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Additional Information

Long-term study of seasonal changes in phytoplankton community structure in the western Mediterranean (Valencian Community)

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1. INTRODUCTION

Phytoplankton is a key component of ocean, sea, and freshwater basin ecosystems. Its production and community composition is closely related to water quality, biogeochemical processes, and the trophic web (Cloern et al. 2014). Studying the composition of the phytoplankton community is of interest since it impacts on the functioning of aquatic ecosystems and global climate (Litchman and Klausmeier 2008). Furthermore, the abundance and community structure fluctuations have profound effects on higher trophic levels and major biogeochemical cycles (Falkowski et al. 1998).

The methods conventionally used to study this shifts in community structure have principally focused on the diatom and dinoflagellate groups, while the smaller planktonic groups—for example the Cryptophyceae or the Prasinophyceae (pico and nanoplankton)—have been overlooked, customarily being included in the group of “other flagellate” (Cerino and Zingone 2006).

This group includes organisms that are the dominant primary producers in vast areas of oligotrophic oceans, although they may also become important in coastal ecosystems (Morán, 2007). Buitenhuis et al. (2012) compiled data on picophytoplankton abundance from several zones with different traits, covering

the North Atlantic, the Antarctic Ocean, the Mediterranean Sea, the South Pacific, the Arabian Sea, the Indian Ocean, the Sargasso Sea, the Equatorial Pacific, the Red Sea, the Tropical Atlantic, the Sea of Japan and the Caribbean Sea. With regard to the Mediterranean Sea, Sammartino et al. (2015) pointed out that picophytoplankton is generally predominant, especially in areas with low nutrient concentrations. Thus, both pico and nano plankton contribute significantly to the bulk of marine phytoplankton (Chen and Liu 2010) and it is important to properly characterise their contribution to the whole phytoplankton community.

General phytoplankton trends establish a bimodal cycle, with an initial peak usually occurring at the end of winter or early spring, and a second peak at the end of summer and early autumn (Casas et al. 1997; Ribera D'Alcalà et al. 2004; Winder and Cloern, 2010). To identify the underlying factors that govern this phytoplankton community assembly and dynamics (both on temporal and spatial scales) is one of the main goals for coastal water assessment when an ecosystem approach is to be adopted. Furthermore, as is well known, this is one of the innovative approaches of the European Water Framework Directive (2000/60/EC), which considers water quality as a function of biological indicators, one of which is phytoplankton composition. For this reason phytoplankton composition dynamics need to be properly characterised prior to any management plan.

Temperature is one of the major environmental factors that governs the distribution and composition of phytoplankton (Moisan et al. 2002; Edwards and Richardson, 2004; Boyd et al. 2013). Photosynthesis, respiration, growth, resource acquisition, motility, and sinking all depend on temperature (Litchman et al. 2008; Reynolds 2006). As major groups of phytoplankton have different temperature optima (Reynolds 2006), temperature also determines phytoplankton seasonal succession in both marine and freshwater habitats (Alvain et al. 2008; Boyd et al. 2013). Another important environmental factor is nutrient content. Macro nutrients, such as nitrogen and phosphorus, and some micro nutrients, such as iron and zinc, determine growth rates and affect the population dynamics.

Most of the environmental factors affecting phytoplankton (composition and abundance) may be modified by human activities in coastal areas. As stated in Micheli et al. (2013), climate (temperature), fisheries, shipping, inorganic pollution, fertilisers, coastal population density, pesticides, and oil spills, among others, are anthropogenic drivers of impacts on the sea. All but the climatic drivers have direct correspondence with environmental status mandated by the Marine Strategy Framework Directive (2008/56/EC).

The Mediterranean Sea ecosystems have been threatened by historical pressures and are threatened by current ones (Lejeusne et al. 2010), leading to major shifts in marine ecosystems and widespread conflict between marine users (Claudet and Fraschetti 2010). In fact, the Mediterranean is characterised as a sea “under siege” (Micheli et al. 2013). Today, the human impact on these ecosystems is still high and is manifested by changes in the hydromorphological characteristics and nutrient loads, among others, which may affect phytoplankton abundance and composition.

In order to assess how the human impact may affect phytoplankton community composition a comprehensive analysis focused on whole ecosystems is needed. It is important to first establish the natural seasonal variability of the plankton community, then to identify the alterations related to human activities in the coastal region.

The coastal waters of the Valencian Community (VC) have not been studied with this approach of taxonomical phytoplankton traits, nor have they been related with anthropogenic pressure. That is why this study represents the first attempt to analyse the general phytoplankton seasonal patterns on the western Mediterranean coast (VC). In addition, it seeks to acquire a deep understanding of the vulnerable coastal ecosystem of VC anthropogenic stressors such as nutrient inputs from human activities and physical factors through the study of a five-year data set (2005–2010).

2. MATERIAL AND METHODS

2.1 Study Area

The study was carried out in the coastal waters of the Valencian Community in eastern Spain. The stretch of coastline (476 km) was divided into several zones (Figure 1) based on a holistic approach. To this end the geomorphology, littoral transport, dominant winds, the area of the fluvial basins, continental inputs, and wet zones were taken into account (Romero et al. 2013).

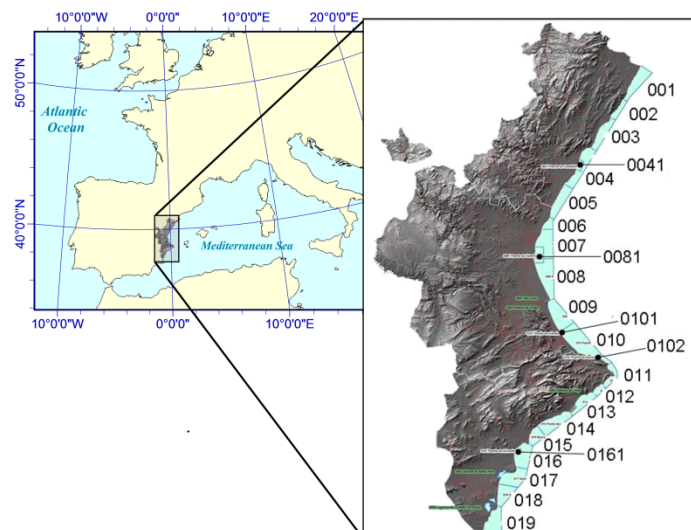


Fig. 1. Location of the 23 zones defined along the coastline of the Valencian Community

2.2 Sampling

The sampling campaigns were performed monthly over a period of five years (from August 2005 to December 2010). The sampling stations were distributed along the coast with three to six stations in each zone. The study period included 64 sampling campaigns and involved 6757 water samples. At each station the samples were taken from the water column at a depth of 10 cm and beyond the wave breakpoint, to avoid extracting samples from areas where the effect of wave motion might generate sediment resuspension and, in turn, affect the biochemical quality of the water. Temperature was measured in situ using a multiparametric probe YSI (6600V2). Samples for nutrients and salinity were collected in 2 L plastic bottles, kept at -4 °C and taken to the laboratory within 12 h of collection. To determine the phytoplankton composition, the samples were stored in glass bottles and fixed in situ with glutaraldehyde (2% final concentration) according to Sournia (1978) for phytoplankton analyses.

The solar irradiance values were obtained from the National Agrometeorological Network (SiAR) (Spanish Ministry of Agriculture, Fisheries and the Environment, 2018).

2.3 Sample analysis

The samples were filtered through 0.45 µm membrane filters (Millipore HAWP) for nutrient analyses. Ammonium, nitrite, nitrate, reactive soluble phosphorus (SRP), total phosphorus, and orthosilicic acid were analysed by an Alliance Instruments Integral Futura auto-analyser, following the methodology described by Treguer and Le Corre (1975), and taking into account Kirkwood et al. (1991) and Parsons et

al. (1984). Salinity was measured with Portasal Guildline 8410 A, calibrated with the appropriate standards (I.A.P.S.O. Standard Seawater, Ocean Scientific International Ltd, $K_{15} = 0.99986$, $S = 34.995\%$). Phytoplankton analyses were carried out by filtering the samples through a $0.2 \mu\text{m}$ membrane filter and then drying the filtered material. Salt was removed by adding 5 mL of distilled water and then filtering and drying the samples again. The material on the filter was then dehydrated by successive washings with 50%, 80%, 90% and 99% aqueous ethanol. Each dried filter was placed on a drop of immersion oil in the centre of a slide, and 2 more drops were then added (Pachés, 2012). Finally, a coverglass was placed on top of the filter. Algal counts were made by epifluorescence microscopy (Vargo, 1978) with a Leica DM2500 microscope, using a $100\times$ oil-immersion lens. A minimum of 300 cells were counted and at least 100 cells of the most abundant species or genera were counted with an error below 20% (Lund et al. 1958).

2.4 Statistical methods

Multivariate statistical analysis of Principal Component Analysis (PCA) was applied to experimental data in order to arrange the taxonomic groups of phytoplankton in response to the environmental variables. The PCA is a useful statistical tool to determine the underlying information and patterns from a multidimensional data set in order to group it into one with fewer named components. The statistical package SPSS (version 16.0) was used to perform this analysis. The Varimax rotational technique was applied to facilitate easier interpretation. Kaiser-Meyer-Olkin (KMO) and Bartlett's tests were also used to check the suitability of the data. The first, KMO, measures sampling adequacy to indicate whether the partial correlations between items are low. A high value, close to 1, generally indicates that PCA may be suitable. The second, Bartlett's test, indicates whether the correlation matrix is an identity matrix, which in turn demonstrates that the factor model is inappropriate. P-values lower than the significance level(0.05) indicate significant differences. The variables included in the matrix were: nutrients (ammonium (NH_4), nitrite (NO_2), nitrate (NO_3), total phosphorus (TP) and orthosilicic acid (SiO_4), temperature, salinity, solar radiation, and the main phytoplankton groups observed along the Valencian Community coastline during the five-year study period in all the sampling stations.

3. RESULTS

3.1 Temporal variability of temperature and solar radiation

The temporal variability of temperature and solar radiation throughout the study period are shown in Figure 2. As can be seen in Figure 2a, the temperature variations in the subsurface layer of the water column followed a clear sinusoidal pattern, with minimum and maximum monthly averages of 12.1 and 28.4 °C, respectively. The lowest mean value was recorded in February 2010 and the highest in July 2006. The highest and lowest punctual temperatures in the study period were reached in 2007 (32 and 7.9 °C respectively). The thermal amplitude recorded for the five-year period varied between 19.7 °C in 2009 and 24.1 °C in 2007. Figure 2b, shows that solar radiation also exhibited a sinusoidal pattern with minimum and maximum monthly averages of 7 MJ·m⁻² in December and 23 MJ·m⁻² in July (Fig 2 b). Between February and March, the increase in solar radiation was highest (mean values of 7 MJ·m⁻²), while the greatest reduction was found between August and September (mean values of 6 MJ·m⁻²).

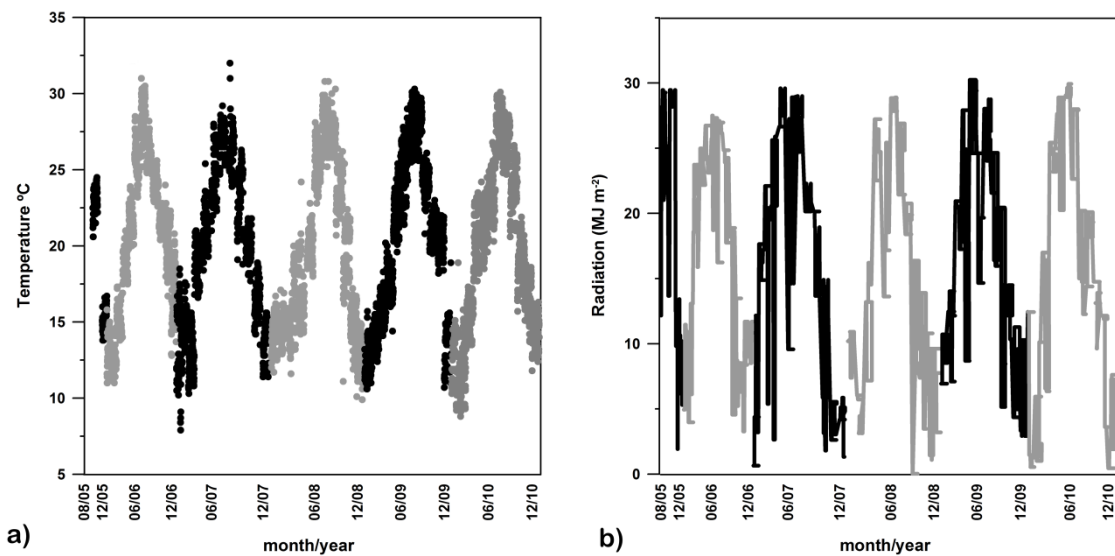


Fig.2. (a) Sub-superficial temperature of water column and (b) solar radiation in the Valencian Community coastal waters (2005–2010)

3.2 Temporal variability of nutrient content /human pressure

Temporal nutrient variability throughout the studied period is shown in Figure 3. As can be seen, there was no seasonal pattern in nutrient release to the coast, but rather there were some zones that were highly stressed by nutrient inputs. Ammonium exhibited high variability, both temporal and spatial. The highest mean ammonium values by zones were found in 161 and 007, at 38 ± 22.8 and 14 ± 25.7 μM respectively. The former receives the inputs from the port of Alicante and the latter is a coastal zone that is affected by the city of Valencia and its metropolitan area. The port of Valencia (zone 0081) also exhibited a high

value ($11 \pm 15.3 \mu\text{M}$) and in these three areas the 90th percentile for ammonium varied between 20–88 μM .

Another form of nitrogen essential for the phytoplankton community is nitrate, which may principally come from the use of fertiliser in the intensive agriculture of the region. The zones displaying the highest values were 007 and 005 (139.6 ± 105.3 and 132.8 ± 60.2 respectively).

Phosphorus is the nutrient with the highest variability along the coast (204.2% variation coefficient). There were two zones with high medium values: zones 007 and 0161 (2.79 ± 2.04 and 3.19 ± 1.67 respectively). As occurred with ammonium and nitrate, zone 007 suffers the impact of diverse anthropogenic activities (agriculture, industrial, urban,) and this is also reflected in phosphorus level. This makes this zone one of the most stressed in the entire VC littoral. Beside this, zone 0161 is also affected by human pressure, although in this case it is due to the port and a desalination plant and sewage outfalls close to it.

With regard to orthosilicic acid, its medium values were $4.69 \pm 8.5 \mu\text{M}$. As occurred for nitrate, the zone displaying the highest medium value was 005, ($17.1 \pm 6.3 \mu\text{M}$) which is highly affected by agricultural pressure. Zones 007 to 009 also exhibited high orthosilicic acid values due to the freshwater inputs (or even intensive agriculture runoff, riverine discharge).

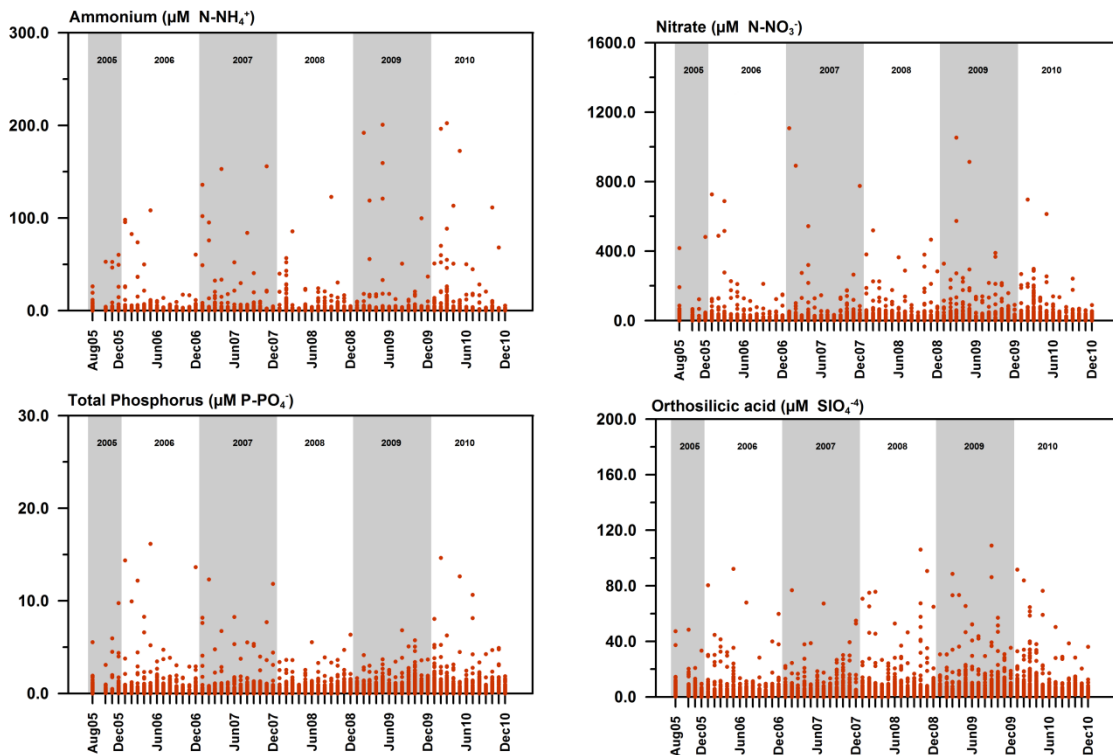


Fig. 3. Spatial variation of ammonium, nitrate, phosphate and orthosilicic acid in the coastal waters of the Valencian Community (2005–2010)

3.3 Temporal variability of phytoplankton groups

Figure 4 shows the abundance (cell L⁻¹) of the main groups of phytoplankton identified for the whole study period along the coast of the Valencian Community.

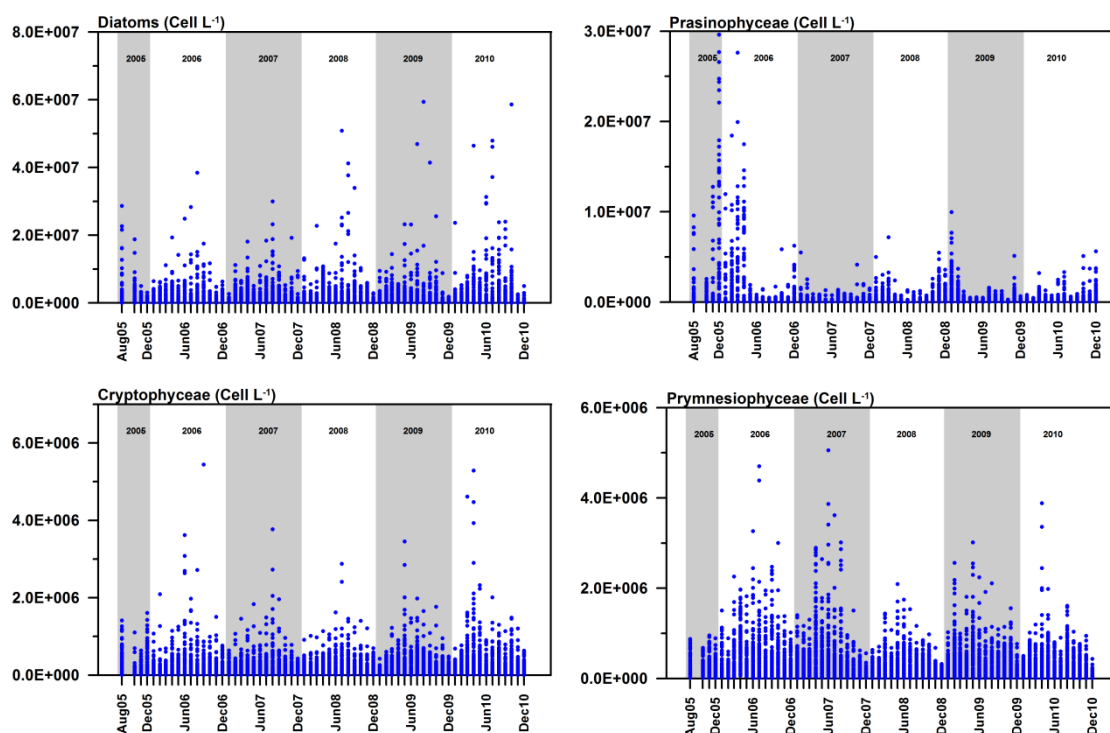


Fig.4. *Diatoms*, *Cryptophyceae*, *Prasinophyceae* and *Prymnesiophyceae* cell abundance in the coastal waters of the Valencian Community (2005–2010)

Diatoms are the most abundant group in the phytoplankton community, with a range from 6.03×10^3 to 5.9×10^7 cell L⁻¹ (Figure 4). On average, this group contributes around 50% of the assemblage composition. Mean maximum values for the five-year period were reached in April and August ($2.5 \pm 0.9 \times 10^6$ cell L⁻¹ and $2.4 \pm 0.6 \times 10^6$ cell L⁻¹ respectively). The most abundant genera found were *Chaetoceros*, *Cyclotella*, *Nitzschia*, *Pseudonitzschia*, *Asterionella*, *Thalassionema* and *Skeletonema*, which make up the bulk of the diatom population that rapidly succeed and overlap each other in the system. The temporal pattern for this group establishes two periods of maximum abundance (seasonally variable). The first peak is in early spring (April) with mean values of $2.55 \pm 0.9 \times 10^6$ cell L⁻¹ with a second lower peak at the end of summer (July–August) with $2.44 \pm 0.6 \times 10^6$ cell L⁻¹. In these periods, this group contributes more than 50% of the

community, whereas in winter this value drops to 40%. This pattern was repeated throughout the entire study series and from 2008 onwards the maximum values increased progressively. The sections of the coast where the spring peak was most evident were located in the area from the north of the coast of Valencia to the south of Gandía (zones 005 to 009). However, the second peak of abundance (summer peak) was observed especially in areas close to the ports, such as the port of Valencia (zone 0081) and the port of Alicante (zone 0161). *Prasinophyceae* are the second most abundant phytoplankton group along the Valencian coast. This group contributed on average 14% of the total community, with cell densities ranging from 1.0×10^3 cell L⁻¹ to 30.0×10^6 cell L⁻¹. The *Prasinophyceae* present in the samples belonged mainly to the genera *Tetraselmis*, *Pseudoscorufieldia* and *Ostreococcus*. The temporal pattern showed an increase in abundance that began at the end of autumn (October–November) and reached a maximum in winter (December, January) when it formed up to 25% of the whole phytoplankton community. These values remained more or less constant until the spring season. This unimodal pattern was repeated throughout the study period on a regular basis. The coastal zones where this behaviour was clear were zones 006 to 009, all located in the province of Valencia. However, as can be seen in Figure 4, in 2005 this group reached exceptionally high abundance values. In fact, in August 2005 the maximum value exceeded those recorded in the winters of the following years. These high values were not limited to a specific area but were found along the entire coastline. The factor responsible for this phenomenon, which appeared in 2005 and the following years, was the massive flowering of organisms of the genus *Ostreococcus*, which dominated the composition of the planktonic community for a few months, attaining 95% of the variability. Two peaks were recorded for this genus in the three years in which blooms occurred, the first in December, reaching up to 40×10^6 cell L⁻¹ and dominated the eukaryotic population with percentages higher than 95%, and the second in March. Although this last bloom was detected in fewer stations (zones 007, 008, and 0081), it sometimes reached abundance values similar to those found in December, with a maximum of 35×10^6 cell L⁻¹. This was the first time that *Ostreococcus* had been identified in coastal water of the VC, which was also repeated in the following years, although the bloom detected was smaller than that of 2005. The *Cryptophyceae* group was also present in the phytoplankton community with abundances ranging from 0.75×10^3 to 5.4×10^6 cell L⁻¹ representing on average 10% of the total community. The most frequent genera found were *Chroomonas* and *Cryptomonas*. The population of *Cryptophyceae* at the beginning of spring rose to almost 30%, with the first peak of abundance in April (average values of $2.9 \pm 1.6 \times 10^5$ cell L⁻¹). These values remained more or less

constant until summer, when they represented almost 12% of the total community. When this period ended, abundance dropped until it reached a minimum. This pattern stayed constant throughout the study period, although from 2007 onwards, the maximum abundance of this taxon was higher. The sections of the coast where this rise was most significantly experienced were in the Valencia coastal oval (zones 005 to 009). The last group with a high contribution was the *Prymnesiophyceae*, which were present throughout the year with average percentages ranging from 12.7 to 28.7%. The abundance of this group ranged from 1.27×10^3 cell L⁻¹ to 5.05×10^6 cell L⁻¹. The main *Prymnesiophyceae* genera found were *Phaeocystis* and *Primnesium*. The temporal pattern followed by this group included a steady increase of abundance in spring (April–May) (mean values, $2.55 \pm 2.8 \times 10^5$ and $4.42 \pm 0.96 \times 10^5$ cell L⁻¹, respectively), maintaining relatively constant values until the beginning of summer. After the summer, the concentration decreased to minimum values in November–December ($2.47 \pm 0.87 \times 10^5$ and $1.69 \pm 0.92 \times 10^5$ cell L⁻¹, respectively). This pattern was repeated in all the years analysed, with the highest values in 2007 and the minimum in 2010. Once again, the areas where these values were found during the spring were in the Valencia coastal oval (zones 005 to 009) and two areas of southern Alicante (zones 018 and 019). Figure 5 shows the abundance (cell L⁻¹) of the less representative phytoplankton groups identified throughout the study period.

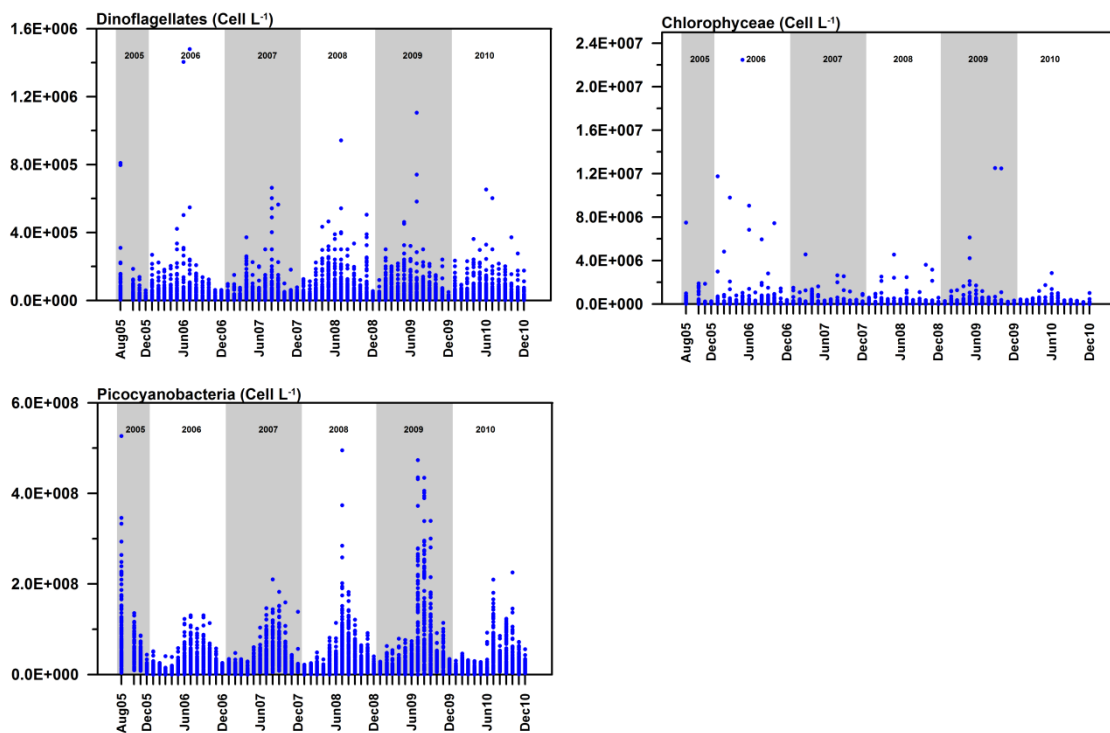


Fig.5. Dinoflagellates, Chlorophyceae and Cyanophyceae (*Synechococcus* spp.) cell abundance in the coastal waters of the Valencian Community (2005–2010)

As can be seen in Figure 5, *Dinophyceae* and *Chlorophyceae* were the least commonly found phytoplankton groups in these waters. Nano and picoplanktonic dinoflagellates that appear on coasts did not exceed 15% in normal conditions. Their relatively slow growth rates mean the dinoflagellates only made a small contribution (cell density between 1.0×10^3 - 1.48×10^6 cell L⁻¹) to the whole community. The dinoflagellates are a complex group and during the study period they exhibited a weak pattern that was repeated every year. Between April and May their abundance increased ($5.67 \pm 1.9 \times 10^4$ cell L⁻¹ average value), then stabilised at a relatively constant value until August ($5.76 \pm 1.6 \times 10^4$ cell L⁻¹). After the summer period the values dropped to the yearly minimum in December. Unlike the rest of the groups analysed, the greater or lesser abundance of dinoflagellates had a marked spatial character, with zone 008 (Port of Valencia-Cullera Cape) and its adjacent zone having the highest registered abundance values. It should be noted that this area is affected by the city of Valencia, in fact by the entire southern area of the city. *Chlorophyceae* were also present in the phytoplankton community, with an average abundance of 5% and cell densities ranging from 1.0×10^3 to 2.24×10^7 cell L⁻¹. The main genera found were *Scenedesmus*, *Ankistrodesmus* and *Pediastrum*. Unlike the other phytoplankton groups studied, *Chlorophyceae* did not seem to have an evident temporal pattern, since high values were found throughout the entire period. The maximum abundance values were recorded in areas between the north coast of Valencia and Gandia (zones 007 to 009). And lastly, the picocyanobacteria group, formed mainly by the genus *Synechococcus*, forms part of the prokaryotic plankton, with much smaller cell sizes than the rest of the groups studied (picoplankton). As can be seen in Figure 5, the picocyanobacteria had a marked temporal pattern; from May onwards, abundance rose to a maximum in July–August $6.28 \pm 2.5 \times 10^7$ and $7.17 \pm 3.8 \times 10^7$ cell L⁻¹, respectively. After the summer period, the concentrations dropped to minimum values in December–January $1.49 \pm 0.08 \times 10^7$ and $1.06 \pm 0.3 \times 10^7$ cell L⁻¹, respectively. This pattern was found to be quite regular throughout the years analysed, with the maximum abundance values in the summers of 2005, 2008, and 2009 (in the Valencia coastal oval, i.e., zones 005 to 010).

3.4 PCA analysis

A PCA model was fitted to the pre-processed data. Five PCs were significant according to cross-validation, with a cumulative variation explained by fitting 0.62. The KMO value was 0.701, which exceeds the recommended value of 0.6 (Hair et al. 2006; Kaiser, 1974) and the Bartlett's Test of

Sphericity was significant ($P < 0.05$). These results indicate that the sampled data set was suitable for proceeding with a PCA.

The results of the fitted model are displayed in Figure 6 in the form of a loading plot that shows how the different variables relate to each other (i.e., correlated variables are close in the plot). The first factor (with an explained variance of 22.5%) is related to nutrients (nitrate, ammonium, nitrite and orthosilicic acid) in the positive part of the axis and inversely with salinity (negative part of the first axis). The second factor (15.2%) is related to temperature and solar radiation. The environmental variables that explain the greatest variability are temperature (0.778), salinity (0.841), orthosilicic acid (0.76) and radiation (0.732).

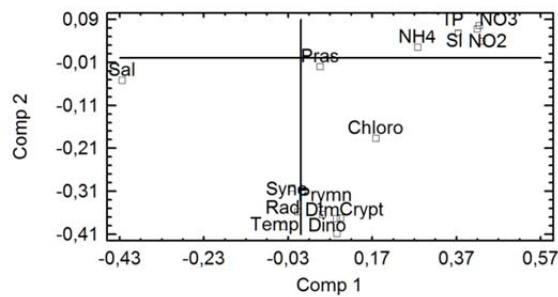


Fig. 6. Loadings of the principal components analysis (PCA) for all data

4. DISCUSSION

The analysis of the five-year period revealed that the composition of the phytoplanktonic community in these waters was formed mainly of the *Diatomeas*, *Prasinophyceae*, *Cryptophyceae*, *Prymnesiophyceae*, *Dinoflagellates*, *Chlorophyceae* and picocyanobacteria groups, although the seasonal composition and timing of the maximum was different for each group.

Diatoms, which often dominate along the coast, were the only group showing a bimodal pattern, with an early spring peak followed by another in late summer. These results agree with the findings of Ribera Ribera D'Alcalà et al. (2004) for this phytoplankton group in the Gulf of Naples (Mediterranean Sea), who also found bimodal patterns (winter–early spring and autumn) driven by different factors, although in our case the second peak was in mid-summer. This summer growth phase was also affected by other factors and was related to zones influenced by proximity to harbours. These harbour zones were able to provide enough nutrients to promote a second phytoplankton growth phase, since they maintained adequate nutrient levels for rapid phytoplankton proliferation at the end of the summer. The same main

genera of Diatoms previously found along the Mediterranean coast (Ribera D'Alcalà et al. 2004) were identified.

Cryptophyceae is a eurihaline group characterised by a wide environment spectrum (Hoef-Emden, 2014) that forms part of the community throughout the year although in spring its abundance increases until summer. Our result supports the findings of Cerino et al. (2006), who found that this group is present in aquatic systems throughout the whole yearly cycle in the Mediterranean and Tyrrhenian Seas. Nevertheless, this temporary pattern is altered (being able to reach high abundance percentages) when there are point source discharges along the coastline. The tolerance of this group to low light intensity and the ability to obtain organic nutrients through phagotrophy (Jones 2000; Lepistö and Holopainen 2003) makes this group the most abundant in zones with submarine outfalls and harbours. In fact, in zone 010 (Port of Gandia-Cape San Antonio), with high anthropogenic pressures by offshore sewage outfall, rivers, irrigation ditch and ravine discharge, this group reached up to 93% of the abundance.

The *Prymnesiophyceae* are mainly nanoplankton difficult to identify by traditional methods (Utermöhl). However, since they have a broad temperature spectrum, the contribution of this taxon to the total phytoplankton community was assured throughout the year. In fact, there was no clear seasonal pattern for this group, since its cell density is relatively constant all year round (smallest variation coefficient 1.2%). Nevertheless, the cell density increased slightly in early spring and kept its values until the end of the summer. Although in general terms this group is abundant in the VC coastal waters, a deeper analysis revealed that its abundance percentage was higher in areas with no terrestrial runoff—i.e., in natural (unaffected) zones like zones 001 to 004 (20.0%), and in zones 012 to 015 (23.3%) and zone 019 (22.6%). This fact may be due to the higher surface/volume ratio exhibited by nanoplankton cell size that allow greater nutrient uptake, thus boosting the growth rate of this microalgae, which can outcompete other groups in terms of abundance (Chisholm, 1992). This makes some *Prymnesiophyceae* genera of special importance in oligotrophic aquatic ecosystems. In other coastal water zones with an anthropogenic nutrient supply this group was abundant—although not in percentage terms—indicating that although nutrient inputs can enhance cell growth, groups better adapted to high nutrient level contribute more to the community composition.

Prasinophyceae displayed the opposite behaviour, predominating mostly in winter. The changes in the abundance of this group did not seem to be subject to variations in solar radiation or temperature, but to continental contributions. The *Ostreococcus* genus has also been detected in the Alborán Sea (southwest

Mediterranean Sea) (Díez et al. 2001) although in most studies its abundance has been insignificant and defined as sporadic or occurring under specific conditions (Marie et al. 2006). Our results do not match these findings, since the presence of *Ostreococcus* was not at all sporadic in the zones examined in the present study. In fact, in all the years sampled, this genus was detected along the entire coast. The *Ostreococcus* bloom in 2005 was unexpected, and thus adds another layer of complexity to phytoplankton seasonal dynamics. Furthermore, it seemed that this group did not show a clear temporal pattern, but was instead more related to continental contributions. Actually, some authors have related the occurrence of this bloom to the selenium concentration in the water (Palenik et al. 2007). Therefore, further studies are needed on the presence of this metal in waters to complete the understanding of its ecological patterns. The picocyanobacteria (*Synechococcus*) group exhibited the clearest and best-defined pattern. This phytoplankton group responded positively to temperature rises and showed a clear unimodal pattern. Picocyanobacterias have also been found to respond positively to temperature in other studies performed on a mesocosmo scale (Sommer and Lengfellner 2008). Furthermore, picoplankton shows higher assimilation rates (and growth rates) than do nano or meso plankton, obtaining most advantage in coastal areas that can become hypertrophic. Nevertheless, some phytoplankton groups do not follow seasonal patterns, clearly suggesting that other environmental factors underlie their abundance. One example is the relative contribution of *Chlorophyceae* to the whole phytoplankton community, which is strongly controlled by freshwater input (Reynolds et al. 2002). This was the only group found in an appreciable percentage in areas with a strong continental influence, such as coastal zone 009, which receives inland waters from the Almenara, Albufera and Estany plumes and the Júcar River.

The statistical model applied (PCA) to analyse the environmental variables (temperature, radiation and nutrients) explained 62% of the variability. The first axis (salinity -0.746, nitrate 0.665, ammonium 0.350, orthosilicic acid 0.693) represents a gradient from low saline brackish water with a high nutrient content to an oligotrophic salty sea water.

The second component provided us with information on seasonal variations since it is highly correlated with temperature and solar radiation (0.335 and 0.339 respectively). The main phytoplankton groups are distributed along these two axes and most of them (Diatoms, Dinoflagellate, *Cryptophyceae*, and picocyanobacteria (Syn)) are closely related to each other as well as to temperature and solar radiation. This indicates that for these groups the (more or less marked) temporal pattern was probably triggered by both factors—i.e., increased solar radiation and temperature values. Since they are located in the middle

of the first axis, they can appear along the saline gradient. Nevertheless, *Prasinophyceae* and *Chlorophyceae* did not show this marked seasonal pattern determined by solar radiation and temperature but seemed to be more related to brackish waters of continental origin with high nutrient content.

Although the response of phytoplankton to nutrients is one of the major concerns of aquatic ecology, physical factors and seasonal gradients derived from anthropogenic activities may better explain its distribution and abundance. Due to the activities being carried out close to the coast (fisheries, shipping inorganic pollution, fertilisers, coastal population density, and pesticides) coastal waters are not nutrient-limited in terms of N or P. As nutrients do not limit phytoplankton growth, other factors become important and essential in the dynamics of the system, such as temperature or light.

In fact, these floristic trends have not been attributed to a single nutrient, but to a combination of temperature, solar irradiation, and nutrient input, salinity gradient, etc. Temperature and solar radiation have conventionally been proposed as major limiting factors in northern aquatic ecosystems, while phytoplankton growth has been said to be limited by nutrient availability along the Mediterranean coast. Our results suggest that although there is a nutrient gradient related to land inputs, phytoplankton abundance also greatly responds to increases in temperature and irradiance. When these phenomena occur, since nutrients are available along the coastline, phytoplankton dynamics undergo changes. Diatoms and *Cryptophyceae* respond to this, due to their efficient growth rates and enhanced nutrient uptake rates (Niemi et al. 2004). The same occurs with picocyanobacteria, although *Chlorophyceae* and *Prasinophyceae* do not seem to be closely related to these phenomena (temperature and solar radiation increase), but to others such as freshwater inputs or inorganic element discharges. The areas where this behaviour can be best seen were in the Valencia coastal oval, which is significantly affected by anthropic activity (tourism, industry and agriculture) with the hydrodynamic alterations caused by harbours (zones 0081, 0101, and 0102), and also the dilution because of the limited coastal geomorphology, ensuring an appreciable nutrient (N and P) contribution. This was corroborated by the nutrient concentration data along the coast, where the SRP concentration was always at the limit of detection, indicating that the P was being rapidly taken up by the microalgae. The five-year series examined may be a good starting point to discern phytoplankton patterns, since the details of the entire seasonal cycle along the VC coastline had not been adequately studied until now. The patterns described in this study cannot be defined as classical. In fact, the characteristic of the coastal zones, the influence of terrestrial runoff and the effect of anthropogenic activities yield annual phytoplankton patterns are far removed from the classical pattern

(Smayda, 1980). However, the specific patterns described in this coastal area seem to reoccur every year around the same dates, despite the natural inter-annual differences in the five-year period. Temporal changes in the water column in temperature and solar radiation were the main environmental forces that governed phytoplankton composition in these coastal aquatic ecosystems replete with nutrients.

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