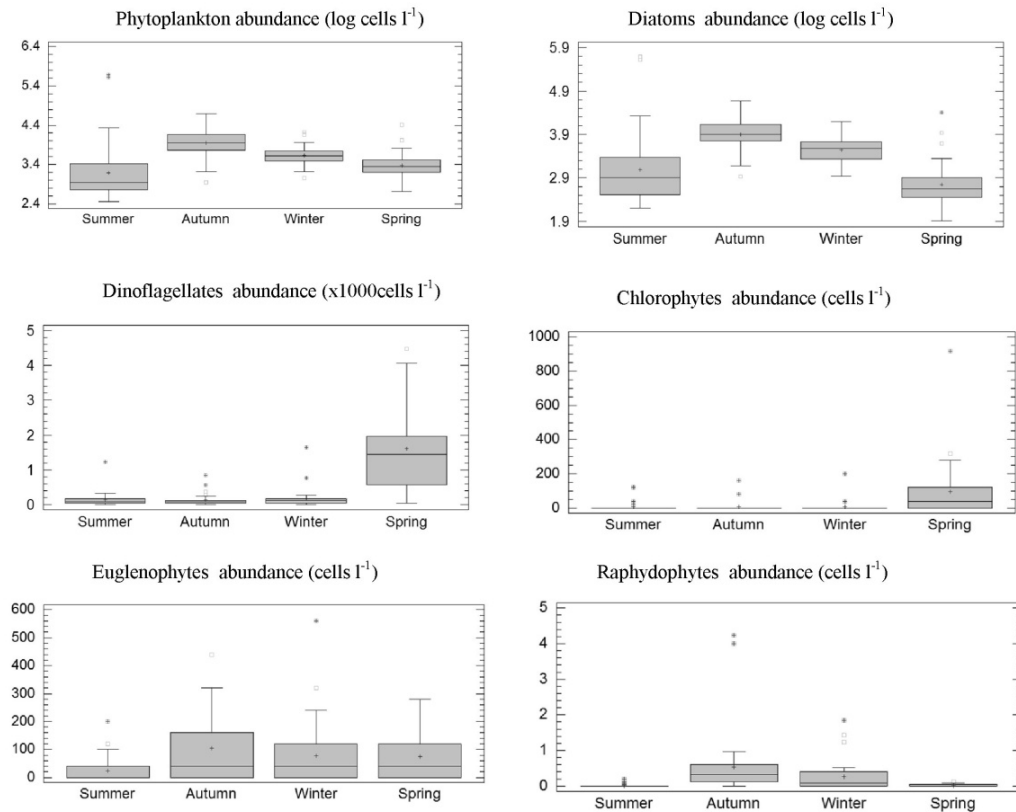
Figure 6:
Box-whiskers diagram of phytoplankton groups.

The most dominant group was diatoms in terms of abundance,and the most dominant species were the diatoms *Bacillaria paxillifera* and *Pseudonitzschia spp* (>500 x 10³ cells l⁻¹ in total phytoplankton). The most dominant in frequency were *Proboscia alata, Pseudonitzschia spp* (72 % and 56 % occurrence respectively), *Cylindrotheca closterium* and *Chaetoceros affinis* appeared in more than 50% of the samples. In contrast, *Asterionellopsis glacialis* had a low frequency (29% occurrence) but contributed in an important way to the density of total phytoplankton (>200 x 10³ cells l⁻¹ in total phytoplankton). Dinoflagellates were not dominant in terms of abundance; the most abundance genus was *Heterocapsa* having 21 x 10³ cells l⁻¹ in total phytoplankton, although it was not the most dominant in terms of frequency (25% of occurrence). The most frequently occurring dinoflagellates were the genera *Gymnodinium* and *S crippsiella* (53% and 44% respectively). Raphydophytes showing a 53% occurrence, but did not contribute greatly to the abundance of total phytoplankton. Finally, euglenophytes appeared frequently during the study (>50 % occurrence) but had a total cell abundance lower than 10 x 10³ cells l⁻¹.

The study of the phytoplankton community in the areas with higher human pressure (table 4) showed that at

Venecia Beach and mainly, at the entrance of the harbour, the diatoms *Guinardia delicatula* and *Pseudo-nitzschia spp* registered the highest values. The dinoflagellates of the genera *Scrippsiella* appeared all over the area, except at the submarine outfall, and they registered the highest values at the entrance of the harbour. The euglenophyte *Eutreptiella gimnastica* only appeared at the entrance of the harbour and Venecia Beach. *Odontella mobiliensis* was only observed in the mouth of the rivers, mainly in the Serpis river and the genera *Chatonella* appeared mostly at the submarine outfall and in the mouth of the Serpis river. On the other hand, *Octactis octonaria*, *Gossleriella tropica* and *Noctilluca scintillans* were only observed at the station control (F1) located at 4000 m from the shoreline.

1. *Seasonal dynamics of phytoplankton community*

In general,the highest values of cell abundance were registered in autumn (fig.6) with median abundance of around 9 x 10³ cells l⁻¹. Generally the lowest values of cell abundance were registered in summer and spring with a median of 880 cells l⁻¹ and 2.240 cells l⁻¹, respectively. On the other hand, the highest cell abundance value was registered in summer, specifically in the southern half of the Serpis estuary, with 492.620 cells l⁻¹. In general,
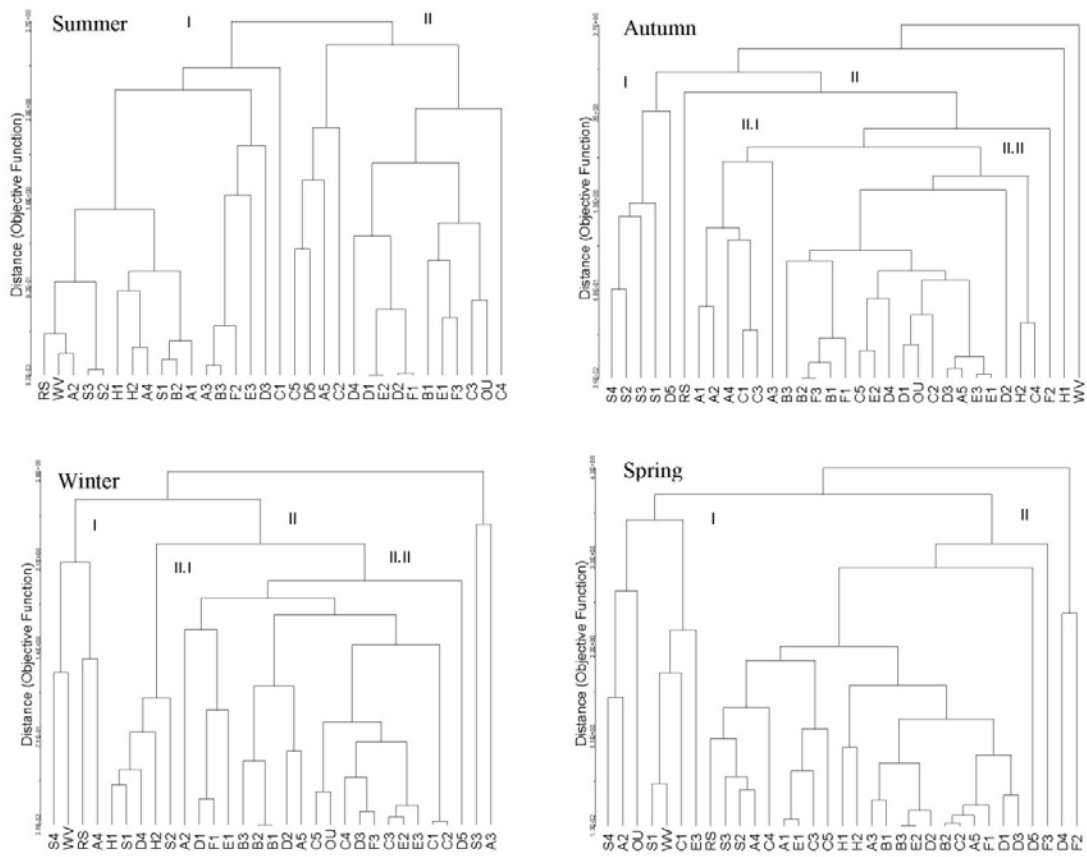
Figure 7:
Dendrogram for the hierarchical clustering of the 33 stations using group-average linking of Bray-Curtis similarities calculated on log-transformed abundance data.

the temporal distribution of cell abundance coincided with temporal distribution of diatoms, which indicates that this group was responsible for the variability of cell concentration. In fact, there was a strong significant correlation (R^2=0.90 p<0.01) between diatom abundance and total abundance of cells. There was a high variability in cell abundance when the temporal distribution of main groups of phytoplankton was examined. Generally, diatoms registered the highest values in autumn (fig. 6). Dinoflagellates and chlorophytes abundance in spring was significantly (p<0.05) higher than the other seasons. Euglenophytes cell density was significantly (p<0.05) lower in summer and raphydophytes were more abundant in autumn and winter than in spring and summer.

In summer (fig.5), the plankton flora was represented mainly by diatoms which represented 99% of cell abundance. The most dominant taxa in terms of abundance were *Bacillaria paxillifera*, *Pseudo-nitzschia spp*. and *Guinardia delicatula*and in terms of frequency, *Bacillaria paxillifera*, *Proboscia alata* and *Rhizosolenia sp*. The genera G*ymnodinium* was the most abundant dinoflagellate in this season. In autumn, the most dominant group

was diatoms (93%) followed by raphydophytes (4.5%) and dinoflaglellates (2%) (fig. 5). The most frequently occurring species were *Thalassionema nitzschioides*, *Asterionellopsis glacialis* and *Pseudo-nitzschia spp*., and these contributed greatly to the density of total phytoplankton. The most frequent and abundant genera of dinoflaglellates were *Scrippsiella* and *Gymnodinium*. The percentage of diatoms decreased in winter (89%) while the percentage of raphydophytes, dinoflagellates and euglenophytes increased. The most abundant and frequent species was *Chaetoceros affinis*,while the most abundant dinoflagellate genus was *Scrippsiella* and the most frequent was *Gymnodinium*. The relative abundances of different groups were significantly different in spring compared to other seasons, the group with the highest relative abundance was the dinoflagellates with 48%; diatoms accounted for 46%, and chlorophytes and euglenophytes 2.8% and 2.2%, respectively. The most abundant species was the diatom *Bacillaria paxillifera* but the most frequently occurring species were the dinoflagellates of the genus *Gymnodinium* followed by the genera *Heterocapsa* and *Scrippsiella*. Spring monitoring had significantly (p<0.05) higher values of diversity (2.03

$\pm$ 0.44 bits individuals-1) and evenness (0.43 $\pm$ 0.09) than the other seasons. In the case of species number, the highest values were registered in autumn and spring (14.12 $\pm$ 4.00 individuals; 14.68 $\pm$ 4.31 individuals, respectively).

2. *Spatial patterns of phytoplankton community.*

The hierarchical cluster showed that different spatial patterns existed according to the season (fig.7 and fig. 8)). In summer, there were significant differences (p<0.05) in the phytoplankton community among stations located nearer the coast up to 1000 m from the shoreline (Group I,except A5) compared to stations more than 1000 m from the shoreline (Group II). Group I presented significantly higher values of abundance, richness, diversity and evenness. In terms of density two zones registered a significantly higher (p<0.05) cell abundance within Group I:Gandia Harbour (H1) 419 x 10^3 cellsl^{-1} and the Serpis mouth (RS y A4) 19770 $\pm$ 1930 cellsl^{-1}. Within Group II, the area south of the breakwater (C5, D5) and in the south of the rivermouth (A5) represented a further subdivision due to their higher values of cell abundance, mainly in A5 (492 x 10^3 cells l^{-1}) and the presence of the genus *Pseudo nitzschia*.

In autumn (fig.8), the surf area (group I) showed greaterdiversity and evenness furthermore dinoflagellates density was significantly higher (p<0.05). The surf area had a lower density (5172 $\pm$ 1068 cells l^{-1}) than the other stations (12293 $\pm$ 6895 cells l^{-1}). Chlorophytes appeared mainly in the mouth of the Serpis river (160 cellsl^{-1}). The mouth of the Vaca watercourse had greater densities of diatoms (47720 cells l^{-1}) and dinoflagellates (840 cells l^{-1}) than the rest of the study area. Dinoflagellates appeared mainly in and around the harbour area and at Ahuir Beach (fig.9).

Winter monitoring showed two different groups (fig.8).The mouth of the Vaca and the Serpis river and Venecia Beach (group I) showed significantly higher values of diversity and evenness; on the other hand, these areas registered lower cell abundances (2500 $\pm$ 551 cell.l^{-1}) than the rest of the study area (group II) (5390 $\pm$ 3270 cells l^{-1}) Diatom and raphydophytedensity was significantly higher (p<0.05) in Group II than in Group I and euglenophytes and chlorophytes groups were more abundant in Group I than in group II. Dinoflagellates appeared mainly at the entrance of the harbour and in the southern part of the mouth of the Serpis river (group II.I) (fig.9).

In spring (fig. 8), it was difficult to establish a pattern of spatial distribution; the hierarchical cluster showed two groups with significant differences (p<0.05) in terms of density but not in terms of richness, diversity and evenness. The Ahuir Beach and the submarine outfall (group

I) registered lower cells abundance (1114 $\pm$ 400 cells l^{-1}) than the rest of the area (group II) (3319 $\pm$ 1942 cells l^{-1}), as a result of the lower concentrations of dinoflagellates observed in group I. The highest abundances of chlorophytes and dinoflagellates were observed at the entrance of the harbour (fig.9).

3. *Potentially harmful and bloom forming species.*

Thirty-one identified taxa could potentially lead to harmful effects (e.g. toxic, fish-killing, or bloom forming). These taxa and their characteristics are shown in table 5. Among them, 12 species are included in the Intergovernmental Oceanographic Commision (IOC) Taxonomic Reference List of Harmful Micro Algae 2009; specifically, 8 dinoflagellates, 2 diatoms, 1 euglenophyte and 1 haptophyte. Higher cell abundance and recurrence of dinoflagellates (*Alexandrium sp.*, *Gymnodinium spp.* *Heterocapsa spp.* *Scrippsiella spp.*, *Noctilluca scintillans*, *Prorocentrum micans* and *Prorocentrum triestinum*) were observed in spring. Among them, the genera *Heterocapsa* and *Scrippsiella* were the most abundant, reaching concentrations as high as 2 x 10^6 cells l^{-1} in the harbour, but the most frequent species were the dinoflagellates of the genus *Gymnodinium*.

Generally harmful diatoms registered their highest abundance and occurrence in autumn and winter *Pseudonitzschia spp.* presented the highest percentage of occurrence (91.7%) in autumn but reached the highest concentration in summer (>400 x 10^3 cells l^{-1}). The largest densities of this diatom were always observed in the surroundings of the harbour. A clear temporal pattern of the haptophyte of genus *Phaeocystis.* was not found from this data.

The results of the Canonical Correspondence Analysis (CCA) are displayed in figure 10. The CCA analysis was performed with the most abundant potentially harmful and bloom-forming species detected. The environmental variables (temperature, radiation, precipitation, light penetration, nitrites, nitrates and dissolved oxygen) explained 31 % of the variance. Potentially harmful and bloom forming species can be divided into two main groups: the first group comprising potentially harmful diatoms and the second comprising potentially harmful dinoflagellates. Abundance of potentially harmful diatoms was higher in waters with a high nitrate concentration and was inversely correlated with radiation and temperature. Among the potentially harmful diatoms, *Pseudo-nitzschia spp* showed a positive correlation with a high nitrite concentration. Potentially harmful dinoflagellate abundance was higher at high radiation and was positively correlated with dissolved oxygen and precipitations events before the monitoring. *Eutreptiella gimnastica* was positive cor-
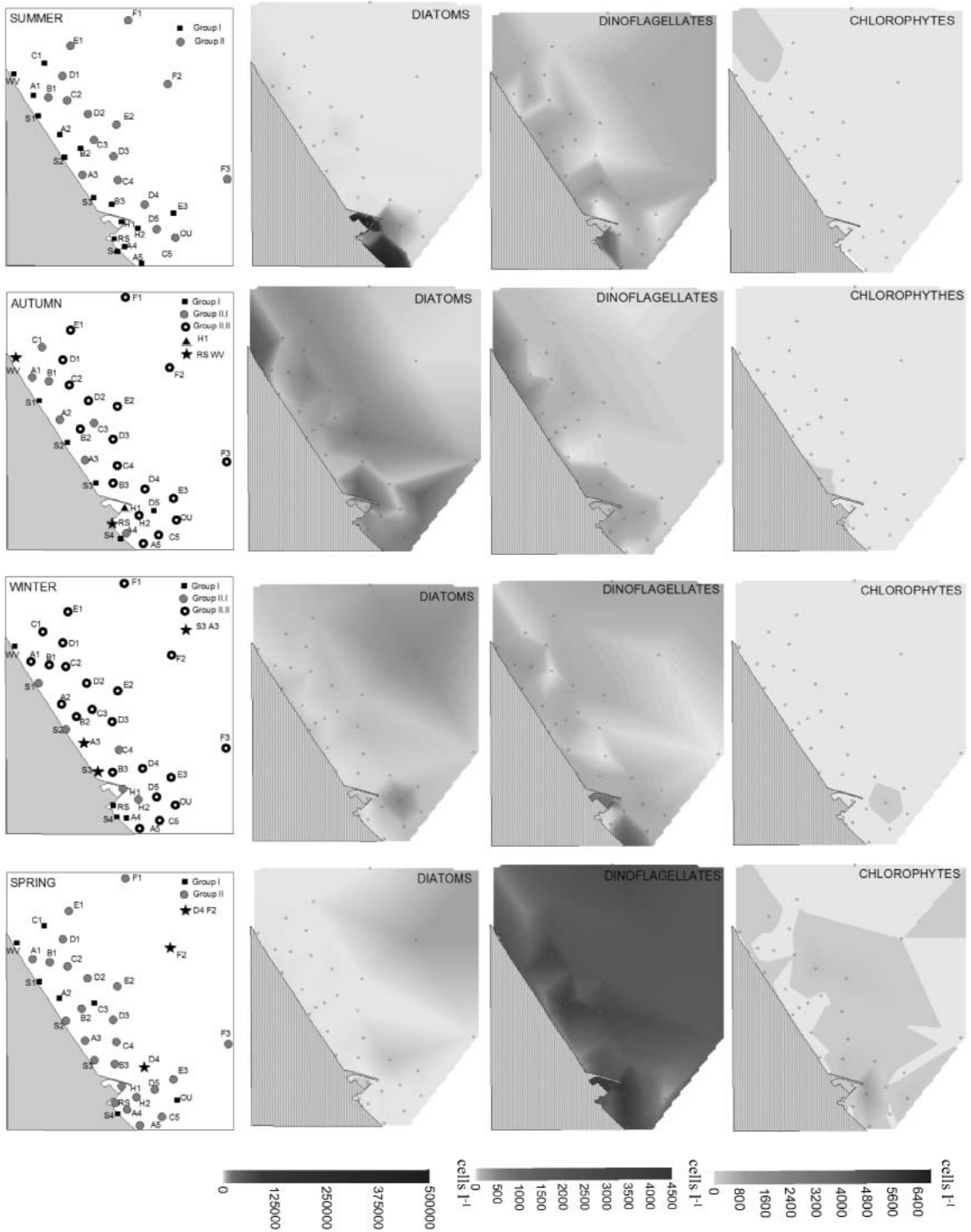
Figure 8:
Distribution of the main phytoplankton groups identified by the cluster analysis.

related with precipitation event before monitoring and dissolved oxygen. *Guinardia delicatula* was the most opportunistic species and *Prorocentrum minimum* and *Prorocentrum lima* had no clear pattern.

Relations between abiotic parameters and phytoplankton community.

Spearman Rank correlation analyses were performed on environmental parameters (water temperature, nutrients, radiation, water transparency, dissolved oxygen and salinity) and phytoplankton groups in order to examine significant relationships (table 6). The assessment of the correlation was based on the scale defined by Torres (2004). Diatoms group showed significantly weak positive correlations with Si and DIP and with DSi:DIN molar ratio, and they had moderately negative correlations with solar radiation and water temperature. Dinoflagellates presented a moderately positive correlation with solar radiation and weak correlation with DIN:DIP and DSi:DIP molar ratios. On the other hand, they showed a weak negative correlation with DIP and DSi:DIN molar ratio. Chlorophytes were weakly inversely correlated with salinity, DIP and light penetration and presented a weak positive correlation with solar radiation, DIN, DSi and DIN:DIP and DSi:DIP molar ratios. Euglenophytes showed weakly positive correlations with DSi and DSi:DIN and DSi:DIP molar ratios and they were weak inversely correlated with salinity and light penetration. Raphydophytes showed negative correlation with solar radiation and weak with water temperature.

Phytoplankton cell abundance had a weak positive correlation with Si and DSi:DIN molar ratio, and this was weakly inversely correlated with water temperature, radiation and salinity. Finally, Chl-*a* showed a moderately positive correlation with dissolved oxygen and weak correlation with TP and DSi:DIP molar ratio and a weak negative correlation with light penetration.

DISCUSSION

The results showed that the coastal area of Gandia is characterized by a high temporal and spatial variability of physicochemical parameters. These changes were particularly striking during the rainy seasons (autumn and spring), which can be explained mainly by the contribution of the Serpis river, surface channels that drain the Safor Wetland and the submarine outfall discharges.

Salinity exhibited seasonal fluctuations (fig. 2) and reached maximum values in the dry seasons (summer and winter) whereas the lowest values were measured in the rainy seasons (autumn and spring). Minimum values appeared at the mouth of the Serpis River, mainly in the

rainy seasons due to the increase in the flow caused by precipitation. Minimum values were also recorded at the submarine outfall plume in summer and autumn. This was a consequence of the increased flow in summer due to the population increase because it is a popular tourist destination, and in autumn because rain water arrives at the sewage treatment plant via the sewer system and then to the sea through the submarine outfall.

On the Gandia coast, during the study period, nutrient concentrations (0.80- 36.52 µM for DIN, 0.03 - 28.11 µM for DSi, <0.01-0.32 µM for DIP) were below those of typical nutrient-enriched areas such as, San Francisco Bay (Cloern, 1996) and Ria Formosa (Newton *et al*., 2003; Brito *et al*., 2010) and Arenys de Mar harbour (Vila *et al*., 2005); and slightly higher than those observed in the Catalan Sea by Olivos *et al*., 2002. Among the three nutrients, DIP 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; Sebastiá *et al*., 2012) and slightly lower than those recorded by Olivos *et al*., (2002).

The highest values of DIN and DSi were observed at the mouth of the Serpis and in Gandia harbour (fig.4), mostly in the rainy seasons when, as pointed out by Sebastiá *et al*. (2012) precipitation events lead to terrestrial runoff and increased river flow and also when the water from Plana Gandia-Denia aquifer, rich in nitrates and DSi, is pumped through the irrigation channels to the harbour to decrease the phreatic level and prevent crop root asphyxia. Thus, the major sources of DIN and DSi in this coastal area, are probably from the Serpis river and the channels that drain the Safor Wetland. In addition to the previous inputs, a diffuse input of freshwater and nutrients is possible, in the swash area, from the Plana Gandia-Denia detritic aquifer through groundwater discharges rich in silica and nitrates (Sebatiá *et al*., 2012), mainly in autumn (fig.2).According to Ballesteros-Navarro (2003), the Ahuir Beach, which is located at the end of Gandia's urban area, receives a discharge from the Plana Gandia-Denia detritic aquifer, quantified at 66 Hm^3 year^{-1} (2.1 m^{3}s^{-1}). Due to continued agricultural practices, nitrate levels in the detritic aquifer have exceeded the limit of 50 mg l^{-1} established by the Nitrates Directive (Directive 91/676/EEC). On the other hand, wetland species of Graminae are characterised by high silica content (typically 10-15% dry shoot weight). This biogenic silica, after decomposition of organic material, remains in the soil and it is lixiviated to the aquifer (Conley 2002). Although, the natural vegetation surface has decreased recent decades, it still remains an important soil use. Thus, biogenic silica is an important element in the terrestrial biogeochemical cycle, and must be taken
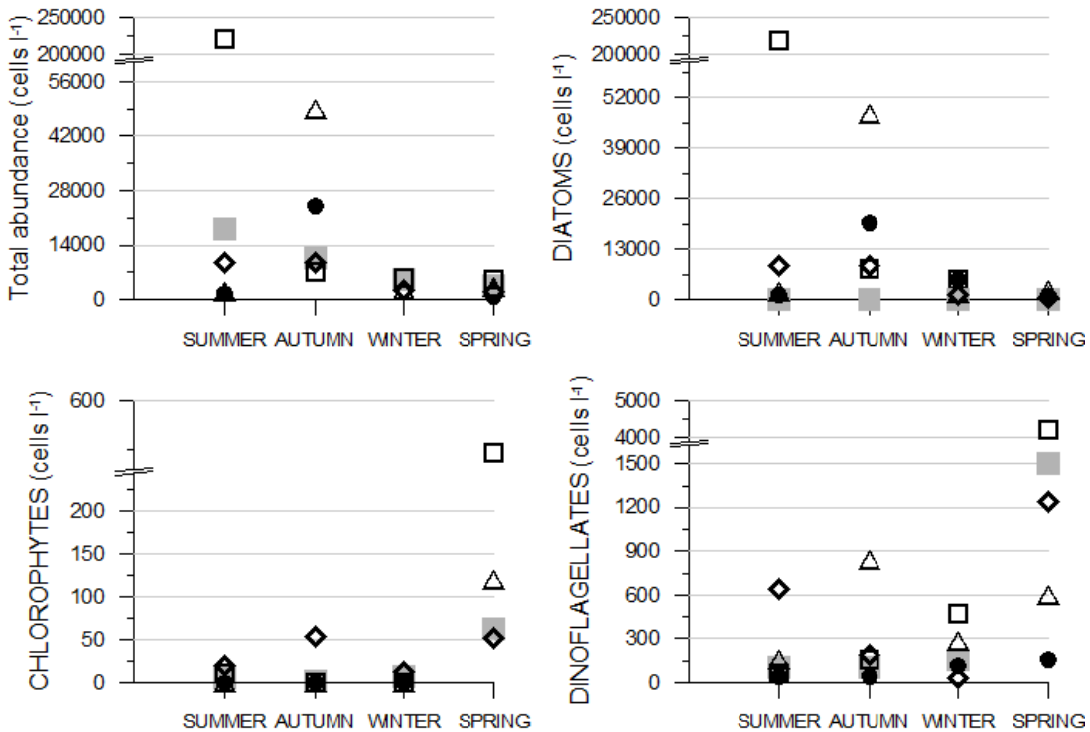
Figure 9:
*Seasonal changes in the main phytoplankton groups from two stations in the entrance of the harbour (□ H1, H2), Ahuir beach (△ WV),
submarine outfall (● OU), river Serpis and Venecia Beach (◊ RS, S4, A4) and from rest of stations (■).*

into account in addition to the chemical weathering of land silicates. Unlike DIN and DSi, in our study the supply of DIP by these sources was not particularly apparent. The submarine outfall is another important source of nutrients in this area, probably the main source of DIP, because the sewage plant of Gandia discharges into the sea through this. The higher values of DIN and DIP observed in summer (fig.4) could be a consequence of the increase in population, which generates a higher flow of sewage in the submarine outfall. Furthermore, the sewage plant, which is located next to the river, was unable to treat all wastewater received and discharged the excess directly into the Serpis river. Moreover, the rainwater that was collected by the collector system in the days before autumn monitoring was also brought to the treatment plant because wastewater and pluvial water are not separated and it was discharged into the sea through the submarine outfall. This fact could explain the high levels of silicate in the outfall plume in autumn because anthropogenic nutrient enrichment usually increases N and P, but not Si (Domingues *et al.*, 2011).

Generally, nitrogen is considered limiting in marine systems (Ryther and Dunstan, 1971) and phosphorus in freshwaters (Schindler, 1977), but these two deeply rooted dogmas have been questioned (Sterner, 2008;

Domingues *et al.*, 2011). The Mediterranean Sea has usually been considered a P-limited basin although a review of the published works on this matter shows that there are important discrepancies (Domingues *et al.*, 2011). Some authors have reported that N-limitation is more probable than P-limitation in areas of the Western Mediterranean (Owens *et al.*, 1989; Denis. Karafistan *et al.*, 1998; Olivos *et al.*, 2002). However, in coastal areas of the Catalan Sea (Olivos *et al.*, 2002) Si acted as the main limiting nutrient. In our study area, as regards nutrient limitations for phytoplankton, phosphorus appeared to be the only potential limiting nutrient in all seasons except during the dry winter which favoured potential Si-limitation

Chl-*a* concentration observed in the study area (0.02-11.71 µg l^{-1}) was similar to that observed by Olivos (2002) in the Catalan sea. In the Mediterranean Sea, phytoplankton biomass, as Chl-*a*, generally displays low values (less than 0.2 µg l^{-1}) over large areas, with a modest late winter increase (up to 3 µg l^{-1}) (Siokou-Frangou, 2010). In our study, the highest values were observed in winter monitoring and minimum values were registered in summer and autumn. A strong Chl-*a* signal in late winter-spring and summer-autumn minima have been detected in many areas of the Mediterranean sea (e.g., Cruzado and Velásquez,
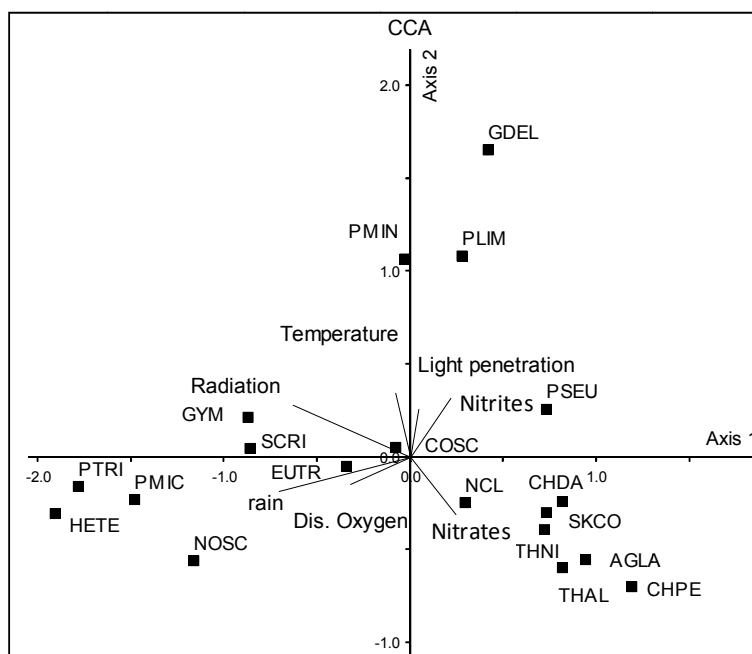
Figure 10:
*Correlations plot of the canonical correspondence analysis (CCA), on the relationship between environmental variables
and species which may be nocive (HAB).*

1990; Lévy *et al.*, 1998 a,b; Ribera d'Alcalà *et al.*, 2004, Siokou-Frangou, 2010).In the Mediterranean, a winter phytoplankton bloom appears to be quite widespread, due to recurrent periods of calm weather in the season, generally associated with the expansion of the Siberian high pressure system towards the West (Duarte *et al.*, 1999; Ribera d'Alcalà *et al.*, 2004). Indeed, the last winter bloom has been defined as the unifying feature for phytoplankton in the Mediterranean Sea (Travers, 1974; Duarte *et al.*, 1999; Ribera d'Alcalà *et al.*, 2004; Siokou-Frangou, 2010).

In the winter monitoring campaign, the highest values of TP and Chl-*a* (average value 2.55 µg l⁻¹) were registered and DIP was very scarce (fig. 3 and fig. 4). In the study area, according to Sebastià *et al.*, (2012) higher phosphorus levels were observed in surface irrigation channels during last winter and spring and have been attributed to diffuse sources, because they coincided with the period of phosphorus fertilizer application. In our study, DIP concentrations are very low (fig. 4), furthermore it is the main potentially limiting nutrient, so any phosphorus discharge through the irrigation channels is rapidly consumed and incorporated as biomass (Falco *et al.*, 2010). Thus,the highest values of Chl-*a* and TP may indicate a winter-early spring bloom. Smith (2006) observed a strong correlation between biomass and TP. In our study, despite higher Chl-*a* and TP values, an increase in cell abundance was not observed. This might be due to the small size fraction of phytoplankton (<20 µm)

and the different chlorophyll content of phytoplankton species. In the Gandia coastal area, the important contribution of picoplankton and nanoplankton to phytoplankton biomass during summer and spring was reported by Sebastià (2012) using diagnostic photopigment analysis. According to Sebastià (2012) the contribution of small forms (<20 µm) to total Chl-*a* could reach 38.9 % in the entrance to Gandia harbour. Similar conditions were observed in the Iskenderun Bay (Northeastern Mediterranean) where the highest values of Chl-*a* and TP were registered in early March (2.78 µg l⁻¹) but not an increase in the cell abundance (Polat, 2001).

The study found a total of 108 phytoplankton species of nine different algal classes (mostly dinoflagellates and diatoms). Diatoms were the most dominant group in terms of abundance: toxic *Pseudo-nitzschia* species were among the most abundant and frequent diatom, and this genus showed the annual diatom trends. This fact was observed in the Balearic Archipelago by Puigserver *et al.*, (2010). Other representative species in terms of abundance were *Bacillaria paxillifera* and *Asterionellopsis Glaciallis* and, in terms of frequency *Cylindrotheca closterium* and *Chaetoceros affinis*. Diatom abundance did not show the typical seasonal cycle of maximum abundances in spring and minimum abundances in summer. Diatoms registered the highest values of abundance in autumn (fig. 6) and the highest relative abundance in summer (fig.5). The key factor supporting the summer population seems to be the

Table 1:
Measured physicochemical and community structure parameters and chlorophyll-a means, standard deviations, minimum and maximum values of stations grouped by seasons.

	SUMMER				AUTUMN				WINTER				SPRING			
	X	δ	min	max	X	δ	min	max	X	δ	min	max	X	δ	min	max
Water temperature (ºC)	27.30	0.87	26.20	30.30	20.85	0.48	19.60	22.20	13.16	0.40	12.70	14.10	20.82	0.90	19.60	23.00
Radiation (W m^{-2})	663	137	378	849	225	95	118	320	642	178	127	686	867	140	577	958
Salinity (g kg^{-1})	37.37	0.69	34.60	37.80	36.66	1.63	27.80	37.50	37.29	0.33	36.00	37.60	36.75	1.36	30.20	37.80
Dissolved oxygen (mgl^{-1})	7.00	0.14	6.55	7.14	7.44	0.36	6.82	8.38	9.65	0.22	9.10	9.87	8.76	0.40	7.94	9.30
pH	7.91	0.06	7.72	8.01	8.15	0.04	8.08	8.22	8.14	0.02	8.08	8.17	8.20	0.03	8.12	8.26
Secchi depth (m)	7.58	5.22	0.50	17.00	2.98	1.50	0.50	5.50	5.28	3.04	0.20	10.00	3.19	1.77	0.20	8.00
Suspended solids (mg l^{-1})	10.42	2.76	7.00	20.43	10.41	1.80	5.50	15.75	16.41	14.61	5.50	63.30	11.34	3.80	6.29	27.67
DIN (µM)	6.95	6.59	2.86	36.52	2.80	3.27	0.80	17.26	2.98	2.51	1.11	14.56	5.79	6.24	1.43	27.42
DIP (µM)	0.16	0.28	0.02	1.06	0.12	0.32	0.01	1.84	0.03	0.03	<0.01	0.15	<0.01	<0.01	<0.01	<0.01
TP (µM)	0.35	0.34	0.09	1.78	0.29	0.29	0.14	1.91	0.39	0.11	0.16	0.68	0.24	0.11	0.06	0.53
DSi (µM)	5.2	2.5	2.3	14.4	3.2	4.8	0.5	28.1	1.0	1.4	0.0	6.2	2.8	2.6	0.4	12.1
DIN:DIP	123.8	119.6	4.0	675.6	42.6	32.1	5.2	129.1	285.7	673.6	13.8	3533.2	236.8	251.8	57.4	1096.8
DSi:DIN	1.0	0.3	0.2	1.4	1.1	0.4	0.3	2.4	0.3	0.2	0.0	0.8	0.5	0.2	0.2	1.5
DSi:DIP	106.8	61.0	4.55	232.0	50.2	45.9	3.8	180.1	75.0	140.8	0.4	695.4	110.6	104.0	17.1	483.2
Diversity (H', bits ind^{-1})	1.35	0.56	0.02	2.40	1.46	0.37	0.67	2.27	1.58	0.37	1.05	2.40	2.03	0.44	1.03	2.70
Evenness (J)	0.30	0.12	0.00	0.51	0.32	0.08	0.14	0.49	0.34	0.08	0.23	0.52	0.43	0.09	0.22	0.57
Richness (S)	8.03	4.10	3	17	14.12	4.00	8	25	11.48	2.75	7	18	14.68	4.31	5	21
Total chlorophyll-a (µgl^{-1})	0.06	0.08	0.02	0.45	0.14	0.06	0.06	0.32	2.55	2.54	0.12	11.71	0.18	0.13	0.02	0.76

continuous availability of silica (even in the dry period) together with the optimal light and temperature conditions. The lowest density and relative abundance of this group was reached in spring in spite of the high concentrations of DSi. In this season dinoflagellates showed the highest densities.

In ecosystems with phosphorus limiting conditions and high nitrate and silicate levels, inputs of phosphorus could trigger the undesired effects of phytoplankton species responsible for the generation of harmful blooms (Sebastiá et al., 2012). In our study, the highest concentrations of dinoflagellates were always observed in the areas where the lowest DIP concentrations were registered despite the fact that at the same time those areas were producing the highest nutrient load (fig. 3 and fig.4). This fact shows that any phosphorus discharge in this area is rapidly consumed and incorporated by phytoplankton, including dinoflagellates.In summer, dinoflagellates appeared mainly in the mouth of the Serpis river (fig. 8 and fig. 9) when the treatment plant capacity is exceeded due to the population increase and the wastewater collector discharges into the Serpis directly. In autumn, they appeared in the Ahuir Beach (fig.8 and fig.9) when groundwater discharges were higher (fig. 2). During the period of phosphorus fertilizer application in the crops an increase in the dinoflagellate abundance was observed in the harbour since it received the discharges from the channels that drain the wetland. In spring dinoflagellates reached their highest abundances and relative abundance (fig.8) in the whole area, but mainly in the harbour. Phytoplankton abundance, including dinoflagellates was triggered by the increase in the incident light, and that increase led to a reduction of the DIP to levels below the detection limit because of nutrient assimilation. According to Fisher *et al.* (1999) light can limit or co-limit algal growth in marine environments that present high nitrogen inputs. This spring increase was characterized by a dominance of genera *Heterocapsa*, *Scrippsiella* and *Gymnodinium*. Some species that belong to these genera are capable of phagotrophy which is advantageous in inorganic nutrient-depleted waters (Stoecker *et al.*, 2006).The heterotrophic dinoflagellates are important in the trophic dynamics of the plankton community due to their various feeding strategies (Jeong, 1994; 1999; Barría and Piccolo, 2008). Dinoflagellates spring bloom was observed in the coastal waters of the Balearic Archipelago (Puigserver *et al.*, 2010), Izmik Bay (Aktan *et al.*, 2005) and in the Catalan Sea, where maximum concentrations were detected during spring and summer (Vila and Masó, 2005).

Table 2:
Percentage when DIN, PO$_4$ or SiO$_2$ act as potential limiting nutrient.

	% limiting nutrient			% without
	%DIN	%PO4	%SiO2	
Summer	6.25	87.50	3.13	3.13
Autumn	0	63.64	3.03	33.33
Winter	0	63.64	33.33	3.03
Spring	0	100	0	0

Table 3a:
Taxa found in the study area. % Np: percentage of total samples where the taxa was recorded; Av>0: averaged density (cells l^{-1}). Abundance total: sum of cell abundance in all samples taken. Number indicates the maximum abundance in each season (cells l-1), n indicates that this species was found in a net sample.

Taxa	% Np	Av>0	Abundance total	SUMMER	AUTUMN	WINTER	SPRING
DINOFLAGELLATES							
Akashiwo sanguinea Hirasaka	5.6	45	360	40		40	80
Alexandrium sp.	8.4	90	1080	40	40	40	200
Centrodinium maximum Pavillard	1.4	40	80				80
Ceratium candelabrum (Ehrenberg) Stein							n
Ceratium extensum (Gourret) Cleve							n
Ceratium euarcuatum E.G.Jørgensen	2.1	40	120	40			
Ceratium furca Ehrenberg	18.2	60	1560	40	40	40	160
Ceratium fusus Ehrenberg	9.8	44	620	40	40	40	80
Ceratium longirostrum Gourret	2.8	40	160	40			40
Ceratium karstenii Pavillard (=C. arcuatum (Gourret 1883) Cleve 1900)	0.7	40	40	40			
Ceratium macroceros Ehrenber				n			
Ceratium trichoceros Ehrenber				n			
Ceratium tripos Müller	3.5	40	200	40	40		
Dinophysis caudata Saville-Kent	0.7	40	40				40
Dinophysis sacculus Stein	0.7	40	40				40
Diplopsalis sp.	4.9	57	400		120		240
Gonyaulax sp.	3.5	56	280				80
Gymnodinium spp.	53.8	174	13380	160	200	240	1200
Gyrodinium sp.	16.8	93	2220	420	80	40	240
Heterocapsa spp.	25.2	584	21040	20	80	40	2120
Heterodinium dispar Kofoid & Adamson							n
Karenia sp.	2.1	53	160			80	40
Noctiluca scintillans (Macartney) Kofoid & Swezy	10.5	82	1240		40		200
Oxytoxum sp.	1.4	30	60	40			
Pentapharsodinium tyrrhenicum (Balech) Montresor, Zingone et Marino	13.3	101	1920			40	280
Peridinium quinquecorne Abé				n			
Podolampas spinifera Okamura	1.4	40	80	40			
Polykrikos kofoidii Chatton	0.7	40	40	40			
Prorocentrum lima (Ehrenberg,) Stein	6.3	58	520	120	40	80	40
Prorocentrum micans Ehrenberg	21.7	87	2680	40	80	40	240
Prorocentrum minimum (Pavillard) Schiller	7.7	49	540	80	80	40	40
Prorocentrum triestinum Schiller	9.1	81	1060	20	40		280
Prorocentrum sp.	2.1	53	160	120			
Protoperidinium claudicans (Paulsen) Balech	1.4	40	80			40	40
Protoperidinium crassipes (Kofoid) Balech	7	44	440		40		80
Protoperidinium diabolus(Cleve) Balech	22.4	180	5760		80		560
Protoperidinium divergens (Ehrenberg) Balech	2.8	40	160		40	40	
Protoperidinium leonis (Pavillard) Balech							n
Protoperidinium murrayi (Kofoid) Hernández-Becerril	0.7	40	40				40
Protoperidinium punctulatum (Paulsen) Balec	1.4	40	80				40
Protoperidinium pyriforme (Paulsen) Balech	9.1	82	680		40	40	160
Protoperidinium sphaericum (Murray & Whitting) Balech	4.9	51	360		40		80
Protoperidinium steinii (Jørgensen) Balec	0.7	20	20	20			
Protoperidinium sp.	4.9	43	300	20	40	40	80
Pyrocystis elegans Pavillard	0.7	40	40			40	
Pyrophacus sp.	0.7	40	40		40		
Scrippsiella spp.	44.1	218	13760	160	200	1120	1960
Spiraulax jollifei (Murray & Whitting) Kofoid	1.4	40	80				80
Dynophyceae spp.	16.1	78	1800	680	120	80	80

Euglenophytes appeared mostly in the rainy seasons and chlorophytes appeared in spring (fig. 6).In general, higher densities of both groups were observed at the mouth of the Serpisat Venecia Beach and in the entrance of the Gandia harbour. Venecia beach is a semi-enclosed bay where the Serpis river and the wastewater treatment plant collector outflow. Furthermore, the breakwater of the harbour reduce the impact of northeast currents, which are predominant in the area in autumn, winter and spring (CEDEX, 1997), this causes a reduction in hydro-dynamics and an increase in the water residence time. Euglenophytes have been observed in other euthrophic systems where they have been related to high nutrient levels and decreasing turbulence (Olly *et al*, 1996; Celik and Ongun, 2007, Sebastiá *et al*., 2012). On the other hand, different chlorophytes are characteristic of brackish and polluted waters, and their distribution has been associated to freshwater plume (Soler *et al*., 1995). Moreover, according to Ramirez *et al*., (2005) high DIN:DIP molar ratios enhance the development of chlorophytes. These conditions exist at Venecia Beach.

Phytoplankton community structure analyses based on the similarity of phytoplankton abundance among station groups showed a clear difference between stations located near the shoreline and the more distant stations. Furthermore, species richness is the simplest way to describe community diversity, and diversity indices have been used to determine spatial and temporal variations

Table 3b:

Taxa found in the study area. % Np: percentage of total samples where the taxa appeared; Av>0: averaged concentration (cells l⁻¹). Abundance total: sum of cell abundancein all samples taken. Number indicates the maximum abundance in each season (cells l⁻¹), n indicates that this species was found in a net sample.

Taxa	% Np	Av>0	Abundance total	SUMMER	AUTUMN	WINTER	SPRING
DIATOMS							
Achnanthes sp.	21	59	1780	80	120	120	120
Amphora spp.	4.9	40	280		40		40
Astartiella sp.	4.8	40	280		200	40	
Asterionellopsis glacialis(Castracane) Round	29.4	5584	234560	40	21640	10200	4600
Asteromphalus spp.					n		
Bacillaria paxillifera (Müller) Hendey	82.5	4722	557220	491580	800	2840	23360
Bacteriastrum sp.	1.4	120	240			200	
Bleakeleya sp.	4.9	43	300	20	80		40
Cerataulina pelagica (Cleve) Hendey					n		
Chaetoceros affinis Lauder	49.7	1125	79920	640	3840	7000	320
Chaetoceros atlanticus (Schütt) Hustedt					n		
Chaetoceros concavicorne Mangin	0.7	40	40		40		
Chaetoceros danicus Cleve	7	56	560		120	80	
Chaetoceros simplex Ostenfeld	0.7	20	20	20			
Chaetoceros socialis Lauder					n		
Chaetoceros peruvianus Brightwell	7	56	560		80	40	
Coscinodiscus centralis Ehrenberg	28	60	2400	40	120	200	120
Cylindroteca closterium (Ehrenberg) Reimann & Lewin	51	114	8340	40	520	560	280
Diploneis sp.	10.5	71	1060	40	280	200	40
Eucampia sp.	2.1	133	400		120		240
Fragilariopsis spp.	0.7	80	80		80		
Gossleriella tropica Schütt	1.4	40	80			40	40
Guinardia delicatula (Cleve) Hasle	28.7	664	27260	8920	440	680	200
Helicotheca tamesis (Shrubsole) Ricard				n			
Hemidiscus sp.	0.7	40	40			40	
Lauderia annulata Cleve	3.5	28	140	40			40
Leptocylindrus mediterraneus (H. Peragallo) Hasle	22.4	198	6340	280	760	800	240
Licmophora spp.	7.7	554	6100	40	5720		80
Melosira spp.	7.0	556	5560		1600	1120	80
Navícula spp.	28.0	53	2120	140	40	80	80
Nitzchia longissima (Brébisson in Kützing)	5.6	260	2080	1680	40		120
Odontella mobiliensis (Bailey) Grunow	12.6	139	2500	760	200	760	120
Planktoniella sp.	0.7	20	20	20			
Pleurosigma sp.	17.5	60	1500	160	120	40	200
Proboscia alata (Brightwell) Sundström	72	167	17240	540	680	800	400
Pseudo nitzschia spp.	55.9	6754	540330	408510	19360	3800	200
Rhizosolenia spp.	18.9	132	3560	480	40		80
Skeletonema costatum (Greville) Cleve	23.8	865	29440	800	2120	2920	2120
Striatella unipunctata (Lyngbye) Agardh	0.7	3760	3760		3760		
Thalassionema nitschioides (Grunow) Merschkowsky	43.4	895	55460	80	4640	480	360
Thalassiosira spp.Grunow	8.4	403	4840		1960	560	40
Thalassiotrix spp.	0.7	40	40		40		
EUGLENOPHYTES							
Eutreptiella gimnastica Throndsen	17.5	86	2140	40	160	480	80
Eutreptia spp.	49.7	115	8160	200	440	240	280
CHLOROPHYTES							
Scenedesmus spp.	1.4	200	400		160		240
Sphaerocistis spp.	1.4	100	200	120			80
Chlorophycea spp.	18.2	138	3600	40	80	200	920
RAPHYDOPHYTES							
Chatonella subsalsa B.Biecheler	0.7	40	40	40			
Chatonella sp.	52.4	405	30400	200	4240	1840	120
HAPTOPHYTES							
Phaeocystis spp. Lagerheim	1.4	40	40	40		40	
Phaeocystis cordata Zingone & Chrétiennot-Dinet				n			
Phaeocystis globosa Scherffel				n			
CHRYSOPHYTES							
Bicosoeca spp.	2.8	60	240		80	40	40
Dinobryon sp.	4.9	57	400	40	120		
Dinobryon balticum (Schütt) Lemmermann	0.7	40	40		40		
DICTIOPHYTES							
Ciliophrys infusionum Cienkowski	0.7	40	40		40		
Dictyocha staurodon Ehrenberg	0.7	40	40		40		
Octactis octonaria (Ehrenberg) Hovasse	4.9	120	840		320		
CRYPTOPHYTES							
Telonema subtile Greissmann					n		

induced by natural and anthropogenic disturbances (He and Legendre, 2002; Gonídez-Dominguez *et al*., 2009; Aktan, *et al* 2011). Spring monitoring presented significantly higher (p<0.05) values of diversity and evenness and, in the case of species number, the highest values were registered in autumn and spring. Coastal waters influenced by continental runoff (natural or anthropogenic) show higher concentrations of Chl-*a* than their surrounding waters as

a result of this fertilization (Magazzu and Decembrini, 1995; Agawin *et al*., 1998; Duarte *et al*., 2000; Olivos *et al*., 2002). This fact has been observed in the coastal area of Gandia near the discharge of the Serpis river and channels that drain the Safor Wetland. However, in our study a discrepancy was observed between high nutrient concentration and low biological response in the submarine outfall plume, as had been previously observed in studies of the effects of sewage discharge through outfalls in Hawaiian coastal waters (Ed Parnell, 2003) and in the Northern Adriatic Sea (Mozetič *et al*., 2008). According to Demir and Kirkagac (2005) and Mozetič *et al.,* (2008) in the case of short water retention time phytoplankton growth is not sustained in spite of high levels of nutrients. The majority of the phosphorus observed in the outfall plume was inorganic, especially at high concentrations of TP. According to Mozetič *et al.,* (2008) due to P-depleted organic substrate, bacteria might have prevailed over phytoplankton for phosphate.

During the study period, thirty-one toxic and harmful species were recorded (table 5). Among them, twelve species are included in the IOC list 2009 (Moestrup *et al*., 2009), mainly dinoflagellates. Higher cell abundance and recurrence of dinoflagellates were observed in spring whereas harmful diatoms registered highest abundance and occurrence in autumn and winter. The CCA analysis (fig. 10) showed that diatoms were favoured by high nitrate concentration and low temperatures. On the other hand, harmful dinoflagellates abundance was higher at high radiation and they were favoured by precipitation events before monitoring. The highest abundances of harmful diatoms and dinoflagellates were observed in Gandia harbour and Venecia Beach (table 4). According to Vila *et al*. (2001) the interaction between high nutrient levels and confinement plays the key role in the occurrence of dinoflagellates in this region. Semi-enclosed gulfs and bays, such as the Venecia Beach, near important harbours and big cities are at higher risk of suffering HABs (EEA 1999, Vila *et al*., 2001). The mainly harmful dinoflagellates observed in the study area were of the genera *Alexandrium* and *Dinophysis*, but they did not reach high densities. The genus *Gymnodinium* proliferates in this area and was the most frequent dinoflagellate. According to Soler *et al*., (1995), blooms of this red tide species seem to be related to continental discharges (low salinities and micro- and macronutrient inputs) and this species blooms in confined and non confined near-shore waters (Vila *et al., 2001*). This group reached 1200 cells l^{-1} in spring, which is much lower than the values recorded in the harbours of the Catalan Sea (Vila and Masó, 2005),in Valencia (Soler, *et al*., 1995) and in the Alboran Sea (Mercado *et al*., 2005). The bloom forming *Heterocapsa spp* and *Scrippsiella spp* were the

most abundant genera, the highest concentrations of these species (2 x 10^6 cells l^{-1}) were observed inside the harbour and were similar to those observed in the harbours of Catalonia (Vila and Masó, 2005). Among harmful diatoms, the genus *Pseudo-nitzschia* showed a positive correlation with high nitrate and nitrite concentration and a negative correlation with precipitations and dissolved oxygen (fig. 10). This genus reached its highest concentration in summer (408 x 10^3 cells l^{-1}) at the harbour entrance. The southeast currents created by wind action are prevalent dominant on the Gandia coast in summer (CEDEX, 1997). This causes marine water to enter into the harbour and produces a turbulent environment in the harbour entrance due to the orientation of its entrance channel. Furthermore, DIN (mainly nitrates) and DSi values were significantly higher (fig. 3) than other areas and they were the maximum values reached in the harbour during the study period. These high levels could be the consequence of wastewater discharges near the mouth of the Serpis river and the water pumped for summer irrigation that outflows through the channels into the harbour. Diatoms tend to dominate in high-nutrient and turbulence environments (Song *et al*., 2009), these conditions may favour diatom bloom, specifically the growth of *Pseudo-nitzschia* species (Puigserver *et al*., 2010). This genera has been observed in Valencia harbour by Assadi *et al*., (2007) in summer and in different harbours of northwestern Mediterranean Sea (Vila and Masó, 2005). The genera *Chatonella* only appeared at Venecia Beach and in the plume of the submarine outfall. This genera has been observed in the Mediterranean Sea in eutrophic coastal areas and brackish coastal areas rich in organic material (Tomas, 1997; Hallegraeff *et al*., 2003). The euglenophyte *Eutreptiella gymnastica* only appeared at Venecia Beach and in Gandia harbour, it has been described by Olly *et al*. (1996) as a bloom species adapted to decaying turbulence and high nutrient environments.

CONCLUSIONS

The coastal phytoplankton of Gandia (Gulf of Valencia) in the surface layers differs in composition and abundance in response to the interaction of regional conditions and the variability imposed by seasonal changes in radiation and precipitation, as well as local conditions such as coastal dynamics and nutrient inputs mainly by the river Serpis and channels that drain the Safor Wetland. On the other hand, the discharge of the effluent from sewage treatment plant through the submarine outfall does not appear to significantly impact the phytoplankton community. During the study period, from summer 2010 to spring 2011, the phytoplankton succession showed a clear transition from a diatom to a non-diatom based assemblage. A relationship between phytoplankton succession, nutrient concentration

Table 4:
Indicator species of anthropogenic pressure in the study area. Av>0: averaged density (cells l⁻¹).

Taxa	HARBOUR ENTRANCE Av>0	MOUTH OF SERPIS RIVER/VENECIA BEACH Av>0	MOUTH OF WATERCOURSE VACA/AHUIR BEACH Av>0	SUBMARINE OUTFALL Av>0	CONTROL STATION (F1) Av>0
Chatonella sp.	200	496	260	1413	106
Eutreptiella gimnastica	180	210	0	0	0
Gosleriella tropica	0	0	0	0	40
Guinardia delicatula	4686	1187	310	240	480
Nitzschia longissima	0	0	0	0	40
Noctiluca scintillans	0	0	40	0	200
Octactis octonaria	0	0	0	0	40
Odontella mobiliensis	40	203	760	0	0
Pseudo-nitzschia spp.	71491	4305	9760	1720	520
Scrippsiella sp.	880	326	340	0	160
Skeletonema costatum	1270	180	520	600	0

Table 5:
Main characteristics of harmful taxa. Code: abbreviation used in CCA.Taxonomic group (Tax group) refers to dinoflagellates (DINO), diatoms (DIAT) and raphidophytes (RAPH), flagellates (FL) and haptophytes (HAPT). Cell type refers to individual cells (i), chain forming o colonial cells (c); Harmful refers to the potencially harmful effect: Paralytic shellfish poisoning (PSP), Diarrhetic shellfish posisoning (DSP), Amnesic shellfish poisoning (ASP), Neurotoxic (NTX), fish-killing and bloom forming (bf), an asterisk * indicates high concentrations. Np: number of occurrences in the season; % Np: percentage of total; Av>0: averaged concentration (cells l⁻¹), calculated from frequency which cells were detected; Max.: Maximum cell concentration (cells l⁻¹)

Taxa	Code	Tax Group	Cell Type	Harmful	SUMMER Np	%Np	Av.(>0)	Max.	AUTUMN Np	%Np	Av.(>0)	Max.	WINTER Np	%Np	Av.(>0)	Max.	SPRING Np	%Np	Av.(>0)	Max.
Akashiwo sanguinea Hirasaka	AKSAN	DINO	i	bf	1	0.2	40	40					1	3.2	40	40	5	15.2	48	80
Alexandrium sp.	ALEX	DINO	i	PSP, bf	1	0.2	40	40	2	6.1	40	40	1	3.2	40	40	8	24.2	115	200
Ceratium fusus Ehrenberg	CFUS	DINO	i	Fish-killing	2	0.3	30	40	1	3.0	40	40	3	9.7	40	40	7	21.2	46	80
Dinophysis caudata Saville-Kent	DCAU	DINO	i	DSP													1	3.0	40	40
Dinophysis sacculus Stein	DSAC	DINO	i	DSP													1	3.0	40	40
Gonyaulax sp.	GON	DINO	i	NTX													5	15.2	56	80
Gymnodinium spp.	GYM	DINO	i/c	bf*	13	40.6	68	160	10	30.3	68	200	20	60.6	78	240	33	100.0	333	1200
Heterocapsa spp.	HETE	DINO	i	bf*	1	0.2	20	20	2	6.1	60	80	2	6.5	40	40	31	93.9	693	2120
Karenia spp. Hansen et Moestrup	KAR	DINO	i	NSP									2	6.5	60	80	1	3.0	40	40
Noctiluca scintillans (Macartney) Kofoid & Swezy	NOSC	DINO	i	bf	2	0.3	100	120	5	15.2	40	40					10	0.3	120	200
Prorocentrum lima (Ehrenberg) Stein	PLIM	DINO	i	DSP	1	0.2	40	40	2	6.1	40	80	3	9.7	40	80	2	6.1	40	40
Prorocentrum micans Ehrenberg	PMIC	DINO	i	bf	3	0.5	60	80	5	15.2	48	80	2	6.5	40	40	24	72.7	104	240
Prorocentrum minimum (Pavillard) Schiller	PMIN	DINO	i	NTX, bf	1	0.2	20	20	2	6.1	60	80	1	3.2	40	40	4	12.1	40	40
Prorocentrum triestinum Schiller	PTRI	DINO	i	bf					1	3.0	40	40					11	33.3	91	280
Scrippsiella spp.	SCRI	DINO	i	bf	6	1.0	67	160	11	33.3	87	200	15	48.4	171	1120	31	93.9	337	1960
Amphora spp.	AMP	DIAT	i	ASP					2	6.1	40	40					5	15.2	40	40
Asterionellopsis glacialis(Castracane) Round	AGLA	DIAT	c	bf*	1	0.2	40	40	29	87.9	6421	21640	7	20.0	1128	10200	1	3.0	4600	4600
Chaetoceros concavicorne Mangin	CHCO	DIAT	c	Fish-killing					1	3.0	40	40								
Chaetoceros danicus Cleve	CHDA	DIAT	c	bf					5	15.2	70	120	5	15.6	50	80				
Chaetoceros peruvianus Brightwell	CHPE	DIAT	i	bf					9	27.3	58	80	1	3.1	40	40				
Coscinodiscus centralis Ehrenberg	COSC	DIAT	i	bf	4	0.7	30	40	14	42.4	69	120	11	35.5	65	200	10	30.3	64	120
Centaulina pelagica (Cleve) Hendey	CPEL	DIAT	c	bf																
Cylindrotheca closterium Reimann & J.C.Lewin	CCL	DIAT	i	bf	1	0.2	40	40	25	75.8	120	520	25	80.6	125	560	13	39.4	55	280
Guinardia delicatula (Cleve) Hasle	GDEL	DIAT	c	bf	19	59.4	1214	8920	9	27.3	156	440	11	34.4	185	680	4	12.1	100	200
Pseudonitzschia spp.	PSEU	DIAT	c	ASP*	13	40.6	37351	408510	33	100.0	2223	19360	29	90.6	731	3800	2	6.1	120	200
Skeletonema costatum(Greville) Cleve	SKCO	DIAT	c	bf	3	0.5	393	800	21	63.6	851	2120	9	28.1	1005	2920	1	3.0	2120	2120
Thalassionema nitzschioides (Grunow) Mereschkowsky	THNI	DIAT	i	bf	1	0.2	80	80	33	100.0	1510	4640	19	57.6	243	480	8	24.2	160	360
Thalassiosira spp Grunow	THAL	DIAT	c	bf					7	21.2	526	1960	4	12.5	307	560	1	3.0	40	40
Eutreptiella gimnastica Throndsen	EUTR	FL	i	bf	2	0.3	30	40	5	15.2	96	160	8	25.8	135	480	10	30.3	52	80
Chatonella subsalsa B. Biecheler	CHSU	RAPH	i	Fish-killing	1	0.2	40	40												
Phaeocystis spp Lagerheim	PHAC	HAPT	i	Fish-killing	1	0.2	40	40					1	3.2	40	40				

Table 6:
Rank Spearman's correlation matrix between phytoplankton groups and environmental variables.

	Diatoms	Dinoflagellates	Chlorophytes	Euglenophytes	Haptophytes	Dictyophytes	Raphydophytes	Cell abundance	Chl-*a*
Water temperature	-0.74[a]	-0.11	-0.03	-0.08	0.06	0.07	-0.28[a]	-0.30[a]	0.02
Radiation	-0.74[a]	0.56[a]	0.40[a]	-0.16	-0.08	-0.17	-0.58[a]	-0.44[b]	-0.14
Saliniy	-0.21[b]	-0.18[a]	-0.42[a]	-0.30[a]	0.08	-0.02	-0.14	-0.35[a]	-0.24[a]
Light penetration	-0.04	-0.31[a]	-0.26[b]	-0.33[a]	-0.06	0.00	0.08	0.12	-0.31[a]
Dissolved oxygen	0.02	0.26	0.01	0.16	0.01	-0.13	0.14	0.17	0.77[a]
DIN	0.24	0.15	0.36[a]	-0.10	-0.02	0.02	-0.12	-0.34	-0.19
DIP	0.31[a]	-0.49[a]	-0.24[a]	-0.04	0.00	0.17	0.22[b]	0.17	0.03
TP	0.01	-0.11	-0.02	-0.05	-0.04	0.03	0.05	-0.03	0.35[a]
DSi	0.32[b]	0.23	0.30[b]	0.30[a]	0.03	0.13	-0.24[a]	0.27[a]	-0.16
DIN:DIP	0.24	0.43[a]	0.42[a]	0.03	0.02	-0.23	-0.28	0.24	0.15
DSi:DIN	0.32[a]	-0.27[a]	-0.09	0.27[a]	0.08	0.16	0.12	0.41[a]	0.14
DSi:DIP	0.04	0.44[a]	0.36[a]	0.29[a]	0.31	0.01	-0.08	0.17[b]	0.35[a]

and nutrient ratios seemed to exist. In the dry winter, high values of TP and Chl-*a* could indicate a last winter bloom. In spring, a dinoflagellate bloom was observed when there was high radiation, very low DIP, high DIN:DIP and low DSi:DIN molar ratios, during the period of phosphorus fertilizer application in the crops of the Safor Wetland. The inputs of phosphorus from agriculture seem to have a significant response in the phytoplankton community. Gandia Harbour and Venecia Beach are areas with high risk of suffering HABs, as a consequence of the continous nutrient inputs from agriculture and wastewater discharges and the reduced water exchange (high water residence time).

ACKNOWLEDGEMENTS

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