



ELSEVIER

Journal of Experimental Marine Biology and Ecology,
197 (1996) 263–278

JOURNAL OF
EXPERIMENTAL
MARINE BIOLOGY
AND ECOLOGY

The effects of a controlled fluctuating nutrient environment on continuous cultures of phytoplankton monitored by computers

Olivier Bernard*, Gilbert Malara, Antoine Sciandra

*Observatoire des Sciences de l'Univers, Station Zoologique, URA CNRS 716, Université Paris VI/INSU/
CNRS, B.P. 28, La Darse, 06230, Villefranche-sur-mer, France*

Received 5 June 1995; revised 15 September 1995; accepted 20 September 1995

Abstract

A completely automatic continuous phytoplankton culturing device is described. This computer-controlled system monitors simultaneously and automatically the basic culture parameters of several chemostats, i.e., limiting nutrient concentrations, cell size spectra, cell densities and temperatures. Culture pH can also be regulated. The dilution rate and the limiting nutrient concentration in the enrichment medium may be programmed to follow different dynamic patterns with time. An example is given with a sinusoidal variation of the dilution rate. Applications are discussed, which concern the advantages to modelling gained by acquiring information from continuously fluctuating systems rather than from nutrient-pulsed systems.

Keywords: *Dunaliella tertiolecta*; Automatic culture system; Chemostat; Dynamic nitrate input; Modelling

1. Introduction

The conception and the validation of phytoplankton growth models from experimental analysis are difficult because data can be biased for two main reasons. On the one hand, stress or uncontrolled artefacts (e.g., deprivation of CO₂, bacteria proliferation) can disturb the algal populations, which then behave erratically. On the other hand, the low sampling rates and the complexity of measurement for living systems that evolve during the sampling give rise to noisy data. These two factors often result in data inconsistent with modelling treatment.

*Corresponding author. Fax: (33) 9376-3834.

Consequently, stringent controls of culturing conditions and automation of measurements are necessary to reduce the variability of the observations. Progress in computer hardware and software has been the starting point for the development of new automatic culture systems, especially in continuous cultures (Miller, 1987; Malara and Sciandra, 1991). Automata have widely increased the knowledge of nitrogen limitation effects on algal physiology. Nevertheless, these systems are not able to reproduce dynamic patterns of variations in nutrients. When studied, nutrient perturbations are generally made at the steady state, and the observations focus on the return to the previous equilibrium. This approach naturally contrasts with oceanic situations where equilibrium is not the rule, due to the spatial and temporal variability of physical forcing. Unlike the effects of dynamic light regimes that have been widely studied with light/dark cycles (Eppley et al., 1971; Kroon et al., 1992), the effects of cyclic nitrogen fluctuations are poorly known.

Besides its capacity to maintain stable equilibria in several continuous cultures of phytoplankton, the automatic system presented here is also able to produce periodic dilution rates or enrichment levels, and, therefore, induce dynamic patterns of nutrient limitation. Such periodic signals can reflect some hydrodynamic processes responsible for oceanic nutrient fluctuations in stratified zones (Klein and Coste, 1984).

Our culturing device comprises 3 control levels. The first one regulates the stability of culture conditions (temperature, pH). The second is devoted to set periodic inputs (dilution rate, enrichment levels, light) and the third monitors the culture components (nutrients, biovolume, mean cell volume). The integrated system works autonomously, with variable control acquisition ranging from minutes to hours. Manual intervention is through interactive software.

2. Materials and methods

2.1. Culture system

The basic culture system has been described by Malara and Sciandra (1991). The chemostats consisted of eight 1.8 l double-jacketed vessels thermostated within 0.05°C (Fig. 1). The sea water, obtained at 3 miles from Villefranche Bay was filtered through 0.22 μm Sartorius filters and autoclaved for 1 h at 115°C. After cooling, sterile f/2 medium, with neither nitrate (NO_3^-) nor silicate was added. The medium was prepared before each experiment and stored in 80 l Nalgene tanks. A sterile concentrate of NO_3^- was also stored for the whole experiment, and mixed to enrichment medium before supplying the chemostat (see below). Magnetic stirrers ensured homogeneous media in the chemostats. Air, passed through a 0.1 μm Whatman filter and activated charcoal, was bubbled with a 0.15 l min^{-1} flow rate into each chemostat. The turbulent energy resulting from bubbling and stirring was sufficient to prevent cells sticking to the vessel walls, at least for 1 month, and to ensure the homogeneity of the culture. This was confirmed by comparing the cell counts from aliquots sampled at different depths in the cultures. The chemostat volumes were kept constant by continuously removing medium from the surface of the cultures. Data acquisition included pH (Ingold electrode) and

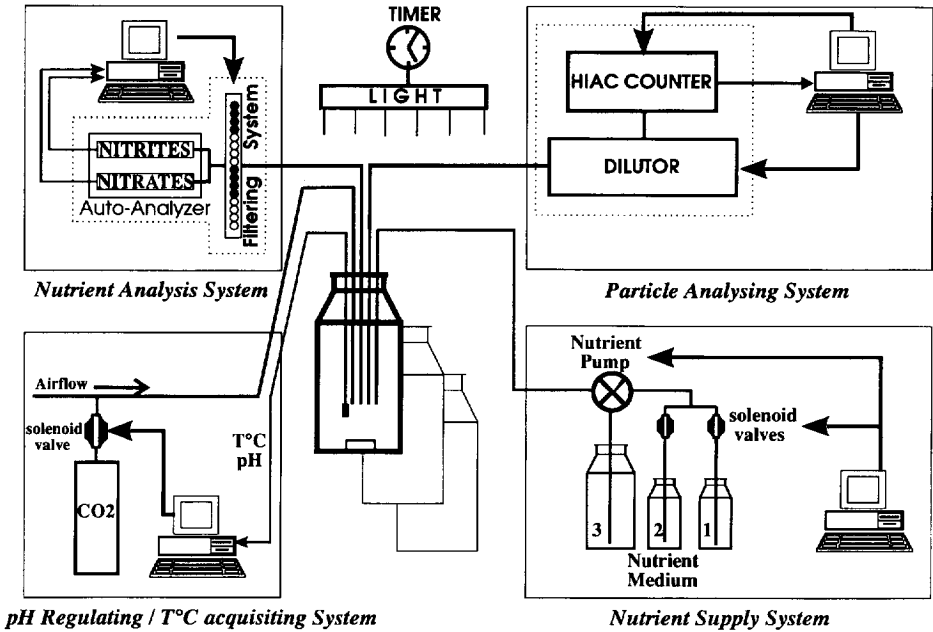


Fig. 1. Synoptic schema of the culturing system. The 3 tanks for the Nutrient Supply System are: 1, sterile *f/2* medium without nitrate; 2, sterile sea water with high nitrate concentration; 3, sterile sea water without nitrate.

semiconductor temperature sensor probes via a multiplexer and an analogue/digital card, whereby pH and temperature values were obtained every minute. If the pH increased above a programmed value, CO₂ was introduced through a solenoid valve. An example of pH regulation is shown in Fig. 2. The cultures were grown on a pre-programmed L:D cycle using a flood-light projector provided with two 150 W metal halide lamps (Osram, HQI) and one anti-UV glass-screen. The possible small remainder of UV energy was definitely cut off by the 1 cm thick Plexiglas covers of the growth chamber. The advantage of this type of lamp is that it provides a high power with a relatively cool light and a flat quantum spectrum (solar simulation, Fig. 3). The growth irradiances were measured with a calibrated 4 π quantum scalar irradiance meter (Biospherical Instruments, QSL 100) immersed into the centre of culture vessels (precision of 5%).

2.2. Nutrient analysis

To ensure the automation of a Technicon Auto-analyser (Tréguer and Le Corre, 1975), a set of pumps and electric valves are controlled by a PC 286 computer. For each chemostat, sampling, filtration through Gelman A/E filters and output signal acquisition from both colorimeters (nitrite (NO₂) and NO₃) are programmed in time with specified intervals. The range of concentrations measured can be easily changed between two sets: 0.01–2 $\mu\text{gat l}^{-1}$ and 1–100 $\mu\text{gat l}^{-1}$. The system standardises itself by calculating the

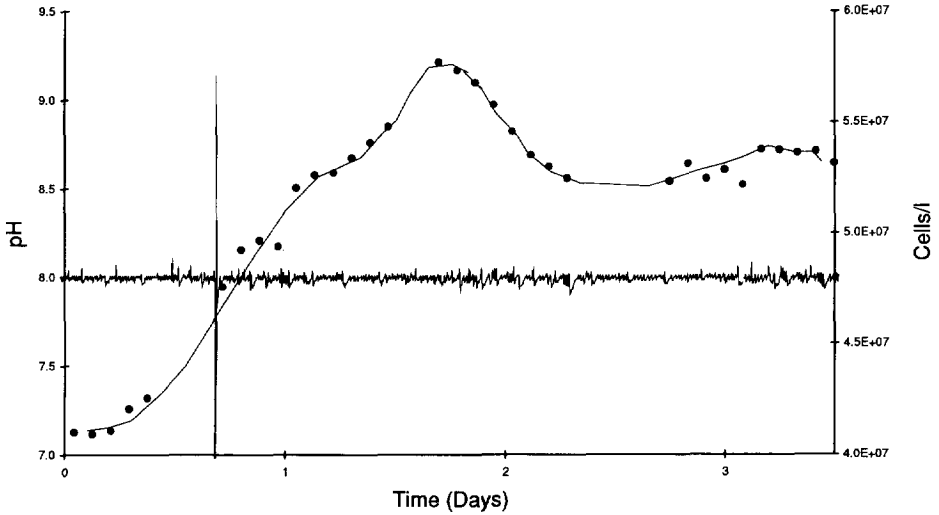


Fig. 2. Time variation of pH in a non-steady continuous culture of *Dunaliella tertiolecta*. The perturbation of pH observed around Day 0.7 is due to probe calibration.

respective gains of the colorimeters and the Cadmium-Copper column efficiency (Garside, 1993).

Two standards were used for the calibration of the measurements. The NO_3 standard had a concentration S_{33} of NO_3 and an unknown (and weak) concentration S_{32} of NO_2 . The NO_2 standard had a concentration S_{22} of NO_2 and a small concentration S_{23} of NO_3 .

The gain on the colorimeter with the column was called G_c and the gain of the 'direct' circuit was called G_d . The efficiency of the column was called E .

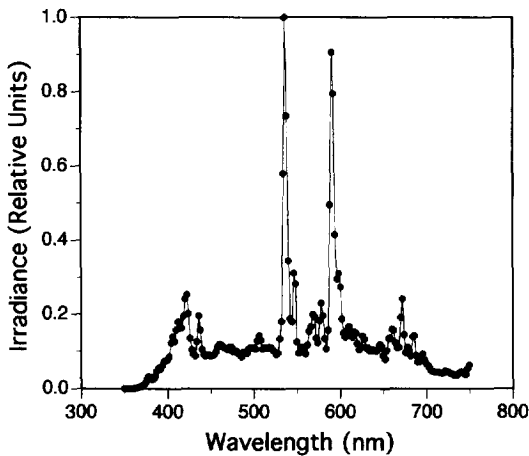


Fig. 3. Spectrum values (relative units) of light provided by a 150 W metal halide lamp (Osram, HQI) and 1 anti-UV glass-screen. Measurements were obtained with the spectro-irradiance meter Li-COR.

In the first step, we measure the signal for the NO_3 standard on the column-circuit (h_{c3}) and on the direct circuit (h_{d3}). We have:

$$h_{c3} = G_c \cdot (S_{32} + E \cdot S_{33}) \quad (1)$$

$$h_{d3} = G_d \cdot S_{32} \quad (2)$$

Then, for the NO_2 standard on the column-circuit (h_{c2}) and on the direct circuit (h_{d2})

$$h_{c2} = G_c \cdot (S_{22} + E \cdot S_{23}) \quad (3)$$

$$h_{d2} = G_d \cdot S_{22} \quad (4)$$

We have, then, 4 equations and 5 unknowns (G_c , G_d , E , S_{23} and S_{32}). We will, therefore, have to make a complementary assumption.

From Eq. (4) we can calculate G_d :

$$G_d = h_{d2}/S_{22} \quad (5)$$

The purity P of the NO_3 standard can be expressed as:

$$P = S_{32}/S_{33} \quad (6)$$

Then, from Eq. (2), Eq. (5) and Eq. (6), we have:

$$P = (h_{d3}/h_{d2}) \cdot (S_{32}/S_{33})$$

We make the assumption that the contamination is the same for both standards, thus:

$$S_{23} = P \cdot S_{22} \quad (7)$$

We can now calculate G_c and E from Eq. (1) and Eq. (3):

$$G_c = \frac{h_{c2} \cdot S_{33} - P \cdot h_{c3} \cdot S_{22}}{S_{22} \cdot S_{33} \cdot (1 - P^2)}$$

$$E = \frac{h_{c3} \cdot S_{22} - P \cdot h_{c2} \cdot S_{33}}{h_{c2} \cdot S_{33} - P \cdot h_{c3} \cdot S_{22}}$$

Note: the assumption in Eq. (7) of equal contamination for the standards can be avoided by measuring a mixing of half of the two standards:

$$h_{c(3+2)} = 0.5 \cdot G_c \cdot (S_{22} + S_{32} + E \cdot (S_{23} + S_{33}))$$

$$h_{d(3+2)} = 0.5 \cdot G_d \cdot (S_{22} + S_{32})$$

But the supplementary manipulations are not justified by a significant gain of precision. For a signal h_c on the column-circuit and h_d on the direct circuit, the concentrations S_2 and S_3 of NO_2 and NO_3 are:

$$S_2 = h_d/G_d$$

$$S_3 = \frac{1}{E} \cdot \left(\frac{h_c}{G_c} - \frac{h_d}{G_d} \right)$$

2.3. Cell counting

Size spectra and cell concentrations are obtained by the particle counter HIAC/ROYCO PACIFIC. The system, constituted by an optical sensor (Laser sensor HRLD-400), a high-speed digital counter (Model 9064) and an automatic sampler (Model 3000), is monitored by a computer (PC 486) using particle distribution analysis software (PDAS). Before counting, dilution of the concentrated phytoplankton cultures is necessary. This is routinely performed by an automatic system consisting of peristaltic pumps (Gilson), solenoid valves and a syringe controlled by another computer. Developed software controls the chemostats to be sampled, the respective dilution rates, the number of replicate samples and the time interval between each series of measurements. A final procedure allows the determination of biovolumes, concentrations and mean diameters from the size spectra (Fig. 4).

2.4. Dilution control

Peristaltic pumps (Gilson) provide axenic enrichment medium into the chemostats. When switched on continuously, a peristaltic pump can ensure a constant dilution rate

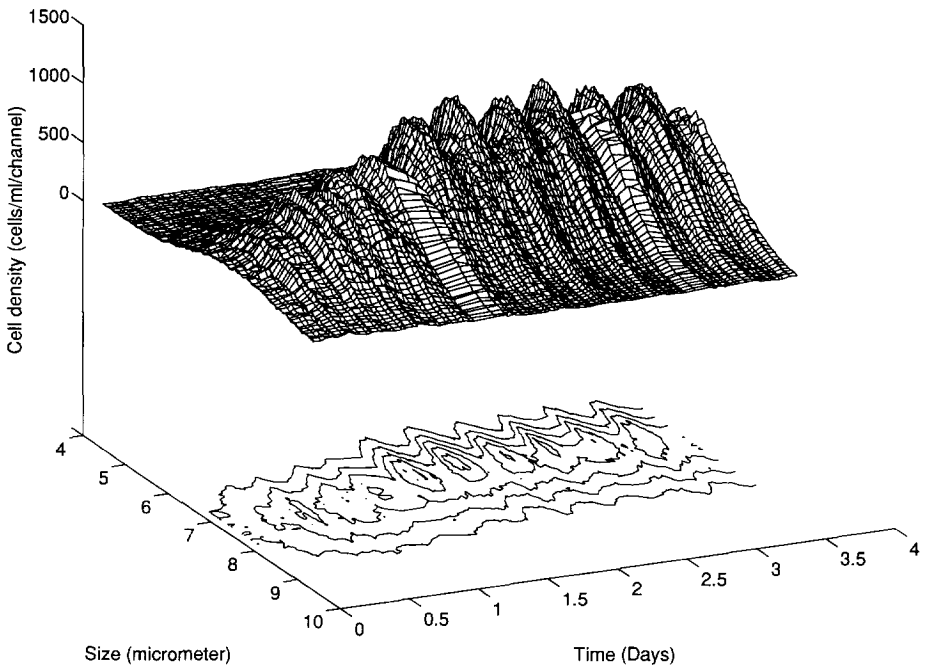


Fig. 4. Example of time series data acquisition provided by the particle analysing system operating on a culture of *Dunaliella tertiolecta* (see also Fig. 7).

D_{\max} . In order to obtain a desired dilution rate D (with $D < D_{\max}$) the peristaltic pump needs to be switched on only for a proportion of time equal to D/D_{\max} . Every p min, the pump is then switched on for $p \cdot (D/D_{\max})$ min. This way of monitoring the pump is used to ensure a time-varying dilution rate $D(t)$: the proportion of time during which the pump needs to be switched on is recalculated every p min. The more p is short, the more the dilution rate is near the continuous mode. The p value is fixed in relation to the variation rate of D . The corresponding mean dilution rate $\bar{D}(t) = D(t)$ over this time period is calculated as follows:

$$\bar{D}(t) = \frac{1}{p} \int_t^{t+p} D(\tau) d\tau$$

For example, the equation used to obtain a sinusoidal dilution rate of mean D_0 , amplitude D_1 and period T , $D(t)$ is:

$$D(t) = D_0 + D_1 \sin\left(\frac{2\pi}{T}t\right)$$

The mean value over p min is thus:

$$\bar{D}(t) = D_0 + \frac{1}{\pi} \frac{T}{p} D_1 \left(\frac{p\pi}{T}\right) \sin\left(\frac{\pi(2t + p)}{T}\right)$$

Finally, the pump has to be switched on during the time $t_c(t)$:

$$t_c(t) = p \frac{D_0}{D_{\max}} + \frac{T}{\pi} \frac{D_1}{D_{\max}} \sin\left(\frac{p\pi}{T}\right) \sin\left(\frac{\pi(2t + p)}{T}\right)$$

Fig. 5 shows that this way of achieving a discontinuously controlled dilution rate gives results which are very close to a theoretical continuous dilution. The possible bias of the

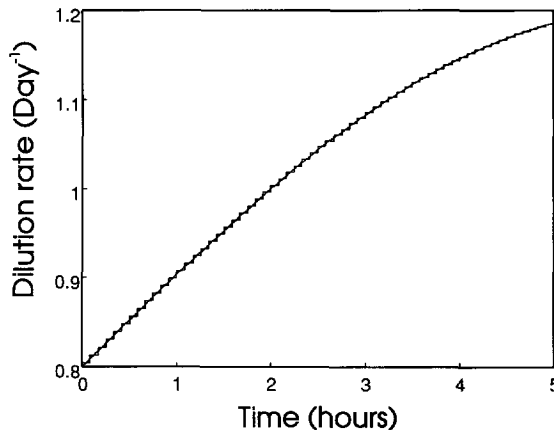


Fig. 5. Comparison of desired (continuous line) with actually obtained dilution rate (steps), with $p=5$ min, $T=24$ h, $D_0=0.8$ day⁻¹ and $D_1=0.4$ day⁻¹.

Table 1
Culturing conditions in Experiments 1 and 2

	Experiment	
	1	2
Dilution mode	Constant continuous/pseudo-continuous	Sinusoidal pseudo-continuous
Dilution rate (day^{-1})	$D = 0.48/D = 0.51$	$D = 1.71 + 1.03 \sin(18.85t)$
Dilution periodicity (h)	∞	8
Command periodicity (min)	$\infty/5$	5
Nitrogen input mode	Constant and continuous	Constant and continuous
[NO ₃] medium ($\mu\text{gat}\cdot\text{l}^{-1}$)	98.7	52.13
[NO ₂] medium ($\mu\text{gat}\cdot\text{l}^{-1}$)	0.14	0.94
Temperature (°C)	19	30
pH control	Free	Free
Light regime ($\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	Constant: 260	Constant: 260

See text for further details.

discontinuous mode compared with the continuous one was studied in Experiment 1 (Table 1).

2.5. Control of nutrient supply

The medium is supplied by mixing the $f/2$ medium without NO₃ (with flow rate d_1) and a concentrated solution S_c of NaNO₃ (with flow rate $d_2 \ll d_1$). The total flow rate of input medium is then $d_1 + d_2$ and the concentration of NO₃ can be expressed as $S_i = S_c d_2 / (d_1 + d_2)$. A double solenoid valve allows the concentrated solution of NaNO₃ to be replaced with another solution of sea water without NO₃ (Fig. 1). If this solenoid valve is commuted for a fraction q of time, the mean concentration of NO₃ in the supplied medium is $S_i = q S_c d_2 / (d_1 + d_2)$. If q varies with time as described in the previous paragraph, we are able in the same way to give to S_i any dynamic pattern by computing every p min the proportion of time sea water replaces concentrated NaNO₃ solution. In Experiment 2, a periodic nutrient supply was used (Table 1).

2.6. Calculation of the growth and uptake rates

The growth rate μ is approximated by the cell division rate:

$$\mu = \frac{1}{n} \cdot \frac{dn}{dt} + D$$

where D is the dilution rate and n and dn/dt are given by a function fitted to the cell count time series. Data were smoothed using spline interpolations (MATLAB). The uptake rate ρ of the nutrient s (NO₃ or NO₂) is calculated as:

$$\rho = \frac{1}{n} \cdot \left[\frac{ds}{dt} - D \cdot (s_i - s) \right]$$

where s_i is the nutrient concentration in the enrichment medium, and s and ds/dt are calculated as for n .

3. Results

This culture system was used to grow the chlorophycean *Dunaliella tertiolecta* (Butcher). We first compared the continuous dilution mode with the pseudo-continuous one in order to verify that both give the same results and that no methodological bias was introduced when using the discontinuous mode. The mean dilution rate was similar and constant in both chemostats C1 and C2, but was continuous in C1 (peristaltic pump always switched on) and pseudo-continuous in C2 (pump intermittently switched on and off, following the mode described previously). Fig. 6 shows the ability of both dilution systems to provide identical steady states for phytoplankton concentrations. The initial conditions were slightly lower in C2, which partly explains the delay with respects to C1.

Fig. 7 shows the response of the population descriptors to sinusoidal variations of the dilution rate (Table 1). Cell numbers oscillate synchronously with biovolumes and asynchronously with mean cell volumes. The cell volume fluctuations were globally sinusoidal, whereas the fluctuations for the 2 other parameters were not. The coupling of the different non-linear processes which control growth can explain the transformation of the initial sinusoid forcing variable (here dilution rate) to a non-periodic signal (Pascual, 1994). The cell volume firstly oscillates around the mean value of $200 \mu\text{m}^3$.

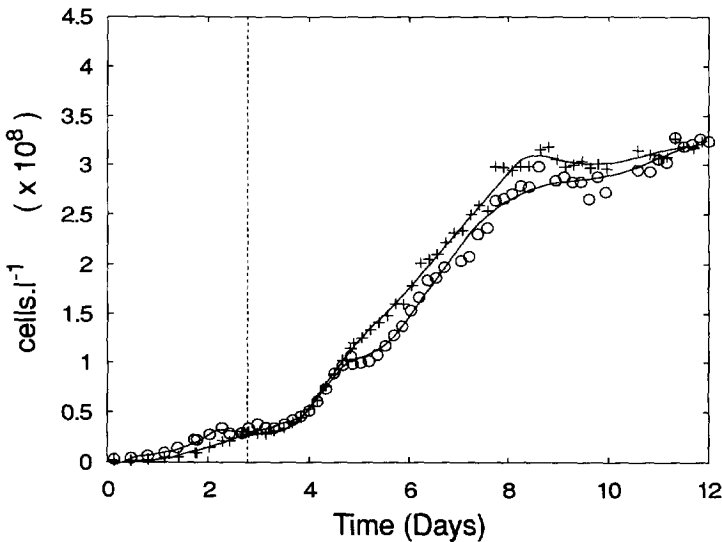


Fig. 6. Experiment 1: Time variation of *Dunaliella tertiolecta* concentration in two chemostat cultures subjected to a continuous dilution rate (C1: +) and to a discontinuous dilution rate (C2: O), respectively. Dilution begins at the dashed line.

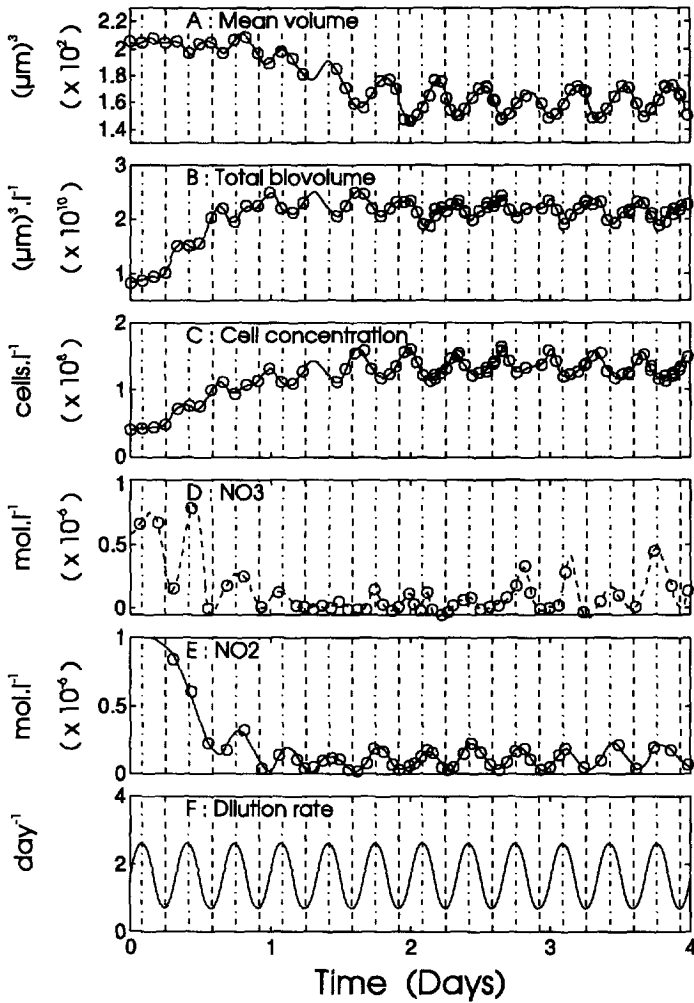


Fig. 7. Experiment 2: Time variation of mean cell volume, biovolume, cell counts, NO_3 and NO_2 in a chemostat culture of *Dunaliella tertiolecta* subjected to a sinusoidal dilution rate (see Table 1). Vertical bars indicate when the dilution was a maximum (— — —) and a minimum (—).

Thereafter, the mean cell volume decreased to a lower level ($160 \mu\text{m}^3$) around which oscillations are sinusoidal. The detail of the size distributions provided by the counter is shown in Fig. 4. NO_2 exhibits quasi-sinusoidal periodic oscillations with a small time lag with the dilution rate (Fig. 7). In contrast, the shape of the NO_3 signal is far from sinusoidal, even if some extremes coincide with those of the NO_2 .

Estimation of the nitrogen fluxes through the cells is presented in Fig. 8. The net NO_3 uptake rate shows sinusoidal variations (Fig. 8A), whereas the NO_2 uptake rate (Fig. 8B), is not so regular. Unlike NO_3 , NO_2 uptake variations are delayed with the dilution rate variations. The cell division rate stops during the maximum of NO_3 assimilation,

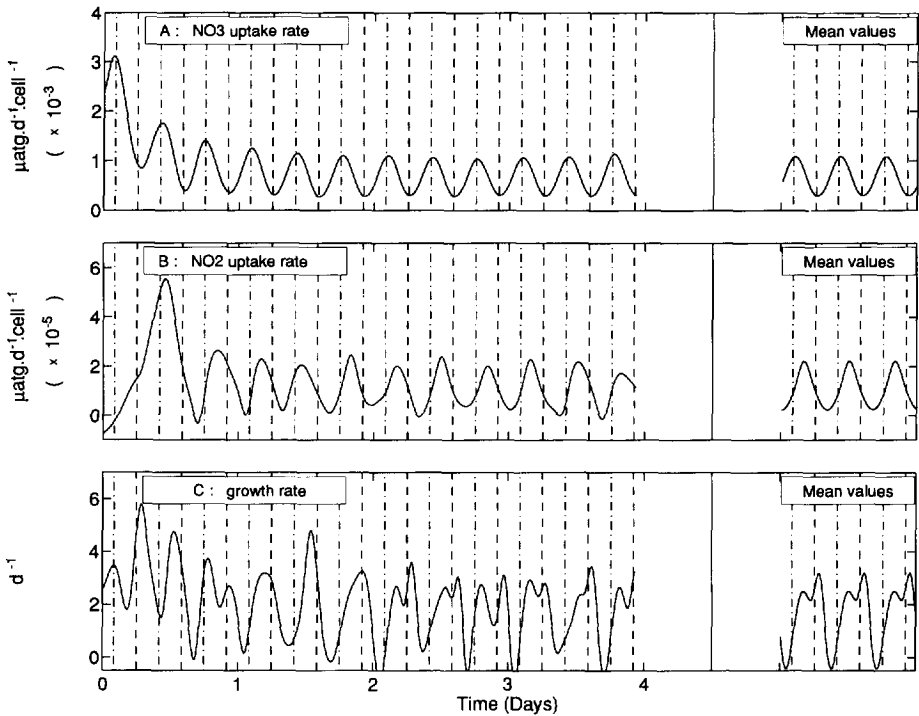


Fig. 8. Experiment 2: Estimation of nitrogen fluxes through the cells. The patterns of the signal represented at the right of the continuous line are the mean periodic values over the last 7 periods. Symbols as in Fig. 7.

and then 2 main peaks of growth per dilution period are apparent (Fig. 8C). As the cell division rate during these peaks is greater than the maximum growth rate for an homogenous population, the cell population is probably synchronised. Consistent with Olson and Chisholm (1983) and Wheeler et al. (1983), where algae were submitted to light–dark cycles and periodic ammonium pulses, we observed an uncoupling between nitrogen assimilation and cell division.

4. Discussion

The experimental determination of dissolved inorganic nitrogen uptake parameters depends largely on enrichment techniques which consist of observing the kinetics of a discrete nutrient quantity after its introduction into a population of cells which are growing at a certain rate. The advantages and disadvantages of this methodology, called a ‘pulse experiment’, have been discussed previously by Harrison et al. (1989). This method is very useful for determining uptake rates and their temporal variation induced by non-linear adaptation processes. Aside from eventual interpretative bias, the ‘pulse experiment’ method has two disadvantages. The first concerns the pulse of nutrients, which is probably non-existent in the ocean, where turbulent and molecular diffusion

tends to smooth over all abrupt variations at the cellular level. It is, therefore, likely that the *in vitro* cellular response is due to artificial physiological phenomena. The second concerns the method cited by Harrison et al. (1989), which measures the variation in uptake rates with time after a large nutrient pulse. The problem is that the measurements of the uptake rates are limited to the pulse duration. The necessity of acquiring sufficient data points implies either that the quantity of substrate introduced must be large, or that the algae population be small. In both cases, initial conditions influence the interpretation of the results. This pulse technique only allows a limited control of the system at the moment of substrate input, and makes the study of the uncoupling between growth and uptake rates limited to a narrow range of data configurations. Because of the non-linearity of adaptation phenomena, it is practically impossible with either a single pulse or sequential pulses, to induce and explore the evolving relation between growth and uptake in the whole range of their variations. The system described in this report makes it possible to exercise continuous and specific control from the beginning of the experiment.

Continuous control lengthens the data acquisition duration of the uptake and growth rates which aids in the understanding of inter-related processes. For example, it is possible to maintain substrate concentrations at a level near half-saturation for a time that is generally short in earlier pulse studies. In this way, more precise data can be obtained on the uptake rates of low-level nutrients. Another example concerns the controversial question of NO_3 – NO_2 interactions, where NO_3 may inhibit NO_2 uptake (Bilbao et al., 1981) or not (Cresswell and Syrett, 1979). This phenomenon is complicated since some measured NO_2 can come from cell excretion. NO_2 can be excreted by *D. tertiolecta* cells, which are not physiologically prepared to assimilate NO_3 in excess (Laws and Wong, 1978; Sciandra and Amara, 1994). During NO_3 pulse experiments, NO_2 is transiently excreted and is reabsorbed when NO_3 levels become low. Excretion of NO_2 , and its re-assimilation with NO_3 , are thus transitory phenomena that are therefore difficult to observe. Here, we have maintained continuous cultures with periodic high NO_3 and low NO_2 supply in such a way that the global NO_3 and NO_2 fluxes in the cells were measurable during the whole experiment. This experimental design provides a great advantage for modelling the relations between nitrogen uptake, assimilation and growth in a continuously changing environment.

The previously described specification of nutrient input control allows us to generate and follow transients of phytoplankton behaviour. These play an important role in the modelling field because of the amount of information they contain. The information involved at equilibrium is very poor: at steady state, distinct models based on differential equations can predict the same equilibria. For example, the Monod model (Monod, 1942) and the Droop model (Droop, 1968; Burmaster, 1979) are equivalent at equilibrium (Goldman, 1977). To choose between these models, the criterion will therefore be the correctness of the dynamic behaviour respective to experimental data. In this sense, the comparison between experimental transients and transients allowed by the model will be the more complete, the longer a system can be maintained far from equilibrium. The observation of transients to test a dynamic model is then a first step for a modelling approach (Bernard and Gouzé, 1995; Bernard and Gouzé, *in press*). A

second step would be the identification of parameter values. Again, the only measure at equilibrium is too poor for estimation of parameters. If we consider a differential system with p parameters, assuming that r quantities can be measured at equilibrium, we obtain r relations to determine p parameters. Generally p is greater than r , such that (at least) $p-r$ complementary measures have to be done, or identification of parameters using transients has to be performed. For example, using Monod modelling in the chemostat there are 3 parameters (the maximal uptake rate, the associated half-saturation constant and the yield coefficient), and at most 2 measurable quantities (biomass and nutrients); for the Droop model, there are 4 parameters (the maximal uptake rate, the associated half-saturation constant, the maximal growth rate and the minimum cell quota) for at most 3 measurements (biomass, nutrients and cell quota). From a practical point of view, the question of the measure of remaining nutrients at equilibrium could be discussed. Therefore, in both cases, 2 relations are lacking to determine all parameters. Complementary measures can be achieved, but the conditions for the new experiment involved can slightly differ from the original one. On the contrary, if transients are monitored, they can allow a determination of parameters and sometimes their evolution with respect to time. To identify linear models, a classical tool of the control science consists in applying a set of controlled inputs which is a mathematical basis for the whole class of inputs that the system can receive. The transient response of the system to this input gives, after calculus, an estimation of parameter values (Davies, 1970; Verbruggen, 1975). The point is that these so-called pseudo-random binary sequences can find an analytical formulation, which leads to estimation of parameters.

With our system, we can generate such inputs that excite the system and maintain a non-steady state in order to facilitate its identification. Two main techniques can then be applied. Either the system is kept around equilibrium, and the parameters can be identified on the linearized system using linear methods. Or, in the general case, methods of non-linear programming (Fletcher, 1987) can be used to minimise the difference between data and model output.

Finally, the choice of control over nutrient input allows simulation of certain forcing characteristics found in the natural environment. For example, it would be of interest to reproduce a nutrient fluctuation by its amplitude and frequency, in this case sinusoidal, as given in the example presented here. As environmental fluctuations engender adaptive processes, it is preferable that the relations between growth and uptake are analysed in a situation where the fluctuations are well specified. For example, the numerical model of Klein and Coste (1984) foresees possible interactions between the Coriolis force and certain conditions of current and sea surface wind, as inducing ascending and regular nutrient supply across the thermocline, with a periodicity approaching several hours. In general, uptake (Michaelis–Menten) and growth models (Monod, Droop) established for systems at equilibrium remain applicable to a fluctuating milieu when the external nutrient variation does not exceed the adaptation rate of the species. It is probable that these models are applicable in the case of a sinusoidal nutrient fluctuation where the correspondence between amplitude and periodicity remains inferior to a given value. However, in the case of a more rapid variation, the models are not applicable because of important uncoupling between uptake and growth. Our system not only allows closer

inspection of the boundary between these two domains but also enables analyses, such as cell division rate and cell quotas, to be made under a large set of physiological conditions.

5. Conclusions

In contrast to numerous culturing systems developed to simulate experimentally nutrient limitation in either constant light (chemostat) or fluctuating light regimes (cyclostat) (Marra, 1978; Cosper, 1982; Kroon et al., 1992), our system has the advantage of maintaining continuous cultures of phytoplankton under various and dynamic controlled patterns of nitrogen limitation. Moreover, automatic high sampling rates allow us to obtain a precise description of biological processes that are triggered under unstable growth conditions. More specifically, our system in its present state provides facilities to study the non-linearities of the processes that are responsible for the change of a periodic input signal (sinusoidal in this experiment) into an output signal, periodic or not. This property characterises non-linear systems, since it is demonstrated that linear systems transform a sinusoidal signal into another sinusoidal signal (after a transient delay). Moreover, the periodic pattern for the input gives a statistical weight to the observations; once the stable cyclic regime is established, the periodic output can be averaged for several cycles. As the response can be measured for a sufficient number of cycles, the significance of the observations is statistically reinforced.

Finally, we project to couple this culture system with another system providing fluctuating light conditions, in order to explore the simultaneous effects of varying patterns of light and nitrogen limitation. From the literature, it is not clear if these limitations act simultaneously (multiplication) or preferentially (minimum law). Some authors have hypothesised that compensation phenomena exist (Rhee and Gotham, 1981; Healey, 1985) because light deficiency could be compensated by more nitrogen. For a set of light–nitrogen (I, N) combinations, one can calculate a set of growth rates $\mu(N)$ and $\mu(I)$ from classical models. In the minimum law, the variable for which μ is the lowest is then considered as more limiting and as the only one to be taken into account for growth calculations, the other one being assumed to be without effect: $\mu = \text{Min}(\mu(N), \mu(I))$. The multiplicative law consists in calculating μ from the product of two functions, respectively, of N and I : $\mu = \mu'(N) \cdot \mu'(I)$. These uncertainties have considerable consequences on the conception of mathematical models, since no consensus has emerged from experiments based on classical chemostat studies.

Acknowledgments

We thank Patrick Chang for improving the English, Jacques Gostan for the measurement of the lamp light spectrum, and the reviewers for their comments. This research was supported by the GDR 1107 of CNRS and by the European MAST II Program MTP-Medipelagos.

References

- Bernard, O. and J.L. Gouzé, 1995. Transient behavior of biological loop models with application to the Droop model. *Math. Biosci.*, Vol. 127, pp. 19–43.
- Bernard, O. and J.L. Gouzé, 1995. Robust validation of uncertain models. *ECC95*, Vol. 2–4, pp. 1261–1266.
- Bilbao, M., J.M. Gabas and J.L. Serra, 1981. Inhibition of nitrite uptake in the diatom *Phaeodactylum tricorutum* by nitrate, ammonium ions and some L-amino acids. *Biochem. Soc. Trans.*, Vol. 9, pp. 476–477.
- Burmaster, D.E., 1979. The unsteady continuous culture of phosphate-limited *Monochrysis lutheri* (Droop): experimental and theoretical analysis. *J. Exp. Mar. Biol. Ecol.*, Vol. 39, pp. 167–186.
- Cosper, E., 1982. Influence of light intensity on diel variation in rates of growth, respiration and organic release of a marine diatom: comparison of diurnally constant and fluctuating light. *J. Plankton Res.*, Vol. 4, pp. 705–724.
- Cresswell, R.C. and P.J. Syrett, 1979. Ammonium inhibition of nitrate uptake by the diatom *Phaeodactylum tricorutum*. *Plant. Sci. Lett.*, Vol. 14, pp. 321–325.
- Davies, W.D.T., 1970. *System identification for self-adaptive control*. Wiley-Interscience, New York, NY, pp. 380.
- Droop, M.R., 1968. Vitamin B₁₂ and marine ecology. IV. The kinetics of uptake growth and inhibition in *Monochrysis lutheri*. *J. Mar. Biol. Ass. UK*, Vol. 48, pp. 689–733.
- Eppley, R.W., J.J. McCarthy and A. Sournia, 1971. Light/dark periodicity in nitrogen assimilation of the marine phytoplankters *Skeletonema costatum* and *Coccolithus huxleyi* in N-limited chemostat culture. *J. Phycol.*, Vol. 7, pp. 150–154.
- Fletcher, R., 1987. *Practical methods of optimization*. Wiley and Sons, New York, NY, 2nd edn., pp. 174.
- Garside, C., 1993. Nitrate reductor efficiency as an error in seawater analysis. *Mar. Chem.*, Vol. 44, pp. 25–30.
- Goldman, J.C., 1977. Steady state growth of phytoplankton in continuous culture: comparison of internal and external nutrient equations. *J. Phycol.*, Vol. 13, pp. 251–258.
- Harrison, P.J., J.S. Parslow and H.L. Conway, 1989. Determination of nutrient uptake kinetic parameters: a comparison of methods. *Mar. Ecol. Progr. Ser.*, Vol. 52, pp. 301–312.
- Healey, F.P., 1985. Interacting effects of light and nutrient limitation on the growth rate of *Synechococcus linearis* (Cyanophyceae). *J. Phycol.*, Vol. 21, pp. 134–146.
- Klein, P. and B. Coste, 1984. Effects of wind-stress variability on nutrient transport into the mixed layer. *Deep-Sea Res.*, Vol. 31, pp. 21–37.
- Kroon, B.M.A., U.M. van Hes and L.R. Mur, 1992. An algal cyclostat with computer-controlled dynamic light regime. *Hydrobiologia*, Vol. 238, pp. 63–70.
- Laws, E.W. and D.C.L. Wong, 1978. Studies of carbon and nitrogen metabolism by three marine phytoplankton species in nitrate-limited continuous culture. *J. Phycol.*, Vol. 14, pp. 406–416.
- Malara, G. and A. Sciandra, 1991. A multiparameter phytoplanktonic culture system driven by microcomputer. *J. Appl. Phycol.*, Vol. 3, pp. 235–241.
- Marra, J., 1978. Effect of short-term variations in light intensity on photosynthesis of a marine phytoplankter. A laboratory simulation study. *Mar. Biol.*, Vol. 46, pp. 191–202.
- Miller, R.L., 1987. A computer based culturing system for measuring the photosynthetic response of phytoplankton to a fluctuating environment. *J. Exp. Mar. Biol. Ecol.*, Vol. 106, pp. 17–29.
- Monod, J., 1942. *Recherches sur la croissance des cultures bactériennes*. Hermann and Cie, Paris, 2nd edn., 210 pp.
- Olson, R.J. and S.W. Chisholm, 1983. Effects of photocycles and periodic ammonium supply on three marine phytoplankton. I. Cell division patterns. *J. Phycol.*, Vol. 19, pp. 522–528.
- Pascual, M., 1994. Periodic response to periodic forcing of the Droop equations for phytoplankton growth. *J. Math. Biol.*, Vol. 32, pp. 743–759.
- Rhee, G.Y. and I.J. Gotham, 1981. The effect of environmental factors on phytoplankton growth: light and the interactions of light with nitrate limitation. *Limnol. Oceanogr.*, Vol. 26, pp. 649–659.
- Sciandra, A. and R. Amara, 1994. Effects of nitrogen limitation on growth and nitrite excretion rates of the dinoflagellate *Prorocentrum minimum*. *Mar. Ecol. Progr. Ser.*, Vol. 105, pp. 301–309.
- Tréguer, P. and P. Le Corre, 1975. *Manuel d'analyse des sels nutritifs dans l'eau de mer. Utilisation de l'Autoanalyser II Technicon*. Université de Bretagne Occidentale, Brest, 2nd edn., pp. 110.

- Verbruggen, H.B., 1975. Pseudo random binary sequences. *Journal A*, Vol. 16, pp. 205–207.
- Wheeler, P.A., R.J. Olson and S.W. Chilsom, 1983. Effects of photocycles and periodic ammonium supply in three marine phytoplankton species. II. Ammonium uptake and assimilation. *J. Phycol.*, Vol. 19, pp. 528–533.