Competition between *Alexandrium minutum* and *Heterocapsa triquetra* : II The role of phosphorus on growth and uptake kinetics

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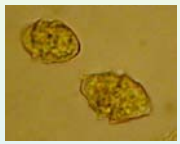
Introduction

The marine dinoflagellate *Alexandrium minutum* (*Am*) has formed regular blooms in the Penze estuary (northern Brittany) with paralytic shellfish toxin contamination of mussels and oysters. Until today the studies undertaken since 1997 have concentrated on the physical conditions of the development of this species, on the determination of the most frequently associated competitors [*Heterocapsa triquetra* (*Ht*) was the most common and abundant] and the role of intracellular nutrients on the induction of sexual reproduction. However the role of nutritional factors on the growth dynamics of *Am* has not been elucidated. A first screening of the environmental conditions favourable for the growth of *Am* to the detriment of that of *Ht* showed initially the importance of a severe phosphorus limitation before a phosphate enrichment (See poster HAB 2004, Erard *et al.*).
Thus the objectives are to specify and parameterize the physiological responses of *Am* and *Ht* to different phosphate supplies in terms of growth rate, phosphate uptake and phosphorus storage capacity. Main questions are (1) What is the influence of the degree of phosphate limitation before a PO_4 pulse on cell growth in monospecific and mixed batch cultures (2) What is the influence of intracellular phosphorus on growth rate and phosphate uptake kinetics (semicontinuous cultures)?

Alexandrium minutum in culture



Heterocapsa triquetra in culture

1- Influence of the degree of PO_4 limitation (nb of days of PO_4 privation) before a PO_4 pulse on growth, PO_4 storage and uptake

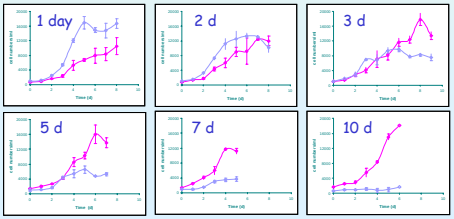


Fig. 1: Evolution of cell concentrations in mixed cultures after 1, 3, 5, 7, 10 days of PO_4 privation and a PO_4 pulse

In mixed cultures (Fig. 1, 2) there was a clear shift between *Heterocapsa triquetra* dominance during the first days (higher specific growth rate μ and maximum cell numbers at the saturation phase) and *Alexandrium minutum* dominance after five days of privation. In monospecific cultures (Fig. 2), μ dropped the first days for both species, then increased and *Heterocapsa triquetra* always dominated.

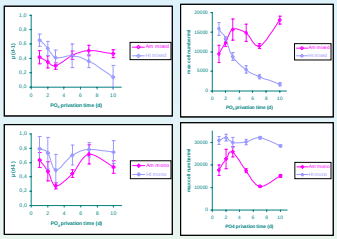
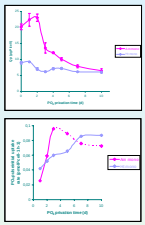


Fig. 2: Evolution of μ and cell concentrations at the saturation phase with PO_4 privation time in mixed and monospecific cultures

Fig.3: Evolution of cell quota Qp and PO_4 pulse initial uptake rate with PO_4 privation time in monospecific cultures



The change in species dominance from the third day corresponded to marked physiological changes observed in monospecific cultures of *Am* (Fig. 3) : the drop in cellular quota of P (Qp) and the increase in pulse PO_4 initial uptake rate over that of *Ht*. In mixed cultures *Am* cells exhausted the phosphate supply preventing *Ht* growth thereafter.

Conclusions

This study enabled growth and phosphate uptake kinetics to be parameterized in relation to Qp (Qp_{min}, Qp_{max}, Ks, Vp_{max}, μ_{max}) in order to calibrate the biological model of *Alexandrium minutum*.
The main physiological characteristics are:

(1) *Am* and *Ht* are not "affinity-adapted" species for PO_4 because of their high Ks. They wouldn't be competitive in severe P limited environment.
(2) P storage capacity of *Am* is twofold that of *Ht*, thus *Am* is a "storage specialist" in comparison with *Ht*, capable of rapid pulse PO_4 uptake, and PO_4 storage for later increase in their growth division rate when PO_4 may be depleted again.
(3) *Ht* is rather a "velocity adapted" species, capable of rapid pulse PO_4 uptake for direct use to increase growth.

Thus *Am* could be more competitive than *Ht* in conditions of high P limitation. In the Penze river algae experience contrasted conditions (from few μM PO_4 to < 0.1 μM). The frequency of nutrient supplies is crucial for competition. The next stage in our studies will focus on the influence of different frequencies (supplies every 1, 2, 4, 6 days) on competition between *Am* and *Ht*.

Material and Methods

Alexandrium minutum (AM89BM) and *Heterocapsa triquetra* (HT99PZ) were cultured in filtered seawater (0.2 μm) at 18°C, salinity of 27 and 200 μE m^{-2} s^{-1} (L:D 14:10).

For batches, the (mixed and monospecific) cultures were inoculated in f/2/4 NO_3 (220 μM) medium (3 L) and with no PO_4 at time to. At 1, 2, 3, 5, 7, 10 days after, $[PO_4]$, cell numbers and cellular P content (cell quota Qp) were measured and a subsample (400ml) was enriched with a 4 μM PO_4 pulse and was examined for cell numbers and $[PO_4]$.

For (monospecific) semicontinuous cultures, the renewal medium contained f/2/4 PO_4 (9 μM) and f/2 NO_3 (880 μM) and the cultures were diluted once per day with dilution rates (D) from 0.05 to 0.5-0.6 d^{-1}. The species were manually shaked once per day only, to avoid physiological stress. At steady state, $[PO_4]$, cell numbers, intracellular quota of P, maximum PO_4 uptake (Vp_{max}) were measured as well as half saturation constant for uptake (Ks) when PO_4 was exhausted at steady state.

Cell numbers were determined in mixed cultures after fixation with an anesthetic. PO_4 was analysed automatically. Intracellular quota of P was determined by the measurement of particulate P divided by the cell number. PO_4 uptake was determined with $^{33}PO_4^{3-}$ at 10 μM $^{31}PO_4^{3-}$ for Vp_{max} and from 0.05 to 6.4 μM $^{31}PO_4$ for determination of Ks. Incubation times from 5min to 6h were sampled.

Results

2- Influence of Qp on specific growth rate (μ) (semicontinuous culture at steady state)

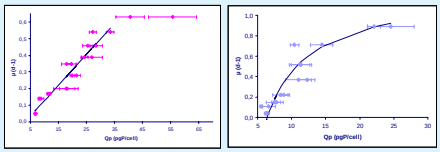


Fig. 4: Relation between μ and cell quota Qp for *Am* and *Ht*

The relation between μ and Qp (Fig. 4) corresponded to the Droop equation [$\mu = \mu_{max}(1-Qp_{min}/Qp)$] for *Ht* (r = 0.89, Qp_{min} = 6.4 pgP $cell^{-1}$ and μ_{max} = 1.25 d^{-1}) and was linear until μ of 0.54 d^{-1} for *Am* (r = 0.95) then an inflexion occurred until 0.63 d^{-1}. The quota range experienced by *Am* (6.8-48 pgP $cell^{-1}$) is twofold higher than that of *Ht* (6.2-23 pgP $cell^{-1}$) with a similar Qp_{min} therefore the storage capacity of *Am* (Qp_{max}/Qp_{min} = 7) is twofold higher than that of *Ht* (Qp_{max}/Qp_{min} = 3.8).

3- Influence of PO_4 and Qp on PO_4 uptake rate (semicontinuous culture at steady state)

Evolution of Vp_{max} with time : there was an acceleration of Vp_{max} with the incubation time (from < 45min to 3h) showing the adaptation of enzymatic equipment to high $[PO_4]$ supply (Fig. 5).

Evolution of Vp with $[PO_4]$: when the shorter incubation time was considered, this evolution followed Michaelis & Menten model with relatively high Ks for both species (Table below).

D (j^{-1})	0.05		0.1		0.2	
	Am	*Ht*	*Am*	*Ht*	*Am*	*Ht*
Qp (pgPcell^{-1})	6.8	6.3	8.5	6.2	17.7	8.7
Ks (μM, st.d.)	0.90 (29%)	2.23 (9%)	0.28 (30%)	1.29 (19%)	1.62 (30%)	0.97 (8%)
Vp_{max} (st.d.) (pmol $cell^{-1}$ h^{-1})	0.0172 (10%)	0.0234 (4%)	0.0034 (8%)	0.0258 (7%)	0.0029 (12%)	0.0217 (3%)
(h^{-1})	0.0804 (10%)	0.1153 (4%)	0.0123 (8%)	0.1288 (7%)	0.0052 (12%)	0.0775 (3%)

Evolution of Vp_{max} with Qp (Fig. 5) : Vp_{max} dropped substantially from Qp_{min} (~ 6 pgP $cell^{-1}$) to 20 pgP $cell^{-1}$ for *Am* and to 12 pgP $cell^{-1}$ for *Ht*. Then it stabilised and tended to increase approaching to Qp_{max}.

Comparison between *Am* and *Ht* (Fig. 5) : For Qp comprised between 10 and 15 pgP $cell^{-1}$, Vp_{max} of *Am* were 4 fold higher than those of *Ht*. They were twofold higher under 10 pgP $cell^{-1}$ and were similar when values were close to Qp_{max}.

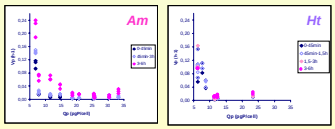


Fig. 5: Relation between Vp_{max} and cell quota Qp for *Am* and *Ht*