Development of a toxic *Alexandrium minutum* Halim (Dinophyceae) bloom in the harbour of Sant Carles de la Rápita (Ebro Delta, northwestern Mediterranean)*

MAXIMINO DELGADO1, MARTA ESTRADA1, JORDI CAMP1, JUAN V. FERNANDEZ2, MERCEDES SANTMARTÍ2, CRISTINA LLETÍ2

1 Institut de Ciències del Mar, Passeig Nacional, s/n 08039 Barcelona, Spain.
2 Centre Tecnic de la Direcció General de Pesca Marítima (DARP, Generalitat de Catalunya), Sant Carles de la Rápita, Tarragona, Spain.

SUMMARY: A bloom of *Alexandrium minutum*, reaching concentrations up to $28 \times 10^6$ cells L$^{-1}$, was observed in the harbour of S. Carles de la Rápita (northwestern Mediterranean) on 4th May 1989. During the following days, Paralyzing Shellfish Poisoning (PSP) toxicity was detected in mussels exposed to harbour waters and in mussels from the neighbouring bay of Els Alfacs, where extensive cultures of bivalves are located. In El Fangar, the other bay of the delta, *A. minutum* was recorded in lower concentrations and no toxicity was detected in mussels. Shellfish extraction was stopped in the delta region and no human illnesses occurred. This paper presents observations on phytoplankton distribution during the outbreak, in relation to hydrographical and meteorological conditions. Possible implications for future toxic blooms in the area are discussed.

Key words: toxic dinoflagellates, PSP, Ebro Delta.

RESUMEN: DESARROLLO DE UNA MAREA ROJA TOXICA DE *Alexandrium minutum* EN EL PUERTO DE SANT CARLES DE LA RÁPITA (DELTA DEL EBRÓ, MEDITERRANEO OCCIDENTAL). — El 4 de mayo de 1989 se observó en el puerto de Sant Carles de la Rápita (Mediterráneo noroccidental) un bloom de *Alexandrium minutum*, que alcanzó concentraciones de $28 \times 10^6$ células L$^{-1}$. Los días siguientes se detectó toxicidad paralizante (PSP) en mejillones expuestos a las aguas del puerto y en mejillones procedentes de la cercana bahí a de E l Alfacs, en la cual se localizan cultivos extensivos de moluscos bivalvos. La presencia de *Alexandrium minutum* fue constatada también en la bahía de El Fangar pero, en este caso, no se detectó toxicidad. La extracción de moluscos bivalvos fue detenida en toda la región del delta y no ocurrieron casos de enfermedad en personas. Este artículo presenta observaciones sobre la distribución del fitoplancton durante el episodio tóxico en relación con parámetros hidrográficos y meteorológicos y en él se discuten las posibles implicaciones futuras de «blooms» tóxicos en la región.

Palabras clave: dinoflagelados tóxicos, PSP, delta del Ebro.

INTRODUCTION

The occurrence of red tides and phytoplankton blooms in the Mediterranean has been revised by JACQUES & SOURNIA (1978-1979). Since 1975, population explosions of diatoms and dinoflagellates seem to have intensified in areas such as the Adriatic Sea (BONI, 1983; BONI et al., 1983). On the Catalan coast, discoloured waters were commonly observed in some polluted harbours such as that of Barcelona, but observed red tides affecting the coastal region had always been caused by *Noctiluca* (LOPEZ & ARTE, 1971). No direct toxic effects of phytoplankton blooms on the Mediterranean coasts of Spain

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were reported until 1988, when Paralyzing Shellfish Poisoning (PSP) toxins were detected in *Venus verrucosa* from Fuengirola (ICES, 1988). The toxicity recurred the following year (BRAVO et al., 1989) and could be related to the presence of *Gymnodinium catenatum*, a species which has been causing toxicity in the Galician Riia since 1976 (LUTHY, 1979; CAMPOS et al., 1982; ESTRADA et al., 1984) and which seems to be presently expanding (HALLEGRAEFF, 1988). In May 1989, a red tide first detected in the harbour of Sant Carles de la Rápita (Ebro Delta, Catalonia, Spain) produced PSP toxicity in mussels from raft cultures in the neighbouring bay of Els Alfacs. This article describes the development of this event in relation to the meteorological and hydrographical conditions in the area.

**Characteristics of the Ebro Delta bays**

The bays of the Ebro Delta are extensions of marine water open to the neighboring Mediterranean Sea. El Fangar is the northern bay, with an extension of 12 km², 2 m mean depth and 16 × 10⁶ m³ capacity. Els Alfacs is the southern bay, with an extension of 50 km², 4 m mean depth and 200 × 10⁶ m³ of capacity. From April to December, both bays receive similar quantities freshwater (about 1 × 10⁶ m³ day⁻¹) through the irrigation channels of the delta.

The bays are positive estuaries (CAMP & DELGADO, 1987). The outgoing surface water (salinity about 33) stands over a wedge of incoming marine water (salinity about 37-38). The water column is generally stratified throughout the year but wind events, frequently with a northerly component, cause partial or total mixing, mainly during the winter months. The temperature of the estuarine water is lower than that of the open sea in winter and higher in summer and reaches surface values of about 24-28 °C in the warmer months. The high nutrient concentrations in the bays (DELGADO & CAMP, 1987) favor the development of dense phytoplankton populations. The chlorophyll concentration in the bays during an annual cycle between 1982 and 1984 ranged between 1 and 15 mg m⁻³ (DELGADO, 1987), but large spatial and temporal differences occur.

The bays of the Ebro Delta are important sites for fisheries and aquaculture. One of the most important activities is the raft culture of *Mytilus galloprovincialis*. Other culture species are Ostrea sp., Crassostrea gigas and Tapes semidecussatus, Murex brandaris, Murex trunculus, Tapes decussatus and Cardium edule are exploited from natural banks.

**MATERIALS AND METHODS**

Following the formation of the Commission of the European Communities, the Generalitat de Catalunya established a monitoring programme to check any possible toxicity in the bivalves before marketing for human consumption. Within this programme, water and shellfish samples have been collected weekly at fixed stations since August 1987. After the red tide outbreak of May 1989 additional toxicity tests and hydrographical surveys were carried out including supplementary sampling points (Fig. 1).

Meteorological data were obtained from the Centre Técnico de Sant Carles de la Rápita. Water temperature and salinity were measured with portable Aanderaa Instruments sensors.

Phytoplankton samples were preserved with Lugol's iodine solution. A 10 ml aliquot was settled in a sedimentation chamber for 1 day and counted with an inverted microscope. One transect of the chamber was examined at 400× magnification to record the smallest forms. Half of the chamber bottom was observed at 100× magnification to record the less-abundant organisms. One supplementary transect was also examined at 100× to enumerate large but abundant organisms. Some samples of water were filtered using 2 µm pore-sized Nuclepore filters. The filters were rinsed with distilled water, critical point dried, moun-

![Fig. 1. — Map of the Ebro Delta showing the irrigation channels and the sampling sites. The bays of Els Alfacs and El Fangar are divided into sectors (A-J).](image-url)
ted and coated with Au/Pd for observation under an Hitachi S-570 scanning electron microscope.

The toxicity tests were carried out following the standard mouse bioassay method (AOAC, 1980). The maximum level of toxin considered tolerable in Spain is 80 μg/100 g of mussel meat.

RESULTS

Sequence of events

All toxicity tests carried out since the beginning of the year until 3 May 1989 had been negative. On 4 May 1989, a red-brown discoloration was observed in the waters of the harbour of Sant Carles de la Rápita. The dominant organism, a typical dinoflagellate from the «tamarensis» complex was later identified as Alexandrium minutum Halim. The same day, a rope with mussels from a neighbouring aquaculture site in Alfacs was placed in the harbour. PSP tests done on 5 May were positive for these mussels (110 μg toxin/100 g mussel meat) and for mussels from sector A of Els Alfacs (65 μg toxin/100 g mussel meat). As a preventive measure, the extraction of shellfish was closed in all the Ebro Delta area until 25 May.

The progression of the results of the toxicity tests in mussels is shown in figure 2. The highest toxicity levels in Els Alfacs (up to 92 μg/100 g mussel meat) were reached between 8 and 12 May, near the mouth of the harbour of Sant Carles. The mussels from El Fangar always gave negative results.

The identification of A. minutum in the red tide samples was based on examination of the plate pattern. Light and scanning electron micrographs are
water column lasting until 23rd May, under strong winds. The hydrographic structure of Els Alfaes during the outbreak is shown in figure 5.

Phytoplankton composition

*A. minuta*, with concentrations ranging from 1 to 28 × 10^7 cells l^{-1}, was the dominant phytoplankter in the samples collected in the harbour of Sant Carles during the early days of the red tide, i.e. between 5 and 11 May. However, other dinoflagellate species were also abundant and *Prorocentrum triestinum* dominated from 11 to 19 May (Fig. 6). Other species or groups which were present in appreciable numbers included *Prorocentrum micans*, *Skeletonema costatum*, *Chaetoceros* spp., *small unidentified dinoflagellates*, *Nitzschia pungens*, *N. delicatula* and *Eutreptiella* sp. The composition of phytoplankton in Els Alfaes was similar to that in the Sant Carles harbour, although with smaller cell concentrations. On the other hand, the phytoplankton of El Fangar, where *Rhizosolenia alata*, *Thalassionema nitzschioides* and *Leptocylindrus danicus* were important components, was significantly different from that of Els Alfaes. Such differences observed between bays are frequent (DÉLGADO, 1987) and are related to hydrographical conditions of the bays and their particular situations.

During the initial stage of the red tide, the concentration of *A. minuta* in the harbour was much higher than in the bay (Fig. 7), in agreement with the findings of the toxicity tests. The overall abundance of *A. minuta* decreased with time, but on 5 June there were still 4 cells/10 ml in the harbour waters. On the two occasions when samples from 4 m depth were taken, the concentration of the organisms was much lower than at surface. However, all samples were taken during day time, and it is likely that vertical migration of the organisms plays a role in modifying their position within the water column.

Meteorological and hydrological conditions during the red tide

The temporal variation of several meteorological and hydrographical parameters in Els Alfaes during April-May 1989, is shown in figure 4. As can be seen, frequent wind events in April contributed to mixing of the water column. At the beginning of May, before the detection of the red tide, water temperature and salinity dropped suddenly due to cold northern winds and the accumulation of freshwater proceeding from the wastes of the delta. This was followed by warm, sunny weather, and resulted in a stratification of the

**DISCUSSION**

In retrospect, meteorological and hydrographical conditions during the red tide were typical of those reported for similar events elsewhere (MULLIGAN, 1975; CANNON, 1989). Mixing by strong winds at the end of April may have caused nutrient enrichment. The following warm weather and stratification of the water column may have favoured the accumulation of motile organisms such as dinoflagellates, that were finally dispersed by strong winds of 23-25 May.
As explained later, it seems clear that the bloom of *A. minuta* developed in the harbour of Sant Carles was the cause of the toxicity. Nevertheless, the available data do not exclude the possibility of an inoculation of the red tide assemblage from outside the harbour.

The taxonomical situation of the dinoflagellates of the "tamarensis" group is complicated (Steidinger, 1989). Recent studies by Balech (1985, 1989) have contributed to clarify the situation, although many problems still remain. Presently, *A. minuta* includes non-toxic and toxic strains. It is difficult to know how many blooms in the past can be attributed to *A. minuta*, since this and other species with similar characteristics were included under the names of *Gonyaulax tamarensis* or *Protopagonaulax tamarensis*. Recently, two PSP outbreaks caused by *A. minuta* have been reported in other marine areas of the world: Australia (Hallegraeff et al., 1988) and the coast of Brittany in France (Nez et

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**Fig. 4.** — Meteorological and hydrological conditions in the bay of Alfas. The hydrological variables (temperature and salinity) correspond to measurements at the central station. The approximate period of PSP event (between 2 and 20 May) is enclosed between solid bars.

**Fig. 5.** — Transect along the Alfacs bay showing the vertical distributions of temperature and salinity during the PSP outbreak (13th May).
Fig. 6.—Temporal evolution of the phytoplankton organisms (log No cells ml⁻¹) in: A) surface water of the S. Carles Harbour, B) the mussel culture zone of Els Alfacs (sector B in fig. 1), C) the mussel culture zone of El Fangar (sector I in fig. 1).

Non-toxic occurrences of *A. minutum* have been reported in Tyrrhenian (Montresor et al., 1989) and Adriatic waters (Boni et al., 1983). The dominance of this species in the zones where mussel toxicity was found, the general agreement between the distributions of *A. minutum* and toxicity and the fact that no other suspicious species were present, suggest that *A. minutum* was responsible for the toxicity in the Els Alfacs outbreak.

We can only speculate about the origin of *A. minutum* in the region. The original description of *A. minutum* by Halim (1960) was based on specimens from a red tide in the harbour of Alexandria (Egypt). Margalef (1969) had noted the presence of Gonionemus tumaresis (name used previously which included many species of Alexandria) on the Spanish Mediterranean coasts. However, no dinoflagellates of this group had been recorded in recent phytoplankton surveys of the Ebro Delta zone (Delgado, personal observation), with the exception of 1-6 cells/10 ml of a similar organism in samples taken from Els Alfacs on 18 April 1989.

A question of interest is the possible presence in the Ebro Delta sediments of *A. minutum* cysts which could provide a concentrated inoculum for development of red tides. This subject is presently being investigated (Blanco, pers. comm.). Further red tides of *A. minutum* in the Delta region are likely to occur, as has happened in other areas after the first detection of dinoflagellate blooms (Prakash, 1975; Marasovic, 1989; Joyce & Roberts, 1975). The hydrological conditions in the Ebro delta, with frequent fertilization events followed by periods of water column stratification, may be specially favourable for red tide development. Another point which should be considered is the possible effect of water eutrophication in increasing the frequency and intensity of phytoplankton blooms (Boni, 1983). Con-

Fig. 7.—Spatial distribution of *Alexandrium minutum* in the surface waters of Els Alfacs on 8th, 13th and 30th of May. The area of the circles is proportional to cell concentrations (scale of abundance is shown). The dotted area in the upper figure represents the concentration of the organism in the harbour of S. Carles.
continuing research is necessary to improve our understanding of the red tide phenomena and to devise adequate forms of action to mitigate possible damages.

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