The Resting Cyst of *Alexandrium catenella*, a Dinoflagellate Responsible for Harmful Algal Blooms in Thau Lagoon (Western French Mediterranean Coast)

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Abstract

For the first time, the sexual resting cyst of the dinoflagellate *Alexandrium catenella* was isolated and described from surface sediments of Thau Lagoon (French Mediterranean coast). During the two periods of January–May 2000 and 2002, *A. catenella* cysts were sampled and quantified from sediments in several locations of the lagoon. This preliminary study has revealed that all the sampling stations contained *A. catenella* cysts. A small embayment (Angle Creek) showed the highest cyst densities and seems to be a favourable area for accumulation of these dormant stages. Different sampling and quantification methods were compared to improve the estimation of cyst abundance.

Introduction

In Thau Lagoon, the first outbreak of PSP caused by *Alexandrium* sp. was reported in 1998 (Masselin *et al.*, 2000). The associated measured toxicity was up to 852 µg STX eq 100 g^{-1} of mussel meat. *A. catenella* has been shown to be responsible for the recurrent blooms since this first toxic event (Barré, 2001; Lilly *et al.*, 2002). In Mediterranean coastal waters, species of the genus *Alexandrium* occur frequently in small embayments or shallow waters (Garcés *et al.*, 1999). The life cycle of *Alexandrium* species is characterised by a sexual phase, producing hypnozygotes which sink and produce benthic resting cysts. The distribution of cysts in sediments is a useful biological parameter for the monitoring surveys of toxic dinoflagellate species (Anderson and Wall, 1978). Resting cysts play an important role not only in dinoflagellate dispersal, initiation and termination of blooms, but also in survival under unfavourable conditions (Dale, 1983). The fate and distribution of deposited cysts are important variables necessary to understand the ecology and bloom dynamics of toxic species (Hallegraeff, 1993). The objectives of the present study were (1) to isolate and to determine systematically *Alexandrium* sp. cysts from Thau, (2) to compare different sediment sampling and cyst quantification methods and (3) to establish a first distribution of *Alexandrium* cysts in some sensitive areas in Thau Lagoon, especially in Angle Creek, where major cell concentrations are regularly recorded.

Material and Methods

Sample Area

Thau Lagoon is a large Mediterranean water mass, 14 km long and 5 km wide. Its mean depth is 4.5 m, with a maximum of 10 m. Three channels link the lagoon to the sea; 80% of exchanges are through the Sète channels (Fig. 1). Sampling of sediment was conducted from January to May in 2000 and 2002. Three locations were selected: (A) Angle Creek, a small embayment to the north where the highest cell concentration of toxic *A. catenella* was recorded (350,000 cells · L^{-1} in 1998), and two locations out of Angle Creek: (B) Bouzigues, an important shellfish production area, and (S) a station near Sète. For this preliminary study, five stations were sampled: A_1, A_2, A_3 (Angle Creek), B (Bouzigues) and S (Sète).

Sediment Sampling, Characterisation and Preparation

To obtain naturally occurring cysts, surface sediment was collected with an Eckman grab sampler (Yamaguchi *et al.*, 1995). The top 3 cm of sediment samples were placed in plas-

Stn	Lat. N	Long. E	Depth
A_1	43°27′364	003°40′509	0.9 m
A_2	43°27′081	003°40′402	2.2 m
A_3	43°26′920	003°40′344	3.3 m
B	43°26′261	003°39′508	8.4 m
S	43°25′460	003°39′359	7.5 m

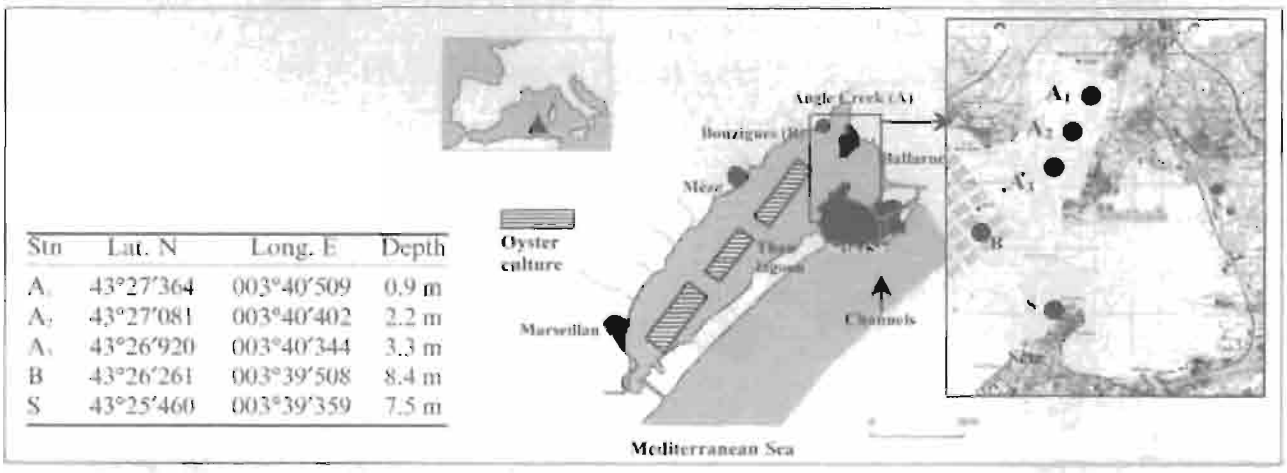

Figure 1 Map of Thau Lagoon and areas where samples were taken. See also geographic coordinates and depths of sampled stations.

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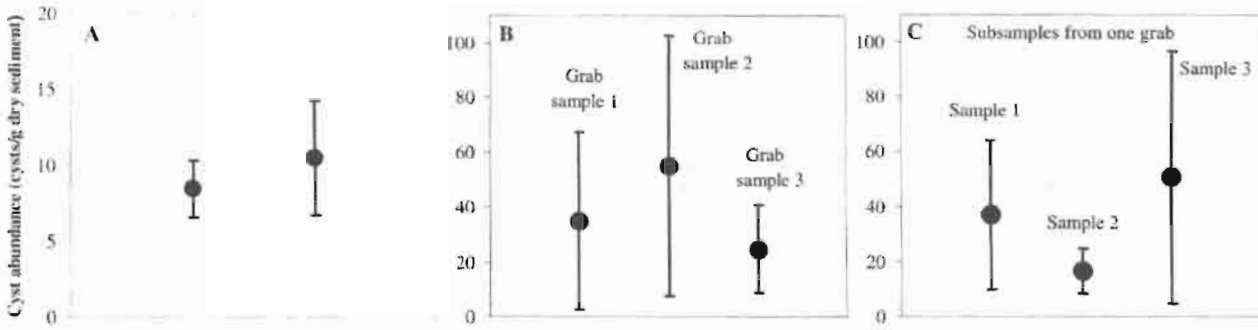

Figure 2 Variation of cyst abundance as a function of sampling method (**A**), number of grabs taken (**B**), or subsamples taken from a grab (**C**).

tic containers and stored in the dark at 4°C. To characterise the sediment, one subsample was oven-dried at 60°C to calculate the water content. The second subsample was lyophilised, weighed and sieved (63-µm mesh). The size fraction <63 µm was reweighed to determine the fine fraction. Two other subsamples were used for cyst counting, using the most probable number (MPN) and density-gradient methods (Erard-Le Denn and Boulay, 1995). After homogenisation, 1-g (wet weight) aliquots of the sediment subsample were suspended in filtered (0.2 µm) seawater, sonicated for 2 minutes to dislodge detritus particles and sieved through plankton nets to obtain the size fraction between 20 and 140 µm.

Germination-Dilution Technique According to the MPN method (Imai *et al.*, 1984), the sediment fraction passing the sieve (140 µm) was suspended in 10 mL filtered seawater. One mL of this suspension was added to five replicate tubes containing 9 mL f/2 medium (Guillard and Ryther, 1962). After three 10th serial dilutions with the same medium, these tubes were incubated at 20°C under 100 µE $\cdot m^{-2} \cdot s^{-1}$ and 12:12 h light : dark cycle. After five days, vegetative cells of *Alexandrium* sp. were examined under the inverted microscope (fifteen settling chambers). In this experiment, any tube with *Alexandrium* sp. motile cells was scored as positive.

Density-Gradient and Centrifugation Technique To separate the cysts from detritus, the density differences between cysts of *Alexandrium* sp. and that of the gradient medium Ludox CLX (d = 1.37 g cm^{-3}) (Erard-Le Denn and Boulay, 1995) were used. The band containing cysts was collected. This fraction was sieved through a 20 µm net and placed in a 5 mL tube containing distilled water and sonicated for 2 minutes. The resultant solution was used to enumerate cysts by the inverted microscope (1 to 3 settling chambers) using the Utermohl (1958) method.

Light Microscope Observations To identify systematically isolated cysts from Thau Lagoon, the morphology of germling cells was studied by light microscopy. Plate tabulation (Balech, 1995) was studied after dissecting a theca using 5% sodium hypochlorite. The length and width of at least 30 germling cells were determined.

Comparison of Sampling Methods We compared samples from an Eckman grab sampler and a gravity core sampler. Sediment samples derived from these methods were taken in A_2. We also compared the variability in cyst abundance within samples from three different grab samplers, and within three samples collected from the sediment of one grab. Each value represents at least three counts corresponding to three replicates.

Results and Discussion

Comparison of Sampling and Quantification Methods

Using the density-gradient method, results showed that the measured abundance of cysts in A_2 were not significantly different in the sediments collected either with an Eckman grab or core sampler (Wilcoxon-Mann-Whitney test, $P > 5\%$) (Fig. 2A). However, core sampling allows information to be obtained about the vertical profile of cyst accumulation. There was no significant difference between cyst abundance measured in the three grab samples (Fig. 2B). Similarly, cyst abundance determined from three subsamples from the sediment of one grab sampler was not significantly different (Fig. 2C) (Kruskal-Wallis test, $P > 5\%$). However, these results underlined the variability (see SD) in cyst distribution within one sediment sample, which implies the need for increased numbers of samples to improve quantification of cyst abundance in a defined area. Our results showed that MPN values were greater than those obtained by enumeration using the density-gradient method, but spatial distribution of cysts determined by the two methods of counting was nearly the same (Fig. 3). Erard-Le Denn and Boulay (1995) suggested that the dilution method could be applied to obtain an indirect count of viable cysts for rapid screening of an area. In conclusion, the two cited methods bring complementary information.

Cyst Features The resting cysts are cylindrical with rounded ends. The clear cyst wall is covered with mucilage, while the cell contains granular material, an orange-red accumulation body and numerous lipid globules (Fig. 4).

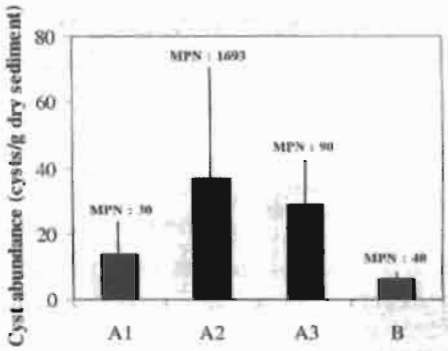

Figure 3 Abundance of *A. catenella* cysts in the stations sampled in Thau Lagoon. Abundance was calculated from counting with the density-gradient (histograms + error bars) and the MPN (MPN : value) methods.

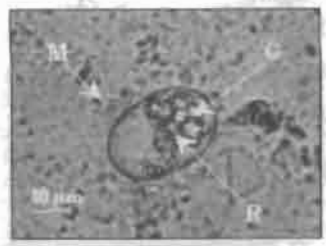

Figure 4 Photographic image of *A. catenella* cyst from Thau. (M) remaining mucilage, (G) granular material and (R) orange-red accumulation body.

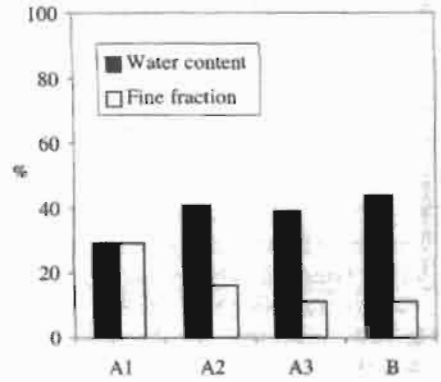

Figure 5 Water content and fine fraction percentages in the sediment sampled in different locations of Thau.

Central body was 41.86 ± 5.32 µm long and 30.4 ± 4.52 µm wide. Cysts of *A. catenella* and *A. tamarense* are indistinguishable in appearance and size (Fukuyo, 1985). The examination of the vegetative cells from germling cells was therefore necessary to determine the taxonomic affinity of the isolated cysts. Germling vegetative cells were round and covered by a cellulosic theca. Mean length and mean width were 26.15 ± 3.2 µm and 30.58 ± 4.28 µm, respectively. Plate formula refers to both *A. catenella* and *A. tamarense* (Fukuyo, 1985). The vegetative cells showed the absence of a ventral pore between the first and the fourth apicals and the ability to form chains of two to eight cells, suggesting that the isolated cysts from Thau Lagoon correspond to *A. catenella*.

Spatial Distribution and Ecological Implications On the basis of cyst densities obtained using the density-gradient method, all the sampling stations contained *A. catenella* cysts. Angle Creek seems to be favourable to deposition and in accumulation of cysts (A_2>A_3>A_1>B) (Fig. 3). MPN counts showed that all the sampled areas including the station S (70 cysts/g^{-1} dry sediment) had *A. catenella* present. The number of cysts was not related to the fine fraction or water content of the sediment in stations $A_{1,2,3}$ and B ($r^2 < 0.03$, $P = 0.05$). However, these results have to be taken with caution because the sampled areas have similar sediment characteristics (Fig. 5) (Erard-Le Denn and Boulay 1995). Further measurements have to be performed throughout Thau Lagoon and must consider hydrographic processes and sediment dynamics.

The presence of *A. catenella* cysts in the sediment of Thau Lagoon may provide a concentrated inoculum for subsequent development of toxic blooms. The maximum observed cyst abundance was 130 cysts per g^{-1} of dry sediment and was found in A_2. Both MPN and density-gradient methods revealed that Angle Creek and particularly A_2 had the highest cyst density. This finding corresponded to the *in situ* distribution of vegetative cells during the recurrent blooms of *A. catenella* in Thau Lagoon (Vaquer, pers. comm). The depth of Angle Inlet is <4 m; this would allow the sedimentation of the new formed hypnozygotes and their subsequent excystment. It seems likely that sediment resuspension locally induces the germination of resting cells and the initiation of a potentially toxic bloom event. Sediment collected from stations B and S contained *A. catenella* cysts. This suggests the extension of distribution of cysts which can be produced locally by motile cells or transported from other previously infected areas. An exhaustive study of cyst abundance and distribution in Thau Lagoon would have to be performed to know the accumulation and dispersion of cyst seedbeds of *A. catenella*.

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