Seasonal relationships between cyst germination and vegetative population of *Alexandrium minutum*

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Introduction

In relation to the initiation of blooms, several investigations have been carried out and have recognised the role of resting cysts as a key responsible for the formation of a new population (Anderson & Wall, 1978). In the estuary of Penzé (Brittany, France) blooms of the toxic *Alexandrium minutum* Halim are a recurring feature. Preliminary studies conducted in the same area of the bay of Morlaix evaluated the distribution of *A.minutum* resting cysts and suggested relationships between their distribution and the sediment transport (Erard-Le Denn *et al.*, 1993). The aim of this study was to evaluate if cysts can play a role in the population dynamic of the toxic dinoflagellates and its competitors and if the germination of one dinoflagellate could be favoured by different environmental conditions. Thus a seasonal investigation was conducted up and down stream of the Penzé estuary to examine temporal changes in the germination ability and time to germinate of the *A.minutum* resting cysts among the other coexistent dinoflagellates *Heterocapsa triquetra* and *Scrippsiella trochoidea*.

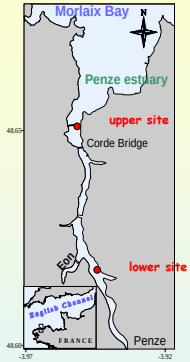


Fig 1. Penzé estuary, sediment sampling sites

Material and Methods

Sampling procedure : sediment samples were collected in the Penzé estuary (Fig.1) at 2 sites (upper and lower) from March to July 2001 every month with a bottom grab sampler (type Ekman). Mud temperature was measured at each sample site and the sediment was stored in the dark until processing. Water samples were collected in subsurface and in the bottom with a horizontal Niskin bottle and temperature and salinity were recorded.

Sampling processing : 3 aliquots of mud were taken.The first sub-sample was for cyst processing and enumeration using the technique previously described (Erard-Le Denn & Boulay, 1995), the second was for germinating experiments. Tests were performed after collecting under 2 experimental conditions : an "*optimal* " condition defined in previous works (t=18°C, I=94µmoles.m^{-2}.s^{-1}, f/2 nutriment) (Erard-Le Denn *et al.*, 1997) and an "*in situ* " condition corresponding to the different field measurements at each mud collecting in place. For that 3 replicates (in each test) of 3 g of sediment mixed with 15 ml of f/2 medium (Guillard & Ryther, 1962) or 15 ml field filtered seawater (5µm) were deposited in Pétri dishes and placed in an incubator. After 3 days of incubation all the water in each dish was collected gently using a syringe to avoid disturbance of the mud sample and fixed with lugol's iodine for dinoflagellate counting under an inversed microscope.

Results

The resting cysts of *Alexandrium minutum* 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. 2). The central body was usually longer (~25 µm) than wide (~20 µm).

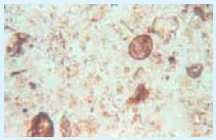


Fig 2. Resting cyst of *A.minutum*. in light microscopy (G) granular material (R) orange-red accumulation body

Fig 3. (L.M.) : Germinating cell of *A. minutum*

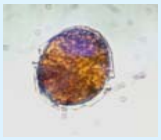


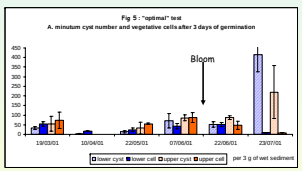
Fig 4. L.M. : vegetative cell of *A. minutum (20-25 µm)*

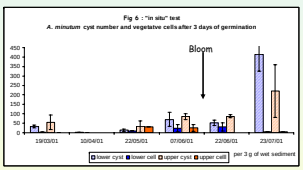
During the seasonal collecting the cyst of *Alexandrium minutum* were particularly concentrated in the upper site of the Penzé estuary (73±46 cysts per gram of wet sediment) except in the end of August (Fig. 5 and 6), in that period the highest concentration was found in the lower site (138±30).

At the end of three days of incubation the results of the different tests have showed that, independently of the experimental conditions, the cysts that were incubated and maintained in their sediments had the ability to excyst (Fig.3 and 4).

- In the "*optimal*" test (Fig. 5) germination of *A.minutum* was very high independently of the site of collecting before the bloom (except on 7th June at the lower site) and exceeded more than 100% due to the division of germinated cells.

- In the "*in situ*" test (Fig. 6) corresponding to each environmental condition (temperature and nutrient) the germination of *A.minutum* was less than 75%, except on 25 th May at the upper site .





In both conditions the germination of *A.minutum* cyst was dependent on the seasonal collecting and the best germination ability was obtained from the samples collected in the end of May and in June, periods during which the temperature of water and sediment reached 17-19°C in the field (Table I). This activity to excyst was also connected with the produced development of vegetative cells of *A. minutum* which densities reached 6 millions cells on 14 th June (Table II).

	Upper site			Lower site		
Date	T° sed.	T° water	S. water	T° sed.	T° water	S. water
19/3	9,9	10,6	33,3	9,9	10,6	30,7
10/4	-	-	-	12,9		
22/5	15,3	16	24,3	18,6	17,4	13,8
7/6	14,8	14,9	33,8	14,7	15,1	18,7
22/6	17,7	17,7	32,9	19,5	19	28,3
23/7	18,4	17,9	30,9	19,5	19,4	25,4

Table I. Environmental conditions during the seasonal collecting of sediment

Sed. collect	Phytopk.collect	A.min nb/L
19/3	2/4	0
	16/4	0
	6/5	0
10/4	13/5	0
	20/5	0
22/5	27/5	0
	28/5	0
7/6	4/6	14400
	11/6	67200
	14/6	6 000 000
	18/6	1 216 000
22/6	25/6	500
	8/7	100
	15/7	0
	16/7	0
	19/7	0
23/7	29/7	0

Table II. Dates of sediment collect, phytoplankton collect and densities of *Alexandrium minutum*

Nevertheless, after the" bloom" in the end of July, in spite of the numerous cysts counted particularly in the lower part of the estuary (138±30 per g of wet sed.), in both experimental conditions the germinations were minimal (<5%) although the temperature was over 18-19°C. The results could lead to a suspicion that temperature was not the major factor influencing the germination and that the cysts were not matured after a bloom as it has been shown by many authors (Anderson & Keafer, 1987; Perez *etal.*,1998; Band *etal.*,2003;...). Thus another factor controlling germination of *A.minutum* cysts - an endogenous annual clock - has been confirmed.

During the germination tests, the vegetative cells of the toxic species were also co-existing with the two dinoflagellates *Heterocapsa triquetra* and *Scrippsiella trochoidea*, two species which are usually competitors of *A.minutum* during a phytoplankton bloom in the Penzé estuary (Posters HAB 2004 133 and 139). Either in "*optimal*" condition or *"in situ"* condition, the 3 species germinated during the same period in the beginning of June (Table. III). In the upper test the density in germinated cells of *H.triquetra* (74 ±72 cells for 3 g of wet sed.) supplanted that of *A.minutum* (87 ± 19 cells for 3 g of wet sed.) predicting in this way the observed "bloom" on June during which *A.minutum* (6 millions cells/L) was dominated by the proliferation of *H.triquetra* (25 millions cells/L) (Fig.7). Thus these seasonal tests have shown the relationship between ability to germination and proliferation of bloom and have brought additional knowledge based on the ecological niches of the coexistent species competing during a toxic bloom.

Upper site

	« Optimal » condition			« In situ » condition		
Date	*Alexandrium minutum*	*Scrippsiella trochoidea*	*Heterocapsa. triquetra*	*Alexandrium minutum*	*Scrippsiella trochoidea*	*Heterocapsa. triquetra*
19/3	72 ± 33	5 ± 1	1 ± 0	0	3 ± 0	0
10/4						
22/5	55 ± 3	7 ± 1	1 ± 1	31 ± 8	6 ± 3	0
7/6	87 ± 19	3 ± 1	74 ± 72	25 ± 4	5 ± 1	22 ± 7
22/6	48 ± 15	22 ± 8	2 ± 2			
23/7	8 ± 1	3 ± 1	2 ± 0	4 ± 1	3 ± 2	0

Lower site

	« Optimal » condition			« In situ » condition		
Date	*Alexandrium minutum*	*Scrippsiella trochoidea*	*Heterocapsa. triquetra*	*Alexandrium minutum*	*Scrippsiella trochoidea*	*Heterocapsa. triquetra*
19/3	54 ± 9	14 ± 3	0	4 ± 1	2 ± 1	0
10/4	16 ± 2	9 ± 4	1 ± 0			
22/5	23 ± 7	5 ± 3	2 ± 2	10 ± 1	3 ± 2	1 ± 1
7/6	42 ± 10	3 ± 4	27 ± 7	24 ± 14	5 ± 2	6 ± 2
22/6	51 ± 7	1 ± 1	5 ± 3	31 ± 16	1 ± 1	6 ± 7
23/7	8 ± 1	2 ± 1	5 ± 6	4 ± 0	2 ± 1	1 ± 1

Table III. Number of germinated dinoflagellates for 3 g of sediment

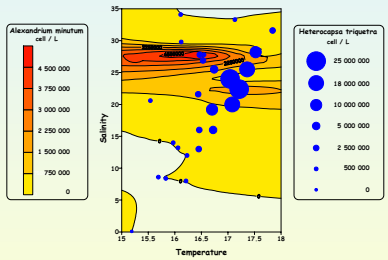


Fig. 7. Competition between the two dinoflagellates *A.minutum* and *H.triquetra* during the bloom in June 2001

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Conclusions

This study shows a relationship between cyst germination and vegetative population of *Alexandrium minutum* which can be explain by :
- the abundance of the benthic stage cells in the bottom mud
- the germination during spring in relation to the toxic events the following summer
- the importance of the role of the cysts as "seed population"
Nevertheless germination rates vary throughout the year and are controlled by a circannual internal biological clock. Non mature cysts are thus able to ignore exogenous character indicators of excystment.
Furthermore the results display the predominance or the competition of the toxic species regarding coexisting competitors.