

Competition between *Alexandrium minutum* and *Heterocapsa triquetra* : I Effects of various environmental and nutritional factors on growth

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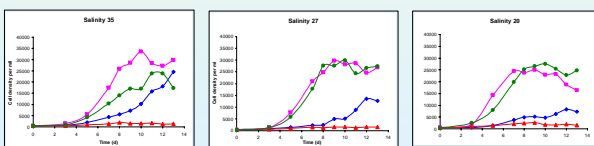
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Introduction

The coexistence of different microalgae species within the same small space was thought to be a paradox, because algae seem to share the same limiting ecosystem. Concerning the proliferation of *Alexandrium minutum*, laboratory experiments were carried out to understand the major mechanisms explaining the appearance or disappearance of this toxic species in the Penzé estuary of Brittany (France). Different bioassays using physical (salinity, light) and chemical (mineral matter) environmental data gathered during blooms in the Penzé river were undertaken concerning the competition between *A. minutum* and a co-existing species, a dinoflagellate *Heterocapsa triquetra*. The aim was to improve our knowledge regarding coexisting competitors during a toxic bloom. These experimental data will be included in the ecophysiological model which is currently in progress.

Results

1- Effect of salinity



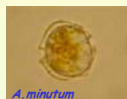
	35	27	20
Am	0.32	0.27	0.25
Am Mixte	0.09	0.11	0.17
Ht	0.45	0.50	0.64
Ht Mixte	0.36	0.51	0.47

Specific growth rate day⁻¹

The results show that *H.triquetra* reference (*Ht*) has a higher growth rate (0.64) at the weakest salinity. In salinity 27, the reference (*Ht*) and the mixed cultures (*Ht mixte*) have approximately the same growth rate (0.50) : thus the presence of *A.minutum* does not influence the growth of *H.triquetra*.

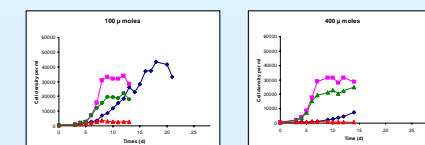
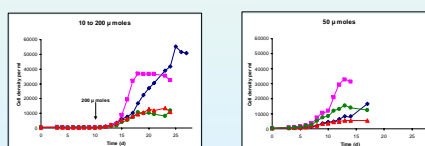
Am reference shows the inverse tendency compared to that noticed for *Ht* reference: the more the salinity decreases, the weaker its growth is (0.25). On the other hand in mixed cultures, the growth of *Am* is affected whatever the salinity : the number of cells not exceeding 2000 cells by ml. Thus experiments showed that a salinity variation (20, 27 and 35) had less influence on toxic species growth than the coexistent effect.

Material and Methods



The experiments were done using *Alexandrium minutum* (AM89BM) and *Heterocapsa triquetra* (HT99PZ) two native dinoflagellates isolated from the Penzé estuary. Before the tests, algae were maintained in f/2 medium (without silicate) at 18°C in salinity 27, values chosen according to the values recorded in the field during the maximum abundance of *A.minutum*. The cells were grown in 2 L flasks with 200 μmoles photons. m². s⁻¹ under a 12-12-h light : dark cycle and no mechanical excitement or aeration was performed on the cultures. Monospecific tests and 3 replicates of mixed culture of *A. minutum*/*H. triquetra* were tested. The same concentration for each, 500 000 cells by litre, was counted daily in a Nageotte chamber for 15 days. The specific growth rate per day was calculated according to the calculation $\mu = (\ln Nt - \ln N0) / (t - t0)$ where N0 represents the number of cells at the beginning of exponential phase in time t0 and Nt the end of the exponential phase in time t. The nutrients were analysed by an autoanalyser.

2- Effect of light



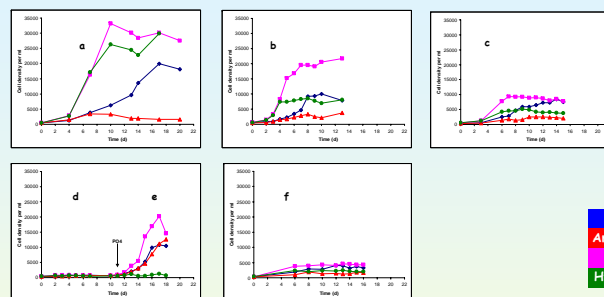
10, 50, 100, 200 and 400 μmoles photons. m². s⁻¹. were experimented. After 10 days of 10 μmoles, a stimulation of 200 μmoles was also tested for 15 days. *H.triquetra* reference presents a preferendum for the average intensities of 100 and 200 (μ=0.5). In competition it shows the same tendency: the growth rates are weaker in 50 and 400 than in 100 and 200. *A. minutum* reference is less affected by the variations of light than *Ht* reference. In competition *Am* is affected by the strongest light, the highest growth rate (0.22) is observed for 50 μmoles. 10 μmoles is not favourable for algae development whatever the species. In competition a stimulation of 200 μmoles after an illumination of 10 μmoles for 10 days seems favourable to *Am* which presents a growth rate (0.33) approximately similar to that of *Ht*(0.37).

	400	200	100	50	10	10/200
Am	0.20	0.27	0.24	0.24	0.05	0.31
Am Mixte	0.09	0.11	0.14	0.22	0.02	0.33
Ht	0.30	0.50	0.55	0.28	-0.02	0.64
Ht Mixte	0.34	0.51	0.47	0.29	-0.02	0.37

Specific growth rate day⁻¹

3- Effect of nutrients

The experiments concerned various combinations of the medium f/2, in limitation of N-NO₃ and P-PO₄ by taking into account values measured *in situ* during *A.minutum* blooms in the river of Penzé : (3a) complete medium f/2, (3b) medium corresponding to the maximal conditions in the nutrient field during a bloom : f/2/4 for NO₃ and f/2/10 for PO₄, (3c) medium corresponding to the minimal conditions : f/2/10 for NO₃ and f/2/20 for PO₄ (3 e-d) a situation of deficiency in PO₄ for 10 days followed by one pulse of PO₄, (3f) estuary filtered water (salinity 27). The various tests were exposed to 200μmoles at 18°C.

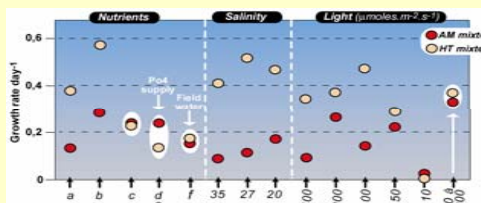


Experiments 3 a, b and c show that whatever the supply, *H. triquetra* growth rates always supplant those of *A.minutum*. Only experiment 3c where the concentration in nitrate is 296 and in phosphorus 3.26 presents a favourable medium for the growth of *A.minutum* in competition (μ = 0.23). The situation of deficiency in PO₄ for 10 days affects *Am* as well as its competitor *Ht* (μ < 0.1). At the end of 10 days a PO₄ supply (Exp. 3e) of 4.05 μM highlights the advantage of *Am* over *Ht*. It is the only case, where *Am* in mixed cultures presents a growth rate (0.24) superior to that of *Ht* (0.14). It tends to show that the growth of *Alexandrium minutum* could be favoured in conditions of alternation : PO₄ limitation and PO₄ pulse.

	a	b	c	d	e	f
Am	0.21	0.46	0.25	0.06	0.41	0.20
Am Mixte	0.13	0.28	0.23	0.06	0.24	0.15
Ht	0.41	0.52	0.49	0.05	0.53	0.24
Ht Mixte	0.38	0.57	0.23	0.02	0.14	0.17

Specific growth rate day⁻¹

Conclusions



This first screening is an attempt to understand bloom dynamics from the relationship between the two major competing species. Within the limits of our experiments it has been shown that regarding its weak growth rates, the toxic species *A.minutum* is a mediocre competitor among the other members of the phytoplanktonic community. Nevertheless some factors seem to favour the "bloom" of *Alexandrium minutum* in the presence of its competitor :

- a brutal stimulation of light intensity, 10 μmoles.m².s⁻¹ for 10 days then 200 μmoles or
- a severe phosphorus limitation for 10 days before a phosphate supply.

Thus the following stage in our study was to specify the physiological responses of these two competitors to different phosphate supplies :

"Competition between *Alexandrium minutum* and *Heterocapsa triquetra*
II The role of phosphorus on growth and uptake kinetics."
poster HAB 2004 Labry *et al.*

