Available online at www.sciencedirect.com
SCIENCE DIRECT®
Harmful Algae 4 (2005) 673–695
HARMFUL ALGAE
www.elsevier.com/locate/hal

A comparative study on recurrent blooms of *Alexandrium minutum* in two Mediterranean coastal areas

Magda Vila[a,*], Maria Grazia Giacobbe[b], Mercedes Masó[a], Ester Gangemi[b], Antonella Penna[c], Nagore Sampedro[a], Filippo Azzaro[b], Jordi Camp[a], Luca Galluzzi[c]

[a]*Institut de Ciències del Mar, Passeig Marítim de la Barceloneta, 37-49, Barcelona 08003, Spain*
[b]*Istituto per l'Ambiente Marino Costiero, CNR, Spianata S. Raineri 86, Messina 98122, Italy*
[c]*Centro Biologia Ambientale, University of Urbino, Vle Trieste 296, Pesaro 61100, Italy*

Received 22 June 2004; accepted 14 July 2004

Abstract

Alexandrium minutum is a toxic dinoflagellate widespread along the Mediterranean coasts. This species is frequently detected year-round at low concentrations within the Mediterranean basin. However, it only proliferates recurrently in some localities. Two affected areas are the Catalan and Sicilian coasts. In order to identify the factors determining the *A. minutum* blooms in the Mediterranean Sea, we compare the bloom conditions in two harbours: Arenys de Mar (Catalan coast, Spain) and Syracuse (Sicily, Italy), during 2002–2003. Arenys de Mar harbour is a fishing and leisure harbour and receives an input of freshwater rich in nutrients. Likewise, the Syracuse harbour – located on the Ionian coast of Sicily – is subject to freshwater inputs. Some points of this site are used for productive activities such as shellfish farming. *A. minutum* from the two areas studied were morphologically and genetically identical. In both sites, recurrent blooms take place from winter to spring. Surface water temperatures and salinities during *A. minutum* bloom events were 12–14.5 °C and 32–38, and 16–24 °C and 32–37.7 for Arenys and Syracuse, respectively. During the blooms, the spatial distribution of *A. minutum* in the two harbours, the physicochemical characteristics and the phytoplankton community were studied. Similarities in composition of the phytoplankton community were evidenced, with a clear dominance of dinoflagellates over the other taxa. In Arenys, the second dominant species was *Prorocentrum micans* followed by *Scrippsiella* spp. and *Dinophysis sacculus*. The same species were found in Syracuse although *P. triestinum*, and alternatively *Lingulodinium polyedrum*, reached cell densities much higher than the other dinoflagellates giving marked water discolourations.

Keywords: *Alexandrium minutum*; HAB; Mediterranean Sea; Phytoplankton assemblages; Toxic dinoflagellates

* Corresponding author. Tel.: +34 932309500; fax: +34 932309555.
E-mail address: magda@icm.csic.es (M. Vila).

1568-9883/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.hal.2004.07.006

1. Introduction

Once the increment in harmful algae blooms (HAB) has been accepted as a common phenomenon in coastal waters (Anderson, 1989; Hallegraeff, 1993), we should aim at investigating the causes of such an increase. Comparative studies will allow us to identify critical processes controlling HAB occurrence and consequently to develop prediction capacities (GEOHAB science plan, Glibert and Pitcher, 2001). For such a type of study, it is important to take advantage of recurrent blooms since they never occur there by chance.

The genus *Alexandrium* includes about 30 species and at least nine of them produce a number of neurotoxins that can lead to paralytic shellfish poisoning (PSP) events. *A. minutum*, which is one of the toxic species, is a small armored dinoflagellate originally described from a red tide in the Alexandria harbour (Egypt, SE Mediterranean Sea; Halim, 1960). This species has been reported over a number of geographical areas and in a wide range of coastal hydrographic regimes (i.e. Hallegraef et al., 1988; Tahri-Joutei et al., 2000; Maguer et al., 2000; Yoshida et al., 2000; Daly Yahia Kefi et al., 2001a,b; Usup et al., 2002). *A. minutum* is an unchained species that is well characterized morphologically (see Balech, 1995). However, some specimens identified as *A. minutum* differ morphologically from the species redescription (Balech, 1989), especially concerning their plate ornamentation (Montresor et al., 1990), and the lack of a ventral pore (Danish specimens in Hansen et al., 2003). This suggests that more than a single species could be included under the name of *A. minutum* (Moestrup et al., 2002). *A. lusitanicum* was described as a distinct species based on the different shape of the s.a. plate (Balech, 1995). However, Franco et al. (1995) suggested that *A. lusitanicum* and *A. minutum* are nonspecific in view of the apparent variability of this feature.

A. minutum is widely distributed in the Mediterranean Sea and events of paralytic shellfish poisoning (PSP) have been frequently associated with this species in different basins such as the Northern Adriatic Sea (Honsell et al., 1996), Eastern Aegean (Koray and Buyukisik, 1988), Tyrrhenian Sea (Giacobbe et al., 2003b), and Catalan-Balearic Basin (Delgado et al., 1990; Forteza et al., 1998). *A. minutum* blooms in the Mediterranean seem to be restricted to coastal enriched sites, particularly harbours, estuaries or lagoons (Belin, 1993; Giacobbe and Maimone, 1994; Vila et al., 2001). The inoculum for bloom initiation in semi-enclosed areas could be the result of offshore advection of cells or excystment of local benthic cysts. Resting cysts in the local sediment of Arenys Harbour (Catalan Sea) have been detected recently (Garcés et al., 2004). These authors highlighted the potential main role of cyst beds in the outbreaks of *A. minutum* in water bodies with restricted water exchange such as harbours.

A lot of effort has been made to understand the ecological preferences and the adaptive strategies of *A. minutum* under laboratory conditions, i.e. nutritional preferences and toxin production in relationship with grazers (Bagøien et al., 1996; Frangópulos et al., 2000; Guisande et al., 2002; Lippemeier et al., 2003), or competition and allelopathy (Cannon, 1996; Arzul et al., 1999; Tillmann and John, 2002; Fistarol et al., 2004). However, there is a gap in comparative field studies in areas which are affected by blooms of the same species. Those are essential for a better understanding of the *A. minutum* bloom conditions.

In this study, we analyze the occurrence of *A. minutum* in two human impacted areas of the Mediterranean: the Catalan coast (North-western Mediterranean) and the Eastern coast of Sicily (Eastern Mediterranean). It is known from the last decade that blooms of *A. minutum* are recurrent in harbours of these two regions. The main objective of the present study is to compare these systems in order to provide further insights into the conditions that make a certain locality susceptible to blooms of this species. First of all, we provide evidence that we are dealing with the same species based on morphological and genetic information of the organism. To identify the factors related to *A. minutum* blooms, we examined the *A. minutum* distribution and related parameters at different scales in both areas. First, we focus on the *A. minutum* distribution and inorganic nutrients concentration on a regional scale during the period 2002–2003. Two target sites were intensively studied: Arenys de Mar harbour (Catalonia) and Syracuse bay (Sicily), where *A. minutum* blooms have been described since 1996 and 2001, respectively. We also studied the small-scale spatial

distribution of *A. minutum*, environmental variables, species competitiveness (phytoplankton community) and potential grazing impact during bloom events at those localities.

2. Methods

2.1. Extensive monitoring

A number of stations were examined along the Catalan coast of Spain (24) and Ionian (14) and Tyrrhenian (11) littoral of Sicily (Italy) to establish the distribution of *A. minutum* in both areas (Table 1, Fig. 1). The stations included different systems (harbours, bays, beaches and lagoons). Temperature and salinity were measured and water samples collected at the surface for phytoplankton, chlorophyll-*a* and inorganic nutrient analyses. In Catalonia, sampling in harbours was carried out on 2–4 occasions per month from March to September and once to twice a month for the rest of the year. Catalan beaches were sampled once a week in summer. Monitorings along the coast of Sicily were conducted on various occasions in 2002 and 2003 (Table 1).

2.2. Study areas

Arenys de Mar harbour is situated on the Catalan coast (NE of Spain) at 41°34′N and 2°33′E. Its extension is approximately 30 ha – total surface – of which 17 ha is water. It is a shallow (0.5–6 m depth), fishing and leisure harbour receiving an input of freshwater rich in nutrients. The Syracuse harbour (37°3′N, 15°17′E), located on the Ionian coast of Sicily (Syracuse bay, extension about 700 ha) is also subject to freshwater inputs (riverine and spring waters). Some spots of this bay (depth: 0.5–8 m at the sampling area; 25–30 m at the entrance) are also used for productive activities such as shellfish farming. Both harbours were intensively monitored over time (2002–2003) and space during *A. minutum* blooms.

2.3. Sampling in Arenys de Mar harbour

Two stations (st. R and T, Fig. 1C) were examined during 2002 and 2003. Station R was sampled all year long and st. T during the *A. minutum* blooms (since mid-January to mid-March). Both st. R and T were sampled twice or even three times a week during that period. Temperature and salinity were measured directly by microprocessor conductivity meter WTW Model LF197. Two temperature sensors that store data every 15 min were placed (st. T) at the surface and bottom (2 m). Surface water samples were collected for phytoplankton, chlorophyll-*a* and nutrient analysis. Hundred and fifty milliliters was preserved with Lugol's iodine solution for microscopic analysis. Sixty milliliters was filtered on Whatman GF/F glass fibre filters, and frozen at −20 °C for chlorophyll analysis, and 60 ml was frozen immediately (−20 °C) for nutrient analyses.

In 2002, a cruise was performed during the maintenance phase of the *A. minutum* bloom (18 February 02), just after it had reached its maximum concentration, to analyze the horizontal variability inside the harbour (30 stations). The phytoplankton and microzooplankton community were analyzed as well as environmental parameters. A vertical transect was repeated at four stations at midday (12–13 h) and in the afternoon (17–18 h, local time) with the aim of analyzing vertical variability. The Pearson correlation coefficient was calculated after logarithmic transformation (log + 1) when necessary (i.e. phytoplankton cell numbers). In 2003, a different sampling scheme was applied: a total of five stations inside the harbour were monitored during the bloom development (same parameters like those of 2002 were analyzed).

2.4. Sampling in Syracuse

Four stations (st. 1–3 inside the harbour; st. 4 in the mussel area, Fig. 1C) were sampled from March to October (2002 and 2003) with a usual sampling frequency of 2–4 times per month. The parameters considered and procedures were the same as indicated for Arenys. Temperature and salinity were measured using a mobile probe (Multiline F/SET-3 WTW). On 11 May 2002 and 2 April 2003, surveys to study the horizontal distribution and composition of phytoplankton inside the area were carried out. Sampling points were increased to 14, including a maximum bloom site that was examined vertically from surface to bottom (0–5 m).

Table 1
A. minutum spatial distribution (maxima cell concentration), chlorophyll-*a* and nutrient relationships (mean, standard deviation and *n*) over the 2002–2003 period along the Catalan and Sicilian coasts

Zone	Code	Station	Latitude (N)	Longitude (E)	Type	Sampled period (month)	Max. Conc.			Chl-*a*			DIN/PO_4			Si/DIN			Si/PO_4		
							2002	2003	Month $> 10^4$	Mean	S.D.	*n*	Mean	S.D.	*n*	Mean	S.D.	*n*	Mean	S.D.	*n*
Catalan coast – Catalanobalear Basin																					
North	Ca1	Roses	42°15′0″	3°10′0″	Harbour	1–12	10^3	$<10^3$		2.6	2.1	57	39	50	57	0.9	0.8	57	34	60	57
North	Ca2	Empuriabrava	42°14′33″	3° 8′6″	Harbour	1–12	$<10^3$	10^5	April	5.7	4.5	36	135	199	36	2.4	3.5	36	232	460	36
North	Ca3	Estartit	42°3′0″	3°12′12″	Harbour	1–12	10^4	10^4	September, July	3.1	2.6	69	56	175	69	2.0	2.9	69	77	182	69
North	Ca4	Platja de l'Estartit	42°2′45″	3°11′56″	Beach	5–9	$<10^3$	$<10^3$		3.8	6.7	23	13	20	24	2.1	2.1	24	16	16	24
North	Ca5	Pals	41°59′18″	3°12′19″	Beach	5–9	n.d.	n.d.		2.3	2.0	12	26	23	19	1.4	0.7	19	33	30	19
North	Ca6	Sa Riera	41°58′27″	3°12′45″	Beach	5–9	n.d.	n.d.		1.0	0.8	21	23	25	21	1.1	1.2	21	19	21	21
North	Ca7	Llafranc	41°53′41″	3°11′45″	Beach	5–9	$<10^3$	$<10^3$		0.4	0.2	26	28	41	27	1.2	0.6	27	40	58	27
North	Ca8	Castell	41°51′48″	3°10′17″	Beach	5–9	n.d.	$<10^3$		1.6	1.4	17	13	22	17	1.8	1.3	17	13	16	17
North	Ca9	Fosca	41°51′29″	3°8′40″	Beach	5–9	n.d.	n.d.		1.7	1.1	3	11	11	30	1.8	2.0	30	20	52	30
North	Ca10	Palamós	41°50′30″	3°7′6″	Harbour	1–12	$<10^3$	$<10^3$		0.7	0.4	35	25	18	36	0.6	0.7	36	13	19	36
North	Ca11	St. Pol	41°47′30″	3°3′14″	Beach	5–9	n.d.	n.d.		7.4	13.6	18	21	30	19	1.2	0.7	19	22	46	19
Center	Ca12	Blanes	41°40′18″	2°47′48″	Harbour	1–12	10^3	$<10^3$		1.2	0.9	36	76	121	36	0.5	0.4	36	26	21	36
Center	st. R	Arenys de Mar	41°34′18″	2°33′18″	Harbour	1–12	10^7	10^6	February, March	4.7	3.7	147	302	367	68	0.4	0.2	68	96	124	68
Center	Ca14	Premià	41°29′6″	2°20′54″	Harbour	1–12	10^3	10^3		4.1	4.4	36	328	727	36	0.4	0.3	36	76	158	36
Center	Ca15	Olímpic	41°23′12″	2°12′6″	Harbour	1–12	10^4	$<10^3$	July	3.1	2.1	47	23	17	48	0.8	0.8	48	16	18	48
Center	Ca16	Barcelona	41°20′6″	2°10′12″	Harbour	1–12	$<10^3$	10^3		3.9	5.3	68	21	27	68	0.6	0.6	68	11	19	68
Center	Ca17	Castelldefels	41°15′52″	1°57′11″	Beach	5–9	$<10^3$	$<10^3$		3.5	2.5	27	12	10	28	2.0	3.7	28	19	22	28
Center	Ca18	Vilanova	41°12′18″	1°43′42″	Harbour	1–12	10^4	10^5	March, April	4.9	4.5	57	61	154	57	0.6	1.0	57	26	61	57
South	Ca19	Torredembarra	41°7′30″	1°24′0″	Harbour	1–12	10^3	$<10^3$		6.4	15.2	36	27	39	36	0.8	0.6	36	15	17	36
South	Ca20	Tarragona	41°5′0″	1°12′54″	Harbour	1–12	$<10^3$	10^3		15.9	50.9	67	85	157	67	0.9	1.0	67	58	129	67
South	Ca21	Cambrils	41°3′42″	1°3′48″	Harbour	1–12	10^5	10^5	May, May	2.1	1.3	36	417	1328	36	0.5	0.2	36	204	670	36
South	Ca22	L'Ametlla	40°52′48″	0°48′12″	Harbour	1–12	10^3	$<10^3$		3.6	7.5	57	199	379	57	0.4	0.2	57	85	249	57
South	Ca23	L'Ampolla	40°48′36″	0°42′48″	Harbour	1–12	$<10^3$	$<10^3$		2.0	1.9	36	304	556	36	0.8	0.3	36	236	421	36
South	Ca24	St. Carles de la Ràpita	40°36′36″	0°36′24″	Harbour	1–12	10^3	10^4	April	10.3	24.0	69	167	347	69	2.0	4.7	69	141	240	69
Sicilian coast – Tyrrhenian Sea																					
Salina	Si1	Salina Rinella	38°32′53″	14°49′45″	Harbour	8		n.d.													
Lipari	Si2	Marina di Porto Salvo	38°28′30″	14°57′30″	Harbour	6		10^3													
Vulcano	Si3	Three stations	38°25′	14°57′	Beach	5–10	n.d.	n.d.		12.6	33.3	42	6	6	42	6.2	6.8	42	23	14	42
Milazzo	Si4	Marina di Nettuno	38°12′58″	15°15′7″	Harbour	3		$<10^3$	March				33	6	2	0.4	0.0	2	12	2	2
Marinello	Si5	Lago Verde	38°8′35″	15°2′45″	Lagoon	3–6		10^5	April				6		1	3.0		1	17		1

Portorosa	Si6	Four stations	38°7′	15°6′	Harbour	2–6		10^4	March, April				164	317	33	0.3	0.1	13	55	62	13
Sicilian coast – Ionian Sea																					
Taormina	Si7	Lido Mendolia	37°51′6″	15°18′17″	Beach	5–6		$<10^3$	May				16	10	2	1.1	0.3	2	16	5	2
Augusta Roadstead	Si8	Foce fiume Mulinello	37°14′16″	15°12′10″	Harbour	2–3		n.d.					242	40	2	0.3	0.1	2	69	27	2
Augusta Roadstead	Si9	Marcellino Mouth	37°12′53″	15°11′0″	Harbour	2–9	10^3	n.d.		0.8	1.2	11	37	43	13	1.1	0.9	13	27	25	13
Priolo	Si10	IAS	37°9′20″	15°12′15″	Harbour	2–4, 6, 9	$<10^3$	$<10^3$	June	0.4	0.5	10	22	16	11	0.8	0.8	11	11	7	11
S. Panagia Bay, Priolo	Si11	Magnisi peninsula	37°9′6″	15°13′46″	Beach	2–9	$<10^3$	n.d.		0.5	0.5	22	58	93	24	1.5	3.3	24	53	98	24
S. Panagia Bay, Priolo	Si12	Lidi	37°8′33″	15°13′20″	Beach	5–9	n.d.	$<10^3$	July	0.5	0.3	22	61	113	23	0.8	0.5	23	26	30	23
S. Panagia Bay, Priolo	Si13	ENEL Power Station	37°8′28″	15°13′13″	Beach	2–9	n.d.	10^3	July	1.1	1.4	22	39	51	24	0.7	0.8	24	17	14	24
Siracusa	Si14	Marmoreo	37°4′5″	15°17′55″	Harbour	4–10		10^5	April	2.2	1.8	8	28	19	7	1.0	1.0	7	16	9	7
Siracusa	st. 1	Sanità Marittima	37°3′51″	15°17′1″	Harbour	3–10	10^5	10^5	June, March	27.6	80.6	53	22	29	26	1.1	1.0	26	14	11	26
Siracusa	st. 8	st. 8	37°3′32″	15°16′39″	Harbour	4		10^6	April				16	8	4	0.8	0.6	4	11	9	4
Siracusa	st. 2	Fonte Aretusa	37°3′25″	15°17′40″	Bay	3–10	10^3	10^4	April	1.1	1.2	31	34	31	32	1.8	2.1	32	28	19	32
Siracusa	st. 3	Foce Anapo-Ciane	37°3′24″	15°16′22″	Harbour	3–10	10^5	10^5	April, March	5.7	7.5	52	21	22	30	0.8	0.7	30	14	24	30
Siracusa	st. 4	Campo Mitili	37°2′16″	15°17′23″	Bay	3–10	10^4	10^4	April, April	2.3	1.8	23	40	44	27	0.5	0.4	27	16	16	27
Noto	Si30	Stagni di Vendicari	36°48′8″	15°5′29″	Lagoon	3		n.d.													

Months with *A. minutum* concentrations above 10^4 cells l^{-1} are indicated. Sampling stations are ordered from north to south. The zone, geographical coordinates, station type and sampled months are indicated. Codes are used as in Fig. 4.

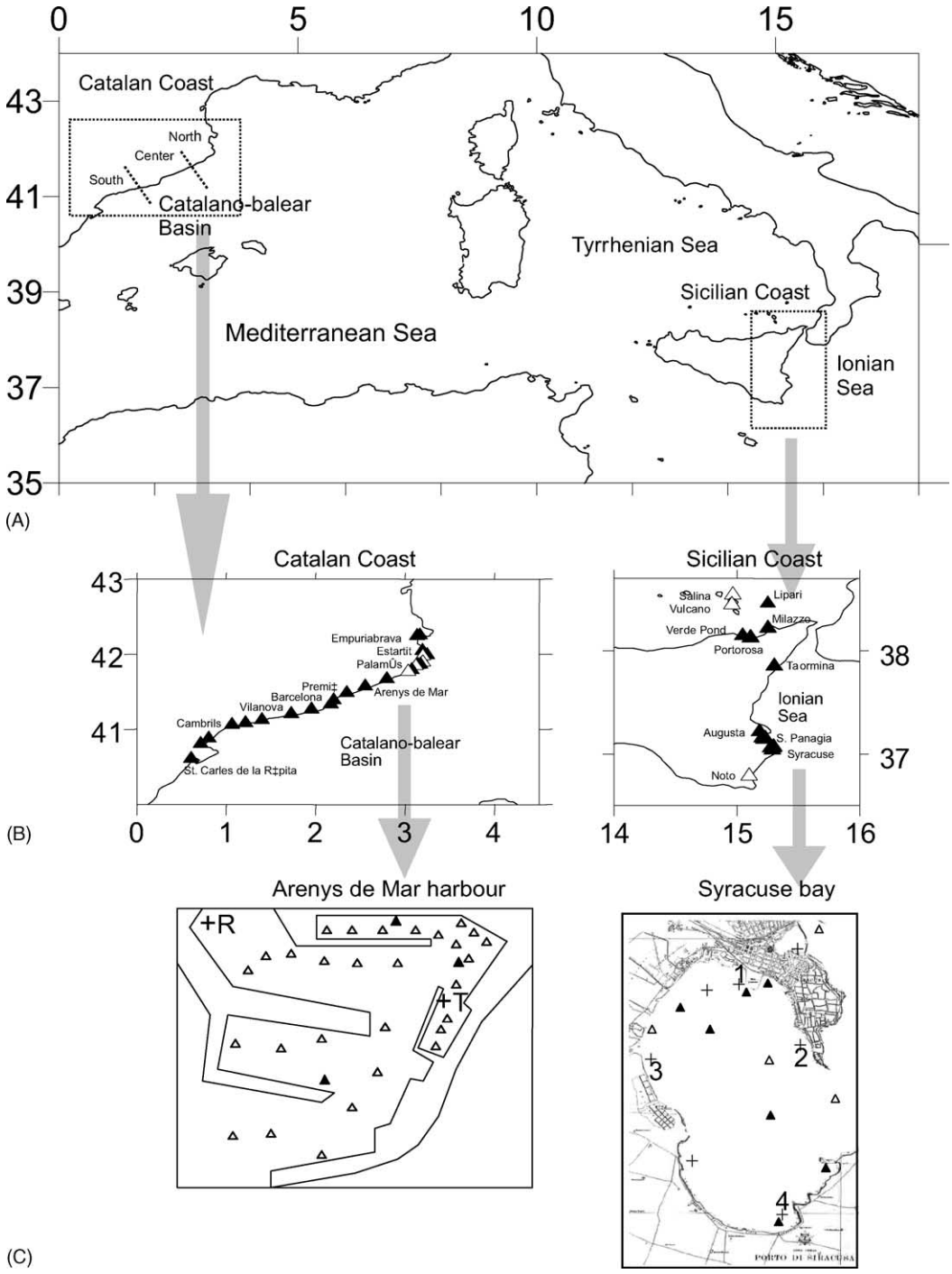

Fig. 1. (A) Study area. (B) Geographical distribution of *A. minutum* along the Catalan and Sicilian coasts (Mediterranean Sea). Presence is indicated by black triangles (▲) and absence by open triangles (△). Further information in Table 1. (C) Sampling points in the Arenys de Mar harbour (Catalan coast) and Syracuse bay (Sicilian coast). Open triangles (△) refer to stations sampled during the 2002 cruise, black triangles

Table 2
List of *A. minutum* strains, sample location and EMBL accession numbers

Species	Strain	Sampling, location and year	Accession number
A. minutum	CSIC-D1	Mediterranean, Catalan Sea, Arenys, Spain, 1995	AJ312945
A. minutum	IEO-AL8C	Mediterranean, Catalan Sea, Arenys, Spain, 2002	AJ532914
A. minutum	IEO-AL9C	Mediterranean, Catalan Sea, Arenys, Spain, 2002	AJ621733
A. minutum	CNR-AMIA1	Mediterranean, Ionian Sea, Syracuse, Italy, 2001	AJ621734
A. minutum	CNR-AMIA4	Mediterranean, Ionian Sea, Syracuse, Italy, 2001	AJ318460
A. minutum	CNR-AMIA5	Mediterranean, Ionian Sea, Syracuse, Italy, 2001	AJ532913

CNR, Consiglio Nazionale delle Ricerche, Messina, Italy; CSIC, Institut de Ciències del Mar, Barcelona, Spain; IEO, Instituto Español de Oceanografia, Vigo, Spain.

2.5. Parameters

Chlorophyll-*a* was extracted in 8 ml of 90% acetone overnight at 4 °C, and the concentration measured with a Turner fluorometer (Turner Designs).

Analyses of dissolved inorganic nutrients (NO_3, NO_2, NH_4 and PO_4) were performed with an Alliance Instruments Evolution II autoanalyzer as described in Grassohoff et al. (1983). Calculations of potential nutrient limitation have been calculated as in Justic et al. (1995). The criteria of probable limitation are as follows: P limitation ($P < 0.1$ μM; $DIN:PO_4 > 22$; $Si:PO_4 > 22$), N limitation ($DIN < 1$ μM; $DIN:PO_4 < 10$; $Si:DIN > 1$), and Si limitation ($Si < 2$ μM; $Si:PO_4 < 10$; $Si:DIN < 1$).

An aliquot of 10–50 ml of the lugol fixed samples was settled in a counting chamber for 1 day. For phytoplankton enumeration, the appropriate area of the chamber was scanned at 63–400× magnification, depending on the cell density of each species, using a Leica-Leitz DM-Il inverted microscope or a Zeiss Axiovert 200 (Throndsen, 1995). Usually, at least three transects were scanned at 100×, one transect at 400× and the complete chamber at 63×. Thus, the minimum concentration detected by this method was 20 cells l^{-1}. *Alexandrium minutum* was identified by thecal plate tabulation (Balech, 1995) after adding some drops of the fluorescent dye Calcofluor White M2R (final concentration 10–20 mg ml^{-1}; Fritz and Triemer, 1985) to the chamber in order to stain the plates. The chambers were examined under 400× magnification in an inverted microscope with UV excitation fluorescent illumination. The phytoplankton nomenclature is used according to Steidinger and Tangen (1997), Daugbjerg et al. (2000) and Moestrup et al. (2002).

For SEM (scanning electron microscopy) cultured cells (strain IEO-AL9C) were fixed with 4% glutaraldehyde, washed in dH_2O, dehydrated in a graded ethanol/acetone series, critical point dried and coated with gold. The microscope used was a Hitachi S-350N (Nissei Sangyo Co. Ltd., Tokyo, Japan), operating at 10 kV.

2.6. Culturing and genetic analyses

Clonal cultures of *A. minutum* were established at the Institut de Ciències del Mar (CSIC-D1), Instituto Español de Oceanografia (IEO-AL8C, IEO-AL9C) and Consiglio Nazionale delle Ricerche (CNR-AMIA1, CNR-AMIA4, CNR-AMIA5) from water samples taken in 1995, 2001 and 2002 at Arenys de Mar and Syracuse bay (Table 2). All marine cultures were maintained in F/20 and F/2 media (see http://ccmp.bigelow.org/), at 17 ± 1 °C and a 14:10 h (light:dark) photoperiod. Illumination was provided by a photon irradiance of 100 μmol m^{-2} s^{-1}.

2.7. DNA extraction

Approximately 5–10 ml of exponentially growing cultures were harvested by centrifugation (4000 rpm) for 10 min at room temperature. The pelleted cells

(▲) to stations sampled during the 2003 bloom. Those stations sampled both years are indicated by (+). Routinely sampled stations are indicated (st. R and T in Arenys, and st. 1–4 in Syracuse).

were rinsed twice with artificial 0.22 μm sterile seawater. Total DNA was extracted using Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA) or DNeasy Plant Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

2.8. PCR amplification, cloning and sequencing

The 5.8S rDNA and ITS regions (ITS1 and ITS2) were amplified as described by Penna et al. (2005). The PCR products were visualized on 1.8% agarose gel. After electrophoresis analyses, the PCR products were excised from the agarose gel and purified with the QIAquick Gel Extraction Kit (Qiagen, Westburg). Purified PCR fragments were directly sequenced or cloned in the vector pDrive Cloning Vector (Qiagen, Valencia, CA) and sequenced. The nucleotide sequences were performed using the ABI PRISM 310 Genetic Analyzer (Perkin Elmer, Applied Biosystems, Foster City, CA) and the dye terminator method was used according to the manufacturer's instructions (ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit, Perkin Elmer Corp., Foster City, CA). The EMBL accession numbers of the 5.8S rDNA and ITS1-ITS2 regions are indicated in Table 2.

2.9. Sequence analyses

Multiple alignments were constructed using the CLUSTAL X program and subsequently rechecked by eye. To determine the ITS and 5.8S rDNA termini for the *A. minutum*, the rDNA coding region and flanking sequences of ITS1 and ITS2 were aligned with those of *Alexandrium* species listed in GenBank.

2.10. Species-specific PCR assay

Species-specific PCR analyses of all six *A. minutum* isolates were performed by using two species-specific designed primers (Galluzzi et al., submitted). The PCR specificity was tested using a plasmid containing the ITS1-5.8S-ITS2 rDNA sequence of *A. minutum*. Further, the primer pairs were tested in the PCR assay for possible cross-reactivity with no-target DNA by including other species of dinoflagellates and diatoms.

3. Results

3.1. Morphological identification and genetic comparison

The identification of *A. minutum* was based on both microscope observations and genetic analyses. Morphologically, cells are rounded and small-sized, 22.5 μm wide (min.: 14.0 μm; max.: 32.7 μm; $n = 112$), and 23.2 μm long (min.: 14.2 μm; max.: 33.6 μm; $n = 112$) on average, although cells in exponentially growing cultures were significantly smaller (Fig. 2). The four main distinctive characters are shown in Fig. 2: direct connection of 1′-Po plates; the presence of a ventral pore (Vp) at the 1′ right-anterior side; plate 6″ narrow; posterior sulcal plate (Sp) quadrangular, lacking a connecting pore. In the six clonal cultures theca was always smooth, without a reticulation pattern (Fig. 2). The morphological identification was further confirmed by molecular analyses. PCR amplification of the *A. minutum* isolates produced a single fragment of 520 bp. Sequence alignment of the 5.8S rDNA and ITS regions of *A. minutum* from Arenys and Syracuse revealed that all six isolate sequences were identical. Selected primers for the conserved 5.8S rDNA and variable ITS-1 region specific for the *A. minutum* species gave a PCR fragment of 212 bp. Molecular weight of the amplified product was as expected and no other aspecific PCR products were visible when total *A. minutum* genomic DNA of each isolate was used as template. Species-specific primers were tested for cross-reactivity PCR amplification using several genomic DNA from other species (data are shown only for one no-targeted species in Fig. 3).

3.2. Regional scale: A. minutum distribution and inorganic nutrients

Table 1 shows the maximum *A. minutum* cell concentrations along the Catalan and Sicilian coasts in 2002 and 2003 and the inorganic nutrient ratios. *A. minutum* cells were widespread along the Catalan and Sicilian coasts (Fig. 1B). They occurred in all the Catalan harbours sampled and in some beach areas. High cell concentrations exceeding 10^5 cells l^{-1} were detected in four Catalan harbours. In Arenys de Mar harbour, water discolourations coincided with high

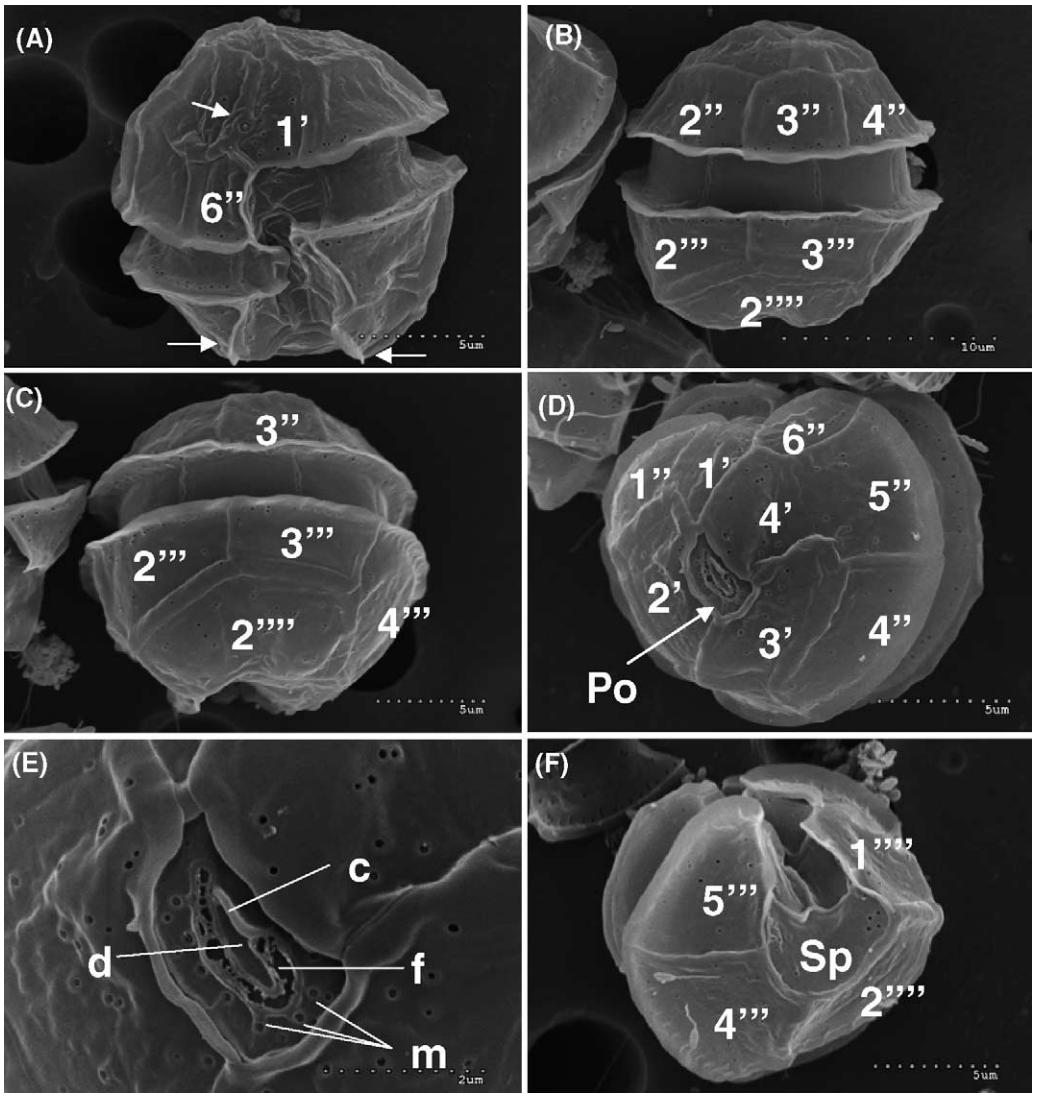

Fig. 2. SEM micrographs of *A. minutum* strain IEO-AL9C from Arenys de Mar (Spain). (A) Ventral view of a specimen with ventral pore (Vp) and well-developed intercalary bands. The connection between Po and 1′ and the sulcal lists (arrows) can be observed. (B, C) Dorsal view. (D) Epithecal plate pattern. Po = apical pore complex. (E) Structure of the Po plate, f = formamen, c = callus, d = canopy, m = marginal pores. (H) Hypothecal plate pattern. Sp = posterior sulcal plate. Note the small size of these cultured cells.

A. minutum concentrations (over 10^6 cells l^{-1}). Various areas of Sicily were also affected by the occurrence of *A. minutum*, both in Tyrrhenian and Ionian coastal waters (Fig. 1B). The maximum cell densities in Sicily did not exceed 7×10^3 cells l^{-1}, with the exception of a single Ionian zone (Syracuse harbour) and a Tyrrhenian brackish site (Verde Pond) where blooms of this species ($>10^5$ cells l^{-1}) were observed in spring.

The median concentrations of nutrients (nitrate, ammonium and phosphate) over the sampling period in the three areas considered were: 5.2, 2.1 and 0.3 μM for the Catalan coast, 5.5, 1.0 and 0.4 for Sicily (Ionian Sea) and 4.0, 0.4 and 0.34 for Sicily (Tyrrhenian), respectively. The comparison of dissolved inorganic nutrients among different localities of the Catalan and Sicilian coasts during the year 2002–2003 indicated

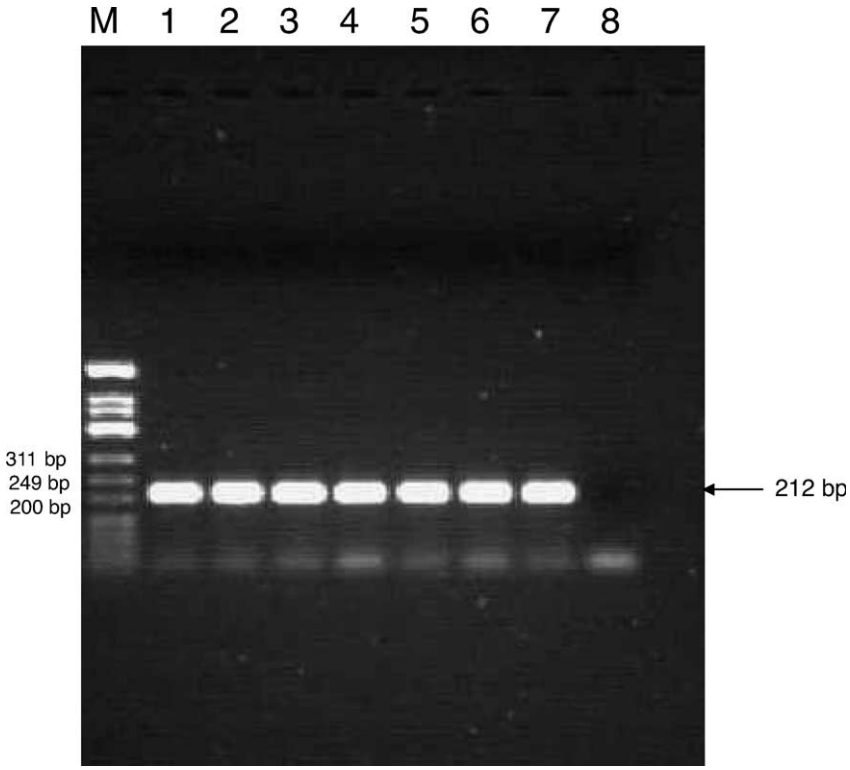

Fig. 3. *A. minutum*-specific PCR assay using genomic DNA from six *A. minutum* isolates, a positive control and *Karlodinium* sp. (= *Gyrodinium corsicum*) IRTA-GC5. Target DNA from each isolate and plasmid was used to test species-specific PCR primer for the *A. minutum* species. The characteristic PCR product sizes generated by species-specific assay are shown by analysis on standard agarose gel. Lane 1, template DNA from the *A. minutum* CNR-AMIA1; lane 2, *A. minutum* CNR-AMIA4; lane 3, *A. minutum* CNR-AMIA5; lane 4, *A. minutum* CSIC-D1; lane 5, *A. minutum* IEO-AL8C; lane 6, *A. minutum* IEO-AL9C; lane 7, template plasmid containing *A. minutum* ITS1-5.8S-ITS2 rDNA; lane 8, *G. corsicum* IRTA-GC5. A clean no-template control was included in the PCR assay (not shown on the gel). M, size standards; arrow indicated reactions that produced a single species-specific PCR product.

that, irrespective of the strong spatial and temporal variability, Catalan sites in general are characterized by higher amounts of dissolved inorganic nitrogen (DIN) than the Italian region considered (Fig. 4). In Catalonia and some Ionian points of Sicily (Syracuse), the median concentration of DIN usually is higher than 5, and concentrations of PO_4–P ranged from 0.1 to 1 μM. Some Spanish harbours, which are located within the south (from Cambrils to St. Carles) and centre (Premià and Arenys de Mar), displayed amounts of DIN 1 or 2 orders of magnitude higher. PO_4–P median values above 0.5 μM were only detected in the centre of Catalonia and in some Ionian (the Syracuse urban area) and Tyrrhenian stations (Vulcano). DIN and PO_4–P levels were often in the lowest range in beach areas of both regions, with a clearest pattern in Catalonia. Consequently, nutrient ratios showed a high temporal and spatial variability in the three areas (Table 1). Most of the average DIN:PO_4 ratios at the coastal sites studied exceeded the Redfield ratio (16:1). The lowest values were found on beach areas, especially in the Tyrrhenian Sea. In almost all the studied coastal sites the average Si:DIN was below the theoretical ratio (1:1), indicating silicate was the most likely inorganic nutrient limiting primary production in these areas. Only the three Tyrrhenian beach areas and some sites in the northern Catalan Sea had ratios higher than 1:1. The average Si:PO_4 along both areas exhibited a great spatio-temporal variability, with values as high as 200 to below 16.

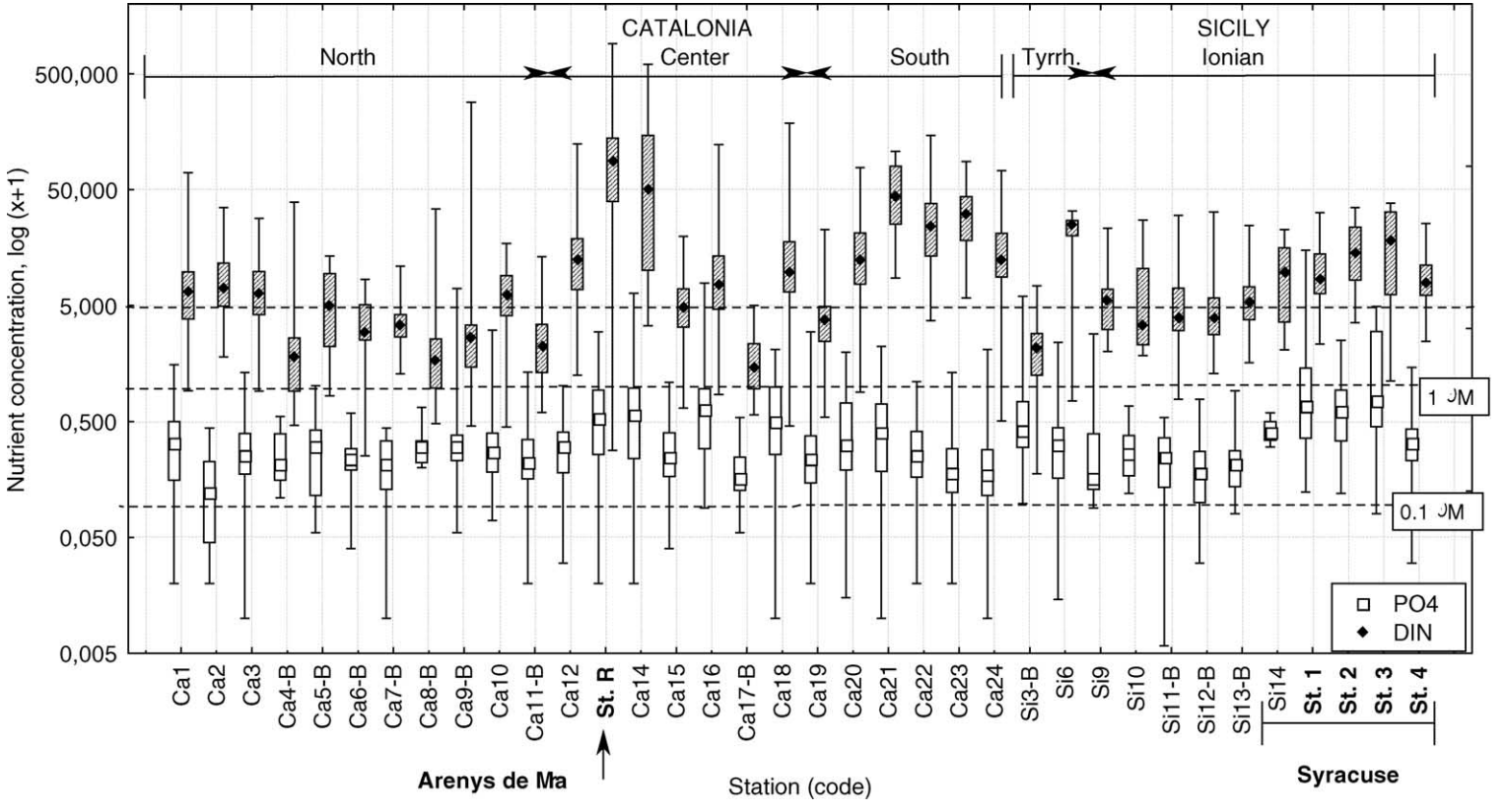

Fig. 4. Box plots of median concentrations of inorganic nutrients (DIN and PO_4) in Catalan and Sicilian harbours and beaches. Box: 25%, 75%; Whisker: Min, Max. Codes as in Table 1. Beaches are indicated adding −B after the code.

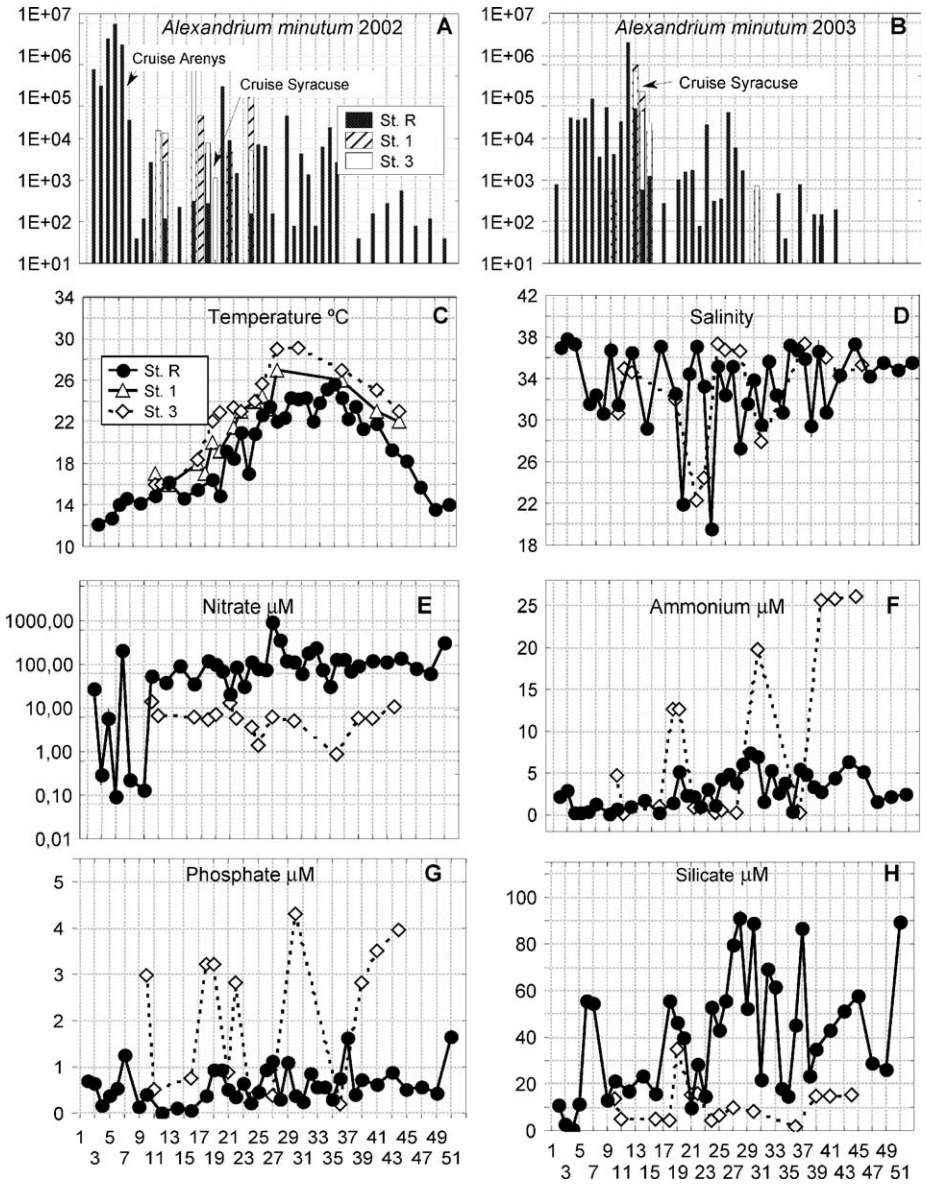

Fig. 5. Temporal distribution of *A. minutum* (log cells l^{-1}) in Arenys de Mar (st. R) and Syracuse (st. 1 and st. 3) during (A) 2002 and (B) 2003. Temporal variability over an annual cycle (2002) in (C) water temperature, (D) salinity, and inorganic nutrients (in µM) as (E) nitrate, (F) ammonia, (G) phosphate and (H) silicate in Arenys de Mar (st. R) and Syracuse (st. 3). Note that nitrate concentrations are plotted on a logarithmic scale. The temporary pattern of inorganic nutrients over the 2003 cycle is not shown since it shows similar nutrient levels to the presented cycle.

Calculations showed that potential limitation of primary production by inorganic nutrient concentrations (DIN, P–PO_4, Si) is quite limited at the sites analyzed in this study (20 and 13% of the cases of potential limitation in the Catalan and Sicilian coasts).

3.3. Temporal distribution of A. minutum in two target localities

Outbreaks of *A. minutum* were observed in Arenys de Mar harbour and Syracuse bay (harbour area)

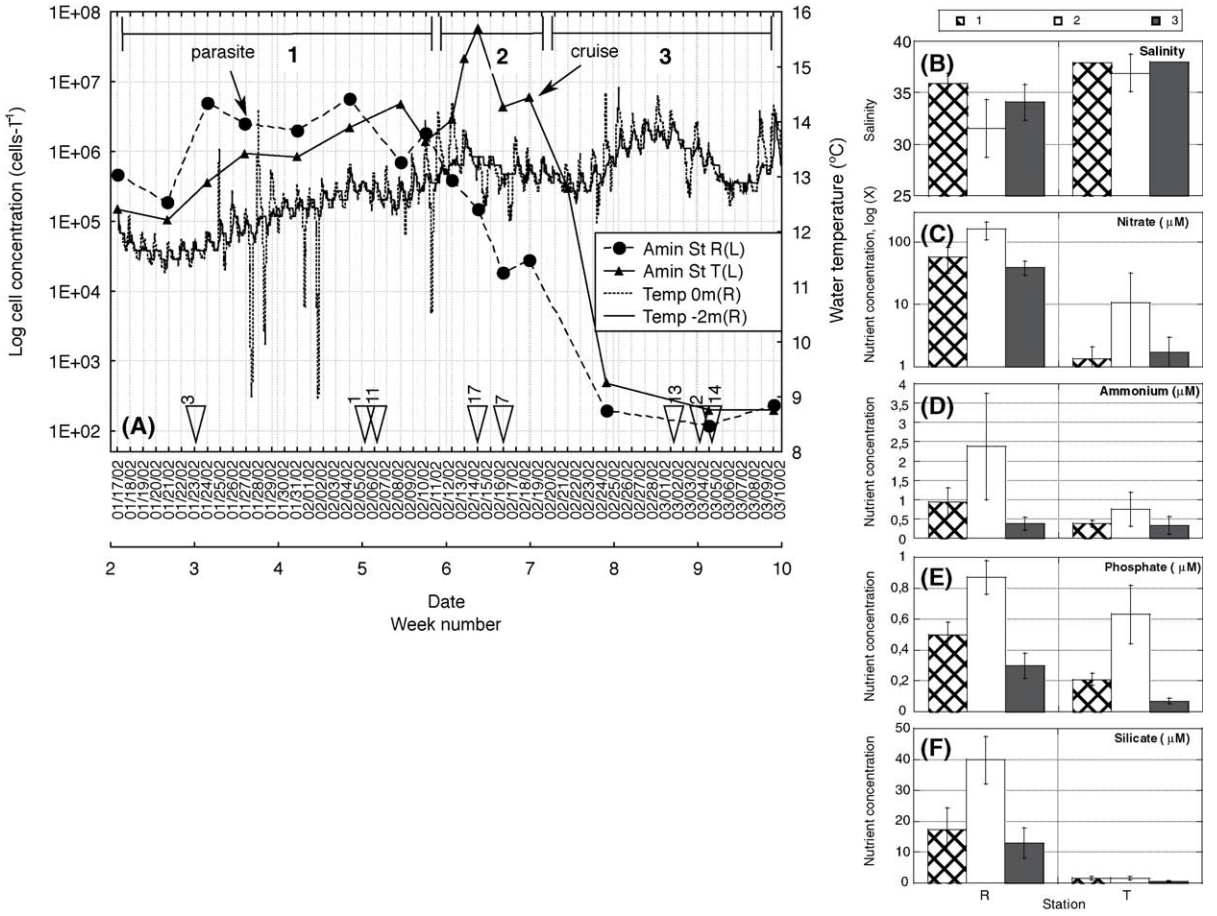

Fig. 6. (A) Small-scale temporal evolution of *A. minutum* in Arenys de Mar harbour during the 2002 bloom event in two stations (st. R and st. T, surface). The difference between water temperature at surface and bottom (st. T) is due to sunny and calm days in front to windy days, indicating water stratification or mixing. Raining events (mm) are indicated by open arrowheads at the bottom. The bloom has been divided into three periods: (1) *A. minutum* blooming concentrations both at st. R and T; (2) bloom declining at st. R, and still increasing at st. T; (3) bloom finished at both stations. (B–F) Comparison of salinity and dissolved inorganic nutrients (NO_3, NH_4, PO_4 and SiO_4) during the three periods at both stations. Error bars: standard error.

during the 2 years analyzed (2002 and 2003) (Fig. 5). Surface water temperatures and salinities during *A. minutum* bloom events were 12–14.5 °C and 32–38, and 16–24 °C and 32–37.7 for the two areas, respectively. Whilst in Arenys *A minutum* vegetative cells were recorded over all the annual cycle, in Syracuse this species was detected only in spring. The highest densities were found in Arenys with winter to early spring peaks ($>10^6$ cells l^{-1}) in February 2002 and in March 2003. A more detailed analysis of the temporal evolution of *A. minutum* densities during the bloom events is presented in Fig. 5. In Syracuse bay, the highest densities occurred in the northern part of the bay (st. 1 and 3, in Fig. 5), being 1–2 orders of magnitude lower than in Arenys de Mar. Both harbours are subject to considerable changes in salinity due to the freshwater inflows supplying a high nutrient load. Concentrations of dissolved inorganic nitrogen (DIN) were much lower in Syracuse than in Arenys (Fig. 5). Nitrate was often the prevalent inorganic nitrogen form, with nitrate peaks (exceeding 200 μM in the Arenys de Mar harbour – st. R), although on some occasions there was a dominance of ammonium–nitrogen in Syracuse,

mostly at the river mouth. Orthophosphates (usually below 1.0 μM in Arenys – st. R) displayed higher values in Syracuse (Fig. 5). Median silicate concentrations were much higher in Arenys than in Syracuse. However, occasional silicate peaks (>30 μM) occurred in both sites (Fig. 5). In both areas, there was no apparent relationship between *A. minutum* biomass and inorganic nutrient concentrations. However, a common trait in nutrient stoichiometry during the course of blooms suggests that DIN and P–PO_4 concentrations were not limiting. In fact, DIN or P–PO_4 did not limit in 90% of the cases.

In both harbours, chlorophyll concentrations were often lower than 8 μg l^{-1}. However, there was an exceptional peak of 380 μg l^{-1} at st. 1 (Sanità Marittima, Syracuse) due to a persistent high biomass bloom of *Lingulodinium polyedrum* (21 May 2002), with a considerable water discolouration (max. 22×10^6 cells l^{-1}).

3.4. *A. minutum* bloom in Arenys de Mar (year 2002): small-scale temporal evolution

The temporal evolution of the *A. minutum* bloom in Arenys de Mar harbour is presented (Fig. 6A). Three phases can be distinguished in the 2002 bloom: (1) *A. minutum* blooming at st. R and T; (2) bloom declining at st. R, and still increasing at st. T; (3) bloom finishing at both stations (Fig. 6A). In the first phase, *A. minutum* cell concentrations were over 10^6 cells l^{-1}, first at st. R. and after approximately 15 days also at st. T.

A parasite infecting *A. minutum* cells was observed on 26 January at st. R. Infected cells were immobile, dark cysts that sometimes conserved the theca broken around. Unfixed water samples showed that the cysts released a lot of small flagellated cells. The bloom started to decline at st. R after two rain episodes, whereas at st. T it continued to increase until a maximum of 4.7×10^7 cells l^{-1} was reached on 14 February. The highest concentration at st. T was achieved after 3–4 days of water warming as shown by the difference in temperature between surface (0 m) and bottom (−2 m).

There was a high variability in salinity and in concentrations of dissolved inorganic nutrients (NO_3, NH_4, PO_4 and SiO_4) in Arenys depending on the sampling point (Fig. 6B–F). Salinity was always lower at st. R since it was directly affected by an inflow of freshwater rich in nutrients, especially nitrate and silicate, explaining the big differences between st. R and T. Ammonium and phosphate, although always higher at st. R, were in the same range. The decrease in salinity, observed at st. R and T during the second period, and the overall nutrient increase, were related to the rain events.

3.5. Spatial variability: Arenys de Mar cruise (year 2002)

A cruise was carried out in the Arenys de Mar harbour (18 February 2002, Fig. 7) just after the maximum bloom (on 14 February, 2002).

3.5.1. Horizontal distribution

A. minutum cells were distributed in the whole harbour, and clearly dominated the planktonic community in all the stations. More than 90% of the phytoplankton cell counts corresponded to *A. minutum*, except in the harbour's mouth (60%). On this occasion, maximum concentrations of *A. minutum* had already been detected in the area of st. T (Fig. 6), from which a dilution gradient was observed towards the harbour mouth. Chl-*a* followed the same pattern as *A. minutum* concentrations ($n = 57$, $r = 0.63$, $p = 0.000$). The horizontal distribution of temperature, salinity, DIN and silicate reflects the inflow of freshwater near st. R. Lower salinities were also detected near the northern pier walls and co-occurred with maximum PO_4 concentrations ($n = 34$, $r = -0.33$, $p = 0.038$) and high silicate levels, indicating freshwater seepage through the harbour walls. Calculations of potential nutrient limitation in the harbour waters during the cruise suggest no limitation by PO_4, only a few cases were limited by DIN (<9%), whereas 47% were limited by SiO_4. During the cruise, the values in phosphate and ammonia were in the usual range (mean values 0.40 and 0.62 μM, respectively). In contrast, NO_3 levels were very low (<2 μM).

The most important co-blooming dinoflagellates were *Prorocentrum micans* and *Dinophysis sacculus*. *P. micans* followed the same pattern as *A. minutum* ($n = 57$, $r = 0.71$, $p = 0$); maximum *D. sacculus* concentrations were detected near st. R whereas *Scrippsiella* spp. were scarce and more homogeneously distributed. High concentrations of microzooplankton were detected (around 10^4 cells l^{-1}).

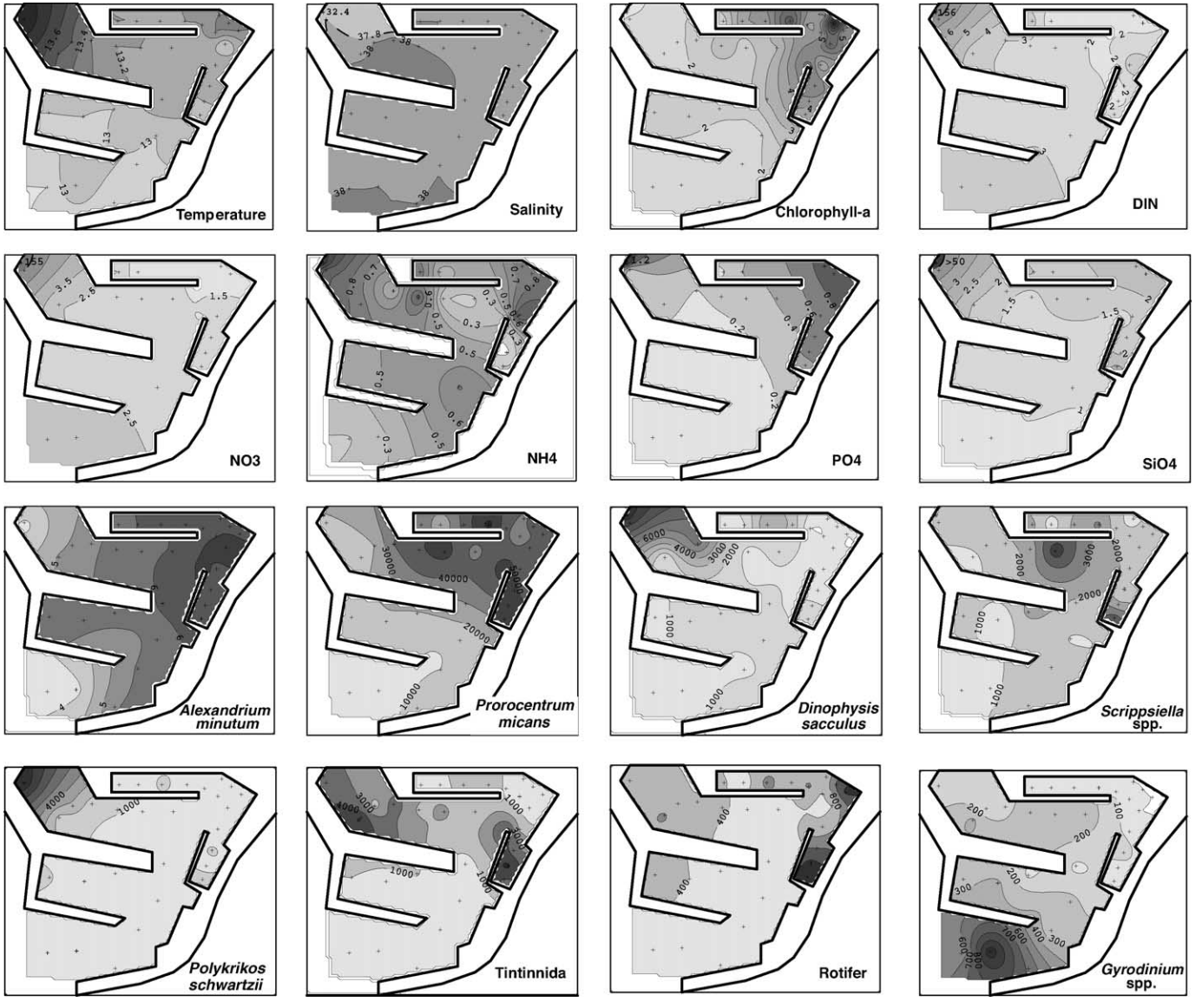

Fig. 7. Surface distribution for the physicochemical parameters and the dominant phyto- and microzooplankton species during the bloom in Arenys (18 February 2002).

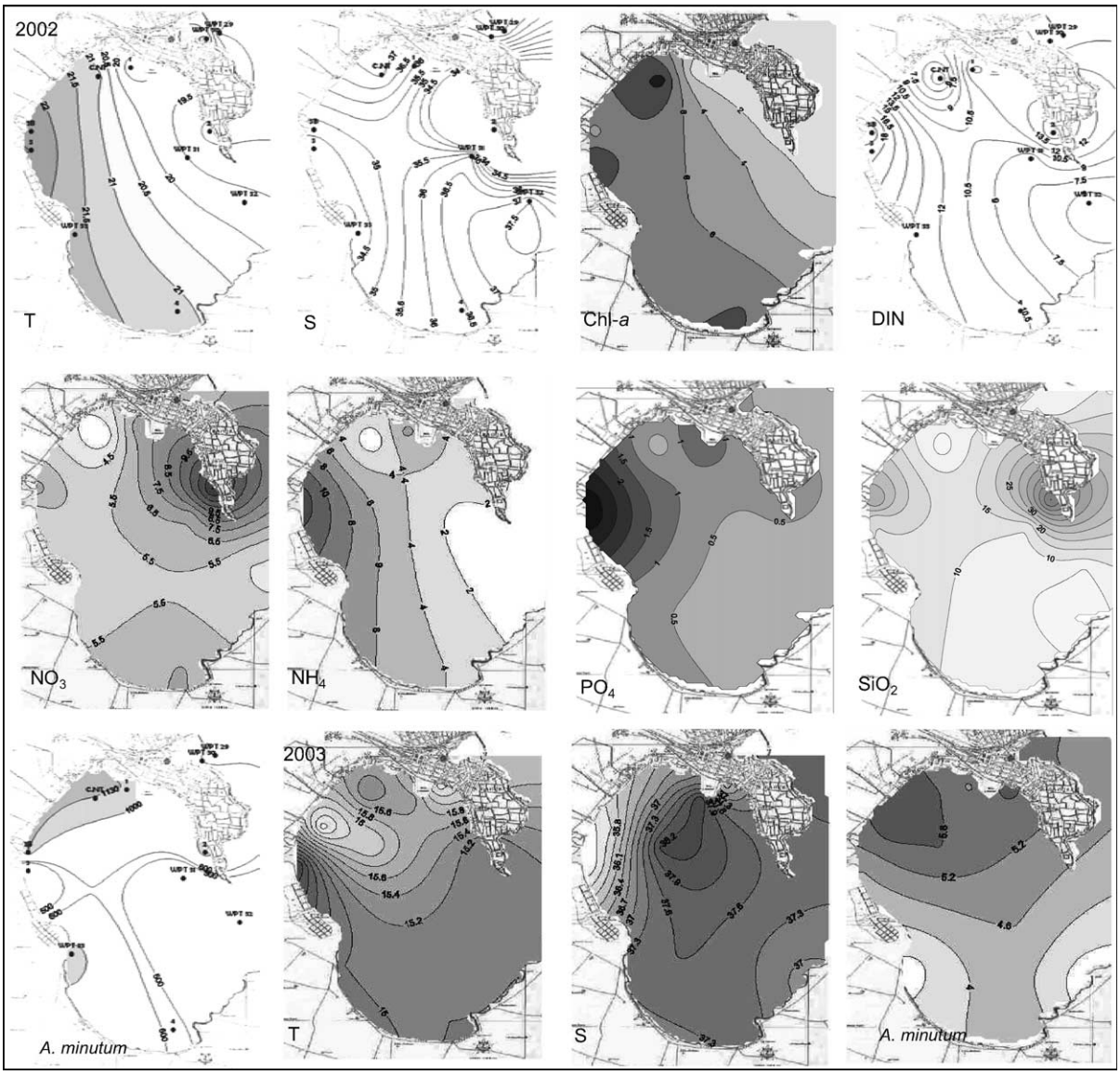

Fig. 8. Surface distribution for the physicochemical parameters and the dominant phytoplankton species during the cruises at Syracuse, 2002 and 2003.

The main organisms were heterotrophic dinoflagellates such as *Polykrikos schwartzii* and *Gyrodinium* sp., loricate ciliates as *Tintinnopsis* spp. and rotifers (Fig. 5) with *Synchaeta triophthalma* the most abundant.

The microzooplankton species distributions were more heterogeneous in the harbour than for the phytoplankton, probably because their swimming speeds were higher than those of the small dinoflagellates, which gave them the chance to exploit phytoplankton patches less exploited by other micrograzers. *S. triophthalma* was the dominant grazer at the st. T area, coinciding with the maximum *A. minutum* concentrations ($n = 57$, $r = 0.50$, $p = 0$). Minimum *A. minutum* concentrations inside the harbour co-occurred with *P. schwartzii* and *Gyrodinium* sp.

3.5.2. Vertical distribution

Phytoplankton and microzooplankton species were almost homogeneously distributed along the water column. *A. minutum* was once again the dominant species at all stations and depths, both at midday and in the afternoon, showing slightly higher cell concentrations at surface. Thus, during the cruise, a thick layer with very high concentrations on *A. minutum* was widespread in the whole harbour. *P. micans* achieved relatively high cell concentrations only in the deepest samples at midday (around 40% of total phytoplankton counts). Other significant differences were not found in the vertical pattern between midday and afternoon profiles.

3.6. Spatial variability: Syracuse cruises (2002 and 2003)

Two cruises were carried out in the Syracuse harbour (11 May 2002 and 2 April 2003). On both occasions *A. minutum* cells assembled in the most confined sector of the harbour (northward the river mouth), characterized by a reduced water circulation. The highest cell densities were found in 2003 ($1.3 \times 10^6\ l^{-1}$) at the surface (Fig. 8), with a sharp decrease (one order of magnitude) in cell numbers at −1 m and still lower densities toward the bottom (−5 m). Concentrations of nutrients (silicate, DIN and PO_4–P) were often maximal near the freshwater inputs with 6.70 μM of inorganic nitrogen (mostly nitrates) and 0.46 μM orthophosphate in the *A. minutum* patch (cruise 2003, DIN:PO_4 = 14.6). In contrast, during the previous cruise in 2002 (Fig. 8), lower cell densities (1.1×10^3 cells l^{-1}) and higher amounts of these nutrients were detected: 20.61 μM DIN – mostly ammonia, 3.21 μM PO_4–P, with a DIN:PO_4 ratio of 6.4. In 2002, simultaneous blooms of the dinoflagellate *Lingulodinium polyedrum* took place within the harbour and persisted during the next spring samplings, accounting for the observed high values of chlorophyll *a*, up to 380 μg l^{-1} on May 21. During the 2003 cruise, the most important, co-blooming dinoflagellate was *Prorocentrum triestinum*, which reached exceptional concentrations (6×10^7 cells l^{-1}), and was mainly responsible for the water discolouration. Thus, in Syracuse harbour *A. minutum* was never found as the dominant phytoplankton species.

4. Discussion

4.1. Organism identification

Cell morphology of *A. minutum* from the two Mediterranean regions fitted the re-description by Balech (1989), with a ventral pore always present in the material studied and thecal plates lacking ornamentations in the cultured strains. However, on very few occasions, some natural Catalan specimens of *A. minutum* showed a reticulation in the hypotheca. Our usual morphotype is the most frequent one among the different geographical isolates around the world (see Hansen et al., 2003, Table 5). The toxin composition in strains from Arenys and Syracuse is basically GTX 1-4 (Van Lenning et al., 2004; Giacobbe et al., 2003a,b). ITS1-5.8S-ITS2 rDNA sequences were identical for the six isolates, confirming the existence of a unique morphotype and genotype in the Mediterranean area. Furthermore, the high specificity of the species-specific PCR primers selected for *A. minutum* was examined against other phytoplankton species and the results obtained allowed detecting the *A. minutum* species unequivocally. Thus, species-specific PCR assays will be further applied in field phytoplankton populations to discriminate the *A. minutum* presence.

Recently, an *A. minutum* morphotype, differing from the typical *minutum* morphotype by the absence of a ventral pore (Vp), has been observed in Denmark (Hansen et al., 2003). Partial large subunit rDNA sequences of Danish specimens clustered together with other European strains of this species having a Vp. Therefore, sequencing of this part of the gene did not resolve intraspecific relationships, as it did not allow the differentiation of populations with or without a ventral pore. These authors suggested that the ventral pore may be a variable feature, making this character questionable. This has important taxonomical implications since it modifies the concept of *Alexandrium* morphological species, which in this case should be revised. In the near future, atypical *A. minutum* strains (lacking the ventral pore and with ornamentations) should be analyzed using the high variable ITS sequences in order to possibly discriminate different genetic populations in the Mediterranean Sea.

4.2. Ecological comparison

The data obtained from the two Mediterranean regions show the extended distribution of *A. minutum* in the Mediterranean. However, high-density blooms of this species always occur in confined or semi-enclosed water areas such as harbours, bays or lagoons. This species was recorded in some beach areas of the Catalan and Sicilian coasts, but densities never exceed 10^3 cells l^{-1}. In both areas, Catalonia and Sicily, *A. minutum* blooms occur in localities affected by local freshwater inputs that could be related to the supply of macro- and micronutrients. In other reports, riverine inputs of selenium after rainfall have been suggested to be a critical trigger factor for blooms of some dinoflagellates such as *A. minutum* and *Gymnodinium catenatum* (Doblin et al., 1999). Studies on batch cultures of *A. minutum* indicated optimal growth rates at salinities between 20 and 37, with peaks at 25 (0.63 ± 0.07 div day^{-1}) (Grzebyk et al., 2003), or even at salinities lower than 15 (Hwang and Lu, 2000). The association between blooms of *A. minutum* and freshwater outflow could be related to the formation of density gradients acting as a retention mechanism (e.g. fronts) or inducing water stability. In both areas examined, the local outflow of continental waters causes the formation of density fronts that could act as a retention mechanism for the phytoplankton population. Obviously, the direct supply of nutrients flowing in a semi-confined area plays a key role in both areas in sustaining high phytoplankton biomass. Giacobbe et al. (1996) reported that *A. minutum* proliferations in the Ganzirri lagoon occurred in May, with DIN:PO_4 atomic ratios close to 16:1 and a minor competitive pressure from other phytoplankton species. In the present study, *A. minutum* bloomed in places that differ markedly in inorganic nutrient concentrations and DIN:PO_4 ratios (Table 1, Figs. 4 and 5). In fact, Arenys was characterized by high inputs of nitrates and silicates, which are mainly associated with a runoff origin, in contrast to the high concentrations of ammonium and phosphate in Syracuse, which reveal a rather urban origin. However, some common traits can be observed (Table 3): (1) throughout the two Mediterranean regions, Si:DIN ratios tend to be lower than 1 in harbours indicating a potential limitation for diatom growth, and suggesting a possible advantage for dinoflagellate growth (Justic et al., 1995; Masó et al., 2000; Anderson et al., 2002). In contrast, on beach areas, the Si:DIN ratio was much higher than 1, indicating a potential advantage for diatom growth; (2) neither in Arenys nor in Syracuse were there cases of potential nitrogen or phosphate limitation and (3) in both areas nitrates were usually the dominant nitrogen form. Measures of nitrate and ammonium uptake during *A. minutum* proliferations in Penzé River estuary (NW France) indicated nitrate to be the main source of nitrogen, representing up to 75% of the nitrogen uptake (Maguer et al., 2000). The evident decrease in nitrates during the bloom in Arenys (weeks 3–4, 2002) also supports this observation (Fig. 5). Furthermore, detrimental effects of NH_4 at relatively low concentrations were found by Su et al. (1993) in lab conditions, while other authors have not confirmed this effect (Arzul et al., 2001). There are many processes implied in nutrient uptake kinetics, as luxury consumption, local inputs and transient nutrient pulses, or the tight relationship between uptake and external concentration. Therefore, it is not realistic to know if a watermass can support a bloom (Smayda, 1997) because the patterns between a given species or a given community and external nutrient concentration (if there are any) can be interpreted in opposite ways. In fact, in the present study there is not any clear pattern between *A. minutum* and external inorganic nutrient concentrations. In a single case (Syracuse, 2002), the *A. minutum* bloom finished simultaneously to an increase in ammonium level. Another example from Arenys suggests that the lack of blooms during the last 6 months of the year could be related to ammonium concentrations higher than 5 μM. On the other hand, a peak of 16 μM NH_4 in Syracuse (2003) coincided with an *A. minutum* peak of 10^5 cells l^{-1}.

During the *A. minutum* blooms in our areas, there was a prevalence of dinoflagellates, despite some slight differences in the phytoplankton species composition and dominance. This observation is consistent with the basic principles of community assembly established by Margalef's classical Mandala (Margalef, 1978; Margalef et al., 1979). According to the Mandala, red tides would occur if high nutrient concentrations coexisted with relatively low turbulence. Thus, a high nutrient load associated with stratified and/or confined waters favours the

Table 3
Similarities and differences between *A. minutum* blooms in Arenys and Syracuse

	Arenys de Mar	Syracuse
Biological factors		
A. minutum maximum density	Over 10^6 cells l^{-1}	Over 10^5 cells l^{-1}; usually 1–2 orders of magnitude lower than in Arenys de Mar
Bloom lifespan	One month	One week
Presence of vegetative cells	All year long	Spring
Recurrency	Known since 1996	Known since 2001
Cystbed	Present and dense	Not found
Dominant phytoplankton group during the bloom	Dinoflagellates	Dinoflagellates
Dominant species (life-form types)	*A. minutum*, *P. micans*, *D. sacculus*, *Scrippsiella* spp. (types I and II)	*L. polyedrum*, *P. triestinum* (*A. minutum*) (types I –II and V)
Bloom decay	Basically grazed by microzooplankton; possible physical dispersion (after raining); parasitic infection (?)	Unknown
Physical factors		
Topography	Shallow, semi-confined; fishing and leisure harbour	Shallow, semi-confined; harbour inside a natural bay; shellfish farming activities
Extension (surface area)	17 ha	700 ha
Depth	0.5–6 m	0.5–8 m at the sampling area; 25–30 m at the entrance
Water temperature	12–14.5 (°C)	16–24 (°C)
Salinity	32–38	32–37.7
Physical structures (fronts/stratification)	Inputs of freshwaters regulate the physical structure	Inputs of freshwaters regulate the physical structure
Chemical factors		
Freshwater influence	Continental (nitrates and silicates)	Urban (ammonium and phosphate)
DIN/PO_4 relationship	Around 300	16–40
DIN and PO_4 limitations	Not limiting	Not limiting
NO_3/NH_4 ratio	Nitrates as the prevalent inorganic nitrogen form	Nitrates as the prevalent inorganic nitrogen form; occasional dominance of ammonium nitrogen near riverine inputs
PO_4	Usually below 1.0 μM	Higher values; around 1.0 μM

development of red tides. *A. minutum* dominated in Arenys de Mar harbour, causing water discolouration. The second dominant species was *P. micans*, followed by *D. sacculus* and *Scrippsiella* spp. In contrast, *A. minutum* never prevailed in Syracuse and *P. triestinum* and *L. polyedrum* were the main responsible for water discolourations. *P. triestinum* is a common species also in Arenys de Mar. However, it seems to show a negative association with *A. minutum*. In years when *P. triestinum* was abundant, *A. minutum* did not reach cell concentrations exceeding 10^5 cells l^{-1} (Vila et al., in press).

A. minutum is described by Smayda and Reynolds (2001) as life-form type I, present in relatively shallow, highly nutrient-enriched habitats, similar to our sites. Also other dinoflagellates such as gymnodinioids and the genera *Heterocapsa*, *Scrippsiella*, *Prorocentrum* are representatives of life-form type I and II.

A. minutum and *L. polyedrum* coexisted in an eutrophicated area on the eastern Adriatic coast with alternating dominance (Marasovic et al., 1995). The last species is, however, adapted to bloom during upwelling relaxations (type V) and therefore able to survive within upwelling habitats (Blasco, 1977). The type V life-form (upwelling relaxation taxa), such as *L. polyedrum* and *G. catenatum* (single cells), swim at rates about six times faster than they sink, and can

readily ascend to avoid sinking (Smayda, 2002). *A. minutum*, *L. polyedrum*, *P. micans* and *Scrippsiella* sp. were the main species of the phytoplankton community in upwelling Atlantic coastal waters of Morocco (Tahri-Joutei et al., 2000). Thus, the lack of *L. polyedrum* blooms in Catalan waters indicates that Arenys and Syracuse are different habitats. Arenys is a harbour much smaller and shallower than Syracuse, with reduced water mixing and limited cell dispersion. Inorganic nutrients, basically nitrates, are highly available in Arenys. The increased confinement of this site is particularly noticeable because it could play an important role in the maintenance of a huge cyst bed (Garcés et al., 2004). In contrast, *A. minutum* cysts in sediments from Syracuse have never been found (Bravo, pers. comm.).

The bloom decay at both sites occurred quickly (in 6–15 days, Fig. 5), after a week (Syracuse) or a month (Arenys) of sustained high cell concentration ($>10^5$ cells l^{-1}). It is necessary to highlight that despite the *A. minutum* parasitic infection at Arenys (st. R), the bloom persisted and some weeks later the whole harbour waters became discoloured. The parasite probably belongs to the new genus *Parvilucifera* (Apicomplexan) recently reported from Scandinavian waters infecting *Dinophysis* (Norén et al., 1999), in estuaries of northern Brittany (ErardLeDenn et al., 2002, France) after an *A. minutum* bloom and, in Tarragona harbour (Spain, Delgado, 1999) after an *A. catenella* bloom. In laboratory cultures, the parasite is capable of removing a significant fraction of dinoflagellate biomass in a short time (Delgado, 1999; ErardLeDenn et al., 2002). However, the effect of this parasite on natural *A. minutum* populations did not induce to the bloom decrease (Probert, 1999; this study). In Arenys, the bloom decline at st. R and successive increase at st. T coincided with two rain episodes (Fig. 6A). The increased freshwater input in st. R after the rainfall could have rapidly washed the cells from st. R and those would be accumulated at st. T, as it could be seen in the cruise carried out some days later (Fig. 7). Otherwise, a dense population of rotifers detected at the end of January near st. R could have participated in reducing the bloom there (Calbet et al., 2003). During the cruise, *P. schwartzii* at the st. R area could have been grazing on *A. minutum*. Rotifers and tintinnida were abundant and co-occurred within the dinoflagellate patch (Fig. 7). As *Synchaeta* spp. are active grazers on dinoflagellates (Egloff, 1988) and *A. minutum* cells have often been observed inside tintinnida during bloom events (personal observation), microzooplankton played, probably, an important role in the bloom termination. Averaged ingestion rates 60 cells ind.$^{-1}$ day^{-1} for rotifers and 45 cells ind.$^{-1}$ day^{-1} for tintinnida (which are in the range reported by Calbet et al., 2003 and references therein) means a daily population decrease of 15%. If no growth in the *A. minutum* population is assumed, which may be quite reliable after 2 months of sustained biomass ($>10^5$ cells l^{-1}), the bloom would be finished in 5 days. That is in agreement with the sharp decrease of the dinoflagellate bloom between 18 and 24 February 2002 (Fig. 6). However, if the dinoflagellate population is actively growing micrograzers can only modulate population dynamics.

5. Conclusion

In summary, the data obtained from the two Mediterranean regions indicates that *A. minutum* blooms occur in confined or semi-confined water areas. This may be attributed to the low flushing rates (tide amplitude is less than 20 cm in the Mediterranean). Long water residence time seems to be a main factor in the bloom maintenance phase, which could differentiate the Arenys bloom from the Syracuse one. The formation of density fronts due to local freshwater outflows could be one of the factors triggering the bloom, especially in Arenys de Mar harbour considering that it initiates near the freshwater entrance. This physical structure could be critical for the bloom initiation, avoiding cell dispersion and assuring high nutrient levels. Once a critical mass is achieved, the bloom expands over the whole harbour. Although Anderson (1998) suggested that *Alexandrium* species does not appear to have particularly high growth rates even under optimal physicochemical conditions, high growth rates were detected for *A. minutum* by in situ measures in Arenys (Garcés et al., 1998) where a dense seedbed has been found (Garcés et al., 2004). According to Probert (1999), the inoculum of a bloom only needs the excystment of a low proportion of the cysts in sediment beds. This explains the high-biomass blooms detected in Arenys de Mar. Thus, local

conditions seem to be a key factor that makes a given locality particularly susceptible to *A. minutum* blooms.

Finally, allelopathy is being considered as a relevant aspect of the *Alexandrium* ecology, playing a non-negligible role in species interaction, succession and, perhaps, bloom formation (Fistarol et al., 2004). Thus, inferring patterns of species interactions, as well as relationships between organism and (environmental) nutrient concentrations is a complex task. This is especially true in areas influenced by continental inputs (as Arenys and Syracuse) – involving a high small-scale, spatio-temporal variability of environmental parameters.

Acknowledgements

The authors thank J. Riba, S. Anglés and P. Tezanos (ICM, Catalonia) and A. Rabito (ARPA, Sicily) for their cooperation and efforts in facilitating the field programme. We thank the support of the Club Nautic Arenys de Mar for facilities during the cruise and sampling and especially Sr. D. Lucena for his enthusiastic collaboration. We thank I. Bravo, M. Fernandez and S. Fraga for supplying culture strains, Dr. F. Andreoni for technical support in sequencing analyses, R. Ventosa for nutrient analysis and Dr. A. Calbet for the rotifer identification. Financial support was provided by the EU project STRATEGY (EVK3-CT-2001-00046), by the Agència Catalana de l'Aigua (Department de Medi Ambient, Generalitat de Catalunya) and CSIC through the contract ''Plà de vigilància de fitoplàncton nociu i tòxic a la Costa Catalana'' and by the Italian Ministery MiPAF, Department of Fisheries and Aquaculture (Projects 5C8 and 6C18).

References

Anderson, D.M., 1989. Toxic algal blooms and red tides: a global perspective. In: Okaichi, T., Anderson, D.M., Nemoto, T. (Eds.), Red Tides: Biology, Environmental Science and Toxicology. Elsevier, New York, pp. 11–16.

Anderson, D.M., 1998. Physiology and bloom dynamics of toxic *Alexandrium* species, with emphasis on life cycle transitions. In: Anderson, D.M., Cambella, A.D., Hallegraeff, G.M. (Eds.), Physiological Ecology of Harmful Algal Blooms. NATO ASI Series, G41, pp. 29–48.

Anderson, D.M., Glibert, P.M., Burkholder, J.M., 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. Estuaries 25 (4b), 704–726.

Arzul, G., Seguel, M., Guzman, L., Erard-Le Denn, E., 1999. Comparison of allelopathic properties in three toxic *Alexandrium* species. J. Exp. Mar. Biol. Ecol. 232, 285–295.

Arzul, G., Seguel, M., Clement, A., 2001. Effect of marine animal excretions on differential growth of phytoplankton species. ICES J. Mar. Sci. 58, 386–390.

Bagøien, E., Miranda, A., Reguera, B., Franco, J.M., 1996. Effects of two paralytic shellfish toxin producing dinoflagellates on the pelagic harpacticoid copepod *Euterpina acutifrons*. Mar. Biol. 126, 361–369.

Balech, E., 1989. Redescription of *Alexandrium minutum* Halim (Dinophyceae) type species of the genus *Alexandrium*. Phycologia 28 (2), 206–211.

Balech, E., 1995. The Genus *Alexandrium* Halim (Dinoflagellata). Sherkin Island Marine Station, Sherkin Island Co., Cork, Ireland.

Belin, C., 1993. Distribution of *Dinophysis* spp. and *Alexandrium minutum* along French coasts since 1984 and their DSP and PSP toxicity levels. In: Smayda, T.J., Shimizu, Y. (Eds.), Toxic Phytoplankton Blooms in the Sea. Elsevier, Amsterdam, pp. 469–474.

Blasco, D., 1977. Red tide in the upwelling region of Baja California. Limnol. Oceanogr. 22, 255–263.

Calbet, A., Vaqué, D., Felipe, J., Vila, M., Sala, M.M., Alcaraz, M., Estrada, M., 2003. Relative grazing impact of microzooplankton and mesozooplankton on a bloom of the toxic dinoflagellate *Alexandrium minutum*. Mar. Ecol. Prog. Ser. 259, 303–309.

Cannon, J.A., 1996. Competition between the dinoflagellates *Alexandrium minutum* and *Prorocentrum micans* in the Port River, South Australia. In: Yasumoto, T., Oshima, T., Fukuyo, Y. (Eds.), Harmful and Toxic Algae. IOC of UNESCO, pp. 381–384.

Daly Yahia Kefi, O., Nezan, E., Daly Yahia, M.N., 2001a. On the presence of the genus *Alexandrium* Halim (dinoflagellates) in the bay of Tunis (Tunisia). Oceanol. Acta 24, 17–25.

Daly Yahia Kefi, O., Nezan, E., Daly Yahia, M.N., 2001b. On the presence of the genus *Alexandrium* Halim (dinoflagellates) in the bay of Tunis (Tunisia). Oceanol. Acta 15, 47–52.

Daugbjerg, N., Hansen, G., Larsen, J., Moestrup, Ø., 2000. Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data, including the erection of three new genera of unarmoured dinoflagellates. Phycologia 39 (4), 302–317.

Delgado, M., 1999. A new ''diablillo parasite'' in the toxic dinoflagellate *Alexandrium catenella* as a possibility to control harmful algal blooms. Harmful Algae News 19, 1–3.

Delgado, M., Estrada, M., Camp, J., Fernandez, J.V., Santmarti, M., Lleti, C., 1990. Development of a toxic *Alexandrium minutum* Halim (Dinophyceae) bloom in the harbour of Sant Carles de la Rapita (Ebro Delta, northwestern Mediterranean). Sci. Mar. 54 (1), 1–7.

Doblin, M.A., Blackburn, S.I., Hallegraeff, G.M., 1999. Comparative study of selenium requirements of three phytoplankton species: *Gymnodinium catenatum*, *Alexandrium minutum*

(Dinophyta) and Chaetoceros cf. tenuissimus (Bacillariophyta). J. Plankton Res. 21 (6), 1153–1169.

Egloff, D.A., 1988. Food and growth relations of the marine microzooplankton, *Synchaeta cecilia* (Rotifera). Hydrobiologia 157, 129–141.

ErardLeDenn, E., Chretiennot Dinet, M.J., Probert, I., 2002. First report of parasitism on the toxic dinoflagellate *Alexandrium minutum* Halim. Estuar. Coast. Shelf Sci. 50 (1), 109–113.

Fistarol, G.O., Legrand, C., Selander, E., Hummert, C., Stolte, W., Graneli, E., 2004. Allelopathy in *Alexandrium* spp.: effect on a natural plankton community and on algal monocultures. Aquat. Microb. Ecol. 35, 45–56.

Forteza, V., Quetglas, G., Delgado, M., Reyero, M.I., Fraga, S., Franco, J., 1998. Toxic *Alexandrium minutum* bloom in Palma de Mallorca harbour, Balearic Islands, western Mediteranean. In: Reguera, B., Blanco, J., Fernándes, M.L., Wyatt, T. (Eds.), Harmful Algae. Xunta de Galicia, IOC of UNESCO, pp. 58–59.

Franco, J.M., Fraga, S., Zapata, M., Bravo, I., Fernandez, P., Ramilo, I., 1995. Comparison between different strains of genus *Alexandrium* of the minutum group. In: Lassus, P., Arzul, G., Erard Le Denn, E., Gentien, P., Marcaillou Le Baut, C. (Eds.), Harmful Marine Algal Blooms. Lavoisier, Paris, pp. 53–58.

Frangópulos, M., Guisande, C., Maneiro, I., Riveiro, I., Franco, J., 2000. Short-term and long-term effects of the toxic dinoflagellate *Alexandrium minutum* on the copepod *Acartia clausi*. Mar. Ecol. Prog. Ser. 203, 161–169.

Fritz, L., Triemer, R.E., 1985. A rapid simple technique utilizing Calcoflour White M2R for the visualization of dinoflagellate thecal plates. J. Phycol. 21, 662–664.

Galluzzi, L., Penna, A., Bertozzini, E., Giacobbe, M.G., Vila, M., Garcés, E., Prioli, S., Magnani, M., submitted. Development of a qualitative PCR method for the *Alexandrium* (Dinophyceae) detection in contaminated mussels (*Mytilus galloprovincialis*). Harmful Algae.

Garcés, E., Delgado, M., Vila, M., Camp, J., 1998. An *Alexandrium minutum* bloom: in situ growth or accumulation? In: Reguera, B., Blanco, J., Fernandez, M.L., Wyatt, T. (Eds.), Harmful Algae. Xunta de Galicia and IOC of UNESCO, Santiago de Compostela, pp. 167–170.

Garcés, E., Bravo, I., Vila, M., Figueroa, R.I., Masó, M., Sampedro, N., 2004. Relationship between vegetative cells and cyst production during *Alexandrium minutum* bloom in Arenys de Mar harbour (NW Mediterranean). J. Plankton Res. 26 (6), 1–9.

Giacobbe, M.G., Maimone, G., 1994. First report of *Alexandrium minutum* Halim in a Mediterranean lagoon. Cryptogamie Algol. 15 (1), 47–52.

Giacobbe, M.G., Oliva, F.D., Maimone, G., 1996. Environmental factors and seasonal occurrence of the dinoflagellate *Alexandrium minutum*, a PSP potential producer, in a Mediterranean lagoon. Estuar. Coast. Shelf Sci. 42 (5), 539–549.

Giacobbe, M.G., Gangemi, E., Masó, M., Milandri, A., Penna, A., Poletti, R., 2003a. First record of shellfish contamination by PSP and DSP toxins in a Mediterranean locality. In: Proceedings of the International Conference on Molluscan Shellfish Safety. Galway, Ireland, June 14–18, (abstract only), p. 36.

Giacobbe, M.G., Azzaro, F., Decembrini, F., Galletta, M., Gangemi, E., Raffa, F., Ceredi, A., Milandri, A., Poletti, R., Masó, M, 2003b. Fioritura tossica del dinoflagellato *Alexandrium minutum* in un'area costiera del Mar Ionio. Biol. Mar. Medit. 11, 703–707.

Glibert, P., Pitcher, G., 2001. Global Ecology of Harmful Algal Blooms, Science Plan. SCOR and IOC, Baltimore and Paris.

Grassohoff, K., Ehrrardt, M., Kremling, K., 1983. Methods of Sea Water Analysis. Verlag Chemie, Germany.

Grzebyk, D., Bechemin, C., Ward, C.J., Verite, C., Codd, G.A., Maestrini, S.Y., 2003. Effects of salinity and two coastal waters on the growth and toxin content of the dinoflagellate *Alexandrium minutum*. J. Plankton Res. 25 (10), 1185–1199.

Guisande, C., Frangópulos, M., Maneiro, I., Vergara, A.R., Riveiro, I., 2002. Ecological advantages of toxin production by the dinoflagellate *Alexandrium minutum* under phosphorus limitation. Mar. Ecol. Prog. Ser. 225, 169–176.

Halim, Y., 1960. *Alexandrium minutum* nov. g., nov. sp., dinoflagellé provocant des eaux rouges. Vie et Milieu 11, 102–105.

Hallegraef, G.M., Steffensen, D.A., Wetherbee, R., 1988. Three estuarine Australian dinoflagellates that can produce paralytic shellfish toxins. J. Plankton Res. 10, 533–541.

Hallegraeff, G.M., 1993. A review of harmful algal blooms and their apparent global increase. Phycologia 32, 79–99.

Hansen, G., Daugbjerg, N., Franco, J.M., 2003. Morphology, toxin composition and LSU rDNA phylogeny of *Alexandrium minutum* (Dinophyceae) from Denmark, with some morphological observations on other European strains. Harmful Algae 2, 317–335.

Honsell, G., Poletti, R., Pompei, M., Sidari, L., Milandri, A., Casadei, C., Viviani, R., 1996. *Alexandrium minutum* Halim and PSP contamination in the northern Adriatic Sea (Mediterranean Sea). In: Yasumoto, T., Oshima, T., Fukuyo, Y. (Eds.), Harmful and Toxic Algae. IOC of UNESCO, pp. 77–80.

Hwang, D.F., Lu, Y.H., 2000. Influence of environmental and nutritional factors on growth, toxicity, and toxin profile of dinoflagellate *Alexandrium minutum*. Toxiconomy 38, 1491–1503.

Justic, D., Rabalais, N.N., Turner, R.E., Dortch, Q., 1995. Changes in nutrient structure of river-dominated coastal waters: stoichiometric nutrient balance and its consequences. Estuar. Coast. Shelf. Sci. 40, 339–356.

Koray, T., Buyukisik, B., 1988. Toxic dinoflagellate blooms in the harbour region of Izmir Bay (Aegean Sea). Rev. Int. Oceanogr. Med. 91–92, 25–42.

Lippemeier, S., Frampton, D.M.F., Blackburn, S.I., Geier, S.C., Negri, A.P., 2003. Influence of phosphorus limitation on toxicity and photosynthesis of *Alexandrium minutum* (Dinophyceae) monitored by in-line detection of variable chlorophyll fluorescence. J. Phycol. 39 (2), 320–331.

Maguer, J.F., Erard-Le Denn, E., Christian, M., Le Grand, J., Morin, P., 2000. Relationships between nitrogen utilization and environmental conditions during an *Alexandrium minutum* bloom in Morlaix bay (NW France). In: Proceedings of the Ninth International Conference on Harmful-Algal Blooms. Tasmania, Australia, (abstract only), p. 170.

Marasovic, I., Nincevic, Z., Odzak, N., 1995. The effect of temperature on blooms of *Lingulodinium polyedra* and *Alexandrium minutum* in Kastela Bay. In: Lassus, P., Arzul, G., Erard Le

Denn, E., Gentien, P., Marcaillou Le Baut, C. (Eds.), Harmful Marine Algal Blooms. Lavoisier, Paris, pp. 187–192.

Margalef, R., 1978. Life-forms of phytoplankton as survival alternatives in an unstable environment. Oceanol. Acta 1 (4), 493–509.

Margalef, R., Estrada, M., Blasco, D., 1979. Functional morphology of organisms involved in red tides, as adapted to decaying turbulence. In: Taylor, D.L., Seliger, H.H. (Eds.), Toxic Dinoflagellate Blooms. Elsevier, North Holland, pp. 89–94.

Masó, M., Camp, J., Garcés, E., Olivos, A., Vila, M., 2000. Nutrient ratios in the near-shore waters of the Catalan Coast: a more realistic scenario for the HABs increment in the NW Mediterranean Sea. In: Proceedings of the Ninth International Conference on Harmful Algal Blooms. Tasmania, Australia, (abstract only), p. 175.

Moestrup, Ø., Codd, G.A., Elbrächter, M., Faust, M.A., Fraga, S., Fukuyo, Y., Cronberg, G., Halim, Y., Taylor, F.J.R., Zingone, A., 2002. IOC Taxonomic Reference List of Toxic Plankton Algae. http://ioc.unesco.org/hab/htmltaxlist.htm.

Montresor, M., Marino, D., Zingone, A., Dafnis, G., 1990. Three *Alexandrium* species from coastal Tyrrhenian waters (Mediterranean Sea). In: Granéli, E., Sundström, B., Edler, L., Anderson, D.M. (Eds.), Toxic Marine Phytoplankton. Elsevier, New York, pp. 82–87.

Norén, F., Moestrup, O., Rehnstam-Holm, A., 1999. *Parvilucifera infectans* Norén et Moestrup gen. et sp. nov (*Perkinsozoa phylum* nov.): a parasitic flagellate capable of killing toxic microalgae. Eur. J. Parositol. 35 (3), 233–254.

Penna, A., Vila, M., Fraga, S., Giacobbe, M.G., Andreoni, F., Riobó, P., Vernesi, C., 2005. Characterization of *Ostreopsis* and *Coolia* (Dinophyceae) isolates in the Western Mediterranean Sea based on morphology, toxicity and ITS-5.8S rDNA sequences. J. Phycol. 41(1), in press.

Probert, I.P., 1999. Sexual reproduction and ecophysiology of the marine dinoflagellate *Alexandrium minutum* Halim. Ph.D. Thesis. University of Westminster, London, p. 99.

Smayda, T.J., 1997. Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnol. Oceanogr. 42 (5, Part 2), 1137–1153.

Smayda, T.J., 2002. Turbulence, watermass stratification and harmful algal blooms: an alternative view and frontal zones as ''pelagic seed banks''. Harmful Algae 1, 95–112.

Smayda, T.J., Reynolds, C.S., 2001. Community assembly in marine phytoplankton: application of recent models to harmful dinoflagellate blooms. J. Plankton Res. 23 (5), 447–461.

Steidinger, K.A., Tangen, K., 1997. Dinoflagellates. In: Tomas, C.R. (Ed.), Identifying Marine Phytoplankton. Academic Press, St Petersburg, FL, pp. 387–584.

Su, H.M., Liao, I.C., Chiang, Y.M., 1993. Mass mortality of prawn caused by *Alexandrium tamarense* blooming in a culture pond in Southern Taiwan. In: Smayda, T., Shimizu, Y. (Eds.), Toxic Phytoplankton Blooms in the Sea. Elsevier, Amsterdam, pp. 329–334.

Tahri-Joutei, L., Berraho, A., Orbi, A., . Harmful algae blooms and the upwelling regime in Atlantic coastal waters of Morocco. In: Proceedings of the Ninth International Conference on Harmful Algal Blooms. Tasmania, Australia (abstract only), p. 145.

Throndsen, J., 1995. Estimating cell numbers. In: Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. (Eds.), Manual on Harmful Marine Microalgae. IOC Manuals and Guides No. 33. UNESCO, Paris, pp. 63–80.

Tillmann, U., John, U., 2002. Toxic effects of *Alexandrium* spp. on heterotrophic dinoflagellates: an allelochemical defence mechanism independent of PSP-toxin content. Mar. Ecol. Prog. Ser. 230, 47–58.

Usup, G., Pin, L.C., Ahmad, A., Teen, L.P., 2002. *Alexandrium* (Dinophyceae) species in Malaysian waters. Harmful Algae 1, 265–275.

Van Lenning, K., Vila, M., Masó, M., Sampedro, N., Anglès, S., Garcès, E., Morales, A., Camp, J., 2004. Monitoring of the 2003 *Alexandrium minutum* bloom in the 'Arenys de Mar' harbour. In: Norte, M., Fernándes, J.J. (Eds.), Proceedings of the VIII Reunión Ibérica sobre Fitoplancton Tóxico y Biotoxinas, CAMPUS, La Laguna, pp. 43–52.

Vila, M., Camp, J., Garcés, E., Masó, M., 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.

Vila, M., Garcés, E., Masó, M., Camp, J., in press. Phytoplankton assemblages and succession changes in communities including *Alexandrium minutum*. In: Steidinger, K.A., Landsberg, J.H., Tomas, C.R., Vargo, G.A., (Eds.), Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

Yoshida, M., Ogata, T., Thuoc, C.V., Matsuoka, K., Fukuyo, Y., Hoi, N.C., Kodama, M., 2000. The first finding of toxic dinoflagellate *Alexandrium minutum* in Vietnam. Fish. Sci. 66, 177–179.