Nutrient and Phytoplankton Analysis of a Mediterranean Coastal Area

Sebastiá, MT, Rodilla, M.

Institut d'Investigació per a la Gestió Integrada de Zones Costaneres (IGIC)

Universitat Politècnica de València

C/ Paranimf 1

46730 Grau de Gandia (Spain)

*Corresponding author

E-mail address: mtsebastia@hma.upv.es

Telephone number: (+34) 962849398

Abstract

Identifying and quantifying the key anthropogenic nutrient input sources is essential to adopting management measures that can target input for maximum effect in controlling the phytoplankton biomass. In this study three systems characterized by distinctive main nutrient sources were sampled along a Mediterranean coast transect. These sources were: groundwater discharge in the Ahuir area, Serpis River discharge in the Venecia area and a submarine wastewater outfall 1900 m from the coast. The study area includes factors considered important in determining a coastal area as a sensitive area: it has significant nutrient sources; tourism is a major source of income in the region; and it includes an area of high water residence time (Venecia area) which is affected by the harbor facilities and by wastewater discharges. We found that in the Ahuir and the submarine wastewater outfall areas, the effects of freshwater inputs were reduced because of a greater water exchange with the oligotrophic Mediterranean waters. On the other hand, in the Venecia area the highest nutrient concentration and phytoplankton biomass were attributed to the greatest water residence time. In this enclosed area harmful dinoflagellates were detected (*Alexandrium* sp. and *Dinophysis caudata*). If the planned enlargement of Gandia Harbor proceeds, this may increase the vulnerability of this system and provide the proper conditions of confinement for dinoflagellate blooms development. Management measures should first target phosphorus inputs, as this is the most potential limiting nutrient in the Venecia area and it comes from a point source that is easier to control. Finally, we recommend that harbor environmental management plans include regular monitoring of water quality in adjacent waters to identify adverse phytoplankton community changes.

Keywords: CHEMTAX, pigments, phytoplankton, nutrients, Harmful Algal Blooms, harbor

1. Introduction

Estuaries and coastal areas are ecosystems of high natural and socio-economic value that are at high risk of suffering the negative impact of human activities (Angelidis and Kamizoulis 2005). One of the main reasons for this risk is the high loads of nutrients that they receive from terrestrial origin, either from point (e.g. submarine wastewater outfalls) or diffuse sources (e.g. atmospheric, leaching from surrounding land) (Paerl 2006). Nutrient inputs in adequate proportions and quantities are essential for primary producers. However, excessive anthropogenic nutrient inputs can lead to undesirable effects associated with eutrophication, including harmful algal blooms (HABs), and alterations in the natural composition of the phytoplankton community, which may, in turn, change the ecosystem food webs and nutrient cycling dynamics (Paerl et al. 2010).

Nevertheless, regardless of the high anthropogenic nutrient loading into coastal systems, phytoplankton biomass may be limited by a specific nutrient due to an alteration in the available N:P:Si nutrient ratios. As Smith et al. (1999) pointed out "the concept of nutrient limitation is the keystone of eutrophication research" and as important as element loading rates and concentrations. Identifying and quantifying the key anthropogenic nutrient input sources is essential to adopting management measures that can target input for maximum effect.

Eutrophication has been described as one of the greatest contemporary threats to the integrity of coastal ecosystems (Vidal et al. 1999). Two key European policy instruments were introduced in the early 1990s to address the most significant pressures leading to eutrophication: the Nitrates Directive (ND, 91/676/EEC), which considers the agricultural contribution and the Urban Waste Water Treatment Directive (UWWTD 91/271/EEC), which focuses on urban sewage discharges. However, implementation of these directives has met with varying degree of success: point sources (UWWTD) are localized and are easier to monitor and control, while diffuse sources (ND) are much more difficult to regulate (Artioli et al. 2008; Maier et al. 2009).

Phytoplankton is a priority item in different national and international water quality and eutrophication assessment policies, such as the National Estuarine Eutrophication Assessment (NEEA) Program (USA) (Whitall et al. 2007), the OSPAR convention (North-East Atlantic) (OSPAR 2003) and the Water Framework Directive (WFD 2000/60/EC). The WFD clearly addresses the status of coastal and transitional waters (such as estuaries, rias and coastal lagoons), integrating these within groundwater and inland surface water management. It bases its assessment on ecological quality status, requiring evidence of impact on biological quality elements as well as supporting physico-chemical data to confirm the likely cause of the impact (Maier et al. 2009). Biological parameters include composition, biomass and abundance of phytoplankton (WFD 2000/60/EC).

Although quality classification schemes for chlorophyll *a* concentrations as a measure of phytoplankton biomass have been developed, the requirement of the WFD for an evaluation system based on the taxonomic composition of the phytoplankton community is more difficult to satisfy (Cartaxana et al. 2009). Furthermore, taxonomic composition is usually assessed by standard microscopy, and accurate species enumeration is laborious, costly and the results can be inconsistent among different laboratories (Duarte et al. 1990; Schlüter et al. 2000). As an alternative approach, the phytoplankton community can be categorized into functional groups on the basis of taxonomic specificity of pigments determined with high performance liquid chromatography (HPLC) (Wright et al. 1996) coupled with the CHEMTAX program (Mackey et al. 1996). However, this technique has been mainly addressed as a complement to standard microscopy techniques (Cartaxana et al. 2009; Seoane et al. 2011). As Latasa (2007) observed "Pigment specialists are confronted with the paradox of requiring information about the composition of phytoplankton in order to learn about the pigment composition of phytoplankton groups and, ultimately, the composition of phytoplankton". In order to overcome this problem a procedure was suggested by Latasa (2007), and appraised by the authors involved in the development of CHEMTAX (H. Higgins and S. Wright pers. comm.).

The objective of this study is to characterize the nutrient levels and the phytoplankton community in a coastal Mediterranean area that can be defined as nutrient-sensitive. According to the UNEP/WHO (1999) definition, nutrient-sensitive areas are "estuaries and coastal waters of natural or socio-economic value that are at higher risk to suffer negative impacts from human activities". In these areas it is necessary to evaluate both the vulnerability of the system and the risk for environmental degradation (Angelidis and Kamizoulis 2005). Vulnerability is determined by natural characteristics (for instance, semienclosed waters are more sensitive to pollution impacts), while risk is determined by human activities (Angelidis and Kamizoulis 2005). In this study, three systems characterized by different hydrodynamic characteristics and a distinctive main nutrient source, linked to a specific human activity, are compared. The aim of this comparison is to prioritize environmental protection actions. This research was triggered by the recent urban development of the area and the projected enlargement of a commercial harbor that may modify both system risk and vulnerability.

2. Materials and methods

2.1. Study area

The study area (Fig. 1) is located in the southernmost sector of the Valencian Gulf (Western-Mediterranean Sea) on the coast of the Safor County (Spain). Three important factors, which determine whether a coastal area is a sensitive area according to Angelidis and Kamizoulis (2005), coincide here.

These factors are: 1) the presence of tourism as major income generation activity; 2) the presence of important nutrient sources in the drainage basin: a submarine wastewater outfall, harbor facilities and important agricultural runoff and 3) a river mouth area with low flushing rate, high water residence time, shallow mean depth and low mean slope of the sea floor.

Tourism is one of the main economic activities in this area. In 2009 the Valencia region received 5.1 million international tourists and 3.1 million national tourists from other Spanish regions (IET -Spain's Tourism Statistics Centre 2010). Tourism development in the Safor County is mainly based on residential development (Mantecón and Huete 2007) and is based on a "leisure and holiday" product type labeled as a "sun and beach" product (Yepes and Medina 2005). The average density in the coastal municipalities of the Safor county (from Gandia to Piles) is 963 inhabitants km^{-2} in winter (1314 inhabitants km^{-2} in Gandia city) (INE 2009). However, that figure triples in summer. Statistical data of tourism accommodation from the Spanish INSTITUTO NACIONAL DE ESTADÍSTICA (INE 2009) did not explain the real summer increase. Published data for the coastal municipalities of the study area are the following: 28 hotels with accommodation for 5496 guests; 4 camp sites with 2822 pitches; 2053 regulated apartments, with 10590 beds. However, beds in non-regulated apartments far exceeded these numbers and explain the summer population increase more accurately. Property ownership increased from 91703 in 2004 to 106384 in 2008 according to the land registry (INE 2009) due to the coastal urbanization process and many of these apartments are devoted to mass tourism.

The impact of mass tourism on the Spanish Mediterranean coast has been addressed by other authors (Vera and Ivars 2003; Yepes and Medina 2005). It is important to highlight that one impact of the summer population increase is the seasonality of the wastewater flow, which is considerably higher during summer. The treatment plant of Gandia is designed to serve a population of 133300 inhabitants and treat 46350 m^{3}day^{-1} of average flow from 17 municipalities of the Safor County. These municipalities have a population of 115328 inhabitants in winter (INE 2009); however, this population triples in summer as described above. The treatment plant is located next to the Serpis River (Fig. 1) and it discharges treated wastewater through a submarine wastewater outfall at an approximate distance of 1900 m from the Serpis river mouth. In addition, the plant has two overflow channels that discharge directly to the river. The first overflow channel discharges next to the plant, mainly during torrential rain episodes because wastewater and pluvial waters are not separated. The other overflow channel discharges near the river mouth. In summer, discharges have been detected through this channel as consequence of the population and wastewater flow in excess of designed capacity.

Another source of economic activity in the Safor County is Gandia Harbor, which is a commercial, fishing and recreational harbor. The main traffic in the harbor is paper reels and wood packages; other merchandise is wood pulp, rice, and mainly citrus fruits. The commercial area is 260000 m^2 and it has an additional 70000 m^2 of storage area with 32000 m^2 of warehouses. It has a nautical club with a 21000 m^2 dock with capacity for 400 sports boats. In 2009, the total traffic in the harbor was 251000 tons (including fisheries and provisioning), mainly of imported products (APV 2009). There is currently a project for enlarging the harbor in order to increase the capacity for commercial and recreational activities, including the construction of a new commercial dock (APV 2009; Decision 28734 BOE nº 214, 6 September 2011) In Fig. 2 the harbor as it is now, and the projected enlargement can be observed.

Two rivers flow into the study area: at the northern end is the Xeraco river mouth, which is a small river, 16.6 km in length, and to the south, the Serpis River, which has a basin of 752.8 km^2 and is 74.5 km in length. These rivers have a Mediterranean regime characterized by a high seasonality, with a dry period during summer, and a wet period with episodes of torrential rain, mainly in autumn and spring (Hermosilla 2005). While the Xeraco River outflows in a more open area, the Serpis River outflows on Venecia Beach, on the southern side of Gandia Harbor. Venecia Beach is a system characterized by high vulnerability because it is a semi-enclosed bay (enclosed by the harbor and the river mouth) (Fig. 2), with shallow water (depth < 10 m) and mean slope < 1%, which are parameters included in the Angelidis and Kamizoulis (2005) definition of marine systems vulnerability. This area is exposed to sea and swell wave from the NE-SSE sector (clockwise) due to the coast configuration (APV 2011). Northeast currents are dominant in the area, followed by southeast currents (NE 9.9%, ENE 7.4%, E 7.4%, ESE 4.8%, SE 4% and SSE 3.4% global frequency for sea and swell wave) (CEDEX 1997; APV 2011). The presence of Gandia Harbor, acts as a man-made barrier for water circulation when wind blows from the SE. In summer, dominant winds come from the NNE-SSE sector and vary from NNE (early morning) to SSE daily creating a circular flow pattern, which increases the water residence time. This effect has also been observed with natural barriers (Cullera Cape) northerly in the Valencian Gulf (Sánchez-Arcilla et al. 2007). The new commercial dock will increase the described barrier effect (Fig. 2). Venecia beach gets flushed out when wind blows from W direction; however this component is more frequent during the wet season, and presents its lowest frequency in summer.

The alluvial plain was totally occupied by the Safor wetland and crops until the seventies. Nowadays, this area shares its agricultural activity, mainly citrus fruits and vegetables, with the tourism of the urban areas. The Safor Wetland 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. The wetland is drained by a network of artificial channels which outflows into Gandia Harbor. Agricultural land use represents 48% of the watershed. 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), and it has been declared a Nitrate Vulnerable Zone. It is likely that the largest groundwater inputs of nutrients to near shore waters will occur in regions of aquifer bearing strata outcropping on the coast and occurring within nitrate vulnerable zones (Maier et al. 2009). In the study area, these conditions are found on Ahuir beach which is located at the end of Gandia’s urban area and which receives discharge from the Plana Gandia-Denia detritic aquifer, quantified at 66 Hm^3 $year^{-1}$ (2.1 m^3 s^{-1}) (Ballesteros-Navarro 2003). According to the aquifer functioning, which is described in Sebastiá et al. (2012), higher water discharges are expected at the end of the wet period (spring) when the phreatic level is higher.

2.2. Sampling design

Sampling was designed in order to characterize the systems receiving the major nutrient inputs along the coast. These systems were each characterized by a distinctive main nutrient source (groundwater discharge, river discharge or submarine wastewater outfall discharge) and by different hydrodynamics (water residence time).

The first study area was Ahuir beach which receives groundwater discharges from the Plana de Gandia-Denia aquifer. Samples were taken in an orthogonal transect (Fig. 1). The first sample was collected in the surf zone (A), the second sample right behind the surf zone (A1), the next sample was collected 50m from the coastline (A2) and subsequent samples were collected every 500 m along the transect (A3 to A6). Samples were collected with Niskin bottles each 5 m of water column depth and with a Van Dorn bottle 50 cm above the bottom. Maximum sampling depth was 18 m in A6.

The Serpis river mouth was selected as the second study area which we call Venecia area. Samples were collected as described below. Only one sample was collected in the Serpis River near its mouth. Four fixed sites were sampled drawing a 200 m square around the mouth (V1 to V4 plume contour stations, Fig. 1). Samples were collected with Niskin bottles near the surface at each fixed site, and an extra sample was collected with a Van Dorn bottle 50 cm above the bottom in V3 (2.8 m depth) and V4 (2.1 m depth). Three variable sites (V5 to V7) were sampled depending on wind and river plume directions. At these sites, water samples were collected at different depths in the water column (0.05, 0.10 and 0.75 m) using a Superficial Water Sampler (SWAS) (Mösso et al. 2008), because density driven vertical stratification was expected.

The third study area was located around the submarine wastewater outfall outlet. Four fixed sites (O1 to O4) (Fig. 1) were sampled around the outlet within 100 m approximate range. At these points, samples were collected with a Niskin bottle at 0.05, 5, 10 and 15 m. Three variable sites (O5 to O7) were sampled depending on wind and submarine wastewater outfall plume directions, water samples were collected at different depths in the water column (0.05, 0.10 and 0.75 m) using the SWAS, because a haline vertical stratification was expected.

Samples were taken in two hydrological periods: the wet one in spring, and the dry one in summer. Intensive samplings were performed in the study areas described above, as close together in time as possible (within a week maximum): in July 2008, in April 2009 and in July 2009. Samples were separated into three subsamples for nutrient, pigment and phytoplankton analysis. Water samples were kept in a cool box (4ºC) and transported to the laboratory.

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), dissolved silicate (DSi) and phytoplankton pigments. Representative samples were microscopically screened to avoid missing taxa following the recommendations of Higgins et al. (2011).

Dissolved inorganic nitrogen (DIN) was calculated as the sum of nitrate, nitrite and ammonium. Salinity was determined by means of an induction conductivity meter Multi 340i/SET WTW, using the Practical Salinity Scale. Nutrients were analyzed colorimetrically using the method of Aminot and Chaussepied

(1983). Dissolved oxygen concentration was measured using the Winkler titration technique (Grasshoff 1983).

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

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.

2.4. Phytoplankton composition and abundance

Once the concentration of important photosynthetic pigments was determined, the phytoplankton community was studied using the CHEMTAX program (Mackey et al. 1996) version 1.95 (S. Wright, pers. comm.) to obtain the contribution to total chlorophyll-*a* (chl-*a*) from the phytoplankton groups identified with microscopy.

To obtain reliable results, CHEMTAX should be applied to a data set where pigment ratios within the different groups do not change (Latasa et al. 2010). In order to identify groups of samples with similar characteristics, a cluster analysis was performed using STATGRAPHICS 5.1 to group samples according to salinity and nutrients (DIN, DIP and DSi). City-block distances were calculated and samples clustered according to Ward’s method. Pigment samples were separated into two subsets following the results of the cluster analysis: the first subset included marine samples, and the second subset included freshwater samples with higher nutrients concentration. Freshwater samples include: Serpis River samples and samples from river plume stations (V5, V6 and V7) from 0.05 to 0.10 m water column depth (only April 2009 and July 2009). CHEMTAX was applied independently to each subset (Latasa et al. 2010) to obtain the contribution of 8 phytoplankton groups to the chl-*a* stock: diatoms, dinoflagellates, euglenophytes, chlorophytes, cryptophytes, prymnesiophytes, prasinophytes and cyanobacteria.

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

2.5. Statistical analysis

A non-parametric one-way analysis of variance (Kruskal-Wallis) was used to test differences in physico-chemical variables and phytoplankton composition and abundance between sampling areas and seasons. The Mann-Whitney test was used to identify the different group.

After studying the phytoplankton data distribution in relation to the physico-chemical variables, the Redundancy Analysis (RDA) was selected from among the different multivariate ordination methods available (Ter Braak and Smilauer 2002). Phytoplankton taxonomic groups were included in CANOCO 4.5 as dependent variables and physico-chemical variables were included as independent variables. The statistical significance of the relationships was evaluated using Monte Carlo permutation tests with manual forward selection procedure, under 499 permutations (Seoane et al. 2011).

3. Results

3.1 Environmental variables

Mean water temperature was 15.7ºC in spring conditions (April 2009) and 27ºC in summer conditions (July 2008 and July 2009). Water temperature showed a longitudinal gradient decreasing from landward to seaward in all the sampling campaigns in the Ahuir study area due to increasing water column depth. The difference in temperature over the gradient was rather small, generally around 1ºC. In the submarine wastewater outfall area, the difference between subsurface temperature and above bottom (approximately 20 m depth) temperature was 0.3ºC in spring 2009 and nearly 1ºC in both summers, so no thermal stratification was observed. In the Venecia area due to the shallow depth and the reduced size of the study area, water temperature differences were minimal.

In the Ahuir area, dissolved oxygen concentration ranged from 6.0 mg L^{-1} in July 2009 to 10.6 mg L^{-1} in April 2009, with higher values in spring near the shore (Table 2). Salinity showed a longitudinal gradient that increased seawards in July 2008 (range 36.7 to 37.6) and April 2009 (range 36.6 to 37.4) (Table 2). In July 2009 salinity was significantly higher than both July 2008 and April 2009 ($p < 0.01$) and no gradient was observed. Suspended solids concentration was not significantly different between sampling seasons ($p > 0.05$); average values were below 11 mg L^{-1} (Table 2), and its value decreased with increasing water column depth.

The Serpis flow, measured in the gauging stations Font En Carrós and Vernisa of the Júcar Hydrographic Confederation (CHJ), which are located approximately 10 km from the river mouth, was null during summer sampling, while in April 2009 it was 5.7 $m^3 s^{-1}$. Summer is the dry period in this Mediterranean area and total monthly precipitation was 31.8 mm in July 2008 and 41.2 mm in July 2009, while in April 2009 (spring) monthly precipitation was 156.6 mm. In spring, a precipitation event of 89.8 mm was registered 3 days before sampling in the Venecia area. Dissolved oxygen concentration was significantly higher in spring 2009 ($p < 0.01$): its values ranged between 6.9 and 9.6 mg L^{-1} (Table 2). This higher DO concentration was linked to the lower temperature values observed in spring. The lowest dissolved oxygen values were observed in summer 2009 (ranging from 5.9 to 7.1 mg L^{-1}) when the Serpis river sample registered the minimum value of 3.1 mg L^{-1} (Table 2). Salinity in the receiving waters did not show statistically significant differences between sampling seasons ($p > 0.05$) given that salinity in the plume contour samples (V1 to V4) was always above 30. However, salinity in the plume sampling stations (V5 to V7) was lower in spring 2009 (under 5 in subsurface samples); while in July 2008 it was above 33, coinciding with a salinity measure in the river sample of 14.9. Suspended solids were significantly higher in July 2009 ($p < 0.01$) and ranged from 9 to 27 mg L^{-1}, with higher values in the plume points (V5 to V7).

In the submarine wastewater outfall area, average dissolved oxygen concentration showed was significantly higher ($p < 0.01$) in spring with an average value of 8.7 mg L^{-1} (Table 2) while in summer average values were 7.6 mg L^{-1} in 2008 and 7.4 mg L^{-1} in 2009. Salinity average values were above 37 in all the sampling seasons (Table 2). Only sampling point O5, located just above the outfall outlet, showed salinity values below 36 (35.2 in O5 at 0.05 m depth in April 2009 and 35.9 in July 2008). Significantly higher salinity values were observed in July 2009 ($p < 0.05$). Maximum suspended solids concentration was under 13 mg L^{-1} (Table 2).

Comparison of the three study areas revealed no significant difference ($p > 0.05$) in the salinity, dissolved oxygen concentration and suspended solids between the Ahuir and the submarine wastewater outfall area. On the other hand, the Venecia area had significantly lower salinity ($p < 0.01$) and dissolved oxygen concentration ($p < 0.01$) and higher suspended solids concentration ($p < 0.01$).

3.2. Nutrients concentration

In the Ahuir area, dissolved inorganic nitrogen (DIN) concentration ranged from 0.15 to 8.36 µM. The highest DIN values were observed in spring ($p < 0.01$), and ranged from 3.24 to 8.36 µM (Table 3). Nitrate was the most dominant nitrogen form in all sampling seasons. Dissolved inorganic phosphorus (DIP) concentration was significantly higher in summer ($p < 0.01$), with the highest values attained in July 2008 (ranging from 0.120 to 0.230 µM), while in spring, DIP values were below the analysis detection limit (Table 3). The lowest dissolved silicate (DSi) concentration was observed in July 2008 ($p < 0.01$), and ranged from 0.70 to 2.30 µM, while the maximum DSi values were attained in spring 2009 with 9.41 µM (Table 3).

In the Venecia area, nitrate was the most dominant nitrogen form in all sampling seasons, as was the case with the Ahuir area. Nitrate ranged from 2.17 to 50.83 µM, and DIN from 2.90 to 55.16 µM (Table 3). In this area no significant differences were observed in nitrate and DIN concentrations among sampling campaigns ($p > 0.05$). However, ammonium concentration was significantly higher in July 2009 ($p < 0.05$); median concentrations were 0.80, 0.30 and 1.75 µM in July 2008, April 2009 and July 2009, respectively. DIP concentration was significantly higher in July 2008 and ranged from 0.170 to 0.860 µM (Table 3). DSi showed no significant differences among sampling campaigns ($p > 0.05$).

In the submarine wastewater outfall area, nitrate was the most dominant nitrogen form in spring (average nitrate concentration was 6.88 µM and DIN was 7.08 µM) when DIN values were the highest ($p < 0.01$). Ammonium concentration was significantly higher in July 2008 ($p < 0.01$), when the DIN concentration was dominated by this ion (average ammonium concentration was 2.45 µM and average DIN was 4.32 µM). The lowest DIN and DIP ($p < 0.01$) values were observed in July 2009. DSi concentration did not show any statistical difference among the sampling campaigns ($p > 0.05$) and ranged from 1.07 to 19.20 µM (Table 3).

When the three study areas were compared, Venecia area showed significantly higher concentrations of nitrate, nitrite, DIN and DSi, than the Ahuir and the submarine wastewater outfall areas ($p < 0.01$ for all the variables). Venecia also showed the highest values for ammonium ($p < 0.01$) and DIP concentrations ($p < 0.01$); although in this case the submarine wastewater outfall area had higher values than the Ahuir area.

3.3. Nutrient ratios

In order to better define potential nutrient control, nutrient ratios between DSi, DIN and DIP concentrations were compared with Redfield ratios (DSi:DIN:DIP = 16:16:1) in Figure 3.

In the Ahuir area, the highest DIN:DIP and DSi:DIP ratios were observed in spring 2009 ($p < 0.01$ for both variables), when average ratios were 11812 and 7823 respectively (Table 3) and clearly indicated a primary potential deficiency of phosphorus. DSi:DIN ratio ranged from 0.2 to 2.7 (Table 3), and in most of the sampling sites it was below the Redfield ratio (1:1) (Fig. 3). In summer, the lower DIN:DIP and DSi:DIP ratios, together with the higher DSi:DIN ratio, caused a change in the main potential limiting nutrient. In summer 2008, nitrogen was the first potential limiting nutrient at all the sampling sites, while in summer 2009 nitrogen was the first potential limiting nutrient only from A4 to A6 (all sampling depths), and phosphorus was the potential limiting nutrient from A1 to A3 (Table 3 and Fig. 3).

In the Venecia area the highest DIN:DIP and the DSi:DIP ratios were measured in the 2009 sampling campaigns ($p < 0.01$ for both variables). In July 2008, when these ratios were lower, DIN:DIP ranged from 7 to 145 (Table 3), and DSi:DIP ranged from 7 to 93 (Table 3). Phosphorus was the first potential limiting nutrient in all the sampling campaigns and sites, except for an isolated instance in July 2008 of a V3 subsurface sample, where the minimum of both ratios was attained (Fig. 3). The DSi:DIN ratio had significantly higher values in summer ($p < 0.01$) than in spring, however this ratio was always close to the Redfield one (Table 3).

In the submarine wastewater outfall area, the three nutrient ratios reached their highest values in July 2009 ($p < 0.01$ for the three ratios). While the DSi:DIP and DSi:DIN ratio showed no significant difference between July 2008 and April 2009, the DIN:DIP ratio was higher in April. Phosphorus was the first potential limiting nutrient at all sampling sites and depths in July 2009 (Fig. 3), while in the other sampling campaigns, the potential limiting nutrient alternated between nitrogen, silicate and phosphorus depending on the sampling site and water depth (Fig. 3).

Among areas, no significant difference was observed in the DIN:DIP ratio ($p > 0.05$). The DSi:DIN and the DSi:DIP ratios reached their highest values in the Ahuir area. While no difference was observed in the DSi:DIN ratio between the submarine wastewater outfall and the Venecia areas, significantly higher DSi:DIP ratio values were detected in the submarine wastewater outfall area.

3.4. Phytoplankton abundance and composition

The chlorophyll-*a* (chl-*a*) mean values and its main descriptive statistics are summarized in Table 2. Comparison of the three areas studied showed that the Venecia area had the highest significant chl-*a* values ($p < 0.01$), while the outfall area had the lowest values, which were mainly below 1 mg m^{-3}. In the three areas, the highest chl-*a* values were attained in spring ($p < 0.01$). The samples taken in the Serpis River reached the maximum chl-*a* values in July 2008 with 76.0 mg m^{-3} and in April 2009 with 71.6 mg m^{-3}, while in July 2009 the chl-*a* value was similar to that measured in the receiving waters (Table 2).

The pigment ratio values of the final matrix used to estimate the contribution of the different groups to chl-*a* stock are presented in Table 1. It is important to highlight that in the microscope screening of representative samples, two potentially bloom forming dinoflagellates were detected in both summers in the Venecia area: *Alexandrium* sp. and *Dinophysis caudata*. In Fig. 4 the spatial and seasonal average contribution of each phytoplankton group to total chl-*a* is represented as estimated with CHEMTAX. In this figure, dinoflagellates, cryptophytes, prasinophytes, chlorophytes, and euglenophytes were grouped as flagellates (prymnesiophytes, which also comprise non flagellate organisms, were represented separately).

In spring 2009, diatoms were the dominant group with a percentage of total chl-*a* higher than 50% in the three studied areas (Fig. 4); no significant abundance difference was observed among areas ($p > 0.05$). Flagellates were the second main group, and its contribution to total chl-*a* was nearly one third at the outfall and Ahuir areas, and nearly one half at Venecia (Fig. 4). Flagellate abundance showed no significant difference among areas ($p > 0.05$), but chlorophytes were more abundant in the Venecia area ($p < 0.01$). The contribution of prymnesiophytes to total chl-*a* was around 10% in the three areas (Fig. 4) (no significant abundance differences were detected, $p > 0.05$). The contribution of cyanobacteria was the lowest with approximately 2% of total chl-*a* (Fig. 4).

In the summer sampling campaigns cyanobacteria abundance significantly increased ($p < 0.01$) in the three areas (Fig. 4). In July 2008, the highest cyanobacteria contribution was found in the submarine wastewater outfall area ($p < 0.01$), where this group was the second main group (36% of total chl-*a*) after flagellates (44%) (Fig. 4). In July 2009, no significant cyanobacteria abundance difference was observed among areas ($p > 0.05$). Diatoms abundance was significantly lower than that observed in spring in the submarine wastewater outfall area in both summer campaigns ($p < 0.01$). In July 2008 the lowest diatom contribution was observed in this area ($p < 0.01$). In the Ahuir area, the summer diatom decrease was only significant in July 2009 ($p < 0.01$), while in the Venecia area the summer decrease, though observable, was not significant ($p > 0.05$) (Fig. 4). In the Ahuir area, a significant increase in flagellate contribution was observed (64% of total chl-*a*) in July 2009, mainly due to an increase in prasinophytes. Due to this increase, these groups' abundance was significantly higher than that observed at Venecia and the outfall areas ($p < 0.01$).

The results of the Redundancy Analysis (RDA) are displayed in Fig. 5. For a more detailed interpretation of the triplots, see Ter Braak (1994). The RDA analysis was performed with the HPLC-CHEMTAX data in terms of chl-*a* biomass. The RDA retained 6 variables: salinity, ammonium, nitrate, dissolved inorganic phosphorus (DIP), dissolved silicate (DSi) and DIN:DIP ratio. These variables together explained 31% of the variance in the phytoplankton composition.

The phytoplankton groups were classified according to their association with the environmental variables. The arrow length indicates the goodness of fit of each group's biomass to the displayed ordination, and acute angles between two vectors indicate a high positive correlation. In general, the phytoplankton groups can be divided into two main groups, depending on their response to a gradient related with nutrient concentration (Axis 1) or a gradient related with salinity (Axis 2) (Fig. 5). In the first group, diatom biomass was higher in waters with a high nitrate concentration and high DIN:DIP ratio, while cyanobacteria, prasinophyte and to a minor extent prymnesiophyte biomass was higher in low nitrate concentration and low DIN:DIP ratio. In the second group, euglenophyte and chlorophyte biomass was higher in low salinity, while cryptophyte and dinoflagellate biomass was higher at high salinity. In addition, the biomass of euglenophytes and chlorophytes was strongly correlated with a high ammonium concentration while cryptophyte and dinoflagellate biomass was strongly correlated with low silica concentration.

Samples taken from the Serpis River and river plume (Venecia area) reflected the salinity gradient in the spring and summer 2009 sampling campaigns; this gradient was more pronounced in spring (Fig. 5 black triangles). In general, the stations sampled in spring were clearly different from the stations sampled in summer: summer samples (Fig. 5, white symbols year 2008 and grey symbols year 2009) were associated with lower nutrient concentration, and spring samples (Fig. 5, black symbols) with higher concentration.

4. Discussion

Higher salinity values were expected in the Ahuir and the Venecia areas in the summer sampling campaigns, due to reduced groundwater and river discharge, respectively, during the dry period (Sebastiá et al. 2012). In this area, summer groundwater discharge is also reduced because the summer population increase makes it necessary to increase pumping from the aquifer to supply drinking water from the aquifer wells. In addition, the aquifer provides freshwater for the citrus crops in the area which need at least two waterings during summer. In the Ahuir, salinity was significantly higher in July 2009. Analysis of all the sampling points in the receiving waters revealed no significant salinity differences among seasons at Venecia but salinity at the Serpis river plume sampling points was lower in spring.

In contrast, the submarine wastewater outfall discharges are lower from autumn to spring and higher in summer, when the tourist population increases and thus the wastewater that needs treatment. In summer, the higher flow of wastewater makes it sometimes necessary to discharge partially through the overflow channel located near the river mouth. A wastewater discharge through the overflow channel could explain the low salinity value, 1.3, measured in the Serpis river sample in July 2009 (Table 2) and the plume samples classified into freshwater cluster (Table 1) when river flow was null at the CHJ gauging stations, which are located 10 km from the river mouth. The lower dissolved oxygen concentration and the higher suspended solids measured in the receiving waters reinforce this hypothesis. In addition, significantly higher salinity values were observed in the submarine wastewater outfall area in this sampling campaign, thus indicating a reduced discharge.

The dominant nitrogen forms followed the expected spatial and seasonal pattern. In the Ahuir and Venecia areas, nitrate was the most dominant form due to agriculture runoff (both groundwater and surface runoff). At the submarine wastewater outfall, a major ammonium contribution was expected in the summer campaigns when the flow was greater due to the population increase. However, this major ammonium contribution was observed only in July 2008. A wastewater release through the overflow channel directly into the Serpis River in July 2009, and then a lower flow in the outfall outlet, could explain the nitrate dominance in this sampling campaign. In fact, samples from the Serpis River and river plume followed the ammonium gradient in this campaign (Fig. 5, grey triangles).

When the three areas were compared, the lowest salinity values, the highest nutrient concentrations (ammonium, DIN, DSi and DIP) and the highest chl-*a* concentration were observed in the Venecia area. In this area, seasonal variations were minimal. Despite the reduced summer discharges of the Serpis

River, no significant differences were observed in the DIN and DSi concentration between seasons, while the ammonium concentration was higher in July 2009 (both in the Serpis River and in the Venecia area). This was attributed partially to the sporadic releases of wastewater through the described overflow channel in the dry periods, which are an additional silica and nitrogen source. In addition, the high residence time of this semienclosed bay leads to higher nutrient accumulation and to greater exchanges with the sediment interface given the shallow water column depth (< 5 m). Silica dissolution at the sediment-water interface could be efficient during summer due to high temperatures and higher bacterial abundance, which has been pointed out as the cause of high silica levels in similar areas (Glé et al. 2008; Garmendia et al. 2010). In this area, phosphorus was always the potential limiting nutrient and phytoplankton composition and abundance has not shown any significant seasonal difference (except for the summer cyanobacteria increase and a minor chlorophyte contribution in July 2009).

In the Ahuir area, DIN and DSi concentrations were highest in April 2009 due to higher groundwater discharge, which is rich in both nitrates and silicates. A seasonal variation in the potential limiting nutrient was observed also: phosphorus was potentially limiting in spring, while a nitrogen potential limitation was found in summer. This summer nitrogen deficiency, which is linked to the decrease of freshwater inputs, has been observed in other coastal areas and has been attributed also to autotroph consumption (Glé et al. 2008). The highest DSi:DIN and DSi:DIP ratios were found in this area, so despite the summer silica depletion, silica was not the potential limiting nutrient. In this area ammonium and DIP concentrations were lower than in the Venecia and the submarine wastewater outfall areas; no direct wastewater discharge was found in the Ahuir, while the other areas received wastewater discharges, rich in both nutrients.

In the submarine wastewater outfall area (located 1.9 km from the coastline and 17.5 m water column depth), the dilution of treated wastewater with marine water results in phosphorus limitation, given that phosphorus has been identified as the most important limiting nutrient in the Mediterranean (Estrada 1996; Olivos et al. 2002).

Previous research conducted further north in the Balearic sea coastal area (NW Mediterranean) focused on the spatial gradient of nutrients and chlorophyll-*a* in relation to their distance from the land (sample stations were grouped into coastal field, middle field and far field) (Olivos et al. 2002). Using the Olivos et al. (2002) sampling point classification, our three study areas fall into the coastal field (approximately within the first 2 km from the coast). Average chl-*a* concentration in the 3 areas falls into the range observed by Olivos et al. (2002) in the coastal field. Olivos et al. (2002) highlighted the silicate limitation in more than 50% of the cases and explained it in terms of the excess of N and P overshadowing the Si abundance. In contrast, in our study area, Si-limited conditions were scarce and that allowed the persistence of the diatom population at high levels even in summer. The Safor wetland location over the detritic aquifer can explain the high silica groundwater discharges in our study area. Wetland species of *Gramineae* are characterized 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). Even if the natural vegetation surface has decreased in the last decades, it still remains an important soil use (Sebastiá et al. 2012). Thus, biogenic silica is an important element in the terrestrial biogeochemical cycle that must be considered in addition to the chemical weathering of land silicates.

The spatial gradient observed in phytoplankton biomass (total chl-*a*) both in spring and summer can be explained by the different factors controlling the dynamics of different phytoplankton groups, mainly nutrient availability and water exchange. In the Venecia area, the continued nutrient inputs and the reduced water exchange (high water residence time) explained the higher biomass, while in the submarine wastewater outfall area, despite the nutrient inputs, the dilution with low phosphorus marine waters determined the lowest chl-*a* values.

In the Ahuir and the submarine wastewater outfall areas, phytoplankton abundance and composition showed the typical seasonal cycle observed in other coastal areas. A shift from diatom-dominated communities and maximum phytoplankton biomass (total chl-*a*) in spring, to flagellate-dominated communities and minimum biomass in summer was observed (Garmendia et al. 2010). This can be observed in the RDA analysis where the stations sampled in spring (Fig. 5, black circles and squares) produced clearly different results from the stations sampled in summer (Fig. 5, white and grey circles and squares). Diatoms are recognized 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 nitrate, phosphorus and silica concentration, and with DIN:DIP ratio and their abundance was higher when these conditions prevailed (RDA, Fig. 5). The cyanobacteria, prasinophyte and to a minor extent prymnesiophyte biomass correlation with low nitrate concentration and low DIN:DIP ratio (RDA, Fig. 5) has been linked to more oligotrophic conditions that mainly happen in summer (dry period) in this area (Sebastiá et al. 2012; Sebastiá et al. unpublished results).

In the Venecia area, flow and salinity play a significant role in regulating phytoplankton communities, as it happens in other estuarine areas (Chan and Hamilton 2001). In the RDA analysis (Fig. 5) two situations can be observed that reflect freshwater inputs: 1) samples taken in April 2009 (black triangles) are linked to higher freshwater discharge after a rain episode with high silica concentration, and 2) samples taken in July 2009 (grey triangles) are linked to a wastewater release with high ammonium concentration. In these samples, both euglenophyte and chlorophyte biomass was higher. Chlorophytes are restricted to freshwater conditions (Chan and Hamilton 2001). A shorter time scale study (fortnightly) was conducted from May 2008 to August 2009 in this area (Sebastiá et al. unpublished results), which revealed that diatom relative abundance did not show the typical seasonal cycle of maximum abundances in spring and minimum abundances in summer. The key factor supporting high diatom abundance even in summer seems to be the continuous availability of silica, together with the optimal light and temperature conditions. This has also been observed in other estuarine areas, such as the Oka estuary (Bay of Biscay, Northern Spain) (Garmendia et al. 2010), which receive wastewater discharges from a treatment plant. However, just after wastewater inputs the flagellate abundance increases and replaces the diatom population, which explains the lowest diatom abundance observed in July 2009 according to the Kruskal-Wallis test.

Dinoflagellates were a minor group in terms of relative abundance and total biomass in the three studied areas. However, their importance should not be disregarded since the dinoflagellates identified in the microscope analysis, *Alexandrium* sp. and *Dinophysis caudata*, are potentially Paralytic Shellfish Poisoning (PSP) and Diarrheic Shellfish Poisoning (DSP) producers respectively (Vila et al. 2001). In addition, potentially toxic diatoms of the genus *Pseudo-nitzschia*, which synthesizes the ASP toxin, have been recorded inside the harbor (Sebastiá et al. 2012). Blooms of this genus have also been observed northerly in the western-Mediterranean (Vila et al. 2001). In the Mediterranean Sea, Harmful Algal Blooms (HABs) are a widespread problem and the proliferation of these harmful dinoflagellates (*Alexandrium* sp. and *Dinophysis* sp.) has been related with conditions of low turbulence and flushing rates (Vila et al. 2001; CIESM 2010). Semi-enclosed gulfs and bays, such as the Venecia area, near important harbors and big cities are at higher risk of suffering HABs (EEA 1999; Vila et al. 2001). In this sense, the construction of new harbors or the enlargement of existing ones may affect coastal hydrodynamics and then enhance these conditions (CIESM 2010).

In this study, we found that at the Ahuir and the submarine wastewater outfall areas the effects of freshwater inputs (groundwater and wastewater discharge respectively) were reduced due to a greater water exchange with the oligotrophic Mediterranean waters. On the other hand, the highest nutrient and chl-*a* concentrations were observed in the Venecia area, which is a semi-enclosed area. This agrees with the observations of Flo et al. (2011) for the north-western Mediterranean Sea. They highlighted that the dominance of urban land and the presence of river inflows are the primary source of coastal inshore waters (CIW, 200 m from the shoreline) variability; but their impact on coastal nearshore waters (200-1500 m from the shoreline, such as the Ahuir area) or coastal offshore waters (> 1500 m, such as the submarine wastewater outfall area) is minimal. In CIW, nutrient enrichment and eutrophication risk are higher in enclosed or semi-enclosed areas (Flo et al. 2011), such as the Venecia area in this study.

Harbors can create semi-enclosed areas partially due to the alteration of coastal morphology and water currents. This alteration has been pointed out as one potential environmental impact of harbors (Gupta et al. 2005; Peris-Mora et al. 2005; Darbra et al. 2009). Sea floor alteration has been selected as the only indicator for monitoring this impact in some environmental management plans (EMP) (Peris-Mora et al. 2005); while others include water quality parameters such as chlorophyll-*a* concentration, phytoplankton biomass and ratio of diatom/dinoflagellates (Xu et al 2004). In Sebastiá et al. (2012) it was stated that chl-*a* levels and phytoplankton composition typical of eutrophicated areas, together with bloom forming species, were mainly restricted to the Gandia Harbor area. In that study, just one point was monitored outside the Gandia Harbor and it was located in an open area. However, the results of this study show that the Venecia area, which is affected by the harbor's alteration of coastal morphology, is prone to suffer eutrophication problems (e.g. detection of harmful dinoflagellates). We suggest that monitoring of the above mentioned water quality parameters should be included in harbors' EMP. In that case, the choice of monitoring point's location should be a key issue and adjacent confined areas should be included. The research of Vila et al. (2001) reinforces this statement as they observed that "the monitoring of confined waters could be used as an early warning system for detection of algal blooms". The use of HPLC analysis and CHEMTAX could be used to monitor chl-*a* concentration, phytoplankton biomass and phytoplankton composition (Seoane et al. 2011). Though CHEMTAX cannot characterize species composition, it makes possible detecting changes in the relative composition of phytoplankton groups (e.g. a decrease in the ratio of diatom/dinoflagellates) that indicate a deterioration of water quality

(Seoane et al. 2011; Xu et al. 2004). In addition, the screening of representative samples (Higgins et al. 2011) with more specific analysis such as inverted microscopy will detect the presence of HABs species.

According to the Water Framework Directive monitoring program, the Gandia Harbor has been classified as a heavily modified water body and it will be studied as an individual case of study. However, the Venecia area is part of a bigger coastal water body, which goes from the Gandia Harbor to the San Antonio Cape (MARM 2009). As Flo et al. (2011) stated it is important that monitoring programs avoid overlooking environmental problems in localized areas. So, we suggest that the Venecia area classification should be revised as it could be classified as a transitional water body like other similar systems in this Mediterranean coast (e.g. Júcar river mouth, Cullera Estany (MARM 2009)).

5. Conclusions

In this study, the nutrient levels and the phytoplankton community of three Mediterranean coastal systems, characterized by distinctive main nutrient sources, have been compared. The results showed that the highest nutrient concentration and phytoplankton biomass were measured in the Venecia area, which is a semi-enclosed area. In this area potentially toxic dinoflagellates were detected, even though its abundance was minor. Two factors coincide in this area that may trigger dinoflagellates blooms: 1) the harbor enlargement will increase the vulnerability of this system and maybe provide the proper conditions of confinement, 2) the wastewater discharges through the overflow channel to the Serpis River will continue supplying phosphorus. Management measures should first target phosphorus inputs, as this is the most potentially limiting nutrient in the Venecia area and it comes from a point source that is easier to control.

Our recommendation is that for successful integrated management, local problems should be taken into account in regional monitoring programs. This is especially important in the Mediterranean Sea and in seas with similar characteristics (e.g., low tidal ranges) (EEA 1999; Flo et al. 2011). More specifically, harbor environmental management plans should include regular monitoring of water quality in adjacent waters to identify adverse phytoplankton community changes. The use of HPLC analysis and CHEMTAX could help to do so at a reasonable cost.

Acknowledgments

We would like to thank Simon Wright and Harry Higgins for providing CHEMTAX software and helpful comments. We would like also to thank Mikel Latasa for his useful help with the CHEMTAX program. We are very grateful to the anonymous reviewers whose accurate revision contributed to improve the original manuscript. This research was supported by the Universitat Politècnica de València.

References

Aminot A, Chaussepied M (1983) Manuel des analyses chimiques en milieu marin. CNEXO, Brest, p. 396.

Angelidis MO, Kamizoulis G (2005) A Rapid Decision-Making Method for the Evaluation of Pollution-Sensitive Coastal Areas in the Mediterranean Sea. Environ Manage Vol. 35, No. 6: 811–820.

APV (2009) Memoria Anual 2009. Autoridad Portuaria de Valencia. http://www.valenciaport.com. Accessed 12 October 2011.

APV (2011) Anteproyecto de ampliación del puerto de Gandía revisado con las medidas correctoras propuestas por la Dirección General de Sostenibilidad de la Costa y el Mar.

Artioli Y, Friedrich J, Gilbert AJ, McQuatters-Gollop A, Mee LD, Vermaat JE, Wulff F, Humborg C, Palmeri L, Pollehne F (2008) Nutrient budgets for European seas: A measure of the effectiveness of nutrient reduction policies. Mar Pollut Bull 56: 1609-1617.

Ballesteros-Navarro BJ (2003) Estado y evolución de los procesos de intrusión marina en la unidad hidrogeológica 08.38. Plana de Gandía-Denia (Valencia-Alicante, España). In: Lopez Geta, JA, de la Orden, JA, Gomez, JD, Ramos, G, Mejías, M, Rodríguez, L, Ed., Tecnología de la intrusión de agua de mar en acuíferos costeros: países mediterráneos. IGME, Madrid, pp. 585-595. ISBN. 84-7840-470-8.

Cartaxana P, Mendes CR and Brotas V (2009) Phytoplankton and ecological assessment of brackish and freshwater coastal lagoons in the Algarve, Portugal. Lakes and Reserv: Res and Manage 14: 221–230.

CEDEX (1997) Atlas de Clima Marítimo. Dpto. Clima Marítimo (Puertos del Estado) - Centro de Estudios de Puertos y Costas.

CIESM (2010) Phytoplankton responses to Mediterranean environmental changes. Nº 40 in CIESM Workshop Monographs. F. Briand Ed., 120 pages, Monaco.

Chan TU and Hamilton DP (2001) Effect of freshwater flow on the succession and biomass of phytoplankton in a seasonal estuary. Mar Freshwater Res 52 (6): 869-884.

Conley DJ (2002) Terrestrial ecosystems and the global biogeochemical silica cycle. Glob Biogeochemical Cycles 16 (4): 1121

Darbra RM, Pittam N, Royston KA, Darbra JP and Journee H (2009) Survey on environmental monitoring requirements of European ports. J Environ Manage 90: 1396-1403.

Duarte CM, Marrasé C, Vaqué D and Estrada M (1990) Counting error and the quantitative analysis of phytoplankton communities. J Plankton Res 12: 295–304.

EEA (European Environment Agency) (1999) State and pressures of the marine and coastal Mediterranean environment. Environmental Assessment Series No 5, 137 pp.

Estrada M (1996) Primary production in the northwestern Mediterranean. Scientia Marina, 60 (supl.2): 55-64.

Fisher TR, Peele EH, Ammerman JW, Harding Jr LW (1992) Nutrient limitation of phytoplankton in Cheasepeake Bay. Mar Ecol Prog Ser 82: 51-63

Flo E, Garcés E, Manzanera M, Camp J (2011) Coastal inshore waters in the NW Mediterranean: Physicochemical and biological characterization and management implications. Estuarine, Coastal and Shelf Science 93: 279-289.

Garmendia M, Revilla M, Bald J, Franco J, Laza-Martínez A, Orive E, Seoane S, Valencia V and Borja A (2010) Phytoplankton communities and biomass size structure (fractionated chlorophyll “a”), along trophic gradients of the Basque coast (northern Spain). Biogeochemistry DOI: 10.1007/s10533-010-9445-2.

Glé C, Del Amo Y, Sautour B, Laborde P, Chardy P (2008) Variability of nutrients and phytoplankton primary production in a shallow macrotidal coastal ecosystem (Arcachon Bay, France). Estuar Coast Shelf S 76: 642-656.

Grasshoff K (1983) Determination of oxygen. In: Grasshoff K, Ehrhardt M, Kremling K Eds., Methods of Seawater Analysis. Verlag Chemie, Weinheim, 419.

Gupta AK, Gupta SK, Patil RS (2005) Environmental management plan for port and harbour projects. Clean Techn Environ Policy 7: 133–141

Hermosilla J (2005) Los riegos de la Safor y la Valldigna. Agua, Territorio y Tradición. Ed. Dirección General de Patrimonio Cultural Valenciano. Conselleria de Cultura y Educación. Generalitat Valenciana. Valencia, p. 239.

Higgins HW, Wright SW and Schlüter L (2011). Quantitative interpretation of chemotaxonomic pigment data. In: "Phytoplankton pigments: characterization, chemotaxonomy, and applications in oceanography", Roy S, Llewellyn CA, Egeland ES and Johnsen G, eds. p: 257-313. Cambridge University Press, Cambridge, UK. ISBN-13: 978-1-107-00066-7

Hooker SB, Claustre H, Ras J, Van Heukelem L, Berthon JF, Targa C, van der Linde D, Barlow R, Sessions H (2000) The JRC Method. In: Hooker SB, Firestone ER (Eds.), The First SeaWiFS HPLC Analysis Round-Robin Experiment (SeaHARRE-1). NASA Technical Memorandum 2000-206892, Vol. 14. NASA Goddard Space Flight Center, Greenbelt, MD, p. 42.

IET (2010) Balance del turismo en España en 2009. http://www.iet.tourspain.es. Accessed 12 October 2011

INE (2009) Hotel industry and tourism. http://www.ine.es. Accessed 12 October 2011

Latasa M (2007) Improving estimations of phytoplankton class abundances using CHEMTAX. Mar Ecol Prog Ser 329: 13–21

Latasa M, Scharek R, Vidal M, Vila-Reixach G, Gutiérrez-Rodríguez A, Emelianov M, Gasol JM (2010) Preferences of phytoplankton groups for waters of different trophic status in the northwestern Mediterranean Sea. Mar Ecol Prog Ser 407: 27-42.

Mackey MD, Mackey DJ, Higgins HW, Wright SW (1996) CHEMTAX – a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Mar Ecol Prog Ser 144: 265–283.

Maier G, Nimmo-Smith RJ, Glegg GA, Tappin AD and Worsfold PJ (2009) Estuarine eutrophication in the UK: current incidence and future trends. Aquatic Conservation: Mar Freshw Ecosyst 19: 43-56.

Mantecón A and Huete R (2007) The role of authenticity in tourism planning: Empirical findings from southeast Spain. Tourism 55 (3): 323-333.

MARM (2009) Documento técnico de referencia: identificación y delimitación de masas de agua superficial y subterránea. Ámbito territorial de la Confederación Hidrográfica del Júcar. http://www.phjucar.com/docs/DTR/DTR_Masas_CHJ_Ed10.pdf. Accessed 7 May 2012

Mösso C, Sierra JP, Rodilla M, Romero I, Falco S, González del Río J, Sánchez-Arcilla A (2008) High vertical resolution sampling in density interfaces of estuaries and river plumes. Estuar Coast 31: 258–268.

Olivos A, Masó M, Camp J (2002) Continental runoff of nutrients and their possible influence over stoichiometric ratios (DIN:P:Si) in the northeastern Mediterranean waters of Spain (Catalan Sea). Cienc Mar 28: 393-406.

OSPAR (2003) OSPAR Integrated Report 2003 on the Eutrophication Status of the OSPAR Maritime Area Based upon the First Application of the Comprehensive Procedure. OSPAR Commission: London.

Paerl HW (2006) Assessing and managing nutrient-enhanced eutrophication in estuarine and coastal waters: Interactive effects of human and climatic perturbations. Ecol Eng 26: 40–54

Paerl HW, Rossignol KL, Hall SN, Peierls BL, Wetz MS (2010) Phytoplankton Community Indicators of Short- and Longterm Ecological Change in the Anthropogenically and Climatically Impacted Neuse River Estuary, North Carolina, USA. Estuar Coast 33: 485-497.

Peris-Mora E, Diez Orejas JM, Subirats A, Ibáñez S, Alvarez P (2005) Development of a system of indicators for sustainable port management. Mar Pollut Bullet 50, 1649–1660

Sánchez-Arcilla A, Mösso C, Mestres M, Cupul L, Sierra JP, Rodilla M, Romero I and González del Rio J (2007) Hydrodynamics of a Coastal Bay. Natural and Man-made Barriers. J Coast Res 47:2-16.

Schlüter L, Lauridesn TL, Krogh G and Jørgensen T (2006) Identification and quantification of phytoplankton groups in lakes using new pigment ratios – a comparison between pigment analysis by HPLC and microscopy. Freshw Biol 51: 1474–85.

Sebastiá M-T, Rodilla, M, Sanchis, JA, Altur, V, Gadea, I, Falco, S (2012) Influence of nutrient inputs from a wetland dominated by agriculture on the phytoplankton community in a shallow harbour at the Spanish Mediterranean coast. Agric Ecosyst and Environ 152: 10-20.

Seoane S, Garmendia M, Revilla M, Borja A, Franco J, Orive E and Valencia V (2011) Phytoplankton pigments and epifluorescence microscopy as tools for ecological status assessment in coastal and estuarine waters, within the Water Framework Directive. Mar Pollut Bull 62: 1484-1497.

Smith VH, Tilman GD, Nekola JC (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environ Pollut 100: 179–196.

Ter Braak CJF (1994) Canonical community ordination. Part I: Basic theory and linear methods. Ecoscience 1: 127-140.

Ter Braak CJF, Smilauer P (2002) CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (Version 4.5). Microcomputer Power, Ithaca, New York.

United Nations Environment Programme/World Health Organization (1999) Identification of priority pollution hot spots and sensitive areas in the Mediterranean. Mediterranean Action Plan Technical Reports Series. No. 124. UNEP, Athens, 102 pp.

Utermohl M (1985) Zur Vervollkommnung der quantitative Phytoplankton-Methodik. Mitteilungen internationale Vereingung fur theoretische und angewandte Limnologie 9: 1–38

Vera JF and Ivars JA (2003) Measuring Sustainability in a Mass Tourist Destination: Pressures, Perceptions and Policy Responses in Torrevieja, Spain. *In*: BRAMWELL, B. (ed.), *Mediterranean Tourism: Impacts and Policy Responses*. J. Sustainable Tourism, 11(2-3): 181-203.

Vidal M, Duarte CM, Sánchez MC (1999) Coastal eutrophication research in Europe: progress and imbalances. Mar Pollut Bull 38: 851–854.

Vila M, Camp J, Garcés E, Masó M and Delgado M (2001) High resolution spatio-temporal detection of potentially harmful dinoflagellates in confined waters of the NW Mediterranean. J Plankton Res 23 (5): 497-514.

Whitall D, Bricker S, Ferreira J, Nobre AM, Simas T, Silva M (2007) Assessment of Eutrophication in Estuaries: Pressure–State–Response and Nitrogen Source Apportionment. Environ Manage 40: 678–690.

Wright SW, Thomas DP, Marchant HJ, Higgins HW, Mackey MD, Mackey DJ (1996) Analysis of phytoplankton of the Australian sector of the Southern Ocean: comparisons of microscopy and size frequency data with interpretations of pigment HPLC data using CHEMTAX matrix factorization program. Mar Ecol Prog Ser 144: 285–98.

Wright SW, Jeffrey SW, Mantoura RFC, Llewellyn CA, Bjoernland T, Repeta D, Welschmeyer N (1991) Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. Mar Ecol Prog Ser 77: 183.

Xu FL, Lam KC, Zhao ZY, Zhan W, David ChenY, Tao S (2004) Marine coastal ecosystem health assessment: a case study of the Tolo Harbour, Hong Kong, China. Ecol Model 173: 355–370.

Yepes V and Medina JR (2005) Land Use Tourism Models in Spanish Coastal Areas. A Case Study of the Valencia Region. J Coast Res 49: 83-88.

Fig. 1 Study area and sampling points location. Sampling point code: first character corresponds to the sampling site (A – Ahuir, V – Venecia (Serpis River outflow), O – submarine wastewater outfall)

Fig. 2 Gandia harbor as it is now (left) and the projected enlargement (right)

Fig. 3 Synthetic graph of DSi:DIN:DIP molar ratios in the coastal waters of Gandia. In each area delimited Redfield ratios (DSi:DIN:DIP = 16:16:1), the potential limiting nutrients are reported in order of priority. Sample symbol correspond to the sampling site (circle –Ahuir, triangle – Venecia, square – Outfall). Sample symbol colors correspond to the season and year (white – summer 2008, black – spring 2009, grey summer – 2009)

Fig. 4 Relative contribution of each phytoplankton group as estimated with CHEMTAX. The average value for each study area and sampling campaign is represented. Dinoflagellates, cryptophytes, prasinophytes, chlorophytes, and euglenophytes were grouped as flagellates

Fig. 5 Correlation plots of the redundancy analysis (RDA), on the relationship between the environmental variables (grey arrows), the biomass of the phytoplankton groups (black arrows) and samples. Sample symbol correspond to the sampling site (circle –Ahuir, triangle – Venecia, square – Outfall). Sample symbol colors correspond to the season and year (white – summer 2008, black – spring 2009, grey summer – 2009)