Alexandrium growth controlled by phosphorus. An applied model

A. Chapelle [a]*, C. Labry [a], M. Sourisseau [a], C. Lebreton[b], A. Youenou [a], Crassous M.P [a]

[a] *Ifremer Dyneco Pelagos, BP70, 29280 Plouzané, France*
[b] *GKSS Research Centre Geesthacht, Institute for coastal research, Max-Planck-Straße 1 21502 Geesthacht*

* Corresponding author, tel. 00 33 2 98 22 43 56 ; fax 00 33 2 98 22 45 48 ; email: annie.chapelle@ifremer.fr

Abstract
Toxic algae is a worldwide problem threatening aquaculture, public health and tourism. *Alexandrium* , a toxic dinoflagellate proliferates in Northwest France estuaries causing PSP troubles. Vegetative growth is a crucial parameter to understand toxic blooms and in particular the role of nutrient uptake and growth rate. This work focuses on phosphorus nutrition and it also explores the most adapted ecophysiological model for *Alexandrium* to reproduce uptake, growth and competition based on laboratory experiments.

Key words
Toxic Algal blooms, Phosphorus uptake, Models, Competition, North-west France

Introduction

The increasing number of toxic algal events threaten regional economies linked to aquaculture and tourism, as well as public health. It is a worldwide phenomenom and in France, in particular, one of the most problematic organism is the dinoflagellate *Alexandrium* which contains toxins that give rise, in humans, to syndromes known as Paralytic Shellfish Poisoning (PSP) (the French monitoring program, Rephy ; the Interreg IIB NEW Final project ; Cembella 1998…). Should these toxic algae be present in areas of aquaculture, the shellfish can, without being harmed, accumulate and concentrate the toxins produced by phytoplankton within their tissues. The ingestion of these contamined shellfish by humans may lead to serious and potentially fatal gastrointestinal and neurological disorders.

The outbreak of *Alexandrium* in France dates back to the late 1980[S] (Belin 1993 ; Erard Le Denn, 1997 ; Probert, 1999). The species is *Alexandrium minutum* (Halim). The first case of toxicity occurred in 1989 in the Bay of Morlaix and in 1992 in the Penzé estuary which are located in Brittany. Toxic events appeared in 1992, 1993, 1994, 1995, 1996, 1997, 1999 and 2001 (Chapelle *et al.*, 2008) and correspond to *Alexandrium* blooms up to 1million cells per litre. As nitrogen concentration in the Penzé estuary is high and the N/P mol ratio is above 100, nitrogen is never limiting primary production. On the contrary, phosphate may be limiting and controlled by river inputs and sedimentary fluxes which are extremely variable (Andrieux-Loyer et al., 2008). *Alexandrium* blooms do not exceed 10 days and may appear from May to July. There are practically never monospecific and not necessarily dominant in

terms of phytoplankton biomass. Although it reached 97% in 1997, its abundance usually remains between 28% and 72% the other years. In the Penzé estuary, *Heterocapsa triquetra*, a non toxic dinoflagellate, is frequently associated with *Alexandrium* and may be a potential competitor, reaching high concentrations, up to 1 million cell per liter, (Labry et al., 2008 ; Maguer et al., 2004 ; Morin et al. 2000).

To understand which environmental factors may favour *Alexandrium* blooms, numerical modeling associated with *in situ* and laboratory data is often used. Environmental models generally simulate the physical environment based on hydrodynamic models. Coupled to *Alexandrium* model, these models give good results in terms of knowledge of the relative influence of physical transport, growth or germination on *Alexandrium* blooms (Anderson et al., 2005 ; Basterretxea et al., 2007 ; Fauchot et al. 2008; McGillicuddy et al., 2005 ; Yamamoto et al., 2002 ; Yamamoto and Seike, 2003). However, the most enduring question - why one specific species – *Alexandrium* - blooms in place at a particular time from among the whole phytoplankton population remains unknown. The relative competitive success of a particular phytoplankton in different growth conditions is at the root of this question. To answer this question, it is probably necessary to explore in depth the physiological responses of *Alexandrium* linked to environmental parameters. But a compromise should be reached between increasing complexity, needing kinetics and parameter knowledge, and a simple approach which is more generic and user-friendly when included in environmental models (Davidson and Gurney 1999).

Applied to *Alexandrium* blooms in the Penzé, this work consists in building a physiological model of *Alexandrium* growth controlled by nutrients. As nitrogen is in excess, only phosphorus is considered. The model is calibrated and validated with *Alexandrium* batch monocultures and semi-continuous monocultures and applied to simulate the *Heterocapsa* – *Alexandrium* competition. The model complexity, directly derived from data results is then analysed so as to keep the simplest description. This can then be incorporated in a more general ecosystem model.

This work is part of an Interreg IIIB NWE project called “Final”.

1. Material and Methods

1.1 Cell culture experiments and subsequent analyses

The data set is based on cell culture experiments performed on non axenic strains of *Alexandrium minutum* (AM89BM) and *Heterocapsa triquetra* (HT99PZ) isolated in northern Brittany (France) from the Morlaix estuary in 1989 and the Penzé estuary in 1999 respectively. Both strains were maintained in prefiltered natural seawater enriched f/2 medium (Guillard and Ryther, 1962)

For all experiments, temperature, light and salinity are constant and optimal. Inorganic nitrogen is in excess. *Alexandrium* or *Heterocapsa* abundance (in cells), PO_4 concentrations and internal quota (P per cell) have been measured.

For cell enumerations, samples were fixed with a few drops of Lugol's iodine and cells were counted with an inversed optical microscope (Utermöhl method). Samples for PO_4 determination were very carefully filtered on glass fiber filters (Whatman GF/F) with a syringe filtration system. PO_4 were analysed on an autoanalyser AACE Bran and Lubbe

following Aminot and Kérouel (2007). Samples for particulate P were filtered on precombusted (12 h at 400 °C) 25 mm Whatman GF/D filters and filters were deep frozen (−20 °C). Particulate phosphorus was determined using high temperature method (Solorzano and Sharp, 1980). P cell quotas (Q_P) were obtained by dividing particulate P by the corresponding cell number taking into account filtered volumes.

Data set for calibration

The growth rate has been calibrated with semi-continuous experiments (Labry et al., 2004). Cells were preconditioned in batch culture then submitted to semi-continuous mode in duplicate with different dilution rate from low value (0.05 d^{-1}) to the maximum without washing out of the culture (0.5 d^{-1} for *Alexandrium* and 0.6 d^{-1} for *Heterocapsa*). In this type of culture, at the equilibrium, the growth rate (μ) reaches a theoretical value linked to the dilution rate (D): $\mu = -Ln(1-D)$

Minimum phosphorus cell quota has been measured with P depleted batch experiments (Labry et al., 2008).

Phosphorus uptake process has been calibrated with semicontinuous experiments (Labry et al., 2004). Half-saturation constant (K_P) have been estimated from the relation between uptake and PO_4 in the medium at the equilibrium. - Rates of PO_4 uptake were measured using the $^{33}PO_4$ incorporation technique. Incubations were performed to estimate maximal uptake rates (V_{Pmax}) for each dilution rate. After adjusting the PO_4 concentration of water samples to 10 mmol m^{-3}, incubations started with the addition of 20 µCi $^{33}PO_4$ and were ended by addition of 4 % formaldehyde final concentration. Different incubation times ranging from 5 min to 6 h were tested and PO_4 uptake rates were calculated on the linear part of the ^{33}P incorporation time series. For kinetics experiments, similar incubations were performed by adding graded PO_4 concentrations (0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 µM KH_2PO_4) and 10 µCi $^{33}PO_4$ to subsamples. At the end of incubations, samples were filtered on 8 µm Millipore filters. Then, filters were stored with 4 ml of scintillation cocktail until they were counted with a liquid scintillation counter.

Data set for validation

Validation of *Alexandrium* model has been performed with various experiments, all of them being nitrogen repleted:

- Batch cultures (Labry et al., 2008) to test the response of *Alexandrium* cultivated in a PO_4 depleted medium with a pulse of 4 µM PO_4 supply after 1, 2, 3, 5, 7 or 10 days of depletion.
- Batch cultures (Erard Le Denn et al., 2003) with *Alexandrium* grown on different phosphorus depleted medium : f/2/10 medium, which is characteristic of the mean P concentration in the Penzé estuary during bloom conditions and f/2/20, which represents the most severe P concentrations during the bloom in the Penzé estuary.
- Semi-continuous mode with a dilution rate varying from 0.05 d^{-1} to 0.5 d^{-1}. The input medium contains a concentration of 8 $mmol.m^{-3}$ of PO_4.
- Semi-continuous mode with a mean dilution rate of 0.15 d^{-1} (Labry et al., 2008) to test four frequencies (every 1, 2, 4 or 6 days) of PO_4 supply (the PO_4 concentration in the input medium is 9 $mmol.m^{-3}$ for pulse every 1 day, 18, 36 and 54 $mmol.m^{-3}$ for the following).

The competition simulations *Alexandrium* /*Heterocapsa* has been performed on 2 different experimentations : PO_4 depleted batch cultures with a pulse of 4 µM PO_4 supply after 1, 2, 3, 5, 7 or 10 days and semi-continuous culture mode with a mean dilution rate of 0.15 d^{-1} and PO_4 supply (9 $mmol.m^{-3}$ for pulse every 1 day, 18, 36 and 54 $mmol.m^{-3}$ for the following), (Labry et al. 2008).

1.2 Building the model

1.2.1 Available models

To simulate algal growth related to nutrient, 3 types ofmodels of different complexity have already been proposed.

Among the simplest is the Monod model (Monod 1942) linking growth directly to extra-cellular nutrient concentrations and considering only 2 state variables :

$$\frac{dAlex}{dt}=\mu\times Alex \quad \text{and} \quad \frac{dPO_4}{dt}=-\mu\times Alex \quad \text{with} \quad \mu=\mu_{max}\times\frac{PO_4}{PO_4+K_P}$$

Alex is the *Alexandrium* abundance (in $cells.ml^{-1}$) and PO_4 the phosphate concentration (in $mmol.m^{-3}$). µ and μ_{max} are respectively the growth rate and the maximal growth rate (in d^{-1}) and K_P the half saturation constant for *Alexandrium* growth (in $mmol.m^{-3}$).

.

The quota model or Droop model (Droop, 1973) links growth to the internal nutrient content (quota, Q_P) which is itself dependant on extra-cellular nutrient concentration. Three state variables are used in this model: *Alexandrium* abundance (*Alex* in $cells.ml^{-1}$), phosphorus quota (Q_P in $pg.cell^{-1}$) and phosphate (PO_4 in $mmol.m^{-3}$) :

$$\frac{dAlex}{dt}=\mu\times Alex$$

$$\frac{dQ_P}{dt}=V_P-\mu\times Q_P$$

$$\frac{dPO_4}{dt}=-V_P\times Alex$$

More complex mechanistic models, based on Droop model, may consider feedback process or multiple internal pools and seek to reproduce more closely the biochemical reality (Flynn 2003 ; John and Flynn, 200). We are going to examine the feedback of phosphorus quota on PO_4 uptake.

1.2.2. Model choice and adjustment

The Monod-model is more suitable to simulate equilibrium features but not transient growth dynamics related to non steady-state environment (Davidson and Gurney, 1999 ; Haney and Jackson, 1996 ; Flynn, 2001 ; Flynn, 2003). As phosphorus may be accumulated within cells so that reasonable growth rates could be maintained for several generations with no or little uptake (Flynn, 2003), the model should consider internal quota, like Droop or Droop-modified models.

- *Alexandrium* growth rate

Growth rate is linked to the internal quota Q_P according to the Droop formula :

$$\mu = \mu_{max} \times (1 - \frac{Q_{Pmin}}{Q_P})$$

The minimum quota measured from batch experiments under P depletion is 6.5 pg.cell^{-1} (table 1).

The maximum growth rate (μ_{max}) is adjusted by the less square method using the Droop formula with data obtained by the semi-continuous P controlled experiments (Labry et al. 2004), giving a μmax of 0.71 d^{-1} (figure 1) which is higher than the maximum measured growth rate (0.63 d^{-1}, table 1).

- *Alexandrium* nutrient uptake

Nutrient uptake is linked to external nutrient concentration, following the Michaelis-Menten relation :

$$V_P = V_{Pmax} \times \frac{PO_4}{(PO_4 + K_P)}$$

K_P is the half saturation constant. It has been calibrated with the semi-continuous culture – Labry et al., 2004 ; table 2). K_P = 0.25 mmol.m^{-3} in the model.

V_P and V_{Pmax} are expressed in pg.cell^{-1}.d^{-1} and correspond respectively to the phosphorus uptake rate and to the maximum uptake rate. V_{Pmax} is considered as constant in Droop formulation.

As Labry et al. (2004) experiments showed that the maximum nutrient assimilation rate is not constant but linked to the internal nutrient quota, the model chosen is Droop-modified (mechanistic model).

Uptake is maximal when the quota is low and then decreases. This type of feedback mechanism is a common feature among phytoplankton species and has already been described and modelled by linear relations (Davidson and Gurney, 1999 ; Ducobu et al., 1998 ; Riegman et al., 2000 ; Roelke et al. 1999 ; Thingstad, 1987), or non linear (Geider et al. 1998 ; Morel, 1987).

So, based on the semi-continuous experiments dataset, we have adjusted PO_4 assimilation rate as a power function against P quota (less-squared method, figure 2) :

$$V_{Pmax} = Min\left(12.286 \times (Q_P - Q_{Pmin})^{-0.3136}; 50\right)$$

A maximum of 50 pg.cell^{-1}.d^{-1} has been fixed in order to avoid very high values when the model runs for quota close to minimum quota (the maximum measured uptake is 39.3 pg.cell^{-1}.d^{-1}).

- *Heterocapsa* model

Heterocapsa growth and uptake model is like *Alexandrium* model a Droop-modified model, see table 2. In fact, Labry et al., 2004 experiments have shown that phosphorus uptake is linked to internal P quota (figure 3).

$$V_{PmaxHt} = Min\left(8.7867 \times (Q_{PHt} - Q_{PminHt})^{-0.3752}; 25\right)$$

The maximal growth rate, μ_{maxHt}, is adjusted from Droop model (r=0,89) and reaches 1.25 d^{-1}, which is higher than the one for *Alexandrium*.

1.3 Running the model

The numeric model has been written with the Stella 9.1 modelling software system. It is a OD model built to reproduce *Alexandrium* growth in different phosphorus environment.

For all simulations we used the Runge-Kutta 4 method with a time step of 0.01 day. The simulation time corresponds to the experiment time (around 15 – 20 days).

To quantify the agreement between model results and data, we have calculated an agreement index I for each variable when data are present : $I=\frac{1}{n}\sqrt{\sum_{i=1}^{n}\left(\frac{simu_i-data_i}{data_i}\right)^2}$. *simui*, *datai* are respectively the value obtained by the model or the dataset and n is the number of data. This index will also allow us to compare different modelling options.

A sensitivity analysis has been performed on *Alexandrium/Heterocapsa* competition, in order to see how the issue of the competition is linked to the *Alexandrium* parameters and formulations employed in the model. The model has been run with each parameter (K_P, μ_{max}, Q_{Pmin} and the formulae V_{Pmax}) varying from 10% of the standard simulation.

2. Results

2.1 Validation of the phosphorus model

2.1.1. Validation on batch experiments (Labry et al., 2008)
The response of *Alexandrium* to different PO_4 pulses is very well simulated, showing an increase of the cell abundance due to growth, delayed 2 - 3 days after the pulse (figure 4). This time lag is due to phosphorus repletion of the internal P quota.
We can notice that 4 µM pulse of PO_4 is not sufficient to have a full repletion of algal cells. Levels of *Alexandrium* at the end of the experiments is almost the same for 1, 2, 3 and 5 days pulses but lower for 7 and 10 days. This is probably because the experiment has stopped before *Alexandrium* reached the stationary phases. In the model, if we continue the simulation after 15 days, the same biomass value is obtained for all experiments.

Simulation of PO_4 is less successful as the model is not able to reproduce the very quick uptake of phosphorus after a long phosphorus depletion (days 3, 5, 7 and 10).

2.1.2 Validation on batch experiments (Erard–Le Denn et al. 2003)
Modelling *Alexandrium* and the phosphate concentrations is quite good (figure 5). We can observe that in the first part of the experiment the uptake and growth are balanced so phosphorus quota is stable. Then, when phosphate disappears in the culture, quota decreases first, and almost 3 days after *Alexandrium* growth decreases. For the f/2/10 medium, the less limited medium, the final *Alexandrium* abundance is slightly overestimates.

2.1.3 Validation on semi-continuous experiments (Labry et al. 2004 ; 2008)
For each dilution rate *Alexandrium* reaches, after 10 days, an equilibrium which is presented in figure 6. The model reproduces fairly well the equilibrium abundance from the low dilution

rate (0.05 d^{-1}) to the maximum one (0.5 d^{-1}). For low dilution rate, *Alexandrium* abundance is maximum and quota minimum, and on the contrary, for high dilution rate, abundance is low, but cellular content is high. For intermediate dilution rates, the simulation slightly overestimate the equilibrium biomass of *Alexandrium*.

The model, applied to semi-continuous experiment with 0.15 d^{-1} dilution rate and different PO_4 supply frequencies, reproduces also successfully the evolution of *Alexandrium* biomass, phosphorus quota and phosphorus figure 7. For 1 and 2 day intervals, cell concentrations increased exponentially as in batch culture mode if we ignored the signal due to the water removal each day. For the 4 and 6 day intervals we can observe biomass increase after the PO_4 pulse then a decrease until the next pulse.

2.2 Application of the *Alexandrium/ Heterocapsa* competition

The *Alexandrium* model, as validated previously is now used to test its capability to reproduce the issue of competition between *Alexandrium* and *Heterocapsa.*

Figure 8 shows the issue of batch competition beween *Alexandrium* and *Heterocapsa*. The model is able to simulate the *Alexandrium* greater biomass as soon as the PO_4 pulse is delayed to 3 days as shown by data. With a pulse after 2 days, *Alexandrium* reaches at the end of the experiment and in the simulation as well the same biomass as *Heterocapsa*.

The *Alexandrium* model fits the data very well whereas *Heterocapsa* model overestimates *Heterocapsa* biomass under high P deficiency (pulse happening day 5 to day 10).
PO_4 is also fairly well simulated but, as in *Alexandrium* monoculture experiments (2.1.1), the model shows a too slow uptake for the most delayed pulses.

The same model is apply to the semi-continuous competition experiment (figure 9). In these conditions, *Alexandrium* always outgrew *Heterocapsa* and this outcome occurred earlier when the PO_4 supply interval was sjorter. Here again the model is fitting data very well concerning *Alexandrium* and the issue of the competition. Like previously, *Heterocapsa* biomass is over-estimated.

The sensitivity analysis has been performed on the batch culture competition. The result of the sensitivity analysis, table 3, shows that the model is not sensitive to the half- saturation constant K_P. The other parameters (μ_{max}, V_P and Q_{Pmin}) variations do not modify the schema of the competition (*Alexandrium* dominant in the case of high phosphorus depletion, *Heterocapsa* dominant in low phosphorus depletion) but may have some impact.
Lowering *Alexandrium* maximum growth rate makes it less competitive and *Alexandrium* overpasses *Heterocapsa* only when pulse is delayed to the day 5. On the contrary, increasing μ_{max}, makes *Alexandrium* more competitive, sooner.
It is the same with the phosphorus minimum quota, lowering Q_{Pmin} makes *Alexandrium* always competitive on *Heterocapsa* and increasing it makes *Heterocapsa* dominant until day 5 pulse.
If the function of phosphorus maximum uptake rate is decreased; then *Heterocapsa* becomes more dominant, and in V_{Pmax} is increased, *Alexandrium* has more advantage on the competition.
The modification of the competition in the case of the day 10 pulse is not commented as the *Heterocapsa* model is good simulated for this condition.

3. Discussion

Representativeness and accuracy of the phosphorus growth model of *Alexandrium*

The model described here fits a various data sets : batch, semi-continuous, Plimited, P pulsed... Such diverse transitory situations are a good indication of its robustness. But we may still wonder whether the choice of such a mechanistic model is appropriate and whether a simpler model would not be more suitable to simulate *Alexandrium.*.

Mechanistic (Droop modified) versus Monod

Using a Monod-model with the same parameters as previously for the calibration pulse experiments (2.1.1) gives exactly the same feature for each experiment (1d pulse, 2 d pulse…) with the same maximum biomass obtained after the pulse of 4 µmol.l^{-1} PO_4 (figure 10). The maximum value obtained is directly linked to the ratio P/cell used in the model (here calibrated at 6.99 pgP.cell^{-1} which is nearly the minimum P quota). The PO_4 uptake is also simulated with the same feature but the Monod model is not able to simulate a quicker uptake when depletion is stronger. The mechanistic model is thus more appropriate even though it fails to simulate this quick uptake. The index calculated for *Alexandrium* and Phosphate displays a better agreement for the mechanistic model (table 4). This result confirms the conclusion drawing by Flynn (2003) that the Monod-type model is not realistic enough to simulate detailed laboratory data series.

Furthermore, if we try to simulate the *Alexandrium/Heterocapsa* competition with Monod-like models for *Alexandrium* and *Heterocapsa*, the model is not able to reproduce the shift in the issue of the competition. *Heterocapsa* always wins the competition, because of its higher growth rate (figure 11). Thus, it is necessary to take into account intracellular pools of nutrients so as to reproduce the issue of competition in a non steady-state environment (see Ducobu et al., 1998). In this case the competition is based on two different ecological strategies, a storage strategy for *Alexandrium* versus a growth strategy for *Heterocapsa*.

Mechanistic (Droop modified) versus Droop.

Nutrient uptake within Droop model has been modified as laboratory experiments have proved that maximum uptake is correlated to quota. This model is compared with a model of V_{Pmax} constant (non dependent on quota) corresponding to the mean V_{Pmax} measured (V_{Pmax} = 8.3 pg.cell^{-1}.d^{-1}, Labry et al., 2008). We have simulated the same experience as in 2.1 (*Alexandrium* growth in batch culture with controlled P pulse).

Simulations seem to be very close (figure 11) and the agreement index gives the same value for *Alexandrium* biomass and a slightly worse agreement for PO_4. Thus the improvement does not seem to be consistent enough. But if we model the *Alexandrium/Heterocapsa* competition (with V_{Pmax}=9.47 pg.cell^{-1}.d^{-1} for *Heterocapsa*) the Droop model can not reproduce the shift between *Heterocapsa* firstly dominant to *Alexandrium* due to high P depletion. As in the Monod model, *Heterocapsa* is always dominant because of its higher growth rate. In conclusion the mechanistic model with V_{Pmax} modified by feedback with the internal quota is necessary to model the issue of competition in a non steady-state environment. This agrees the Flynn (2003) conclusion regarding competitive advantage that it is more a function of uptake kinetics rather than internal resource utilisation kinetics.

Versus a new version of PO_4 uptake

If we look back at experiment 2.1.1, the uptake of PO_4 is not quick enough in the event of low P quota. The P pulse disappears in less than 1 day in the case of 3, 5, 7 and 10 day pulses but in the model it still takes between 4 and 5 days. By calculating the PO_4 uptake from PO_4 disappearance in these experiments we obtain values from 20 $pg.cell^{-1}.d^{-1}$ for day 1 pulse to 67 $pg.cell^{-1}.d^{-1}$ for day 3 pulse. Values calculated for a greater pulse are under-estimated as PO_4 measurement was not made immediately after the pulse but one day after and there might be uptake of the whole 4 $mmol.m^{-3}$ pulse in less than one day. Such values are far higher than those calculated from adjustment in the semi-continuous experiment. Another uptake process is tested in order to allow a greater uptake for low P quota. The function V_{Pmax} is adjusted with the V_{Pmax} deduced from the PO_4 uptake in the pulse experiment. For that we can use only the 1d, 2d, and 3d pulse measurements. Based on these 3 points we obtain :

$$V_{P\max} = \left(188.66 \times \left(Q_P - Q_{P\min}\right)^{-1.0729}\right)$$

Figure 13 shows a better agreement for the PO_4 simulation with an uptake of the whole PO_4 pulse in 4 days in the case of low P depletion (1d pulse) and in 2 days in the case of high P depletion (10 d pulse). We note that even with V_{Pmax} adjusted in this experiment, the model is not able to simulate the very fast uptake (less than one day. In this experiment, PO_4 uptake was estimated by the difference over time between PO_4 concentrations of the culture medium. Since cultures were non axenic, the disappearance of PO_4 may also be due to bacterial uptake leading to an overestimation of PO_4 uptake due to algal species. By contrast, for the semi-continuous experiments used to calibrate PO_4 uptake (1.2), uptake was estimated by the ^{33}P incorporation technique ended by a filtration onto 8 µm filter, thus focusing on algal species.

If we apply this new formulation of PO_4 uptake in the competition experiment, *Alexandrium* is always dominant, thus this formula is not adequate to simulate the competition issue.

Conclusion

In conclusion to this calibration exercise, a better adjustment was carried out with independent and consequent measurements. Its robustness was tested by applying it to various experiments. It is recommended to keep this model for later *Alexandrium* modelling exercise, for exemple the inclusion in an environmental model (with light, temperature, nutrient, and physical circulation). But, as the conclusion of the competition experiment shows that only the mechanistic model can reproduce the issue, in a more general ecosystem, the competition concerns *Alexandrium* and various phytoplankton species (diatoms, dinoflagellates). The same complexity for all phytoplankton groups appeared to be essential. This means a large and complex model with a huge amount of unknown data. Thus, the *Alexandrium* blooms should be simulated without any feedback on the ecosystem. Modelling should also evaluate the relevance of the large other factors such as physical control (dilution, stratification, …), hydrological factors (temperature, light, nitrogen…) and plankton community (competition with other phytoplankton, grazing, parasitism…)

Acknowledgements
This research was supported by Interreg IIB NWE project called Final

Litterature

Aminot, A., Kérouel, R., 2007. Dosage automatique des nutriments dans les eaux marines: méthodes en flux continu. Ed. Ifremer, Méthodes d'analyse en milieu marin. 188 p.

Anderson, D.A., Stock, A.C., Keafer, B.A., Nelson, A.B., Thompson, B., McGillicuddy, D.J., Keller, M., Matrai, P.A., Martin, J., 2005. *Alexandrium fundyense* cyst dynamics in the Gulf of Maine. Deep-Sea Research II. 52, 2522-2542.

Andrieux-Loyer, F., Philippon, X., Bally, G., Kérouel, R., Youenou, A., Le Grand, J., 2008. Phosphorus dynamics and bioavaibility in sediments of the Penzé estuary (NW France) in relation to annual P-fluxes and occurrences of *Alexandrium minutum*. Biogeochemistry. 88, 213-231.

Basterretxea G., Garces E., Jordi A., Angles S., Maso, M. 2007. Modulation of nearshore harmful algal blooms by *in situ* growth rate and water renewal. Mar. ecol. Prog. Ser. 353, 53–65.

Belin, C., 1993. Distribution of *Dinophysis* spp. and *Alexandrium minutum* along French coasts since 1984 and their DSP and PSP toxicity levels, in: Smayda, T.J., Shimiziu, Y. (Eds), Toxic Phytoplankton Blooms in the Sea. Elsevier, Amsterdam, pp. 469-474.

Cembella, A.D., 1998. Ecophysiology and metabolism of paralytic shellfish toxins in marine microalgae, in: Anderson, D.M., Cembella, A.D., Hallegraeff, G.M. (Eds), Physiological ecology of Harmful Algal Blooms. Springer-Verlag, Heidelberg, pp. 381-403.

Chapelle A., Andrieux-Loyer F., Fauchot, J., Guillaud, J.F., Labry, C., Sourisseau, M., Verney, R., 2008. Understanding, predicting and tackling algal blooms. “What is the current situation concerning *Alexandrium minutum* in the Penzé estuary?”. Rapport Ifremer. 23 p.

Davidson, K., Gurney, W.S.C., 1999. An investigation of non steady state algal growth. II. Mathematical modelling of co-nutrient limited algal growth. J. Plankton Res. 21, 839-858.

Droop, M.R., 1973. Some thoughts on nutrient limitation in algae. J. Phycol. 9, 264-272.

Ducobu, H., Huisman, J., Jonker, R.R., Mur, L.R., 1998. Competition between a prochlorophyte and a cyanobacterium under various phosphorus regimes : comparison with the Droop model. J. Phycol. 34, 467-476.

Erard-Le Denn, E., 1997. *Alexandrium minutum*. Efflorescences toxiques des eaux côtières françaises, in : Berland, B., Lassus, P. (Eds.), Ecologie, écophysiologie, toxicologie. Repères Océan. 13, 53-66.

Erard-Le Denn, E., Crassous, M. P., Youenou, A., Martin-Jézequel, V., Harang, B., Mézière, V., 2003. Etude de la compétition entre espèces lors du développement d'une efflorescence toxique à *Alexandrium minutum*. Rapport Ifremer PNEC–ART3. 25 p.

Fauchot, J., Saucier, F.J., Levasseur, M., Roy, S., Zakardjian, B., 2008. Wind-driven river plume dynamics and toxic *Alexandrium tamarense* blooms in the St Lawrence estuary (Canada): a modeling study. Harmful algae. 7, 214-227.

Flynn, K.J., 2001.A mechanistic model for describing dynamic multi-nutrient, light, temperature interactions in phytoplankton. J. Plankton Res. 23, 977-997.

Flynn, K.J., 2003. Modelling multi-nutrient interactions in phytoplankton, balancing simplicity and realism. Prog. Oceanogr. 56, 249-279.

Geider, R.J., Mac Intyre, H.L., Kana, T.M., 1998. A dynamic regulatory model of phytoplanktonic acclimation to light, nutrients, and temperature. Limnol. Oceanogr. 43, 679-694.

Guillard, R.R.L., Ryther, J.H., 1962. Studies on marine plankton diatoms. I *Cyclotella nana* (Husted) and *Detonela confervacea* (Cleve). Gran. Can. J. Microbiol. 8, 229-239.

Haney, J.D., Jackson, G.A., 1996. Modelling phytoplankton growth rates. J. Plankton Res. 18, 63-85.

John, E.H., Flynn, K.J., 2000. Modelling phosphate transport and assimilation in microalgae: how much complexity is warranted? Ecol. Model. 125, 145-157.

Labry, C., Erard-Le Denn, E., Chapelle, A., Youenou, A., Crassous, M.P., Martin-Jézequel, V., Le Grand, J., 2004. Etude écophysiologique et paramétrisation du rôle du phosphore sur la croissance d'*Alexandrium minutum*, espèce responsable d'eaux colorées toxiques en estuaire de Penzé, et de son principal compétiteur *Heterocapsa triquetra*. Rapport Ifremer PNEC–ART3. 32 p.

Labry, C., Erard-Le Denn, E., Chapelle, A., Fauchot, J., Youenou, A., Crassous, M.P., Le Grand, J., Lorgeoux, B., 2008. Competition for phosphorus between two dinoflagellates: A toxic *Alexandrium minutum* and a non-toxic *Heterocapsa triquetra*. J. Exp. Mar. Biol. Ecol. 358 (2), 124-135.

Maguer, J.F., Wafar, M., Madec, C., Morin, P., Erard-Le Denn, E., 2004. Nitrogen and phosphorus requirements of an *Alexandrium minutum* bloom in the Penze Estuary, France. Limnol. Oceanogr. 49, 1108-1114.

Maguer, J.F., Madec, C., Caradec, J., Mage C. P., 2005. Mesures des constantes cinétiques d'adsorption des différentes formes de l'azote chez *Alexandrium minutum* par l'utilisation des isotopes 15N. Convention Ifremer 2004 2 24 31 405, 21p.

Monod, J., 1942. Recherches sur les croissances des cellules bactériennes. Hermann, Paris, 211 p.

Morel, F.M.N., 1987. Kinetics of nutrient uptake and growth in phytoplankton. J. Phycol. 23, 137-150.

Morin, P. Erard-Le Denn, E., Maguer, J.F., Madec C., Videau, C., Le Grand, J., Mace, E., 2000. Etude des causes de proliférations de microalgues toxiques en mer : cas d'*Alexandrium*. Rapport Université Bretagne Occidentale, Ifremer Brest. 135 p.

Probert, I. P., 1999. Sexual reproduction and ecophysiology of the marine dinoflagellate *Alexandrium minutum* Halim. Ph D thesis, University of Westminster, London**:** 99p.

Riegman, R., Stolte, W., Noordeloos, A.A.M., Slezak, D., 2000. Nutrient uptake and alkaline phosphatase (EC 3:1:3:1) activity of *Emiliana huxleyi* (Prymnesiophyceae) during growth under N and P limitation in continuous cultures. J. Phycol. 36, 87-96.

Roelke D.L., Eldridge P.M., Cifuentes L.A. 1999. Estuaries 22, 92-104.

Solorzano, L., Sharp, J.H., 1980. Determination of total dissolved phosphorus and particulate phosphorus in natural waters. Limnol. Oceanogr. 25, 754-758.

Stock, C.A., McGillicuddy, D.J., Anderson, A.M., Solow, A.R., Signell, R.P., 2007. Blooms of the toxic dinoflagellate *Alexandrium fundyense* in the western Gulf of Maine in 1993 and 1994 : a comparative modelling study. Cont. Shelf Res. 27, 2486-2512.

Thingstad T.F. 1987. Utilization of P, N, and organic C by heterotrophic bacteria. I. Outline of a chemostat theory with a consistent concept of 'maintenance' metabolism. Mar. Ecol. Prog. Ser. 35, 99-109

Yamamoto, T., Seike, T., 2003. Modelling the population dynamics of the toxic dinoflagellate *Alexandrium* in Hiroshima Bay, Japan. II. Sensivity to physical and biological parameters. J. Plankton Res. 25, 63-81.

Yamamoto, T., Seike, T., Hashimoto, T., Tarutani, K., 2002. Modelling the population dynamics of the toxic dinoflagellate *Alexandrium tamarense* in Hiroshima Bay (Japan). J. Plankton Res. 24 (1), 33-47.

Yamamoto, T., Tarutani K., 1996. Growth and phosphate uptake kinetics of *Alexandrium tamarense* from Mikawa Bay, Japan, in : Yasumoto, T., Oshima, Y., Fukuyo Y. (Eds), Harmful and Toxic Algal blooms, Intergovernmental Oceanographic Commission of Unesco, pp. 293-296.

Figure captions

Figure 1 : Calibration of *Alexandrium* maximal growth rate following Droop formulae. Data are provided from semi-continuous experiments at the equilibrium (Labry et al., 2004)

Figure 2 : Adjustment of maximal phosphorus uptake (V_{Pmax}). against internal quota. Data from Labry et al., 2008

Figure 3 : : Phosphorus model for *Heterocapsa.* Data from Labry et al. 2008.
a : growth rate calibration
b : P uptake calibration

Figure 4 : Simulation of *Alexandrium* growth in PO_4 depleted batch with various P pulse.
— simulation, ◆ *Alexandrium* data, ✕ PO_4 data. (Data from Labry et al., 2008)

Figure 5 Simulation of *Alexandrium* growth in PO_4 depleted batch.
— simulation, ◆ *Alexandrium* data, ✕ PO_4 data. (Data from Labry et al., 2008)

Figure 6 :: Simulation of *Alexandrium* growth in semi-continuous experiments with various dilution rate.

◆— simulation,  *Alexandrium* data with error bars illustrating the standard deviation. (Data from Labry et al., 2008)

Figure 7 : Simulation of *Alexandrium* growth in semi-continuous experiments with four frequencies of PO_4 supply.
— simulation ; ◆ Alexandrium data ; ▲ Phosphorus quota data

Figure 8 : Simulation of *Alexandrium/Heterocapsa* competition (line) and data (dots) under different phosphorus pulse events (after 1, 2, 3, 5, 7 10 days) or under P depleted culture

Figure 9 : *Alexandrium/Heterocapsa* competition (line) and data (dots) with semicontinuous experiments.

Figure 10 : Simulation of *Alexandrium* and PO_4 Monod model

Figure 11 : Competition modelled with the Monod model

Figure 12 : Simulation of the *Alexandrium/Heterocapsa* competition with Droop model (V_{Pmax} constant)

Figure 13 : New simulation with V_{Pmax} calibrated from this experiment
— simulation, ◆ *Alexandrium* data, × PO_4 data. (Data from Labry et al., 2008)

Table 1 : *Alexandrium* parameters measured from laboratory experiments

Paramterss	Value	Units
Maximum growth rate at 20°C	0.63	d^{-1}
Maximal phosphorus cell quota	48	pg $cell^{-1}$
Minimum phosphorus cell quota	6.5	pg $cell^{-1}$
Half saturation constant for PO_4 assimilation	0.25 – 1.62	µmol l^{-1}
Max PO_4 uptake rate	39.3	pg.$cell^{-1}$.d^{-1}

Table 2 : Parameters for *Heterocapsa* phoshorus model

Parameters	Value	Units
Maximum growth rate at 20°C	0,89	d^{-1}
Maximal phosphorus cell quota	23	pg $cell^{-1}$
Minimum phosphorus cell quota	6.2	pg $cell^{-1}$
Half saturation constant for PO_4 assimilation	0.97 – 2.23	µmol l^{-1}
Max PO_4 uptake rate	22.8	pg.$cell^{-1}$.d^{-1}

Table 3 : Results of the sensitivity analysis of the *Alexandrium/Heterocapsa* competition

	std	K_P^-	K_P^+	μ_{max}^-	μ_{max}^+	Q_{Pmin}^-	Q_{Pmin}^+	V_{Pmax}^-	V_{Pmax}^+
day 1	Ht	Ht	Ht	Ht	Am=Ht	Am	Ht	Ht	Am=Ht
day 2	Am=Ht	Am=Ht	Am=Ht	Ht	Am	Am	Ht	Ht	Am
day 3	Am	Am	Am	Am=Ht	Am	Am	Ht	Am=Ht	Am
day 5	Am	Am	Am	Am	Am	Am	Am	Am	Am
day 7	Am	Am	Am	Am	Am	Am	Am	Am	Am
day 10	Am	Am	Am	Am=Ht	Am	Am	Ht	Am=Ht	Am

Table 4 ; Agreement index for simulations with P uptake adjusted with P quota, Puptake constant (Droop), or without quota (Monod)

	Alex			PO4		
	Vp ajusté	vpconstant	monod	Vp ajusté	vpconstant	Monod
J1	7	6	21	162	30	27
J2	13	14	19	199	35	37
J3	4	5	12	463	416	535
J5	7	7	7	1123	1090	1345
J7	12	13	16	310	732	1368
J10	12	12	10	423	419	526
Carency	13	13	16	35	35	29
MOYENNE	**10**	**10**	**14**	**388**	**394**	**552**

Table 5 : Agreement index for simulations with NO3 and NH4 uptake adjusted with N quota or NO3 and NH4 uptake constant

		VN constant			VN ajusté	
Agreement Index (%)	Alex	NO3	QN	Alex	NO3	QN
Probert B	55	29	30	51	28	15
ProbertC	18	38	10	15	38	9

Figure 1

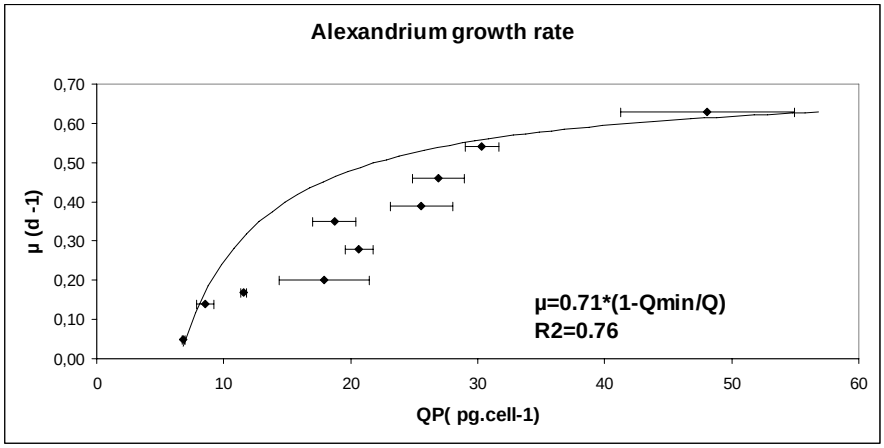

Alexandrium growth rate
0,70
0,60
0,50
0,40
0,30
0,20
0,10
0,00
μ (d -1)
0
10
20
30
40
50
60
QP(pg.cell-1)
μ=0.71*(1-Qmin/Q)
R2=0.76

Figure 2

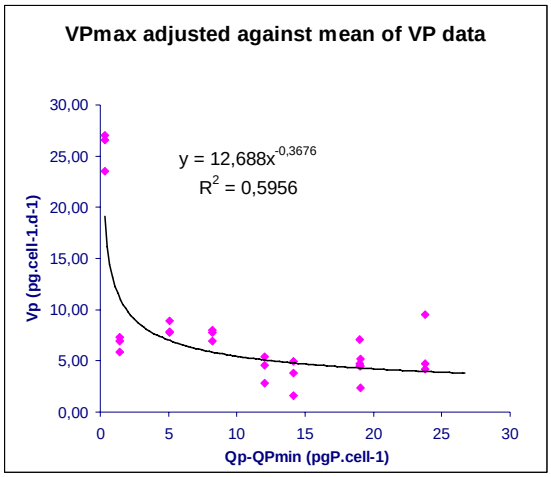
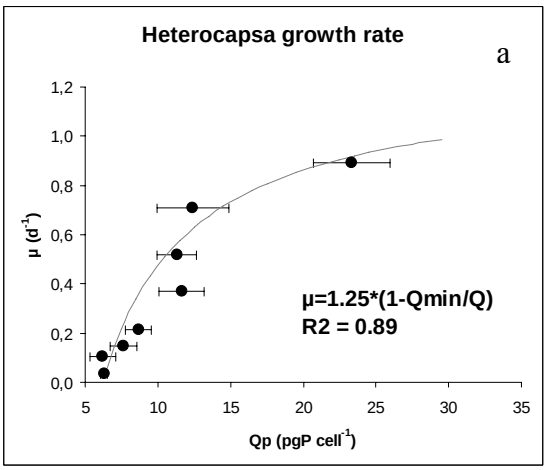

VPmax adjusted against mean of VP data
y = 12,688x-0,3676
R2 = 0,5956
Vp (pg.cell-1.d-1)
Qp-QPmin (pgP.cell-1)
30,00
25,00
20,00
15,00
10,00
5,00
0,00
0
5
10
15
20
25
30

Figure 3:.


Heterocapsa growth rate
a
μ (d-1)
Qp (pgP cell-1)
μ=1.25*(1-Qmin/Q)
R2 = 0.89

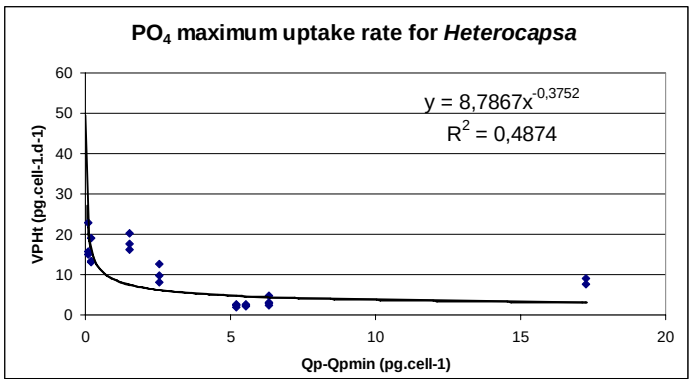

PO4 maximum uptake rate for Heterocapsa
VPHt (pg.cell-1.d-1)
Qp-Qpmin (pg.cell-1)
y = 8,7867x-0,3752
R2 = 0,4874

Figure 4 :

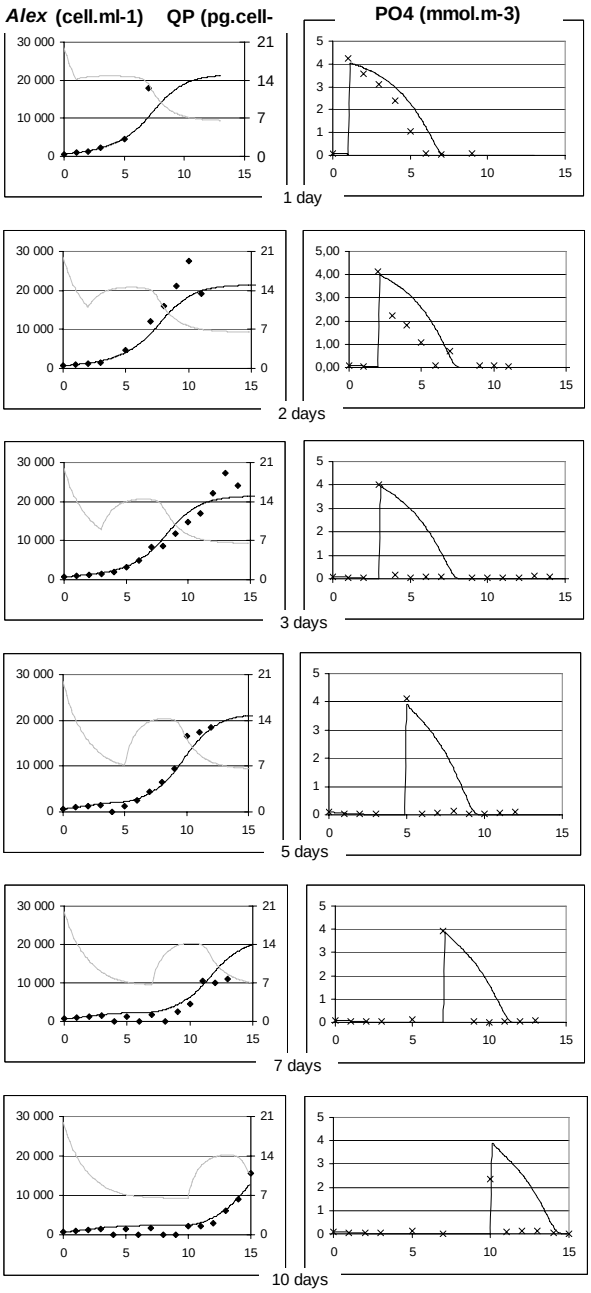

Alex (cell.ml-1)
QP (pg.cell-
PO4 (mmol.m-3)
1 day
2 days
3 days
5 days
7 days
10 days

Figure 5

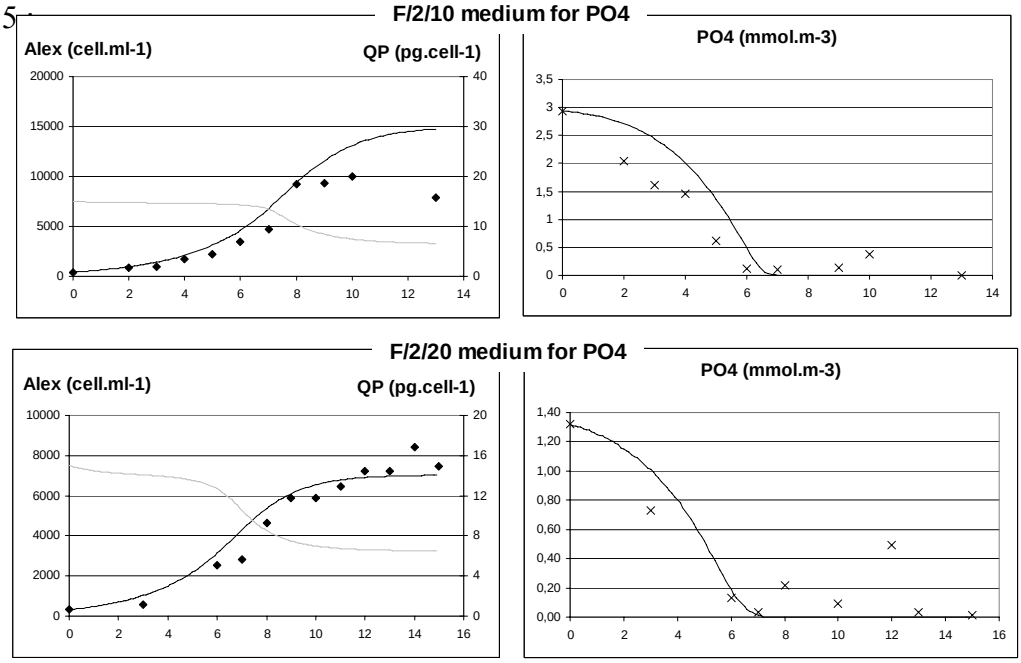

F/2/10 medium for PO4
Alex (cell.ml-1)
QP (pg.cell-1)
PO4 (mmol.m-3)
F/2/20 medium for PO4
Alex (cell.ml-1)
QP (pg.cell-1)
PO4 (mmol.m-3)

Figure 6 :

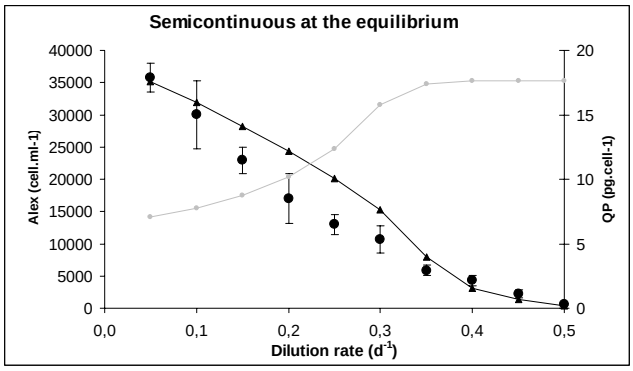

Semicontinuous at the equilibrium
Alex (cell.ml-1)
QP (pg.cell-1)
Dilution rate (d-1)
40000
35000
30000
25000
20000
15000
10000
5000
0
20
15
10
5
0
0,0
0,1
0,2
0,3
0,4
0,5

Figure 7 :

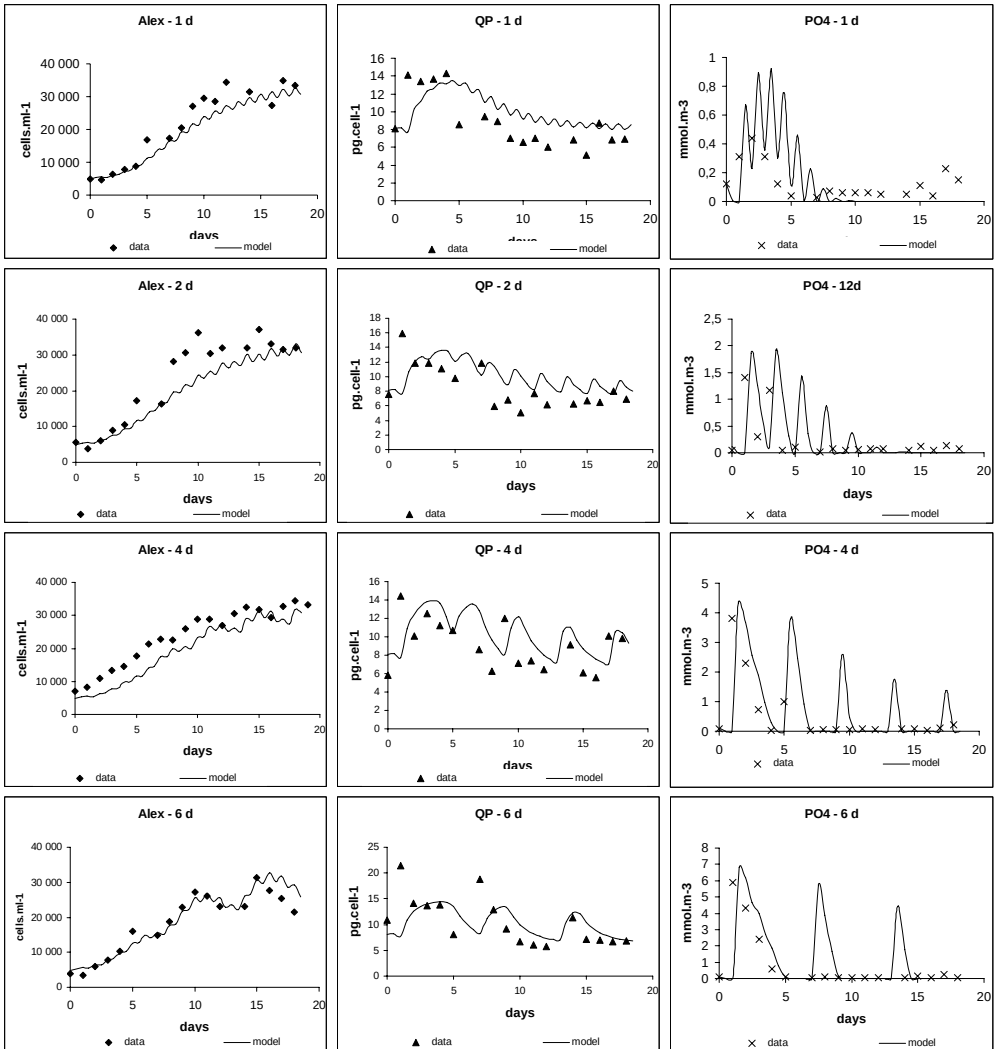

Alex - 1 d
cells.ml-1
days
data
model
QP - 1 d
pg.cell-1
days
data
model
PO4 - 1 d
mmol.m-3
data
model
Alex - 2 d
cells.ml-1
days
data
model
QP - 2 d
pg.cell-1
days
data
model
PO4 - 12d
mmol.m-3
days
data
model
Alex - 4 d
cells.ml-1
days
data
model
QP - 4 d
pg.cell-1
days
data
model
PO4 - 4 d
mmol.m-3
data
model
Alex - 6 d
cells.ml-1
days
data
model
QP - 6 d
pg.cell-1
days
data
model
PO4 - 6 d
mmol.m-3
days
data
model

Figure 8 :

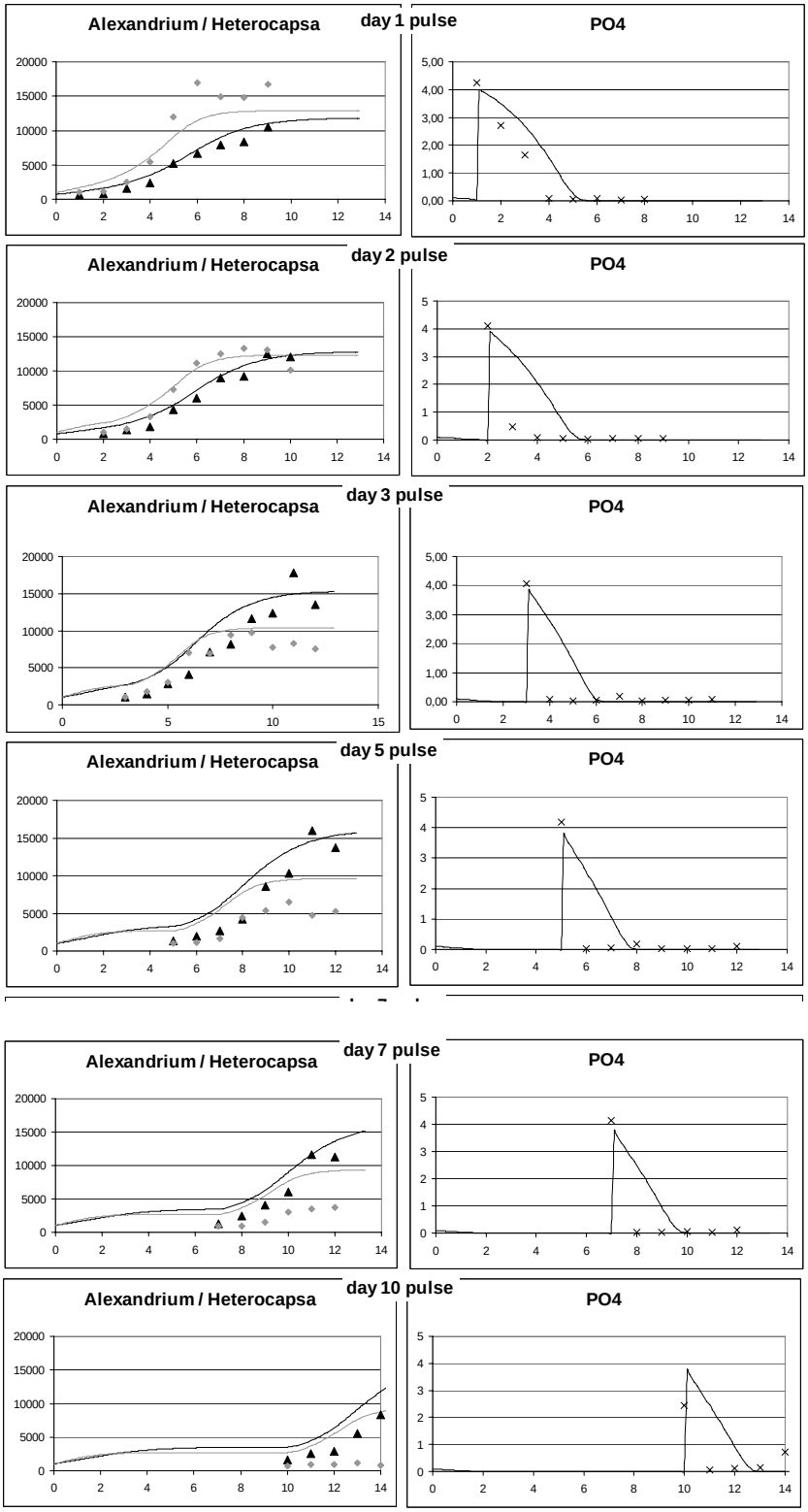

Alexandrium / Heterocapsa
day 1 pulse
PO4
Alexandrium / Heterocapsa
day 2 pulse
PO4
Alexandrium / Heterocapsa
day 3 pulse
PO4
Alexandrium / Heterocapsa
day 5 pulse
PO4
Alexandrium / Heterocapsa
day 7 pulse
PO4
Alexandrium / Heterocapsa
day 10 pulse
PO4

Figure 9

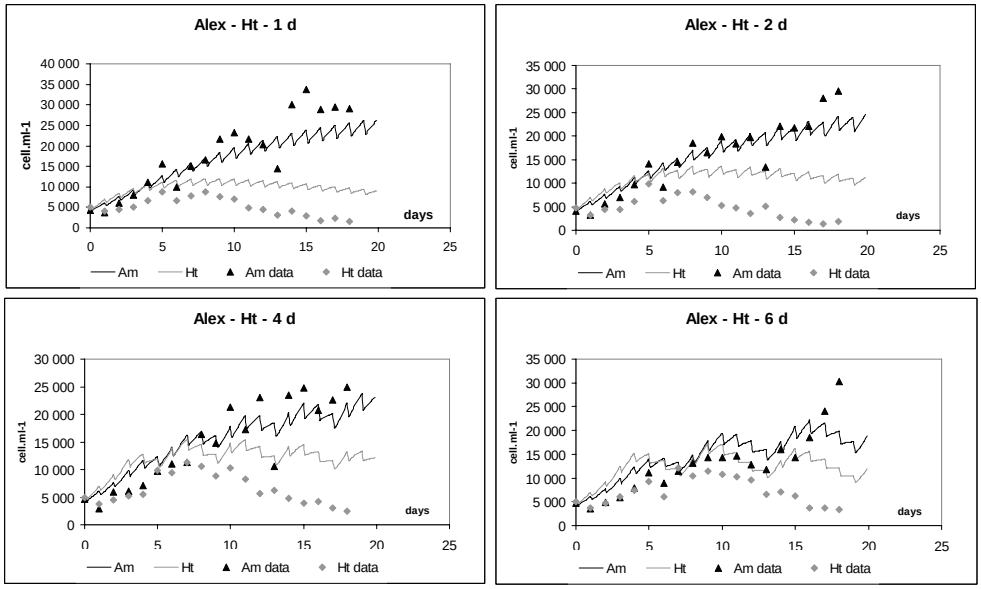
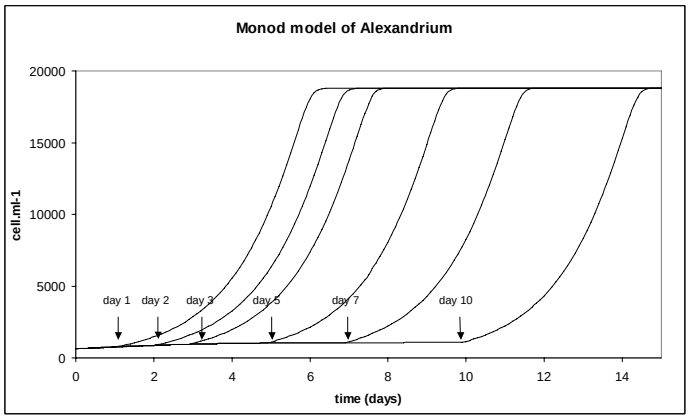

Alex - Ht - 1 d
Alex - Ht - 2 d
Alex - Ht - 4 d
Alex - Ht - 6 d
cell.ml-1
days
Am
Ht
Am data
Ht data

Figure 10


Monod model of Alexandrium
cell.ml-1
20000
15000
10000
5000
0
day 1
day 2
day 3
day 5
day 7
day 10
0
2
4
6
8
10
12
14
time (days)

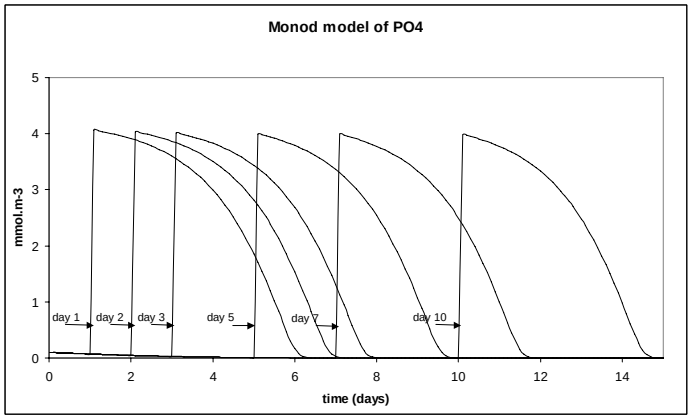

Monod model of PO4
mmol.m-3
5
4
3
2
1
0
day 1
day 2
day 3
day 5
day 7
day 10
0
2
4
6
8
10
12
14
time (days)

Figure 11 :

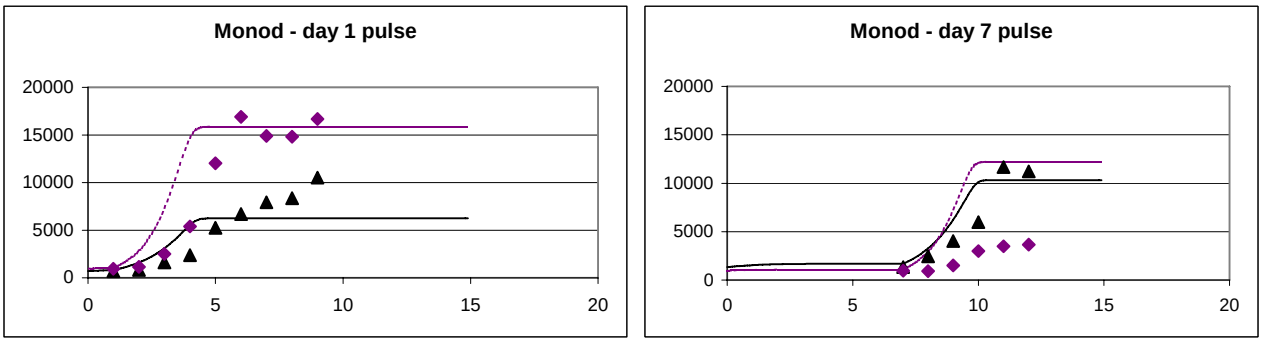

Monod - day 1 pulse
20000
15000
10000
5000
0
0
5
10
15
20
Monod - day 7 pulse
20000
15000
10000
5000
0
0
5
10
15
20

Figure 12

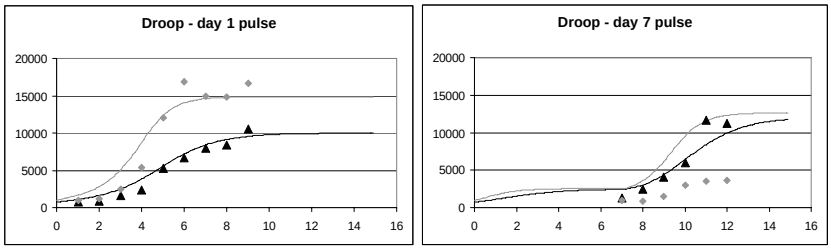
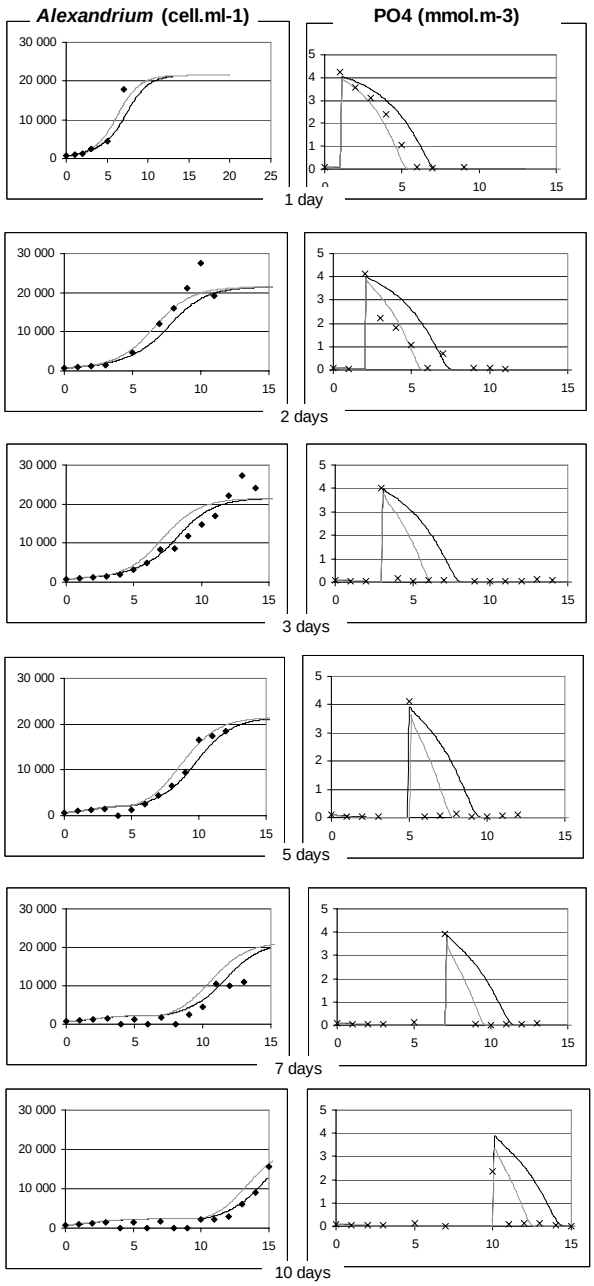

Droop - day 1 pulse
20000
15000
10000
5000
0
0 2 4 6 8 10 12 14 16
Droop - day 7 pulse
20000
15000
10000
5000
0
0 2 4 6 8 10 12 14 16

Figure 13


Alexandrium (cell.ml-1)
PO4 (mmol.m-3)
1 day
2 days
3 days
5 days
7 days
10 days