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Relationship between vegetative cells and cyst production during *Alexandrium minutum* bloom in Arenys de Mar harbour (NW Mediterranean)

ESTHER GARCÉS*, ISABEL BRAVO[1], MAGDA VILA, ROSA ISABEL FIGUEROA[1], MERCEDES MASÓ AND NAGORE SAMPEDRO
INSTITUT DE CIÉNCIES DEL MAR (CSIC), BARCELONA, SPAIN AND [1]INSTITUTO OCEANOGRAFICO VIGO, VIGO, SPAIN

*CORRESPONDING AUTHOR: esther@icm.csic.es

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A recurrent Alexandrium minutum bloom in the Arenys de Mar harbour (Catalan coast, North Western Mediterranean) was monitored in order to establish the relationship between vegetative cells and cyst production. The bloom lasted from January 21 to February 24, 2002 and reached cell concentrations of up to 47 × 10^6 cell L^{-1}. Two aspects related to the resting cysts deposition were studied: (i) production of resting cysts during the bloom period (by means of sediment traps) and (ii) distribution of resting cysts in the sediment after the bloom (May 2002). Cyst formation in Arenys clearly started in a period with high vegetative cell densities in the water column. Once production was initiated encystment fluxes remained constant for two weeks, and covering the periods of maintenance and decline of the bloom. High cyst fluxes (up to 6000 cysts cm^{-2} day^{-1}) were quantified as a result of the high vegetative cell concentration. Moreover, encystment occurring in less than 1% of the total population indicates that most of the cells are not involved in resting cysts formation. A comparison of the resting cyst flux values obtained from the sediment traps and the resting cyst concentrations in surface sediment (628–3270 cysts cm^{-3}) three months later, revealed that the number of cysts in the sediment decreased during that time. The studies of excystment showed a high germination percentage (91%) and germling viability (100%). These data, together with the resting cyst distribution in the sediment, are important in assessing the role of resting cysts in the bloom dynamics of A. minutum in confined waters.

INTRODUCTION

Since Halim (Halim, 1960), who originally described *Alexandrium minutum* forming proliferations in the Alejandria harbour, blooms of this species have been reported in confined areas (Delgado *et al.*, 1990; Giacobbe *et al.*, 1996; Vila *et al.*, 2001) in the Mediterranean Sea. Most studies evaluated bloom development in relation to hydrographic conditions, i.e. low salinities and the stabilization of the water column. However, studies on the population dynamics of *A. minutum* and other dinoflagellates rarely take into account phases of life histories that include not only the vegetative phase but also dormant resting stages. The dinoflagellate hypnocyst, which is a dormant stage resulting from sexual fusion of two gametes, is produced in response to physical, chemical and biological conditions. The hypnocysts remain in the sediment, survive for several years and constitute a 'seed bank' which allows further blooms at the region where they are present.

Alexandrium minutum resting cysts have previously been described by Bolch *et al.* (Bolch *et al.*, 1991). Since then, a few studies have reported distribution of resting cysts of this species in sediment of the Atlantic coast (Erard-Le Denn *et al.*, 1993; Nehring, 1994; Blanco, 1995). For the Mediterranean Sea studies on *A. minutum* cysts are scarce. In addition, morphological details of other life stages (gametes and planozygotes) and other features of asexual and sexual reproduction have been characterized in cultures of this species (Probert *et al.*, 2002). The author also reported sexual reproduction and subsequent encystment in natural populations in two estuaries on the French coast (Probert, 1999).

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Despite an increasing awareness of the role that sexual reproduction and the subsequent cyst formation play during bloom decline, the controlling factors and physiological mechanisms involved have not been clearly defined. Moreover, the limited amount of data available for laboratory experiments and field observations provide conflicting information at this point, although life cycles have important implications for bloom development and dynamics of the harmful species involved. Only a few studies have attempted to address the relationship between concentrations of cyst and vegetative cells in marine environments. Some of these studies are long term where one sediment trap has been sampled for a long period of time. The number of cysts deposited in the sediment trap has been compared with the number of planktonic dinoflagellates found in the water over the year, and has given valuable information on the seasonal pattern of cyst formation of several species (Ishikawa and Taniguchi, 1996; Montresor *et al.*, 1998). The problems involving quantitative trap measurements (including configuration induced variations in efficiency) are well known (Gardner, 2000). Sediment traps are nevertheless widely used to study vertical flux of particles in the water column, and they have proved to be useful tools for cyst flux estimations, when employed with caution (Heiskanen, 1993). Knowledge on vertical cyst fluxes is needed to estimate *in situ* cyst production (patterns and rates) and to understand better dinoflagellate life strategies.

Here we present the results of a short-term *in situ* study of dinoflagellate cyst sedimentation in the Catalan harbour of Arenys de Mar, located on the eastern Spanish coast. This is one of the local harbours affected by recurrent blooms of *Alexandrium minutum*. Since 1996, vegetative cells of this species have been present throughout the year and the yearly blooms have always reached concentrations of up to 10^6 cells L^{-1} (January–May) (Vila *et al.*, 2001). In addition to enumeration of planktonic dinoflagellate cells and cysts, a wide range of physical, chemical and meteorological parameters were included in the study. The main objective of this study was to investigate whether there is a direct relationship between dinoflagellate density and production of cysts, taking into account the environmental factors that may influence the percentage of cyst formation.

METHOD

Arenys de Mar harbour (41°34.3′N and 2°32.4′E) has a maximum depth of 2.5 m at the dockside and 5–6 m at the entrance. The harbour provides a sheltered ecosystem with a total volume of ~134 000 m^3.

Sediment traps procedures and dinoflagellate cyst identification

Sediment traps were placed at harbour stations A and B (Figure 1). Each trap consisted of two cylindrical collection vessels (height 15 cm, diameter 5 cm, aspect ratio 3) moored 0.5 m from the bottom (the depth at both stations was 2 m). Settled material was collected every 2–4 days. Samples were kept in the dark at 4°C, without adding preservatives. Aliquots of 10 mL were sonicated for 20 s (Cole Palmer Ultrasonic Homogenizer, 50 W) and 1–3 mL subsamples were subsequently examined with an inverted microscope at 400× magnification. *Alexandrium minutum* resting cysts were classified according to shape, colour, wall thickness and size. Counted cysts from the traps were used to estimate *in situ* cyst flux and encystment percentages. Cyst fluxes are expressed as the number of cysts sedimented per m^2 per day. The percentage of encystment was calculated for each trap sample using the following equation: $\%E = [2Cy/(Ce + 2Cy)] \times 100$, where 'Cy' represents the cysts collected in the trap per day (Cyst m^{-2} day^{-1}). This number was multiplied by 2 because each cyst is produced from two motile cells. 'Ce' represents the standing stock of vegetative cells above the traps (Cells m^{-2}), in a water column of 1.5 m depth. This number corresponds with the mean cell concentration between the days that the trap was placed and removed. '2Cy' was added to 'Ce' to estimate the total number of

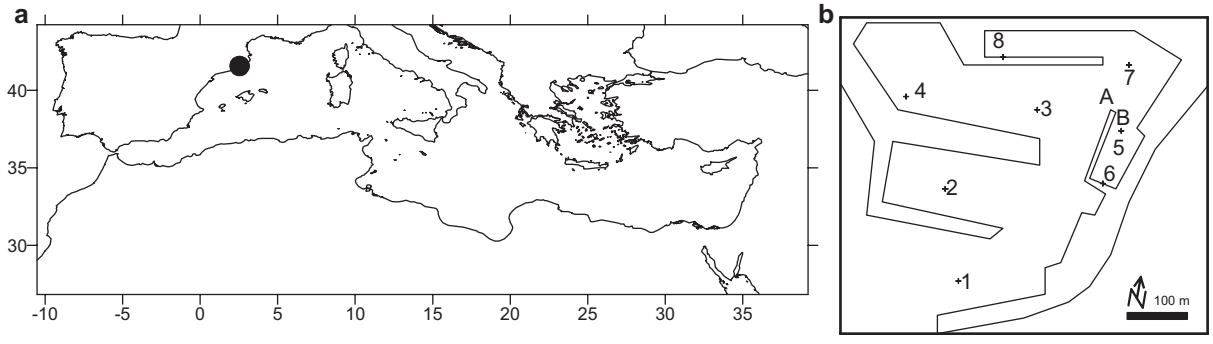

Fig. 1. (**a**) Location of the study area and (**b**) sampling stations in Arenys de Mar harbour. Stations A and B are the sites for the traps. Numbers show the sampling stations for core sediment samples.

cells involved in the encystment for each period (Montresor and Marino, 1996; Lewis, 2002). To relate the number of cysts of all intervals to the vegetative cell number, a cumulative plot is presented. The cumulative curve was obtained by totalling the daily cysts flux values.

Total organic matter was measured in a subsample (10 mL) collected from the sediment trap. The value was obtained from the difference in weight of the dried material before and after desiccation in a muffle furnace (5 h at 450°C).

Meteorological data (air temperature, rainfall, wind velocity and direction) were obtained from a coastal meteorological station located at ~30 km from the Arenys de Mar harbour. Two temperature sensors storing data at 15 min intervals were placed at the surface and at 0.5 m from the bottom using the same stations as the sediment traps.

Vegetative cell counts and water characterization

Surface samples (150 mL) were collected from the location of the traps at 2–4 day intervals (from 17 January until 9 March) to evaluate variations in *A. minutum* cell numbers and the overall phytoplankton biomass proxy chlorophyll *a* (Chl *a*). Phytoplankton samples were immediately fixed with Lugol iodine solution for cell counts using an inverted microscope. Samples (60 mL) for quantification of nutrients (NO_2, NO_3, NH_4 and PO_4) were stored at −20°C until analysed on an autoanalyser (Grassohoff *et al.*, 1983). Samples (60 mL) for Chl *a* measurements were vacuum filtered on 25 mm Whatman GF/F glass fibre filters, and kept frozen at −80°C until analysed with a fluorometric approach (Welschmeyer, 1994). Chl *a* was passively extracted in 8 mL 90% acetone (12 h at 4°C). Extraction tubes were subsequently turned upside down once before determining Chl *a* concentrations with a Turner fluorometer (Turner Designs).

Spatial sampling

To evaluate spatial distribution of *A. minutum* in the Arenys harbour a total of 30 additional stations (Figure 3) were sampled during the period of maximum cell concentrations (February 18, 2002) and just before the bloom declined. These phytoplankton samples were fixed with Lugol and examined on an inverted microscope following procedures outlined below.

Quantification of sediment cyst

Sediment samples were collected on May 15, 2002 at eight stations in Arenys de Mar harbour (Figure 1).This task was performed by a scuba diver inserting plastic cylindrical corers (20 cm long × 2.5 cm base diameter) into the sediment in such a way that the vertical sediment profile resulted in a depth range from 0 to 10 cm. To prevent possible losses of sediment surface cysts, the 12–10 cm water column over the sediment was also sampled and brought to the laboratory. Samples were stored in the dark at 4°C until being processed 1 week later.

The first step was to remove the remaining water using a siphon, being careful not to disturb the interface of the sediment. The dried profiles were cut at 1 cm intervals to quantify vertical cyst concentrations. Sediment samples of 1.5–3 cm^3 were suspended in filtered sea water, disaggregated with a Cole Palmer Ultrasonic Homogenizer (50 W) for 2 min, sieved to retain the 10–100 μm size fractions and collected material was resuspended in 10–15 mL distilled water. A 5 mL aliquot of this suspension was centrifuged at a density gradient using a Ludox TM Colloidal Silica Tech (density of 1.4 g cm^3). This gradient was carried out according to a modification of the technique described by Blanco (Blanco, 1986). This modification included the use of distilled water containing 24% saccharose (instead of distilled water) to avoid osmotic changes in processing cysts during sediment cleaning and gradient formation. The density gradients were carried out in 15 mL centrifuge tubes, where step gradient solutions of 100%, and 60% Ludox TM were carefully delivered. To avoid possible bias in the results of concentrations of different types of cysts due to the different densities, all cysts of the whole fraction over 1.4 g cm^{-3} density were counted. To evaluate the accuracy of the density gradient procedure, direct counts of the initial suspension were also carried out in seven samples with relatively high cyst concentration. Significant correlation ($r = 0.8$) between both procedures was obtained.

Viability of cysts from the sediment

Alexandrium minutum cysts from the May 15 sampling were analysed on a fluorescence microscope (450–490 nm). Detection of Chl *a* by this means was considered to be a sign for a good germination tendency. To study the viability of cysts from the sediment and to confirm their assumed *A. minutum* origin, a total of 22 isolated cysts were transferred to 100 μL L20 medium in 96-well tissue culture plates. Plates were incubated at 22°C using a 12:12 h light:dark cycle provided by cool white illumination tubes at 120 μmol m^{-2} s^{-1}. Cysts and germlings were carefully examined for germination and viability for a maximum of one month.

RESULTS

Bloom development

Concentrations of *A. minutum* in the Arenys de Mar harbour ranged from 150 × 10^3 cells L^{-1} (Figure 2a) mid January to a maximum of 47 × 10^6 cells L^{-1} one month later, provoking a clearly visible intense discoloration of the water mass. During the first part of the bloom

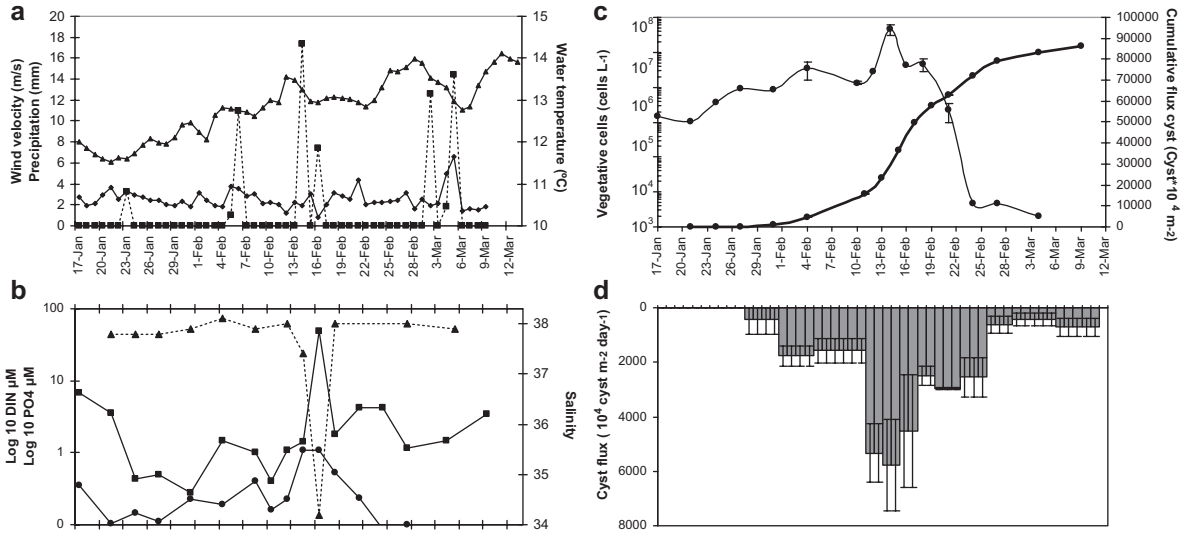

Fig. 2. (**a**) Daily mean of surface water temperature (°C) calculated from the surface sensor data, daily maximum wind velocity (m s^{-1}), daily cumulative rainfall (mm). (**b**) Dissolved inorganic nitrogen and phosphorus (µM) and water surface salinity. (**c**) Vegetative cell numbers of *A. minutum* (cells L^{-1}), and cumulative curve of cyst flux (cyst 10^4 m^{-2}). (**d**) Cyst flux (cyst 10^4 m^{-2} day^{-1}) estimated from the cysts collected in the sediment traps; values are average ± standard deviation (n = 2).

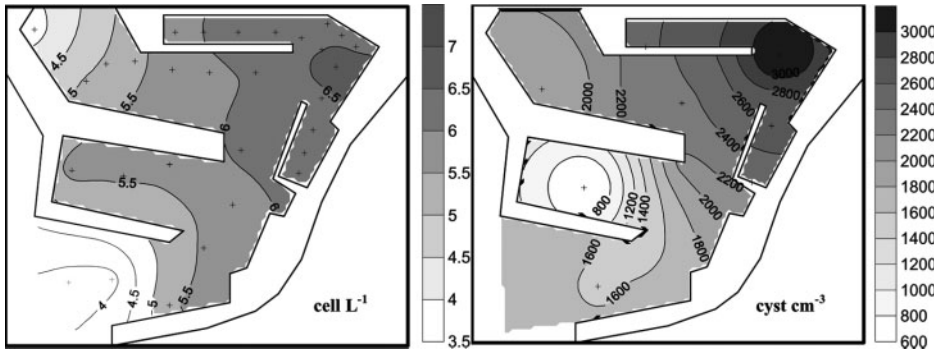

Fig. 3. (Left) Vegetative cell concentration (cells L^{-1}), February 18, 2002. Crosses show the sampling station. (Right) Cyst distribution in the sediment (cysts cm^{-3}) May 15, 2002.

(January 17–24) observed increments of *A. minutum* cell numbers were related to water temperature of 12°C, decreasing inorganic nutrients (Figure 2a,c) and development of other phytoplankton. These additional groups included diatoms, other dinoflagellates and nanoflagellates (results not shown). Growth of these groups of microalgae coincided with an important decrease in concentrations of nutrients, with minimum levels on January 21–24 (Figure 2b). The period of nutrient minima, in combination with a slight increase in water temperatures, coincided with the exponential phase of *A. minutum* on January 24. Maximum cell concentrations coincide with maximum temperature (13–14°C). During bloom development surface temperatures were almost identical to that attained near the bottom, but during the 3 days prior to maximum cell densities they were 1°C higher. Observed cell maxima coincided with a remarkable decline of salinity between February 12 and 18 (Figure 2a,b), although a correlation between cell concentration and salinity was not observed. Observed salinity decline was attributed to rainfall. This event increased nutrient values to maximum levels observed. Cell densities of *A. minutum* in January coincided with concentrations of dissolved inorganic nitrogen (DIN) as low as 0.3 µM. In February, both phosphate and DIN showed a slight increase concomitant with cell concentrations during both exponential and maintenance phases. However, phosphate concentrations showed a sharp decline, which followed that of cell concentrations, whereas concentration levels of DIN remained stable until the end of the bloom.

Correlation between meteorological data and cell densities and cyst production was not observed, but there was not any noticeable wind during bloom decline (Figure 2a,c).

Spatial distribution of *A. minutum* cell numbers and associated cysts in the Arenys harbour (based on February 18 samples) revealed clearly localized maxima (up to 10^7 cells L^{-1}) along the dockside (near station 7, Figure 1), whereas concentrations remained relatively low (10^3 cells L^{-1}) at the entrance (station 1). Low cell densities were also observed between station 4 and the dockside.

Cyst deposition

Traps collected on January 27 already revealed the presence of cysts (Figure 2). The collecting period of this trap coincided with the exponential phase of bloom development and minimum nutrient levels (Figure 2b,c). A cumulative curve for the cyst flux showed two different shapes or scenarios: (i) flat scenario and (ii) increasing scenario (Figure 2c). A flat cumulative curve is observed from the first sampling to February 4. The estimated encystment percentage for that period remained below 1% (Table I). The cumulative curve increases from February 11 to February 24 coinciding with cell concentrations of 10^6 cells L^{-1}. Maximum cyst flux was observed on February 10–15, coinciding with maximum cell concentrations in the water column. The increasing scenario shows a period where cyst production was constant and positive. A flat curve is observed from February 27 when the cells decreased to levels of 10^3 cells L^{-1}. Cyst flux values decreased during the decline phase of the bloom but remained over 500 cysts cm^{-2} day^{-1}despite the fact that cells dropped below 480 cells L^{-1}. During this period, the flux of cysts does not have any effect on cumulative results because the added amounts were low. The cumulative curve indicates a total cyst concentration of 86 000 (cyst $\times$ 10^4 m^{-2}) at the sediment trap stations.

Table I: The standing stock of cells above traps (cells $\times$ 10^4 m^{-2}), the cyst flux (cyst $\times$ 10^4 m^{-2} day^{-1}) as determined from sediment trap deployments and the percentage of encystment from January 17 to March 9, 2002

Sampling date	Standing stock of cells above trap (cells $\times$ 10^4 m^{-2})	Cyst flux (Cyst $\times$ 10^4 m^{-2} day^{-1})	Percentage of encystment (%)
17 Jan	10 898		
21 Jan	18 574	0	
24 Jan	26 785	0	
27 Jan	69 046	0	
31 Jan	130 097	813	1.2
4 Feb	221 070	1497	1.3
11 Feb	103 207	1884	3.5
13 Feb	208 353	6096	5.5
15 Feb	2 725 176	6952	1.0
17 Feb	528 150	5989	2.2
19 Feb	3 054 409	2246	0.1
21 Feb	329 713	2995	1.8
24 Feb	35	3066	99.4
27 Feb	35	856	98.0
4 Mar	33	599	97.3
9 Mar	19	941	99.0

Total organic matter from sediment traps showed a maximum of 74 mg m^{-3} day^{-1} and a mean of 40 mg m^{-3} day^{-1}. Deposition of the organic matter showed little variation throughout the bloom event. A correlation with organic matter or cyst deposition was, therefore, not observed.

Resting cysts observed in the traps usually had an almost circular (23–23 μm diameter) shape in apical view and reniform in the lateral view (Figure 4), and showed a distinct thick wall. These morphological characteristics corresponded to those described by Bolch *et al.* (Bolch *et al.*, 1991). These cysts produced *A. minutum* vegetative cells when isolated to L20 medium. Other clearly smaller round cells (20–22 μm in diameter) were also observed, mainly on February 12–14, coinciding with the period of maximum cell densities. These cysts were equally viable and also produced *A. minutum* cells when incubated in L20 medium. However, these cysts, probably temporary cysts, did not present the characteristic thick wall of resting cyst and were thus not considered in the quantification of encystment percentages.

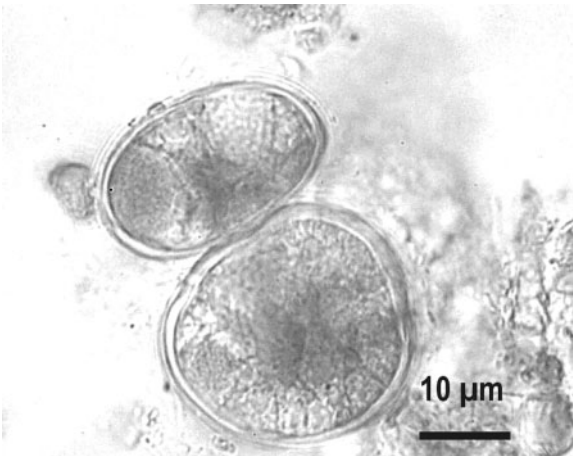

Fig. 4. Light micrograph of *Alexandrium minutum* cysts from the sediment trap (March 4–9).

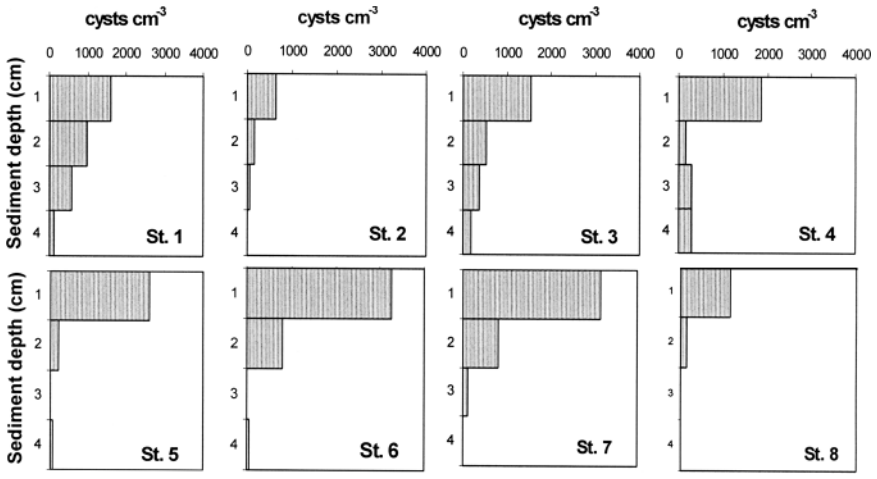

Fig. 5. Vertical cyst distribution in the sediment (cysts cm^{-3}) at the different sampling sediment stations.

Cyst distribution in the sediment

Concentrations of *A. minutum* resting cysts in the surface sediment (first cm) of Arenys de Mar reached concentrations up to 3270 cysts cm^{-3} of wet sediment (Figure 3b). The concentrations decreased notably with sediment depth in such a way that cysts were not detected below 5 cm depth (Figure 5). The highest densities were observed in the inner part of the harbour, where the major concentration of cells was also detected (Figure 3a,b). The morphology of cysts from the sediment closely matched the description of *A. minutum* cysts given by Bolch *et al.* (Bolch *et al.*, 1991) and also with those of the traps.

Chlorophyll fluorescence examination confirmed that the sediment cysts presented chloroplasts concentrated in the middle of the cells. The 91% of isolated cysts germinated within the 3 days at 20°C and all of them produced viable cultures of *A. minutum*.

DISCUSSION

Encystment and excystment processes have been used to explain the marked seasonal changes observed in the phytoplankton population in temperate waters. We, herein, present data on encystment during a bloom of *A. minutum*, establish the existence of seedbeds in the sediment of the area, and also, demonstrate the high viability for those cysts. Our data show that in the case of massive proliferation, characterized by high cell concentrations, high quantities of cysts are produced without requiring high encystment percentages (<1%). Cyst formation in Arenys clearly started in a period with high vegetative cell densities in the water column. Once production was initiated encystment fluxes remained constant for 2 weeks, and covering the periods of maintenance and decline of the bloom.

Laboratory studies suggest that induction of sexuality in *Alexandrium* occurs as a result of macronutrient limitation (Anderson *et al.*, 1984; Anderson and Lindquist, 1985; Probert *et al.*, 2002). However, there are few field measurements to confirm that statement. The major problem associated with such field studies is how to measure the sexual stages in the plankton population since they are, in most cases, indistinguishable from vegetative cells. Anderson *et al.* (Anderson *et al.*, 1983) and Probert (Probert, 1999) used the cell size to distinguish planozygotes of *A. tamarense* and *A. minutum*, respectively. The first authors did not link encystment to any obvious environmental cue (including inorganic nutrients), whereas Probert (Probert, 1999) found that induction of gametes (estimated by calculation of the gamete index) was related to physiological macronutrient stress. Data from sediment traps published in recent years contributed to the calculations of cyst fluxes and encystment rates during dinoflagellate blooms (Heiskanen, 1993; Kremp and Heiskanen, 1999; Ichimi *et al.*, 2001). However, only the last authors show data that relate inorganic macronutrients to cell concentrations and cyst fluxes. They found a maximum cyst flux just after recording maximum cell abundance. Limiting phosphate concentrations detected at this moment might have induced cyst formation.

In our study, the appearance of the first resting cyst in the traps, between January 27 and 31, coincided with minimum concentration of inorganic N and P and exponential growth phase of the bloom. The depletion of the macronutrients could trigger the encystment but not seem to be a determining factor in the subsequent cyst production. Cumulative curve of encystment showed a constant and positive increase from February 11 to February 24 and was not influenced by simultaneous decline of cellular concentrations and inorganic P initiated on February 16.

Contrary to the general accepted idea that the nutrient status plays a triggering role for encystment, Wall *et al.* (Wall *et al.*, 1970) suggested that encystment is a naturally occurring stage in the dinoflagellate life history. Based on this hypothesis, encystment is favoured by optimum growth conditions, rather than reflecting a reaction to adverse conditions. Moreover, endogenous or 'clock'-regulated sexuality has also been proposed similar to that shown to regulate excystment in *A. tamarense* (Anderson and Keafer, 1987). In the case of *A. minutum* bloom, continuous encystment during the maintenance phase would be consistent with the hypothesis of encystment with optimum growth conditions and/or an endogenous clock.

Other possible factors inducing encystment have also been documented, including iron stress (Doucette *et al.*, 1989; Blanco, 1995) and cell densities (Uchida, 2001). In addition, Wyatt and Jenkinson (Wyatt and Jenkinson, 1997) reported the necessity of a threshold level in cell concentration (>10^4 cells L^{-1}). Our data showed that encystment occurred at cell densities of 10^5 cells L^{-1} reaching the maximum at cell densities of 10^7 cells L^{-1}. These data support that, in the case of *A. minutum* bloom, encystment started during the development phase at high cell densities.

Several methodological aspects deserve an extended explanation. (i) Reported periods of progression of planozygotes to cyst formation in *Alexandrium* genus (Walker and Steidinger, 1979; Fritz *et al.*, 1989; Probert, 1999) are the same order of our interval of sediment trap experiment. However, delays between planozygote formation in the water column and detection of cysts in the traps could bias our estimated encystment percentages. The high percentages found at the end of the bloom (up to 96% after February 24), probably resulted from a combination of low vegetative cell numbers at that moment, and high concentrations of cysts produced in a previous interval with much higher cell concentrations in the water column. (ii) A second inconvenience of sediment traps is the risk of resuspension reported for cysts. Resuspension is definitely a factor to consider, but its influences on the results presented in the present study were not likely to be significant as traps were located in a very well-protected harbour, whereas wind speeds remained low during the period of sampling. (iii) We estimated encystment percentage following the method used by Montresor (Montresor and Marino, 1996) in cultures for *A. pseudogonyaulax*. Our results were therefore based on cysts and not on planozygotes, as considered in the Kremp and Heiskanen (Kremp and Heiskanen, 1999) approach. These authors estimated the number of resuspended cysts from the amount of cysts detected in the traps during periods that planozygotes were not detected in the water column. These procedures are well suited to study cyst formation over long periods of time, but might lead to underestimations in situations as detected in the Arenys harbour, characterized by short blooms with high concentrations of vegetative cells and low encystment percentages. During the present study a coherent pattern of cell sizes allowing differentiation of sexual stages, as suggested by Probert (Probert *et al.*, 2002) was not observed. However, this hypothesis, based on cultured *A. minutum*, has not yet been confirmed during other field studies either. Moreover, our culture experiments based on *A. minutum* showed a great variability in vegetative cell sizes, and values were inversely correlated to growth rates (Bravo *et al.*, 1997). Based on these results differentiation of sexual stages based on cell sizes should be used with caution.

The *A. minutum* bloom described herein showed an abrupt decline after 1.5 months of exponential growth. Cellular concentrations dropped from 6×10^6 to $<1 \times 10^5$ cells L^{-1} in a 3 day interval only (February 18–21). Similar patterns have been observed for other *Alexandrium* blooms in bays and salt ponds (Anderson *et al.*, 1983; Delgado *et al.*, 1990; Forteza *et al.*, 1998; Garcés *et al.*, 1999). The strong decline of *A. minutum* observed in the Arenys harbour could not be attributed to encystment as encystment rate remained extremely low (>1%). The high rates observed for blooms of *A. tamarense* in Cape Cod [20% (Anderson, 1998)] and *A. minutum* in northern Brittany [40% (Probert *et al.*, 2002)] were by no means attained. Moreover, grazing, cell mortality and advection should also be taken into consideration to having contributed greatly to the rapid decline in *A. minutum* cell numbers. In our study, the community of potential grazers, including heterotrophic dinoflagellates, rotifera and tintinnida, were found at concentrations up to 22×10^3 cells L^{-1} (data not shown). These high figures supported the hypothesis of grazing as a main reason for a rapid bloom termination as previously suggested by Calbet *et al.* (Calbet *et al.*, 2003).

The comparison of the resting cyst flux values obtained from the Arenys sediment traps and the resting cyst concentrations in the sediment suggest that the number of resting cysts remaining in the sediment decrease to 10% in an interval of three months only. These unexpected declines are not related to analytical errors as the technique of cyst quantification in the sediment was carefully checked. Published records on temporal variability of cyst concentration in the sediment are rare, but a few coincided with the Arenys results. Erard-Le Denn (Erard-Le Denn *et al.*, 1993) for example, detected up to 24 000 and 16 000 cysts g^{-1} wet sediment after the occurrence of an *A. minutum* bloom on the coast of Brittany (France) in July, 1989, but one month later, concentrations had already declined below 500. The marked decreases in concentrations of deposited cysts are probably influenced by a number of factors, including predation, dispersion and degradation. Tsujino

et al. (Tsujino *et al.*, 2001) indeed found a correlation between the decrease of *Alexandrium* cysts and the density of the macrobenthos in the sediment. Giangrande *et al.* (Giangrande *et al.*, 2002) found that the polychaeta *Naineris laevigata* plays a relevant role in the vertical transport and germination success of resting cysts. However, the cysts in the Arenys de Mar sediments showed a clear vertical distribution, and the influence of redistribution by polychaeta can, therefore, be discarded. Dispersion by other means can, of course, not be excluded. Even though a high survival percentage of cysts in the sediment has been mentioned (Keafer *et al.*, 1992), this largely depends on the biological, physical and chemical conditions in the sediment studied. In the case of the Arenys harbour, germination should also be considered as a possible reason for rapid cyst declines in the sediment, because results obtained in the present study suggest dormancy periods of less than three months.

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