JOURNAL OF PLANKTON RESEARCH | VOLUME 24 | NUMBER 7 | PAGES 681–686 | 2002

Role of temporary cysts in the population dynamics of *Alexandrium taylori* (Dinophyceae)

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Although temporary cyst stages are common in dinoflagellates, their role remains unclear. Every year Alexandrium taylori (Dinophyceae) forms dense patches (10^6 cells l^{-1}) along La Fosca beach (Spain, northwest Mediterranean), which last for 2 months (July, August). One of the characteristics of the life history of A. taylori is the shift from a vegetative motile stage to non-motile temporary cysts. Here we present the temporal changes in the abundance of temporary cysts in sediments and their in situ encystment and excystment rates. The in situ encystment rate of temporary cysts from the water column to the sediment ranged from 1.8×10^6 to 4.4×10^6 cysts m^{-2} day^{-1}, whereas the excystment rate was between 0.9×10^6 to 2.7×10^6 cysts m^{-2} day^{-1} during the bloom period. Some of the temporary cysts in the sediment took more than 1 day to produce vegetative cells and remained viable for at least 4 days. We propose that temporary cyst formation in this species is a tool for reducing population losses. The production of temporary cysts can be an advantage since part of the population is stored in the sediments.

INTRODUCTION

A priority of harmful algal bloom research is to identify the life history stages of species, to determine the factors that control transitions between these stages, and to establish the role of each stage. Species of the dinoflagellate genus *Alexandrium*, which cause paralytic shellfish poisoning (PSP), spend a brief period in the plankton where they undergo vegetative growth and produce resting cysts. In recent years, many studies have focused on the resting cysts, which form the dormant stage of the organism and the potential 'seed' for initiating new bloom events (Anderson, 1997; Ellegaard *et al.*, 1998; Hallegraeff *et al.*, 1998; Rengefors and Anderson, 1998). Less attention has been paid to the other transitional cell stage, the temporary cyst, which has been described in less than 15 dinoflagellate species (Garcés, in press). Adverse conditions have been considered to trigger the formation of temporary cysts, such as ageing cultures (Jensen and Moestrup, 1997), deficiencies in specific nutrients (Anderson and Wall, 1978; Doucette *et al.*, 1989; Fritz *et al.*, 1989), changes in temperature (Schmitter, 1979), and bacterial attack (Nagasaki *et al.*, 2000). However, *in situ* ecological studies of this stage, the factors that trigger transitions or their role in population dynamics, are scarce.

For some species, this stage is unrelated to reproduction, but some species of the subgenus *Gessnerium*, such as *Alexandrium hiranoi* (Kita *et al.*, 1985, 1993), *A. pseudogonyaulax* (Montresor, 1995) and *A. taylori* (Garcés *et al.*, 1998) show asexual reproduction. It has been suggested that these benthic stages contribute to the growth of dinoflagellate populations (Kita *et al.*, 1985) and are a strategy to avoid competition (Uchida *et al.*, 1999).

Alexandrium taylori blooms have been occurring for at least 10 years along La Fosca beach (Catalan Coast, northwest Mediterranean). These blooms are widespread and recur every summer (Garcés *et al.*, 1999). Of the biological factors that contribute to the population dynamics, the role of temporary cysts for *in situ* population growth is thought to be significant. This study reports the *in situ* temporal and spatial distribution of temporary cysts and the rates of excystment and encystment. We use the terms 'encystment' and 'excystment' to refer to the formation of temporary cysts from the vegetative cells by ecdysis and the formation of vegetative cells from temporary cysts, respectively. Temporary cyst abundance was quantified throughout the bloom period over 4 years (1996–99).

Moreover, *in situ* experiments were performed to quantify encystment and excystment rate of the cells on a daily basis since previous results indicated that these processes occur within a 24 h period (Garcés *et al.*, 1998). Here we discuss the hypothesis that the formation of temporary cysts is a strategy which allows *A. taylori* to form persistent dense patches along an open beach.

METHOD

The study was carried out along La Fosca beach in northeastern Spain (Costa Brava, Catalonia) (Figure 1). This beach measures 525 × 300 m and faces southeast. The average and maximum depths are 3 m and 7 m, respectively, with a fairly uniform and gentle slope between 2 and 7 m. More information is given elsewhere (Garcés *et al.* 1999).

To choose a sampling station to quantify the abundance of temporary cysts and granulometry on the beach, 10 stations were sampled. The mean concentration of temporary cysts at the 10 stations was 558 ± 386 cysts g^{-1} (n = 10) and the mean diameter of the sand was 0.34 ± 0.05 mm (n = 10). One of these ten stations was selected which had values of cyst numbers and grain size (at 1.5 m depth) close to the mean values.

Surface water samples (150 ml at 0.25 m depth) were collected for quantitative determination of vegetative cells during the summers (June to September) of 1996, 1997, 1998 and 1999. At the same time, sand samples from the upper 3 cm of sediment were also collected to quantify temporary cysts. Samples were obtained at the same time each day (between 13:00 and 17:00 h GMT) every 2–3 days. To relate the two data sets (vegetative cells in water and temporary cysts in sediment) and compare the cell concentrations, we calculated the total vegetative cells and total temporary cysts as the cell numbers in a cylinder containing 150 cm of water and 1 cm of sediment.

Encystment and excystment fluxes between water and sediment (temporary cysts m^{-2} day^{-1}) were calculated by means of PVC sediment traps (5 cm diameter and 10 cm high) installed at the sampling station between August 19 and 20, 1996, August 10 and 11, 1999 and August 13 and 14, 1999. The experimental design was based on previous information on the vegetative cell cycle and temporary cyst formation in the field (Garcés *et al.*, 1998). In each experiment, four traps were placed at the bottom (1.5 m depth) at 16:00 h GMT (before maximum temporary cyst formation) and collected at 4:00 h GMT (before germination of the temporary cysts into vegetative cells). Light and dark periods were 14 and 10 h, respectively, with sunrise at approximately 5:00 h GMT.

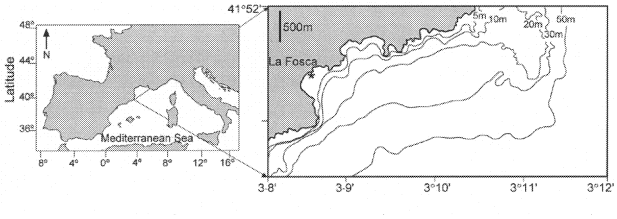

Fig. 1. Map of the study area showing the sampling station at La Fosca beach, northwestern Mediterranean.

To distinguish newly-formed temporary cysts and temporary cysts of known age, two traps were filled with 1 kg of sterile sediment from the beach on each sampling date (EnEx1 for August 19–20, 1996, EnEx2 for August 10–11, 1999 and EnEx3 for August 13–14, 1999) and the other two were filled with *in situ* sediment as a control for each experiment (Control1, Control2, Control3, respectively). When the traps were collected, two samples were obtained for vegetative cell quantification in the water and temporary cysts from the sediment. Cells formed clusters of vegetative cells and temporary cysts in the water immediately in contact with the sediment; clusters were counted separately from the cysts within the sediment from August 10 to 11, 1999.

To obtain the excystment rates of temporary cysts, four subsamples of 25 g of the surface layer from the sediment traps (EnEx and Control) were incubated for 12 h in 50 ml culture chambers with filtered sea water (Iwaki filter of cellulose acetate 0.20 μm pore size) from La Fosca beach. The samples were incubated under *in situ* conditions of temperature and light. After 12 h incubation, motile cell concentrations were estimated by sub-sampling the culture chambers.

Since some temporary cysts in sediment take more than 1 day to produce vegetative cells, their viability was studied using 1-day-old cysts. The traps from August 13–14,1999 (EnEx3) were used for this purpose. Four subsamples of 25 g of sediment were incubated for 4 days in 50 ml culture chambers with filtered sea water from La Fosca beach under *in situ* conditions. Water subsamples were collected daily to quantify the new vegetative cells that appeared in the incubations during the following days.

The general procedure for counting the vegetative cells after fixation with formaldehyde solution (1% final concentration) was applied via 24 h sedimentation in a 50 ml settling chamber. An appropriate area of the chamber was scanned for dinoflagellate enumeration using a Leica-Leitz inverted microscope. Temporary cysts in the sediments were quantified in samples preserved in formaldehyde solution (1% final concentration). Sediment samples (10 g) were sonicated for 5 min to separate cysts from sand and sieved; the 10–60 μm fraction was kept and

transferred into filtered sea water. Microscopic cyst identification and counts were performed on this fraction using a Palmer–Maloney counting chamber.

Computation of the population net growth rate was calculated as the growth constant K_e, which is the number of units of increase per day (Guillard, 1973).

RESULTS

Vegetative cell concentrations in water, temporary cyst concentrations in sediment, and water temperature at La Fosca beach shared common characteristics during the 4-year sampling period (Figure 2a and 2b). An initial phase of vegetative growth, which lasted approximately 20–25 days, was a common characteristic, although it varied in time. In summer 1998, this phase took place 1 month earlier than in 1996 and 1999. There was a clear relationship between water temperature and vegetative cell increase. Calculations of the vegetative population net growth rates were around 0.2 day^{-1} at the beginning of the bloom, although some higher rates were found, e.g. 1.48 day^{-1} in the last week of July 1996. The stationary phase was variable between years (July or August). Average concentrations were 10^3–10^4 temporary cysts g^{-1} of sediment during this phase. Maximum concentrations of temporary cysts in the sediment lagged about 15 days behind vegetative cell concentrations in the water column in late July and August (Figure 2b).

The vegetative population decrease at the end of the bloom did not show a common pattern. In 1996, high concentrations were maintained while the temperature began to decrease. Population decrease in 1996 occurred more quickly than in 1998, when a gentle slope described the fall in temperature and cells. The decrease in temporary cyst population in the sediment followed the decline in vegetative cells, but with less fluctuation (Figure 2b). Moreover, in some cases vegetative cells decreased considerably (e.g. the last 2 weeks of August 1997), but this was not followed by a decline in the number of temporary cysts, which remained at the same concentration in the sediment.

Vegetative cell concentrations in the water and temporary cysts in sediment during a 5-day period between August 10 and August 14, 1999 are shown in Figure 3. The vegetative population at the water surface increased each morning, reached 10^6 cells l^{-1} by midday or early afternoon, and then began to decrease at the surface in the evening or early night (10^2 cells l^{-1}). At the same time as the fall in the numbers of vegetative cells caused by daily vertical migration, formation of clusters near the sediment was detected, with concentrations of 10^6 cells l^{-1}. Temporary cyst concentration in the sediment was 10^2 temporary cysts g^{-1} with a maximum of 10^3 temporary cysts g^{-1} on the afternoon of August 12. The concentration of 10^3 temporary cysts g^{-1} was maintained during the following day and showed a significant decrease on the 5th day.

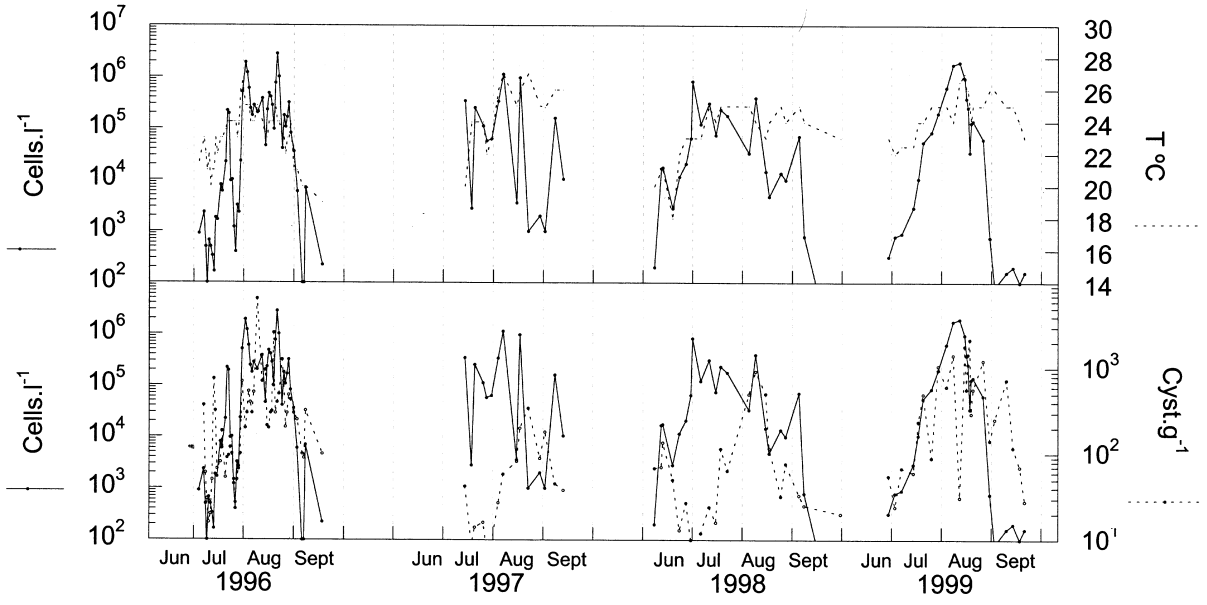

Fig. 2. (**a**) Water surface temperature and vegetative cells l^{-1} of *A. taylori* at La Fosca fixed point during the summer months of 1996–99. (**b**) Total vegetative cells and total temporary cysts of *A. taylori*, expressed as the number of cells in a cylinder with a 150 cm height of water and 1 cm of sediment during the summer months of 1996–99. Arrows show the periods of the experiments.

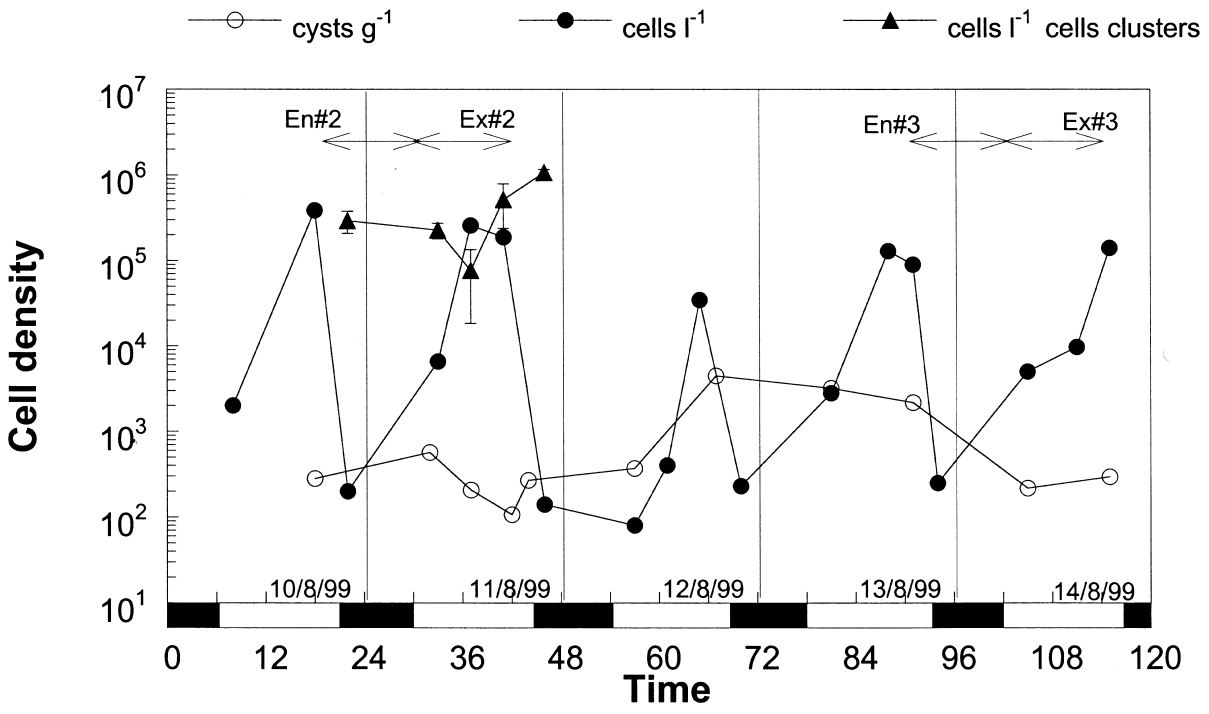

Fig. 3. Vegetative cells in the water, temporary cyst concentrations in the sediment and cell concentration in the clusters of *A. taylori* on La Fosca beach for the daily cycle from August 10 to August 14, 1999. Horizontal bars at bottom indicate period between sunset and sunrise.

In the sediment trap experiments, the initial temporary cyst concentration in the control was from 1.4×10^6 to 4.1×10^6 cysts m^{-2} (Table I). When these traps were collected after 12 h (Figure 2) this concentration had almost doubled. The new temporary cysts formed in the sterile sediments (EnEx1, EnEx2, EnEx3) showed an encystment rate between 1.8×10^6 to 4.4×10^6 cysts m^{-2} day^{-1}, similar to the control traps (1.2×10^6 to 6×10^6 cysts m^{-2} day^{-1}).

After 12 h of sediment incubation in filtered sea water, the excystment rates of 1 day temporary cysts (EnEx) was between 0.9×10^6 and 2.7×10^6 cysts m^{-2} day^{-1}. If the

Table I: Results of the sediment trap experiments in 1996 and 1999

Sediment traps	Initial concn	Collected cysts	Encystment rate	Final concn	Excystment rate
August 20, 1996					
EnExn 1	–	1.8 ± 0.3	1.8 ± 0.3	0.9 ± 0.3	0.9 ± 0.6
Control 1	1.4 ± 0.3	3.0 ± 0.7	1.6 ± 0.9	0.5 ± 0.2	2.5 ± 0.6
August 11, 1999					
EnExn 2	–	4.4 ± 2.5	4.4 ± 2.5	1.7 ± 1.2	2.7 ± 1.8
Control 2	4.1	10.1 ± 6.4	6.0	1.1 ± 0.3	9 ± 6.5
August 14, 1999					
EnExn 3	–	2.4 ± 0.4	2.4 ± 0.4	1.0 ± 0.3	1.4 ± 0.6
Control 3	3.1	4.3	1.2	1.3 ± 0.9	3.0 ± 0.9

Values are average ± SD ($n = 4$).
Initial temporary cyst concentration (cysts $\times 10^6$ m^{-2}) in the control sediment. Temporary cyst concentration (cysts $\times 10^6$ m^{-2}) after sedimentation (from 16:00 to 4:00 h GMT). Encystment rate (cysts $\times 10^6$ m^{-2} day^{-1}). Final temporary cyst concentration (cysts $\times 10^6$ m^{-2}) after the incubation period (from 4:00 to 16:00 h GMT) and excystment rates (cysts $\times 10^6$ m^{-2} day^{-1}).

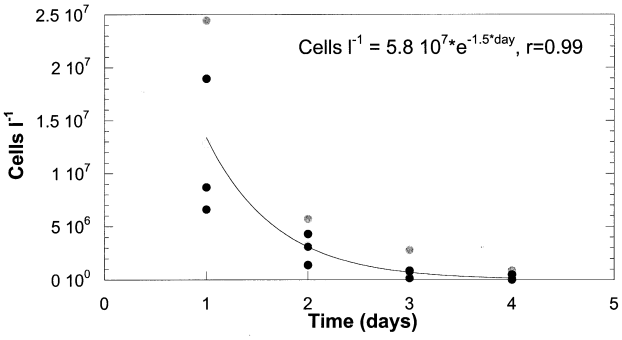

Fig. 4. Viability of temporary cysts monitored by quantification of motile cell concentration in the water during continuous 4 day sediment incubation. One-day-old cysts were used from August 13–14, 1999 traps.

previous temporary cysts in the sediment are taken into account (control), the excystment rate was between 2.5×10^6 and 9×10^6 cysts m^{-2} day^{-1}. This latter rate comprised germination of new and previous temporary cysts.

The percentage of temporary cysts remaining in the sediment without excystment in 24 h was 30 ± 15% for the sediment trap experiments on August 19–20, 1996 (EnEx1), 29 ± 9% on August 10–11, 1999 (EnEx2) and and 33 ± 11% August 13–14, 1999 (EnEx3).

The viability of the temporary cysts was calculated for 1-day-old cysts from samples obtained on August 13–14, 1999 (EnEx3 trap). An excystment success of 90 ± 5% was recorded over 4 days (Figure 4). The temporary cyst germination, monitored as the number of vegetative cells in the water incubations, followed an exponential curve (cells $l^{-1} = 5.8 \times 10^7 \times e^{-1.5 \times day}$, $r = 0.99$). More than 50% of temporary cysts germinated in 24 h.

DISCUSSION

Alexandrium taylori produces temporary cysts as a part of its life cycle, which may be essential to its population dynamics. The temporary cyst stage is related to the vegetative reproduction of the species and not to a dormant stage. The relatively short 'lag' period between temporary cyst formation and formation of vegetative cell allows for a rapid shift between benthic and planktonic stages of *A. taylori*. The shift is an active process as we see with the encystment and excystment rates and like the vegetative cells, the temporary cysts present an *in situ* cell division pattern (0.14 day^{-1}) (Garcés *et al.*, 1998). We suggest that temporary cysts participate in two main functions: reducing population loss and as population stock.

Temporary cyst formation in this species may represent a means for reducing population losses. The temporary cyst population reaches high concentrations in the sediment (10^3–10^4 cysts g^{-1}) during the stationary phase, which lasts at least 4 weeks. In addition, the formation of clusters of cysts reaching concentrations up 10^6 cells l^{-1}, was often observed in the water layer close to the sediment. These clusters could further contribute to avoid population losses from the area, as Margalef (Margalef, 1997) suggested for cell aggregations. Cell aggregations of *A. taylori*, together with the physical characteristics (relatively low water renewal) of La Fosca beach (Garcés *et al.*, 1999) favour population growth and persistence.

The production of temporary cysts can be an advantage since in this way, some of the stock is stored in the sediments. In fact, about 30% of the temporary cyst population in the sediment did not divide within a 24 h period. Furthermore, we demonstrated viability of these temporary cysts for at least 4 days, and temporary cysts remain viable for 1 month in the laboratory (data not show). Encystment probably allows *A. taylori* to withstand short-term perturbations, for instance storms or swells, and contribute to restore the population after a few days of calm weather, during the beginning and maintenance of the bloom.

Contributions to the population growth rate by excystment can participate in the sudden increases in the vegetative population on some days (e.g. 1.48 day^{-1} in August 1996). These high growth rates cannot be explained by vegetative cell potential division rates only, as measured by cell cycle *in situ* (0.4–0.5 day^{-1}) (Garcés *et al.*, 1998) and extrapolated over a diluted population (cell concentrations below 10^2 cells l^{-1}). Thus, sudden increases may be due to the combination of vegetative cell and temporary cyst division and excystment from the cyst pool in the sediment, which contributes to the overall growth rate of the population in the water column. This indicates an adaptive life history strategy of *A. taylori*, which allows the organism to form long-term dense patches for as long as 2 months. These features are exceptional, since *Alexandrium* blooms often reach only moderate biomass levels and are not particularly long-lasting (Wyatt and Jenkinson, 1997).

The temporal variability of temporary cyst concentrations in the sediments indicates that encystment and excystment rates vary considerably during the different bloom phases (beginning, stationary and end). At the beginning of a bloom, temporary cyst accumulation rates increase in the sediment and may exceed the excystment rate, thus forming a cyst stock in the sediment. This is contrary to the general view of the role of temporal cysts in dinoflagellates, which is that their formation is mainly due to environmental stress in the population (Hallegraeff *et al.*, 1995). During the stationary phase of the bloom, encystment and excystment rates compensate each other and maintain constant concentrations, as shown by the temporal distribution of the population.

Environmental conditions probably modify encystment and excystment fluxes, but in the same sense that they modify cellular division, since daily encystment in *A. taylori* is part of its cellular growth.

ACKNOWLEDGEMENTS

The authors thank the personnel in charge of routine monitoring at the *Junta de Sanejament, Medi Ambient de la Generalitat de Catalunya* for their co-operation and for facilitating the field programme. The CIRIT grant FI/948003 and the project of the *Department de Medi Ambient, Junta de Sanejament* (*Generalitat de Catalunya*) funded this study.

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Received on November 3, 2000; accepted on February 2, 2002