An ecophysiological model of *Alexandrium minutum*. Effect of nutrients on vegetative growth and sexuality induction.

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Introduction

Alexandrium spp are poison-producing dinoflagellate species which are known to produce near-shore blooms and which are harmful to shellfish production due to harvesting bans. Along French coasts, *Alexandrium minutum* (Halim) blooms were first recorded in estuaries of northern Brittany in June 1988 and since, the phenomenon is believed to have spread. In the Penzé estuary, a tidal flat estuary connected to the west part of Morlaix bay (Northern Brittany, France), proliferation events are strongly linked to neap tides of late spring/early summer. There, cell density may reach a high level (44,6 10^6cell.l^{-1} up-estuary in June, 1997).

Two major biological processes are involved in the observation of a bloom : first, the synchronous germination of cysts from sediment beds ; second, the ability of *A. minutum* to compete for nutrients in nutrient enriched coastal waters. For a better understanding of the phenomenon, numerical models have to reproduce both vegetative growth and sexual induction leading to cyst formation ; the latter is a major cause of breakdown and of survival of the species from one year to another. From batch culture data (Probert, 1999), we propose a model of growth for *A. minutum* derived from a quota-type model that includes co-limitation of nitrogen and phosphorus and sexual induction.

Methods

The data set

The Probert batch culture experiments were conducted on the AM89BM strain of *A. minutum* isolated from the Morlaix Bay. The data set refers to time-course experiments in optimal forcing conditions (T=20°C ; I=150 μE.$m^{-2}.s^{-1}$) but varying nutrient initial regimes (K medium, Keller, 1987). Five initial nutrient regimes were tested in time-course experiments : N-P replete (K concentration for N and P), N-limited (K/4-N ; K-P) ; N strong limited (K/10-N ; K-P) ; P-limited (K-N ; K/4-P) and P-strong limited (K-N ; K/10-P). All the batches were grown in a 14L:10D regime at salinity S=32.

Batch	Motile cells 10^3 cell.ml^{-1}	QN pgN.$cell^{-1}$	QP pgP.$cell^{-1}$	NO_3 µmol.l^{-1}	NH_4 µmol.l^{-1}	PO_4 µmol.l^{-1}
A (K-NP)	1,82	245	27,5	884	10	36
B (N/4)	2,19	128	25	221	2,5	36
C (N/10)	2,19	128	25	88	1	36
D (P/4)	2,76	186	25	884	10	9
E (P/10)	2,76	186	25	884	10	3,6

Table 1 : Initial culture conditions

Daily measurements of cell number (motile cells and zygotes), dissolved and particulate nutrients were done.

Results

1- The growth model

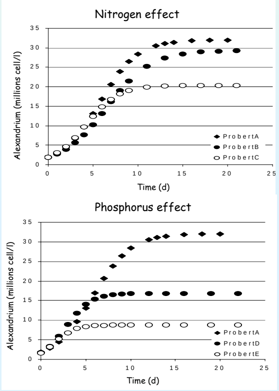

Figure 1 : Nutrient limitation effect on Alexandrium growth

The growth rate (μ) is plotted against the NP cellular status : $\mu = \mu_{max}$. statusNP

Cell quota control is derived from Droop limited between 0 and 1 :
$statusN = (1 - QN_{min}/QN) / (1 - QN_{min}/QN_{max})$
$statusP = (1 - QP_{min}/QP) / (1 - QP_{min}/QP_{max})$
With QN : Nitrogen quota, QP : Phosphorus quota.

We use a combination of threshold and multiplicative approaches (Flynn,2001) between QN and QP control :

If statusN > statusP :
statusNP = statusP.(1-statusP+statusN.statusP)

If statusP > statusN :
statusNP = statusN.(1-statusN+statusN.statusP)

Uptake rate is plotted against nutrients following Michaelis – Menten law and quotas :
$VS = VS_{max}. (S/(S+K_S)) . (QS_{max}-QS)/(QS_{max}-QS_{min})$
with S = NO3, NH4, P and QS = QN, QP

To explain the decline in the non limiting nutrient assimilation we add a control by the limiting nutrient (Roelke *et al.*, 1999):
$VNO3 = VNO3 . PO4/(aP.K_P+PO4)$
$VP = VP . (NO3+NH4)/(aN.K_{NO3}+NO3+NH4)$

2- The sexuality induction model

Sexual induction proceeds from nutrient stress which consists in intracellular depletion rather than in extracellular nutrient availability. In Probert's batch experiments both N limitation and P limitation induced zygote yielding . The relative importance of N and P limitation is not obvious and the same weight for sexual induction as for growth was attributed to N and P limitation by modelling sexual induction versus the NP status.

Experimental data were fitted by the mean values of non-linear regression with the least squares method using the Microsoft EXCEL Solver and suitable values for constants α and KNP were obtained :
$ISEX = 1 - statusNP^{\alpha}/(statusNP^{\alpha}+KNP^{\alpha})$

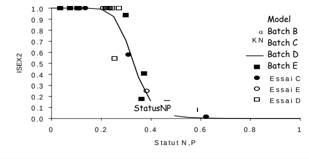

Figure 2 : Relation of the sexuality Index with N and P quota limitation

Conclusions

The ecophysiological model of *Alexandrium minutum* takes into account the influence of nutrient stress on vegetative growth as well as on sexual reproduction. This means that environmental factors like nutrients play a significant role in the formation and decline of toxic blooms. In terms of further exploration, parameters and processes should be better estimated with culture experiments (batch or semi-continued) in order to test separately the different physiological mechanisms (like the relation between uptake and intracellular quotas…). We also need to consider the effect of light and temperature on these physiological responses. To understand *in situ* the occurrence of *Alexandrium* blooms, the physical environment should be considered, such as currents, water stability, the coexistence/competition with other planktonic algae as well as grazing.

3- The batch culture simulations

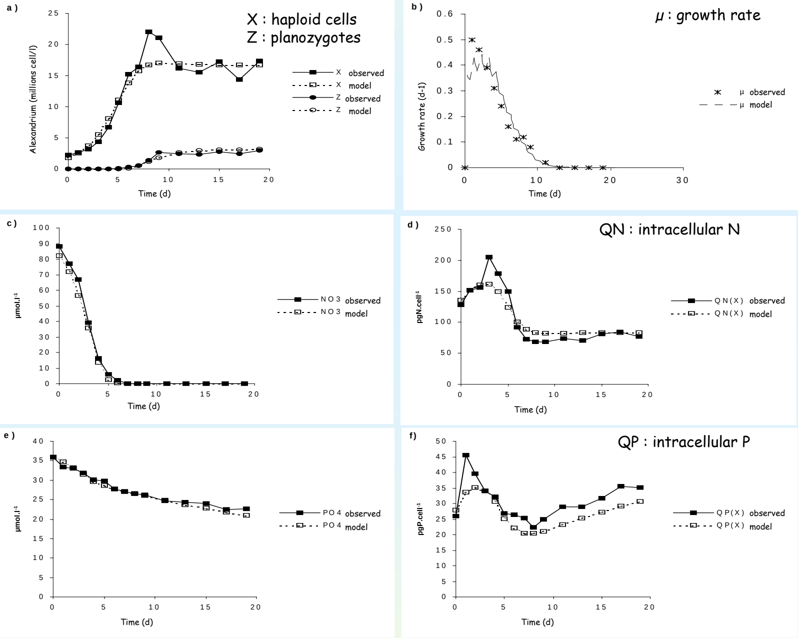

Figure 3 : Simulation of Probert's batch C (N limited)

	Parameter	Unit	Value
µmax	Maximal growth rate	d^{-1}	1,15
QNmin	N minimum quota	pgN.$cell^{-1}$	64
QPmin	P minimum quota	pgP.$cell^{-1}$	15
QNmax	N maximum quota	pgN.$cell^{-1}$	450
QPmax	P maximum quota	pgP.$cell^{-1}$	50
α	Adjusted value for sexual induction	sd	8,34
KNP	constants	sd	0,32
V_{NH4}MAX	NH4 maximum uptake	pgN.$cell^{-1}.d^{-1}$	47
V_{NO3}MAX	NO3 maximum uptake	pgN.$cell^{-1}.d^{-1}$	3
a_N	Influencing factor of NO3 on PO4 uptake	sd	2
V_PMAX	PO4 maximum uptake	pgP.$cell^{-1}.d^{-1}$	35
a_P	Influencing factor of PO4 on NO3 uptake	sd	0,3
K_{NH4}	Half saturation constant for NH4 uptake	µmol.l^{-1}	1
K_{NO3}	Half saturation constant for NO3 uptake	µmol.l^{-1}	25
K_P	Half saturation constant for PO4 uptake	µmol.l^{-1}	5

Table 2 : Parameters used in the model

The model reproduces correctly the population growth as well as the nutrient uptake. It is also suitable for the other N limited batch (D) as well as for the two P limited batches (D and E). The model does not reproduce population starvation in case A with no nutrient limitation which could point to a biomass effect or limitation by another substance.

References

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