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Phytoplankton functional groups and harmful algal species in anthropogenically impacted waters of the NW Mediterranean Sea*

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SUMMARY: Phytoplankton assemblages were analysed by multivariate analysis techniques in highly modified waterbodies (harbours) of the northwestern Mediterranean Sea over an annual cycle. The phytoplankton functional groups that characterise these anthropogenically impacted waters were identified and related to HAB events. Four functional groups can be distinguished from the analysis: (1) *bloom-forming dinoflagellates*, (2) *winter diatoms*, (3) *summer-autumn diatoms*, and (4) *large dinoflagellates and elongated diatoms*. The species that characterise these functional groups occurred at all sampling stations. Potentially harmful species are distributed through all the clusters. However, *bloom-forming dinoflagellates* occur particularly in large harbours and during summer months. The results indicate that: (1) functional groups indicate where and when there is a high risk of blooms, but they cannot determine the risk of a toxic or fish-killing event; and (2) large harbours in tideless seas must be considered as areas with a high risk of high-biomass bloom occurrences.

Key words: phytoplankton assemblages, functional groups, toxic dinoflagellates, Mediterranean Sea, HAB.

RESUMEN: GRUPOS FUNCIONALES DE FITOPLÁNCTON Y ESPECIES TÓXICAS O NOCIVAS EN AGUAS DEL MEDITERRANEO NORDOCCIDENTAL IMPACTADAS ANTRÓPICAMENTE. – Se presentan los resultados de un analisis multivariante de asociaciones fitoplanctónicas en aguas confinadas (puertos) del Mediterráneo nordoccidental a lo largo de un ciclo anual. El objetivo del estudio es identificar los grupos funcionales de fitopláncton que caracterizan aguas impactadas por efectos antrópicos y determinar su relación con las incidencias de especies tóxicas o nocivas (HAB). Se han identificado cuatro grupos funcionales: (1) *dinoflageados formadores de proliferaciones*, (2) *diatomeas de invierno*, (3) *diatomeas de verano-otoño*, y (4) *dinoflagelados y diatomeas de gran tamaño*. En todas las estaciones muestreadas se detectan las especies que caracterizan los cuatro grupos funcionales. Sin embargo, los *dinoflageados formadores de proliferaciones* aparecen principalmente en puertos grandes y durante los meses de verano. Los resultados indican que: (1) Los grupos funcionales indican donde y cuando hay riesgo de proliferaciones, pero no pueden determinar el riesgo de un determinado evento tóxico. Y (2) los puertos grandes situados en mares con escasa amplitud mareal deben considerarse con un elevado riesgo de proliferaciones de elevada biomasa.

Palabras clave: asociaciones fitoplanctónicas, grupos funcionales, dinoflagelados tóxicos, mar Mediterráneo.

INTRODUCTION

Since the 1980s, an increase in Harmful Algal Bloom (HAB) events has been noted on a world-scale (Anderson 1989; Smayda 1990; Hallegraeff 1993), including Mediterranean waters (Honsell *et*

al., 1995; Vila *et al.*, 2001a). Currently, HAB events are recognised as one of the main problems of coastal areas that affect the environment and coastal populations in many different ways. They lead to fauna death and the deterioration of water quality, and can have direct or indirect negative effects on human health (GEOHAB 2003). Anthropogenic influences such as nutrient over-enrichment of

coastal waters, transport of harmful phytoplankton species via ballast-water vessels, and the translocation of shellfish stocks (Hallegraeff, 1998; Caroppo *et al.*, 1999; Velikova *et al.*, 1999; Moncheva *et al.*, 2001) are considered to be the primary cause of the increase. The increase in man-made sheltered waters, as in harbours, jetties, etc. to provide protected embayments for vessels in tideless seas, has scarcely been considered in a similar manner (Vila *et al.*, 2001a, c). In tourist destinations such as the Mediterranean coastline, there is a current trend towards an increase in sheltered water areas through construction of harbours, jetties and groins, and an increasing number of restricted-flow embayment areas. Although this type of shoreline development is global, there are major efforts in some Mediterranean areas that are now a major concern. For example, in Catalonia (NW Mediterranean coast) the number of harbours has increased from 12 in 1950 to 46 in 2003. These harbours are located along 575 km of coast, or approximately one harbour every 12.5 km, or even less in some places. More harbours are being built, so the mean distance between harbours will decline in the future.

From the late 1980s to the present, the number of HAB monitoring programmes has dramatically increased throughout the world (EUROHAB, 2002). However, most of them focus on just a few harmful species. This approach considerably reduces the predictability and understanding of the factors responsible for the blooms locally and fails to explain the current global increase in HABs (Estrada *et al.*, 2003). Phytoplankton functional groups have been analysed in association with physical structures (Estrada *et al.*, 1999; Pitcher *et al.*, 2000; Roegner *et al.*, 2002), and have been related to HAB species in upwelling systems (Fraga *et al.*, 1988; Figueiras, 1994; Estrada, 1995). The relationship between anthropogenic activities and changes in phytoplankton composition and diversity is one of the main objectives proposed in HAB research (De Vries, 1998; Glibert *et al.*, 2001; Anderson *et al.*, 2002). Long time series on phytoplankton communities have recently been used to analyse HAB increase related to anthropogenic forcings (Solic *et al.*, 1997; Beliaeff *et al.*, 2001; Annevielle *et al.*, 2002). However, the phytoplankton community approach has some handicaps: e.g. an important fraction of the species in natural samples cannot be identified and counted with routine methods, and also, one species name may hide a number of cryptic species (Zingone *et al.*, 2003). Also, the availability of time

series is scarce or inexistent in numerous regions where the anthropogenic pressure is highly significant. The purpose of the present paper is to analyse the spatial variability of the phytoplankton composition in an area where modification of the coastline's morphology is the most dramatic anthropogenic impact. The approach consists of examining phytoplankton functional groups by means of multivariate statistical techniques. The principal taxa of concern are potentially toxic species for humans (Paralytic shellfish poisoning (PSP), Diarrhetic shellfish poisoning (DSP), Amnesic shellfish poisoning (ASP) and Neurotoxic (NTX) producing species) as well as fish killers and other potentially bloom-forming dinoflagellates and diatoms. Functional groups are defined as non-phylogenetic associations of organisms that are based on physiological, morphological or other features that respond to recurrent patterns or factors similarly (Margalef, 1978; Gitay and Noble, 1997). This is the premise of the following text: the predictability of HAB is substantially increased when the focus is based on the identification of functional groups rather than on the identification of species.

MATERIAL AND METHODS

During 1996, phytoplankton species composition from nine different stations along the Catalan coast was analysed. Stations were selected to include: (1) anthropogenically impacted (harbour) areas, and (2) area coverage across the coast. These sites included eight harbours representing a range of sizes and a highly anthropogenically affected beach (near Barcelona City) (Fig. 1). Those harbours greater than 15 ha. were considered large whereas those less than 15 ha. were considered small. The harbours' properties and activities are presented in Table 1.

Weekly sampling was conducted from May to October and twice a month from November to April. Associated temperature and salinity were measured with a mercury thermometer and a microprocessor conductivity meter WTW Model LF197. Bucket phytoplankton samples (150 ml) were taken at the surface from piers in each harbour. The non-confined station was sampled at the surface from the coast (near-shore waters, 0.5 m depth). Samples were preserved with formaldehyde (1% final concentration). A 50 ml aliquot was settled in a counting chamber for 1 day. For phytoplankton enumeration, the appropriate area of the chamber was
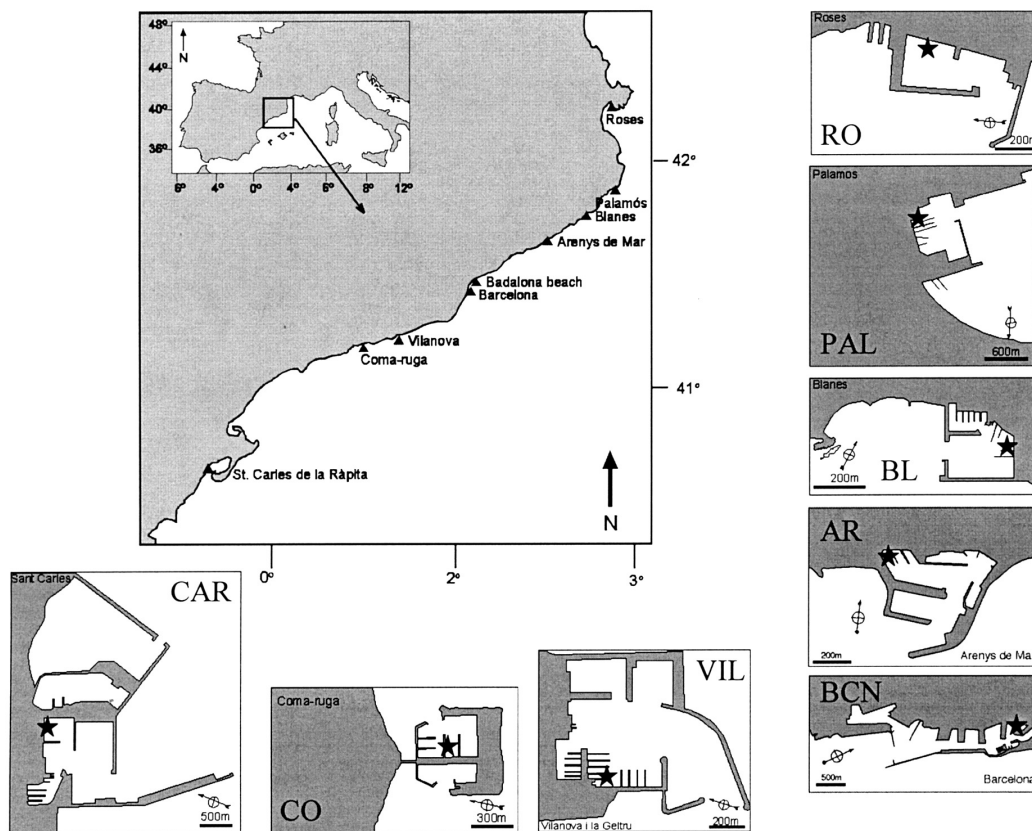
FIG. 1. – Study area showing the location of the nine stations. One beach (Badalona) and eight harbours are indicated as: RO: Roses; PAL: Palamós; BL: Blanes; AR: Arenys; BCN: Barcelona; VIL: Vilanova; CO: Coma-ruga; CAR: Sant Carles de la Ràpita.

scanned at 63-400X magnification using a Leica-Leitz DM-Il inverted microscope (Throndsen, 1995), depending on the abundance of each species. Usually at least three transects were scanned at 100X, one transect at 400X, and the complete chamber at 63X. The minimum concentration that can be detected by this method is 20 cells l^{-1}. The entire phytoplankton community was identified to species or genus according to Tomas (1997), Daugbjerg *et al.* (2000), and the IOC list (2002); when identification was impossible, the different taxa were grouped into categories such as "small unidentified dinoflagellates" or "centric diatoms". Ciliates, tintinnids and rotifers were grouped as microzooplankton.

Similarities and differences within the phytoplankton community were analysed through the classical taxonomic approach (list of species, taxonomic ratios, abundance, diversity index, seasonal patterns) and also by means of Principal Component Analysis (PCA, STATISTICA for Windows, StatSoft, Tulsa). Margalef's and Pielou's evenness diversity index were calculated by means of

TABLE 1. – Sampling stations ordered from north to south, type of station (harbour or beach), type of each harbour (C-commercial, F-fishing, M-marina), and surface area. Harbours larger than 15 ha are considered large while those smaller than 15 ha are considered small.

Station	Code	Lat.	Long.	Type of station	Type of harbour	Surface area (ha.)	Size
Roses	RO	42°15'0" N	3° 10'0" E	Harbour	FM	12	small
Palamós	PAL	41°50'5" N	3° 07'1" E	Harbour	CFM	5	small
Blanes	BL	41°40'3" N	2° 47'8" E	Harbour	FM	6	small
Arenys de Mar	AR	41°34'3" N	2° 33'3" E	Harbour	FM	17	large
Badalona	BAD	41°26'5" N	2° 15'7" E	Beach			
Barcelona	BCN	41°26'4" N	2° 10'2" E	Harbour	CFM	114	large
Vilanova	VIL	41°12'3" N	1° 43'7" E	Harbour	CFM	55	large
Coma-ruga	CO	41°10'5" N	1° 30'8" E	Harbour	M	2	small
Sant Carles de la Ràpita*	CAR	40°36'6" N	0° 36'4" E	Harbour	CFM	17	large

* This harbour is situated in a bay

TABLE 2. – Main characteristics of the taxa used in the multivariate analysis. The most common synonym of some species is indicated between brackets. The Code gives the key to the species names used in Figure 5. Taxonomic group (Tax Group) refers to dinoflagellates (DINO), diatoms (DIAT), coccolithophorids (COCO), flagellates (FL), silicoflagellates (SFL), and ciliates (CIL); Trophic State (auto-, hetero- or mixotrophic); Cell Type refers to individual cells (i), chain-forming or colonial cells (c); Harmful refers to the potentially harmful effect: Paralytic shellfish poisoning (PSP), Diarrhetic shellfish poisoning (DSP), Amnesic shellfish poisoning (ASP), Neurotoxic (NTX), fish-killing and bloom-forming (bf; cell concentrations over 10^6 cells l^{-1}; an asterisk (bf*) indicates known bloom-forming species but blooms were not observed in 1996). Habitat Type refers to the dinoflagellate habitat classification proposed by Smayda and Reynolds (2001). Np: number of occurrences in one year (total = 327 samples); %Np: percentage of total; Av.>0: averaged concentration (cells l^{-1}), calculated from frequency which cells were detected; Max.: Maximum cell concentration (cells l^{-1}).

Taxa	Code	Tax Group	Trophic State	Cell Type	Harmful	Habitat Type	Np	%Np	Av. (>0)	Max
Alexandrium minutum	AMIN	DINO	auto	i	PSP, bf	I	77	24	32238	2.0E+06
Ceratium furca	CFUR	DINO	mixo	i		VI-VIII	92	28	69	400
Ceratium fusus	CFUS	DINO	mixo	i	Fish-killing	III	66	20	58	360
Dinophysis rotundata	DROT	DINO	mixo	i	DSP	VII	30	9	149	1500
Dinophysis sacculus	DSAC	DINO	mixo	i	DSP	VII	103	31	852	40950
Akashiwo sanguinea (=*Gymnodinium splendens*)	GSPL	DINO	auto/mixo	i	bf*		55	17	111	910
Gyrodinium cf. *spirale*	GYRO	DINO	hetero	i			143	44	1371	116935
Gymnodinium impudicum (=*Gyrodinium impudicum*)	GIMP	DINO	auto	c	bf*		48	15	33207	388143
Heterocapsa sp.	HETE	DINO	auto	i	bf*	II	117	36	9349	481770
Prorocentrum micans	PMIC	DINO	auto	i	bf*	II	203	62	2949	76440
Prorocentrum minimum	PMIN	DINO	auto	i	NTX, bf*	II	86	26	4074	165620
Prorocentrum triestinum	PTRI	DINO	auto	i	bf	II	185	57	65504	4.0E+06
Protoperidinium diabolum	PDIA	DINO	hetero	i			70	21	95	910
Protoperidinium spp. small	PRTP	DINO	hetero	i			164	50	984	15453
Scrippsiella spp.	SCRI	DINO	auto	i	bf	II	196	60	31335	1.7E+06
Asterionellopsis glacialis	AGLA	DIAT	auto	c	bf*		74	23	14217	177905
Cerataulina pelagica	CPEL	DIAT	auto	c	bf		111	34	31482	1.8E+06
Coscinodiscus spp.	COSC	DIAT	auto	i			58	18	53	455
centric diatom sp. 1	CEN	DIAT	auto	i	bf		107	33	24722	1.0E+06
Chaetoceros curvisetus+ pseudocurvisetus	CHCP	DIAT	auto	c			70	21	8879	85540
Chaetoceros lorenzianus	CHLO	DIAT	auto	c			59	18	1831	10010
Chaetoceros spp. large (>20 μm)	CHG	DIAT	auto	c			72	22	6412	94640
Chaetoceros spp. small	CHP	DIAT	auto	c	bf		132	40	70424	2.7E+06
Dytilum brightwellii	DBRI	DIAT	auto	i			45	14	1012	13195
Eucampia zodiacus	EZOD	DIAT	auto	c			31	9	3030	17745
Lauderia sp.	LAUD	DIAT	auto	c			48	15	4868	41405
Leptocylindrus danicus (+*minimus*)	LDAN	DIAT	auto	c	bf		180	55	26916	1.8E+06
Cylindrotheca closterium+ Nitzschia longissima	NCL	DIAT	auto	i	bf		260	80	10483	487301
Odontella spp. (*Biddulphia*)	ODON	DIAT	auto	c			98	30	646	22295
Pleurosigma spp.	PLEU	DIAT	auto	i			206	63	132	9100
Proboscia alata	PALA	DIAT	auto	c			54	17	313	2275
Pseudo-nitzschia spp.	PSEU	DIAT	auto	c	ASP, bf		198	61	29889	1.5E+06
Rhizosolenia cf. *imbricata*	RIMB	DIAT	auto	c			76	23	3312	44590
Dactyliosolen fragilissimus (=*Rhizosolenia fragilissima*)	RFRA	DIAT	auto	c			45	14	4642	61812
Guinardia striata (=*Rhizosolenia stolterfothii*)	RSTO	DIAT	auto	c			41	13	548	2727
Skeletonema costatum	SKCO	DIAT	auto	c	bf		107	33	111373	5.0E+06
Thalassionema nitzschioides	THNI	DIAT	auto	c	bf*		159	49	5902	230230
Thalassiosira spp.	THAL	DIAT	auto	c	bf*		94	29	21932	447372
Syracosphaera pulchra	SYRP	COCO	auto	i			29	9	973	4550
Eutreptiella gymnastica	EUTR	FL	auto	i	bf*		175	54	12853	325611
Dictyocha fibula	DFIB	SFL	auto	i	Fish- killing		52	16	558	1365
Mesodinium cf. *rubrum*	MESO	CIL	mixo	i	bf*		126	39	6300	299061

PRIMER 5 for Windows (Plymouth Routines In Multivariate Ecological Research). PCA was based on the correlation matrix of all data collected over an annual cycle at all nine stations (327 samples) and 42 species as descriptors (from a total of 171 taxa identified) (Table 2). Cell densities were logarithmically transformed prior to analysis. Those taxa which had been lumped into the broadest categories as well as those that only occasionally appeared in samples (<9%) were excluded.

In order to interpret the PCA derived axes, correlation coefficients were calculated between the values of the factor scores of the samples for the first three axes and relevant parameters such as taxonomic groups, diversity index, or other factors that could affect phytoplankton distribution. A K-means
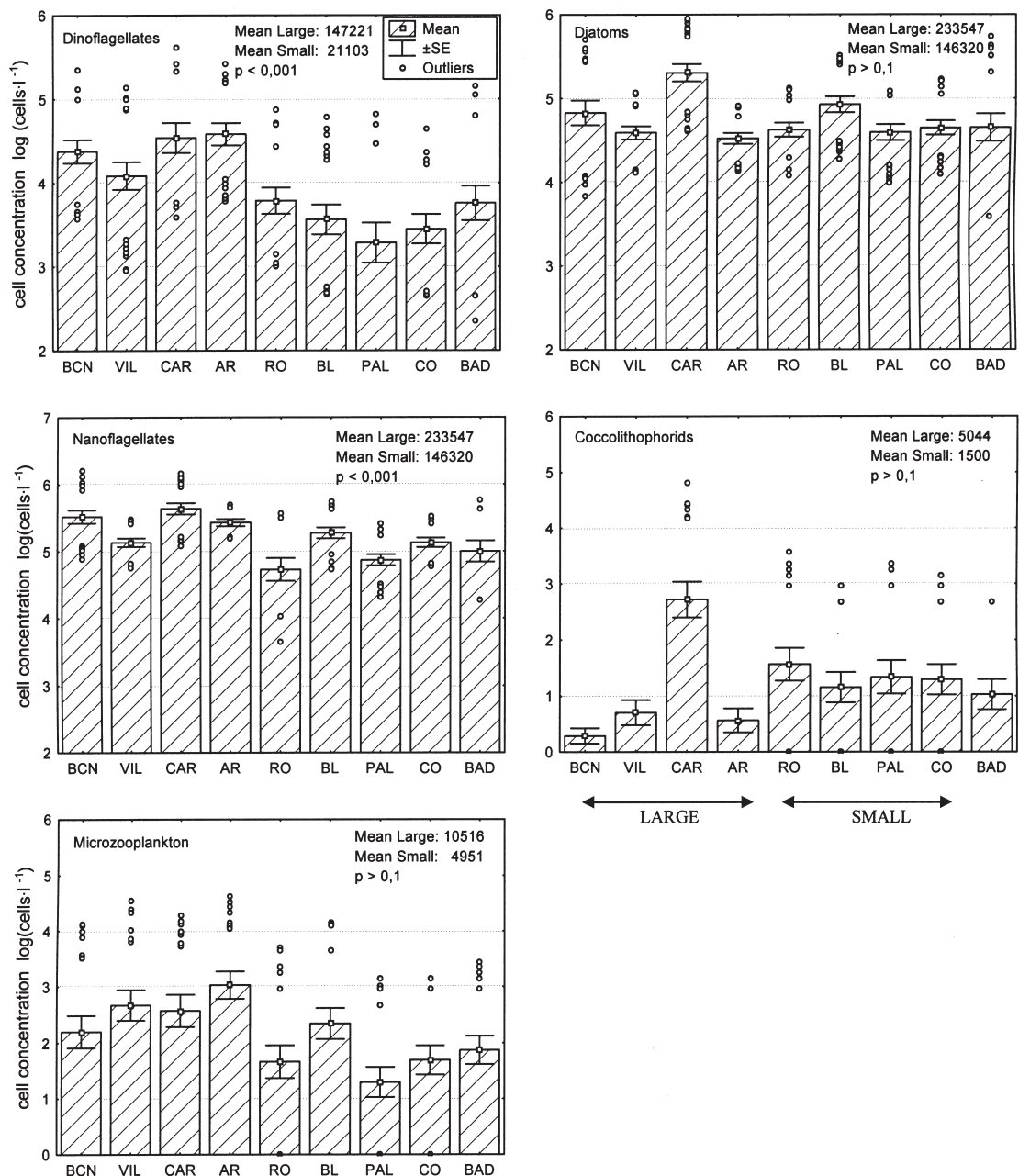
FIG. 2. – Annual average concentrations (±SE) of dinoflagellates, diatoms, nanoflagellates, coccolithophorids and microzooplankton at each of the stations from north to south (station codes as in Table 1). The results of the t-student analysis comparing cell concentrations in large harbours vs. small ones are indicated.

clustering (Euclidean distances) was applied to the PCA factor loadings (3 first axes) to identify groups of species objectively (k=4).

RESULTS

Spatio-temporal distribution

General patterns are shown in annual concentrations of dinoflagellates, diatoms, coccolithophorids, nanoflagellates and microzooplankton for each of the stations studied (Fig. 2). The average annual concentration of dinoflagellates and nanoflagellates was significantly higher in large harbours than in small ones (t-student, p<0.001). The microzooplankton showed the same tendency although it was not significant. The average annual concentration of diatoms was high everywhere (10^4-10^5 cells l^{-1}), with no significant differences between the stations. The concentration of coccolithophorids was low compared to that of the other groups, and the maxi-

mum concentrations were detected in the sourthern-most harbour (St. Carles).

The seasonal variability of dinoflagellates and diatoms is shown in Figure 3, where five selected harbours are arranged from north to south. In general, maximum dinoflagellate concentrations were detected during spring and summer. However, some latitudinal gradient is apparent. Maximum concentrations were noted in the southernmost harbours (St. Carles, Coma-ruga and Vilanova) in spring-early summer, whereas in the northern harbours (Blanes and Roses), maximum concentrations were noted in summer. Dinoflagellate concentrations were relatively low during winter (10^3-10^4 cells l^{-1}) and did not reach higher concentrations than 10^5 cells l^{-1}, except for St. Carles where *Gyrodinium corsicum* reached 43000 cells l^{-1} in January. There was no clear seasonal pattern for diatoms. However, if the relative abundance of both groups is examined, dinoflagellates predominated during the warm months, whereas the relative concentrations of diatoms were highest during the rest of the year (Fig. 4).

In the 327 samples counted, 171 taxa were identified. The most common and abundant species among the thecate dinoflagellates were *Scrippsiella* spp., *Prorocentrum triestinum, P. minimum, P. micans,* small *Protoperidinium* spp. and *Heterocapsa* sp. Other species such as *Ceratium furca,* and *C. fusus* were frequently recorded but cell concentrations were low (max. 400 cells l^{-1}). The non-thecate *Gyrodinium* cf. *spirale* was recorded year-round and attained the highest cell concentrations (max. 10^5 cells l^{-1}) during the summer. *Alexandrium minutum* (PSP-producer) was also observed year-round but its highest concentrations (>10^5 cells l^{-1}) occurred in winter and spring. *Dinophysis sacculus* (DSP-producer) and *Gymnodinium impudicum (=Gyrodinium impudicum)* bloomed in spring and summer. *Alexandrium catenella* (PSP-producer) was detected for the first time in the study area in 1996, particularly in the Barcelona harbour during the summer months. *Prorocentrum rhathymum* and *Pratjetella medusoides* were frequently detected during autumn. The later is a rarely reported species which exceptionally was abundant along the Catalan coast in 1996 (pers. obs.). *Gyrodinium corsicum* (fish-killing species) was only recorded at one harbour in the southern area (St. Carles harbour) and reached its highest concentrations during the winter-spring months.

The most commonly recorded and abundant diatoms were *Asterionellopsis glacialis, Chaeto-*
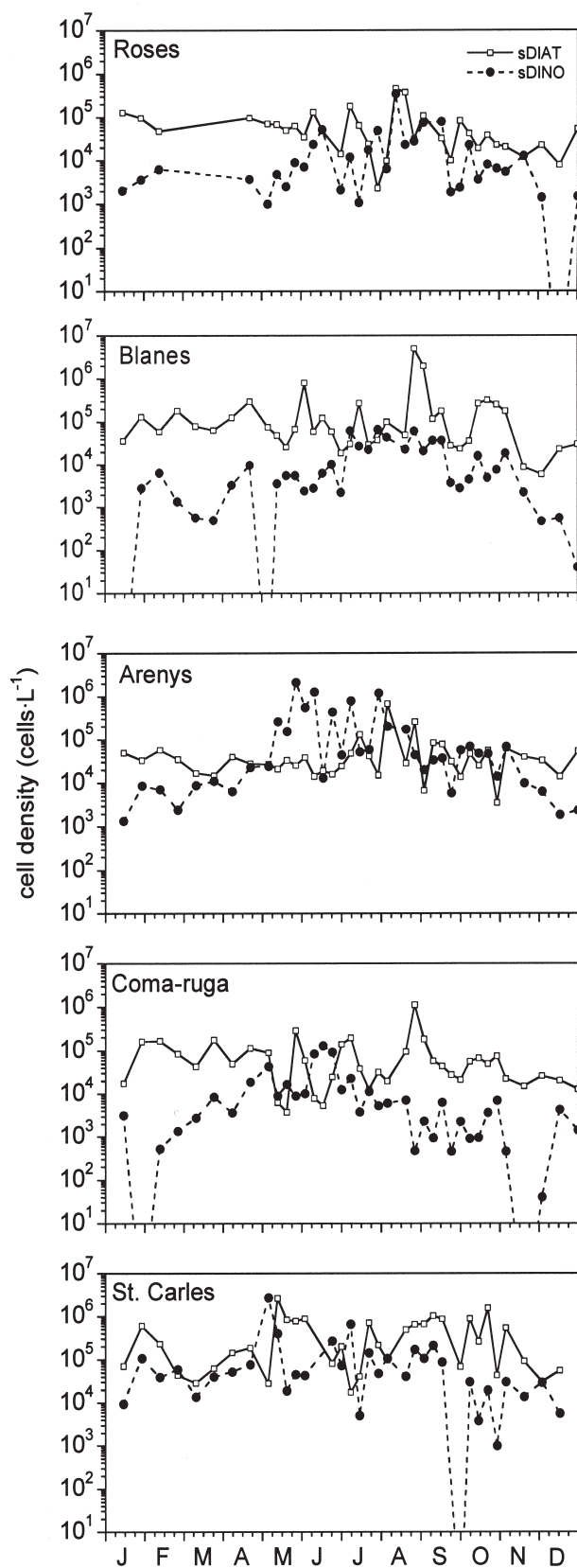

FIG. 3. – Total dinoflagellate and diatom cell densities at five selected stations in 1996 (sampled weekly from May to October and twice a month during the rest of the year).
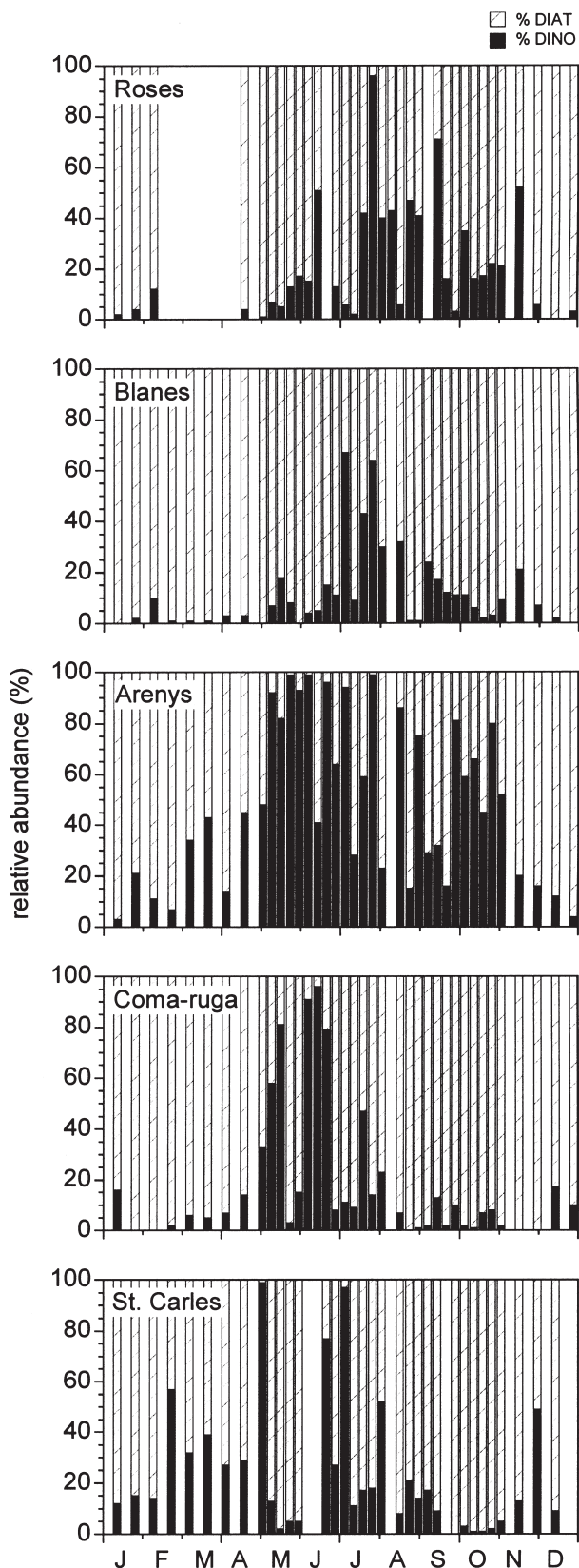
FIG. 4. – Relative abundance of dinoflagellates (black) and diatoms (striped) at five selected stations. The phytoplankton relative abundance is examined here as if it was only composed by these two groups. (White bars are the weeks which were not sampled).

ceros spp., *Cylindrotheca closterium (+ Nitzschia longissima), Leptocylindrus danicus (+ minimus), Pseudo-nitzschia* spp. and *Thalassionema nitzschioides. Ditylum brightwellii* and *Eucampia zodiacus* were recorded during the winter and *Skeletonema costatum* bloomed during the summer and autumn.

Few coccolithophorids were recorded except in the St. Carles harbour. Within this group, *Acanthoica quattrospina, Calciosolenia murrayi, Rabdosphaera claviger*, and *Syracosphaera pulchra* were identified. The silicoflagellates *Dictyocha fibula* and *D. speculum* were also present all along the coast although concentrations were low (<1500 cells l^{-1}). As for nanoflagellates, cells similar to the *Cryptomonas, Pyramimonas, Chrysohcromulina*, and *Calycomonas* genera were also recorded throughout the region. The flagellate *Eutreptiella gymnastica* and the ciliate *Mesodinium* cf. *rubrum* were also common and abundant in the plankton.

HAB species

Twenty-seven identified taxa could potentially lead to harmful effects (e.g. toxic, fish-killing, or bloom-forming); data on their relative contribution (HAB index) are presented in Table 3. Among them, 11 species are potentially toxic to humans. The occurrence of potentially toxic dinoflagellates (IOC list 2002) such as *Alexandrium minutum, Dinophysis sacculus*, and *Prorocentrum minimum* was quite high, and present in the 24, 31, and 26 % of samples respectively. Some taxa were bloom-forming species that characterise surface water discoloration, e.g. *A. minutum* reaching concentrations as high as 10^6 cells l^{-1} in the Arenys Harbour in the spring of 1996. As the dominant species, it contributed up to 96% of the microphytoplankton community. *Prorocentrum rhathymum* never reached high concentrations (<5·10^3 cells l^{-1}) but it accounted for 12-22% of the microphytoplankton community in autumn in Blanes and Palamós harbours. Since Steidinger (1983) *P. rhathymum* has been assumed to be synonymous of *Prorocentrum mexicanum*. However, it has been separated into two different species recently (Cortés-Altamirano and Sierra-Beltran, 2003). Although the chemical toxin structure of *P. mexicanum* is undefined, it is known that it does not produce okadaic acid or dinophysistoxin. However, many toxic effects have been observed. Although there are no direct evidences, when both species were considered synonymous, *P. mexicanum* was

TABLE 3. – List of potentially harmful species (HAB) observed on the Catalan coast during 1996. It includes potentially toxic species for humans (with syndromes indicated), potentially fish-killing species, and potentially bloom-forming dinoflagellates and diatoms. The taxonomic group (Tax Group) is indicated: dinoflagellates (DINO), diatoms (DIAT), and silicoflagellates (SFL). %Np: percentage of the number of occurrence (≥9 species included in the PCA analysis). Max.: maximum abundances (cells l^{-1}). HAB index: percentage of the harmful species with respect to the total microphytoplankton community (dinoflagellates plus diatoms plus silicoflagellates). The HAB index of the two major events (Highest concentrations reached by the harmful species during the annual cycle) are shown.

Species	Tax Group	%Np	Max Cells l^{-1}	HAB index
Human potentially toxic species[1]				
Paralytic Shellfish Poisoning (PSP)				
*Alexandrium minutum**	DINO	24	2026332	63-96
*Alexandrium catenella**	DINO	3	36400	< 10
Diarrhetic Shellfish Poisoning (DSP)				
Dinophysis acuta	DINO	1	20	<1
Dinophysis caudata	DINO	6	460	<1
Dinophysis rotundata	DINO	9	1500	<5
Dinophysis sacculus	DINO	31	40950	<5
Dinophysis tripos	DINO	0.3	20	<1
Prorocentrum lima	DINO	2	240	<1
*Prorocentrum rhathymum***	DINO	7	4368	12-22
Amnesic Shellfish Poisoning (ASP)				
Pseudo-nitzschia spp.*	DIAT	61	1459854	85-90
Neurotoxic (NTX, mouse assay)[2]				
*Prorocentrum minimum**	DINO	26	165620	20-45
Potentially fish-killing species[3]				
*Ceratium fusus**	DINO	20	360	<1
*Gyrodinium corsicum**	DINO	4	43225	10-17
*Noctiluca scintillans**	DINO	1	20	<1
*Dictyocha fibula**	SFL	16	1365	<1
*Dictyocha speculum**	SFL	2	910	<5
Potentially bloom-forming species[4]				
Akashiwo sanguinea (=Gymnodinium splendens)	DINO	17	910	<1
Cerataulina pelagica	DIAT	34	1841840	72-92
Centric diatom sp. 1	DIAT	33	1026324	63-79
Gymnodinium (=Gyrodinium) impudicum	DINO	15	388143	48-69
Heterocapsa sp.	DINO	36	481770	33-59
Leptocylindrus danicus	DIAT	55	1796067	65-92
Prorocentrum micans	DINO	62	76440	6-12
Prorocentrum triestinum	DINO	57	4000000	81-96
Scrippsiella spp.	DINO	60	1671066	71-77
Skeletonema costatum	DIAT	33	5000040	90-97
Small *Chaetoceros* spp.	DIAT	40	2671074	82-93

[1] Known species that are potentially toxic: PSP, DSP, ASP, NTX (IOC list 2002).

[2] Responsible for mouse mortality via a water-soluble toxin. However, there are no data indicating impacts to human consumers (Grzebyk *et al.,* 1997)

[3] Those species that are known or suspected to cause fish losses in mariculture (Anderson *et al.*, 2001).

[4] Those species that are known to produce blooms elsewhere (Anderson *et al.*, 2001) or that occasionally reached abundances exceeding 10^6 cells l^{-1} in our samples.

* Species that are potentially toxic or fish-killing and also known to reach cell concentrations of 10^6 cells l^{-1}. In this study, blooms >10^6 cells l^{-1} were only detected in *Alexandrium minutum* and *Pseudo-nitzschia*.

** It is not a true DSP-producing species as it does not produce okadaic or dinophysistoxin, but it has been related to diarrhetic symptoms (Levasseur *et al.*, 2003).

related to diarrhetic symptoms (Levasseur *et al.*, 2003 and references therein).

The genus *Dinophysis* was usually present in low concentrations (<1.5·10^3 cells l^{-1}) and with a low HAB index (<5%), except for occasional peaks in *D. sacculus* where cell concentrations of up to 4·10^4 cells l^{-1} were noted. However, the HAB index for *D. sacculus* remained low even at this time, as it generally coincided with *A. minutum* blooms of several million cells per litre.

The genus *Pseudo-nitzschia* spp. (known to contain several toxic species) was present in 61% of the analysed samples. Three potentially toxic species have been identified from this region, *P. pungens, P. delicatissima*, and *P. pseudodelicatissima* (Vila 2001). In 1996, the main *Pseudo-nitzschia* spp. blooms occurred during May, July, September and October.

Harmful phytoplankton species that are known or suspected to cause fish mortalities in mariculture (Anderson *et al.*, 2001) have also been detected in the study area, generally with a low HAB index (<5%), except for *G. corsicum,* which reached 10-17% of the microphytoplankton community.

Species known to produce generally harmless water discolorations (Anderson *et al.,* 2001) or those that surpassed 10^6 cells l^{-1} in our samples were considered potentially bloom-forming. Bloom-forming dinoflagellates were present in 87% of the samples. Considering the definition of this group, almost all of these taxa (e.g. *Gymnodinium impudicum, Prorocentrum triestinum, Scrippsiella* spp.) have a high HAB index (>60%), as was true with bloom-forming diatoms such as *Cerataulina pelagica, Leptocylindrus danicus* and *Skeletonema costatum.* There were some exceptions, such as *A. sanguinea* and *P. micans,* described as potentially bloom-forming in other areas but not in our area of study, where they showed consistent low HAB indexes.

Phytoplankton functional groups

The PCA was undertaken by using a matrix made with the samples as objects (327 samples) and species as variables (42 taxa). Codes and main features of the species included in the PCA are presented on Table 2. Five potentially toxic species, two suspected fish-killing taxa, and all of the potentially bloom-forming species were included in the analysis. The other detected harmful species were not included in the PCA analysis because they occurred in less than 9% of the samples (Table 3). In order to define the main phytoplankton assemblages objectively, a K-means cluster analysis (k =4) was applied to the factor coordinates of the species of the three first axes.

The three main axes of the multivariate analysis (PCA) account for 28.9% of the variance (f1=13.5%; f2=8.8%; f3= 6.5 %). Factor 1 shows the highest positive correlation with total dinoflagellates (Table 4). Factor 1 also has a high and significant positive correlation with temperature, total nanoflagellates, and microzooplankton. Factor 2 showed a strong negative correlation with the two diversity index (TOTAL SP, MARGALEF) and biomass (TOTAL IND). Its correlation is also negative with all of the analysed groups, but particularly diatoms. Factor 3 had a significant negative correlation with temperature and total diatoms, and a significant positive correlation with the Margalef index. The correlation between harbour size and the three factors (1 positive, 2 and 3 negative) should be noted (Table 4).

Figure 5a give the factors' loadings of the variables (species) in the space defined by factors 1-2. Negative values of the first axis are associated with some diatoms which are present most often during cold months *(Ditylum brightwellii, Eucampia zodia-*

TABLE 4. – Correlation between the first three factors of the principal components analysis and other related variables: surface area of the harbour (SIZE), water temperature (TEMP), salinity (SAL), number of species (TOTAL SP), number of individuals (TOTAL IND), Margalef's index (MARGALEF), Pielou's evenness index (PIELOU), sum of coccolithophorids (sCOCO), diatoms (sDIAT), dinoflagellates (sDINO), nanoflagellates, (sNANO), and microzooplankton (sMICROZOO). Correlations with * are significant at p < 0.05, those with ** are significant at p < 0.01. N ranged between 290 and 327.

	F1		F2		F3	
SIZE	0.14	*	-0.18	**	-0.31	**
TEMP	0.61	**	0.09		-0.25	**
SAL	-0.10		0.08		0.02	
TOTAL SP	0.15	**	-0.92	**	0.09	
TOTAL IND	0.23	**	-0.95	**	-0.02	
MARGALEF	0.11	*	-0.87	**	0.13	*
PIELOU	-0.02		-0.33	**	-0.07	
sCOCO	0.08		-0.11		0.00	
sDIAT	-0.01		-0.56	**	-0.14	*
sDINO	0.71	**	-0.33	**	0.06	
sNANO	0.35	**	-0.30	**	-0.05	
sMICROZOO	0.30	**	-0.43	**	-0.04	

cus, Rhizosolenia cf. *imbricata, Odontella* sp.) and positive values with most dinoflagellates (*Alexandrium minutum, Dinophysis sacculus, Akashiwo sanguinea, Gyrodinium* cf. *spirale, Gymnodinium impudicum*). The second axis separates rich samples (high abundance and diversity) from poor samples. The most negative values were associated with diatoms such as *Thalassionema nitzschioides,* Centric diatom sp. 1, *Pseudo-nitzschia* spp., *Chaetoceros* spp., *Cerataulina pelagica,* and *Leptocylindrus danicus (+ minimus).*

In Figure 5b, the species are presented in the space defined by factors 1-3, jointly with the results of the K-means cluster analysis (4 groups). Factor 3 discriminates better between diatoms detected in winter and those detected in summer-autumn, and it also discriminates these species from the spring ones. Cluster 1 basically contains dinoflagellates that can reach very high concentrations, the *bloom-forming dinoflagellates.* Diatoms typical of winter are found in cluster 2, while those that bloom in summer and autumn are found in cluster 3. The groups were designated *winter diatoms* and *summer-autumn diatoms* respectively. Finally, cluster 4 contains, among others, a mixture of *large dinoflagellates and elongated diatoms.*

In Figure 6, scatterplots of the values of the coordinates of the objects (samples) of Barcelona and Palamós, as representative of large and small harbours, are presented in the space defined by factor 1 and 2. Small harbours were typified most frequently by positive values of factor 2 whilst showing very few
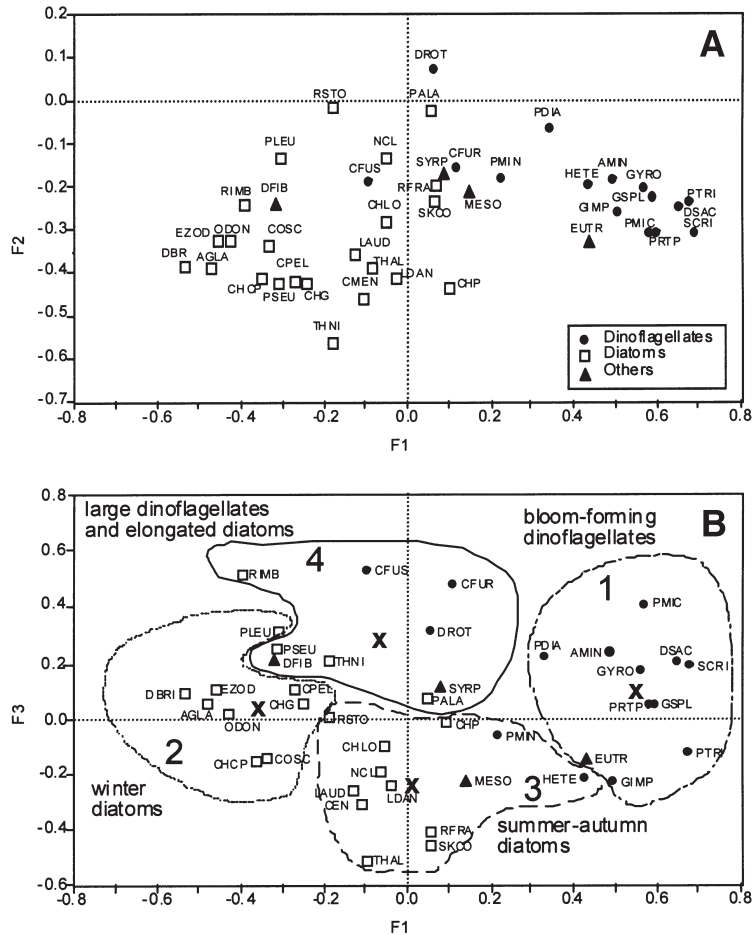
Fig. 5. – Scatterplot of the factor loadings of the variables (species) in the space determined: a) by the first and second principal components and b) by the first and third principal components. The results of a K-means clustering (Euclidean distances) were applied to the factor scores of the three first axes to objectively identify functional groups. The cluster center coordinates are indicated (X). The functional groups identified are *bloom-forming dinoflagellates* (cluster 1), *winter diatoms* (cluster 2), *summer-autumn diatoms* (cluster 3), and *large dinoflagellates and elongated diatoms* (cluster 4). Identification codes for species are found in Table 2.
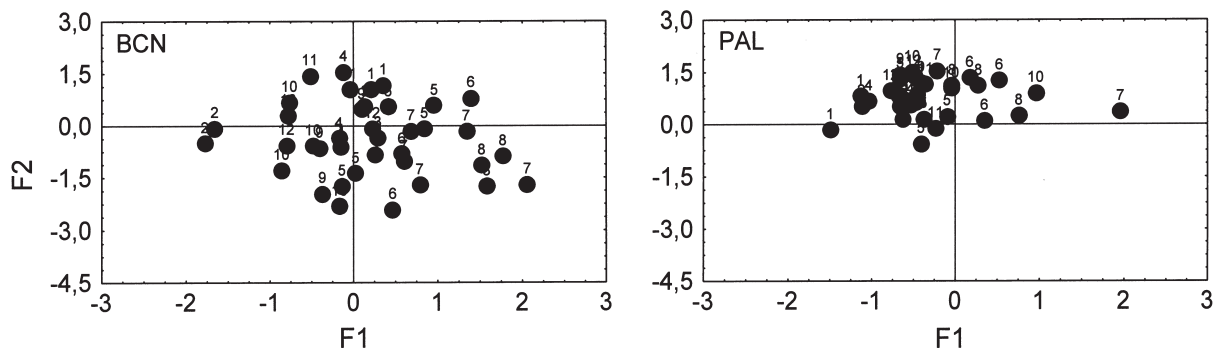
Fig. 6. – Scatterplot of factor score coefficients of the objects (samples) in the space defined by factors 1 and 2 in a large (Barcelona-BCN) and a small (Palamos -PAL) harbour (numbers indicates months). In general, the largest harbours are characterised by high values of factor 1 and low values of factor 2.

cases of high positive values of factor 1. On the other hand, large harbours (> 15 ha.) were represented by high positive values of factor 1. In relation to factor 2, large harbours had samples scattered along the axis, quite different to those noted for the small harbours.

The space defined by factors 1 and 3 clearly shows phytoplankton seasonal succession. The seasons of the year can be observed in a clockwise direction (Fig. 7a). It is clear from Figure 7b that this seasonal pattern is displaced in relation to the har-
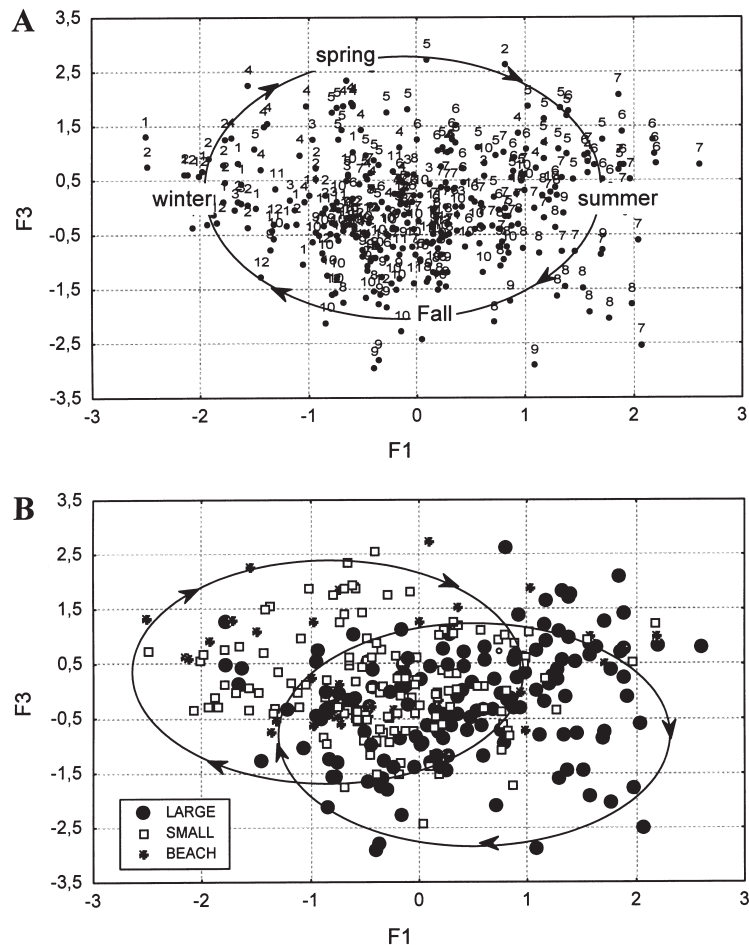
FIG. 7. – Scatterplot of factor score coefficients of the objects (samples) in the space defined by factors 1 and 3: a) Sampling month is noted and seasons are presented clockwise; b) Harbour size. The largest surface-area harbours tend to be dominated by dinoflagellates (right side), while diatoms dominate the smallest surface-area harbours (left side). The seasonal sucession in the phytoplankton community is displaced to the right in large and to the left in small harbours.

bour size. Cluster 1 (*bloom-forming dinoflagellates*) is related to summer (specially in June-July) and principally large harbours. The small harbours are mostly related to cluster 2 and 4 (*winter diatoms* and *large dinoflagellates and elongated diatoms*).

DISCUSSION

The general trends in annual phytoplankton succession found in the present study coincided with those that have been previously described, as well as with recent studies for the region (e.g. López and Arté, 1972; Estrada, 1980; Mura *et al.,* 1996; Cebrián and Valiela, 1999).

In general, all species detected in this study were distributed along the entire coast, with only two exceptions, *Gyrodinium corsicum* and *Alexandrium catenella*. The former was only detected in St. Car-

les harbour, as previously reported (Garcés *et al.,* 1999; Vila *et al.,* 2001a). At the same harbour the maximum cell concentrations of coccolithophorids were also found. The fact that *Gyrodinium corsicum* is only found in this harbour and that coccolithophorids are found in their highest concentrations there is probably related to the fact that St. Carles is the only harbour which is situated in a bay.

A. catenella is possibly a new introduced species in the Mediterranean Sea. After it had been detected for the first time in Barcelona harbour in 1996, it colonised the nearby harbours, increasing its geographical distribution (Vila *et al.,* 2001c; Lilly *et al.,* 2002). Apart from these exceptions, no latitudinal spatial pattern was found with regard to species composition.

In general, large dinoflagellates are characteristic of the summer (stratification and low nutrient availability), and diatoms are associated with winter and

TABLE 5. – Concentrations of chlorophyll (μg·L^{-1}) and major inorganic nutrients (μM). Data were derived from an extensive environmental monitoring programme for 2 annual cycles in the near-shore Catalan surface waters. Even though the sampling period for the nutrient data does not coincide (1998-2000) with the period of the present study, the data presented were collected at the stations sampled in the present study (n=250). Frequency of single nutrient limitation (% of observations) was calculated as in Justic *et al.* (1995) (criteria detailed below).

P limitation (Lim PO$_4$):	P < 0.1 μM	DIN:P > 22; Si:P > 22
N limitation (Lim DIN):	DIN < 1 μM	DIN:P < 10; Si:DIN > 1
Si limitation (Lim Si):	Si < 2 μM	Si:P < 10; Si:DIN < 1

	Chl-a Median	PO$_4$ Median	SiO$_4$ Median	DIN Median	Lim PO$_4$	Lim DIN	Lim Si	n
Open	1.30	0.35	3.56	13.41	0	0	1	50
Small	0.87	0.39	2.15	4.55	0	2	2	83
Large	2.64	0.60	3.56	9.64	0	1	2	117
All Grups	1.38	0.42	3.02	7.90	0	3	5	250

spring, a period with no stratification and relatively high nutrient content (Margalef, 1978). However, in contrast to open Mediterranean waters known to be nutrient-depleted during the summer months, near-coastal waters are rich in nutrients year-round, even in summer (Olivos *et al.*, 2002). Moreover, phytoplankton production rarely appears to be potentially limited by nutrient defficiency in confined waters along the Catalan coast (Table 5). During the summer months, most dinoflagellate blooms appear as a clear property of these near-coastal enriched waters and HAB species are also very frequent and abundant (Table 3 and Fig. 5b).

Functional groups and HAB species

The classical definition of Margalef's Mandala (Margalef, 1978; Margalef *et al.,* 1979) is an attempt to define functional groups in marine waters. The Mandala, which describes marine phytoplankton succession, maps the distribution of different phytoplankton species in an ecological space defined by turbulence and nutrient levels. R-selected species (basically diatoms) are followed by k-selected species (large dinoflagellates) in the main sequence of species succession. In this conceptual model, there is a coincidence between primary phylogenetic groups and functional groups. According to Margalef, red tides would only occur if high nutrient concentrations co-occurred with relatively low turbulence. The species growing under these conditions would out-compete their neighbors through adaptations such as vertical migration, grazer deterrence, and allelopathic inhibition of potential competitors (Cullen *et al.,* 2002 and references therein).

The groups that characterise the anthropogenic impacted waters of the Catalan coast in our analysis were: *bloom-forming dinoflagellates* (cluster 1), *winter diatoms* (cluster 2), *summer-autumn diatoms*

(cluster 3), and *large dinoflagellates and elongated diatoms* (cluster 4) (Fig. 5b). Cluster 1, the *bloom-forming dinoflagellates*, is typified by small, more or less rounded, autotrophic, neritic species with many cyst-formers (e.g. *A. minutum, G. impudicum, P. triestinum, Scripsiella* spp.) or r-strategists. Cluster 1 also contained heterotrophic dinoflagellates which are suspected to prey on these small species (*Gyrodinium* sp., *P. diabolum*). Cluster 4 was typified by large mixotrophic dinoflagellates (*Ceratium* spp.) with their horns/morphology yielding a high surface to volume ratio; elongated diatoms were also distinct to this group. Both have relatively low growth rates (Garcés and Masó 2001) and can be considered K-strategists. The remainder of the diatoms fall into two different groups according to the season. Thus, we distinguish *summer-autumn diatoms* (cluster 3) and *winter diatoms* (cluster 2).

Smayda and Reynolds (2001) have focused exclusively on dinoflagellates. They distinguished a range of nine different habitats or environmental situations associated with different dinoflagellate life-forms. A combination of two distinct gradients (onshore-offshore and mixing-nutrient) is used in this classification. Although diatom blooms are generally associated with high turbulence and high nutrient concentrations (Margalef, 1978), our results indicate that they could also be situated across a similar gradient to that proposed by Smayda and Reynolds (2001) for the dinoflagellates. *Summer-autumn diatoms* (e.g. *Skeletonema costatum, Leptocylindrus danicus* and small *Chaetoceros* spp.) is a group of bloom-forming diatoms differentiated by the multivariate analysis that, in fact, coexists with the *bloom-forming dinoflagellates* although in lower concentrations (Fig. 5b).

The coexistence between *bloom-forming dinoflagellates* and s*ummer-autumn diatoms* implies a common response to the same environmental conditions.
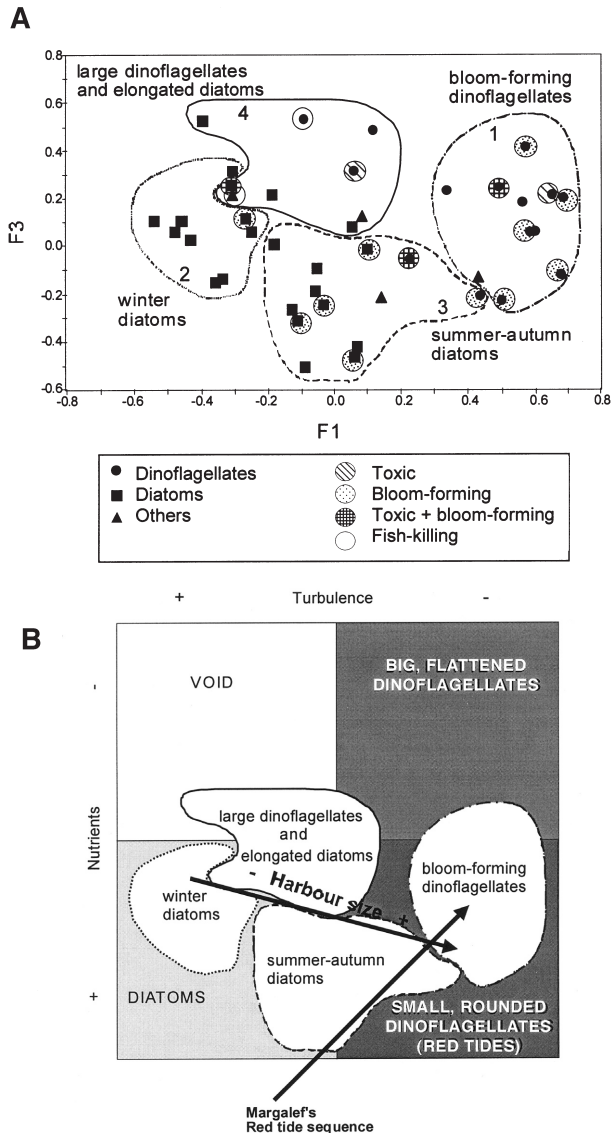
A

B

FIG. 8. – A) Functional groups identified by the cluster analysis (Fig. 5b), in which the potentially harmful species have been pointed out according to their potential harm (toxic, bloom-forming, fish-killing and toxic plus bloom-forming) (Table 3). B) Schematic representation of the functional groups incorporated in Margalef's Mandala, rotated 180°. Only the two nutrient-rich quadrants of the Mandala are represented by our study habitats, and harbour size defines the diagonal of these quadrants.

In Figure 8a, the different harmful species have been pointed out according to their potential harm (toxic, bloom-forming, fish-killing, and toxic plus bloom-forming) (Table 3). Most bloom-forming species are grouped in two clusters, basically cluster 1 for dinoflagellates and cluster 3 for diatoms. On the other hand, the toxic species are scattered across all the functional groups. Thus, the most probable species associations are predictable in the analyses employed in this study. However, there is little probability of predicting a toxic event across the spectrum of conditions considered in this assessment.

The importance of the harbour size

The functional groups defined in this study have been overlapped on Margalef's Mandala (rotated 180°, Fig. 8b). Only the two nutrient-rich quadrants of Margalef's theory include our study habitats. Margalef's quadrant of flattened dinoflagellates is not well represented in the habitats sampled in the present study, as the habitats along the coast are characterised by high nutrient concentrations and a wide range of turbulence related to seasonality.

From results along the Catalan coast, there appears to be a relationship between the harbour size and the frequency of and cell concentration in dinoflagellate blooms (cluster 1). The main difference found between large and small harbours is that dinoflagellate abundances are higher in the largest harbours than in the smallest embayments (Fig. 2). The same assemblages are detected at all stations but the *bloom-forming dinoflagellate* group is less represented in small harbours. Additionally, this group is also less represented in winter than in summer (Fig. 7b). Harbour size, therefore, seems to be critically important to the occurrence and recurrence of dinoflagellate blooms in the region. The probable explanation for this relationship is through the hydrodynamics of a harbour with perhaps larger harbours characterised by deeper basins, longer distances from the mouth to headwaters, and potentially longer water residence times relative to smaller harbours in the area. Although large harbours seem to be characterised by higher nutrient levels than small ones, there is no indication of differences concerning the frequency of potential nutrient limitation (Table 5). Therefore, the interaction between high nutrient levels and confinement plays the key role in the occurrence of dinoflagellate blooms in this region.

The largest harbours are also typified by high commercial shipping traffic, important vectors in translocating cysts of harmful phytoplanktonic species (Hallegraeff, 1998; Hamer *et al.*, 2001; Marangoni *et al.*, 2001). After a dormancy period, these cysts germinate to yield vegetative populations and potential bloom precursors. The cysts, ballast transported and locally produced cells experience recurrent germination over 10-20 years (Hallegraeff, 1998), potentially reseeding blooms for decades. Confined waters areas (e.g. harbours) have recently been shown to be reservoirs of large beds of resting cysts (Garcés *et al.*, 2004).

After species settlement and colonisation in a harbour, the species has a new array of suitable habi-

tats to colonise, forming a basis for establishment in near neighbours. In fact, coastal currents in the study area approximate 10-20 cm·s^{-1} (Masó and Tintoré, 1991), favouring advection of cells to adjacent harbours in 1 day. The expansion of *A. catenella* on the Catalan coast (Vila *et al.*, 2001b, c) is probably a result of this process. During the widespread bloom of *A. catenella* in 1998 and 1999, huge patches of cells were noted inside the harbours (>10^6 cells l^{-1}), with far lower concentrations in open near-coastal areas (<10^4 cells l^{-1}). The series of small harbours along the coast therefore play a key role in cell dissemination coastwide via the very short distances between sheltered waters.

These characteristics indicate that large harbours in tideless seas should be considered as areas with a high probability of high-biomass blooms. They are rich in nutrients and, as cell dispersion is limited, actively growing cells would accumulate in the harbour. This model of dinoflagellate blooms in semi-closed or closed bays, estuaries or harbours is not new and, in fact, is widely known in some areas. For example, inshore-offshore differences in phytoplankton biomass were found in the Ria de A Coruña (NW Spain); cell concentrations were much higher in the harbour area (inner part of the Ria), where residence was long and could be considered an accumulation area (Varela *et al.*, 2001). Estuaries and coastal waters with high flushing rates have significantly less algal production than similar systems that are poorly flushed, consistent with the general pattern that shallow systems typically support more algal growth than deeper systems (Anderson *et al.*, 2002 and references therein).

The increase of man-sheltered structures associated with harbour construction may be important in cell dissemination along the coast, particularly with the limited distances between sheltered areas of the region. Therefore, proliferation of blooms in the area might be expected as shore development proceeds in this highly valued coastal system.

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