

A multiparameter phytoplankton culture system driven by microcomputer

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Abstract

A computer-controlled phytoplankton culture system is described which is regulated as a chemostat. Measurements of temperature, pH, size distribution, culture density, *in vivo* fluorescence, nitrate and nitrite, are automatic and programmable, thus obviating manual errors. The system has been developed successfully to survey long-term cultures of the dinoflagellate *Prorocentrum minimum*.

Introduction

Production of unicellular autotrophic organisms is an essential step in providing nutrition for large-scale food farming such as fish sprey or small crustaceans. The nutritive value of the phytoplankton can be influenced by the culture conditions, as has been shown with diatoms and dinoflagellates; their sugar, lipid and protein contents can be modified by different illumination conditions (Hitchcock, 1982; Claustre & Gostan, 1987), or by varying inorganic nitrogen supplies (nitrates or ammonium) to the culture (Dortch *et al.*, 1984). Hence, attempts to control and optimize primary food production necessitate automation of production modes in addition to acquisition of culture parameters.

The advantage of open culture systems compared to batch cultures, is that inorganic components necessary for phytoplankton growth are at low concentrations in the culture medium and therefore have little influence on the larvae fed. We present here an autonomous culture system working on the principle of a chemostat which automatically collects biomass parameters (*in vivo*

fluorescence, size distribution, culture density) while measuring nitrate and nitrite concentrations, temperature and pH.

Materials and methods

The culture system shown in Fig. 1 consists of a 3.3-litre incubator (volume can be varied) with a thermostated water jacket assuring temperature to 0.1 °C. The culture is stirred with a motorized glass helix and receives bubbled air passed first through a 0.22 µm Millipore filter. A 300-watt quartz iodide halogen lamp supplies light on a pre-programmed basis. The culture can receive nutrients from three independent inlets, thereby allowing continuous or pulsed regiments which are also microprocessor controlled. Culture volume is stabilized via an overflow.

The autonomy of the system and culture data acquisition are controlled by two apple IIe computers programmed in BASIC. System I controls acquisition of the culture data mentioned above. An ADALAB card (Interactive Microware, Inc.) programmed in machine language interfaces data

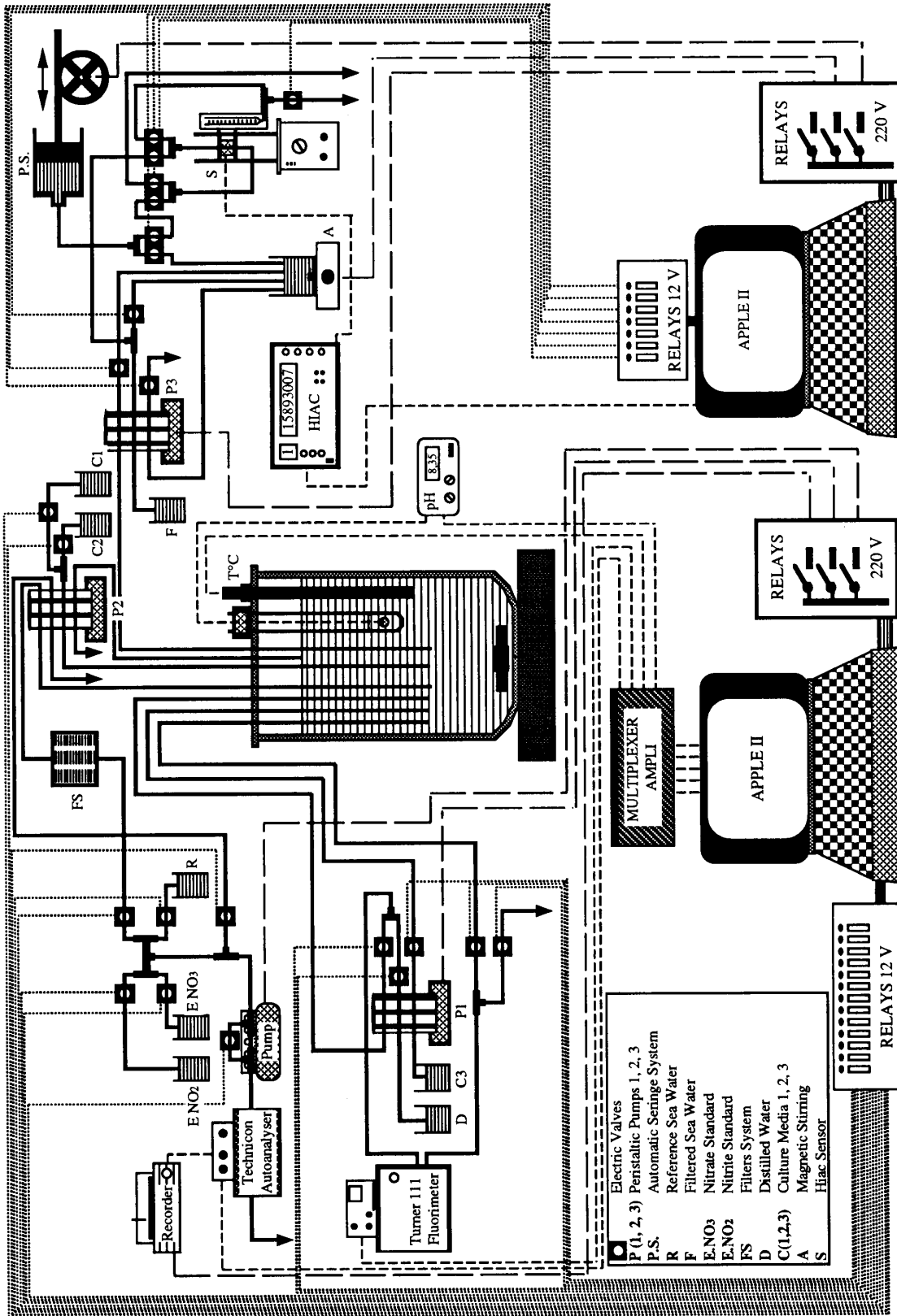


Fig. 1. Schematic diagram of the automated culture system.

Table 1. Measurements monitored by the automated cultures system.

Variables	Measurement delays (mn)	Sampled volumes (ml)	Precision
T °C	Instantaneous	0	0.1 °C
pH	Instantaneous	0	0.01 unit
Nitrogen	NO ₃	4	0.05 µg-atl ⁻¹
	NO ₂	4	0.05 µg-atl ⁻¹
	NO ₃ & NO ₂	8	0.05 µg-atl ⁻¹
Cell counts	8	5	1%
Fluorescence	6	10	3%

collection and permits accessing, via simple BASIC instructions, the following modules: 2 timers, 1 analog digital input, and 2 parallel input and output sockets. An 8-channel multiplexer (HEF 4051B) received measurements from four sensors:

- (i) semiconductor temperature sensor (Analog Devices AC 2626-K4) which delivers 300 µA at 20 °C and 1 mA per °C;
- (ii) pH probe (pH meter KNICK 646) with a combination electrode INGOLD which delivers 1 mV per 0.01 pH units (700 mV at pH 7.0);

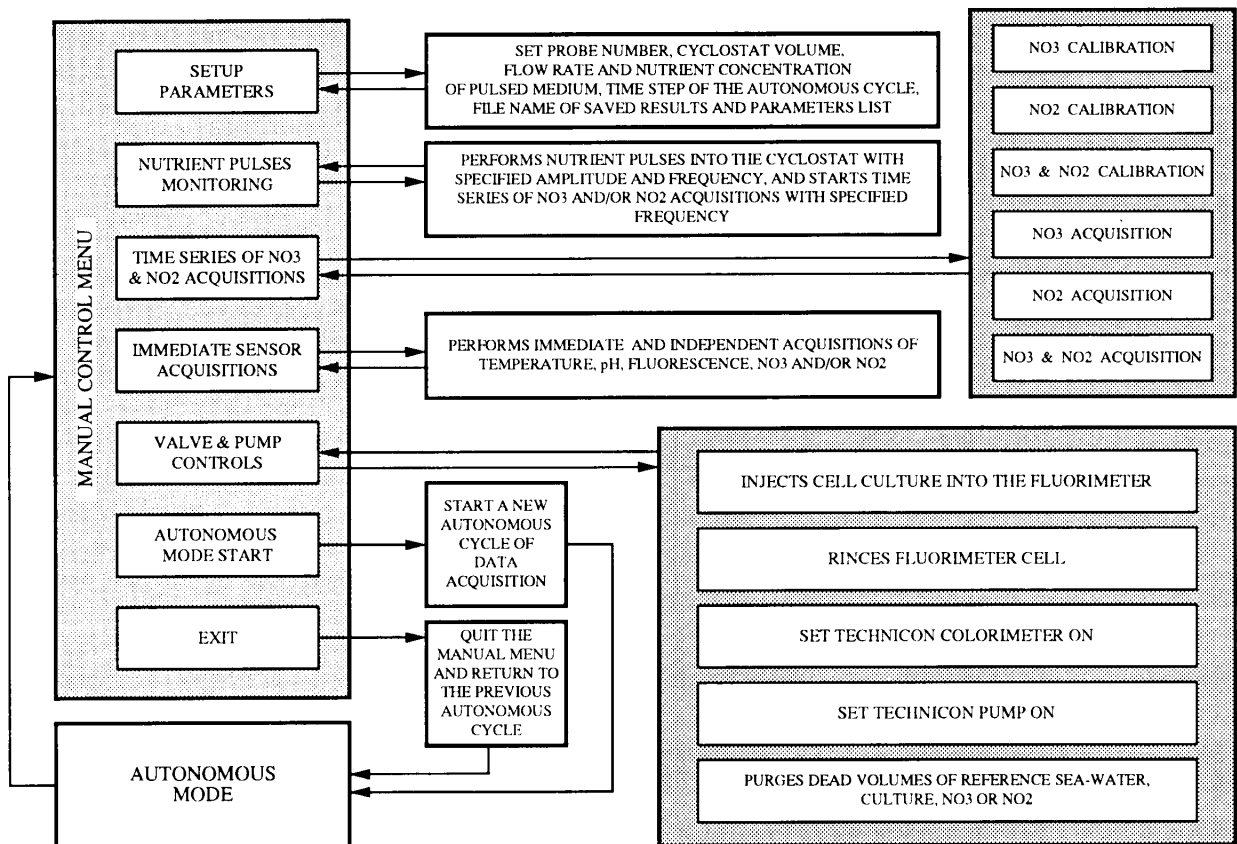


Fig. 2. Principle commands in the manual mode.

- (iii) fluorimeter (Turner III) which allows fluorescent measurements *in vivo*. Output voltage of the device is 0–10 mV before amplification and before analog input the ADALAB card;
- (iv) colorimeter associated with a Technicon Autoanalyser, which measures nitrate and nitrite. Output signal amplification is also necessary.

The parallel input/output sockets on the ADALAB card allow activation/inactivation of relays, peristaltic pumps, and recorders via opto-relay electronics. In addition to automatic sampling, titration and addition of media into the culture, it is also possible to select different modules of the system (for example, rinsing the fluorimeter measuring cell). System I can function autonomously to survey culture parameters (Table 1), or can be manually operated to effectuate a particular function (Fig. 2). During the

autonomous mode, cycles of culture parameter acquisition are performed with a programmable periodicity ranging between 30 and 100 min. The system automatically continues a program in case of a power failure. The automatic routine between 2 measurement cycles can be interrupted by one keyboard command. Finally, if a manual operation such as nitrate calibration exceeds the re-institution of a programmed cycle, the latter will be retarded until the manual operation has been terminated.

System II controls size (in 12 increments) and acquisition of data on phytoplankton concentration. Its function is to take culture aliquots, diluting them if necessary, and to transfer them to the particle counter (HIAC, Pacific ROYCO). The results are then stored. The microprocessor has an internal clock and a VIA card with 4 parallel ports. There are 32 individually programmable input/output bits, which allow interfacing with the particle counter. System II also operates on manual or automatic with a 8-min delay step allowing sufficient time to carry out the required operations.

Results

The culture system presented here allowed us to study the growth characteristics of *Prorocentrum minimum*, a dinoflagellate associated with red toxic blooms. Fig. 3 shows nitrate and nitrite concentrations and cellular counts of a culture, whose growth was controlled by the dilution rate (d) and the concentration of nitrate in the injected medium (c). Our system allowed us to follow in real time the influence on the culture when one of the parameters was changed. During the first part of phase 1, cell growth is superior to the culture dilution rate (i.e. positive biomass change), and the nitrate concentration was in excess. As nitrate is consumed, the biomass decreases. During phase 2, the injected nitrate concentration is increased (c) which results in an increased energy supply to the culture (even if the nitrates are not detectable). The phytoplankton culture now tends towards equilibrium (arrow in phase 2 of Fig. 3).

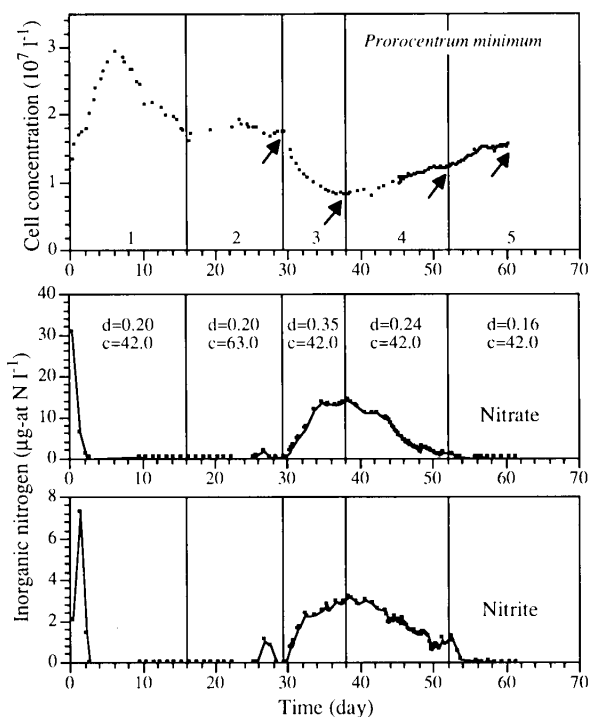


Fig. 3. *Prorocentrum minimum* culture. As of day 45, the particle counter has doubled measurements from 2 to 4 per day. The arrows indicate the different stationary phases of the culture in respect to different dilution rates (d) and nitrate concentrations (c) in the injected milieu.

The new values of (c) and (d) during phase 3 have immediate repercussions on the cell density of the culture. The increase in dilution rate contributes to the decrease of the culture and as a consequence, nitrate accumulation. A new stationary equilibrium is reached on day 38. The excess of nitrate during this phase induces nitrite excretion. During phase 4, the reduction in dilution rate induces a phenomenon opposite to the preceding phase. As a new state of equilibrium is reached at day 52, the dilution rate is reduced, permitting the culture to reach a new equilibrium on day 60.

Figure 4 shows a graph of the data obtained with the automatic particle counter during the first 10 days. This system provides evidence for a variation in average cell diameter during the exponential growth phase followed by a size stabilization.

The culture system also allows automatic addition of a known concentration of nitrate (or any other limiting component) in discrete pulses. The system then collects concentration data and finally provides absorption kinetics. Figure 5 shows the effects on *P. minimum* of two different culture conditions. In case A, the cells were not deficient in nitrate. They are in a stationary phase where the salt depletion to zero is linear with time. In case B, the cells were deficient in nitrate for 4 days prior to a nitrate pulse, and one sees a time

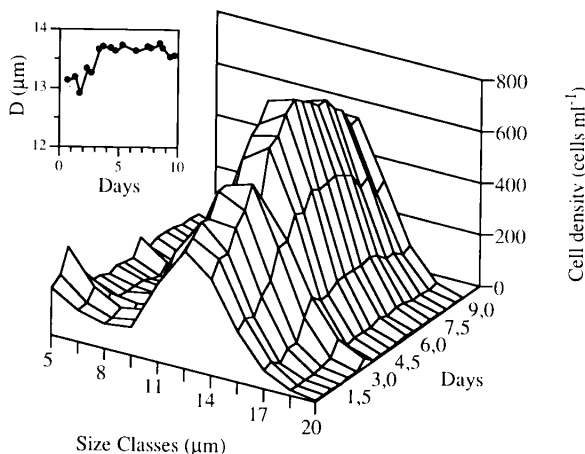


Fig. 4. Size distribution and mean diameter of *Prorocentrum minimum* at the beginning of the culture experiment.

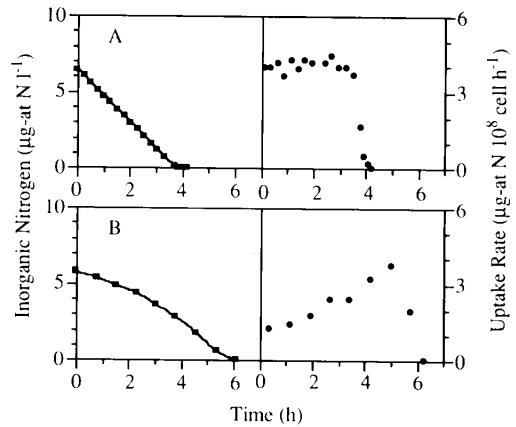


Fig. 5. Case A: *Prorocentrum minimum* cells non-depleted in nitrate: the rate of nitrate absorption measured after one nitrate pulse is linear with time. Case B: *Prorocentrum minimum* depleted in nitrate: the absorption rate is low after the pulse and then augments showing an induction time lag characteristic of a physiological adaptation of the cells in an environment which has changed abruptly.

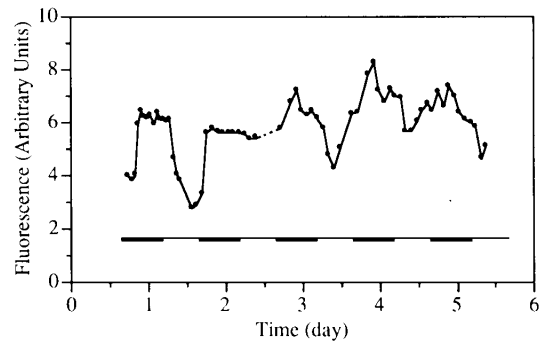


Fig. 6. Automatic *in vivo* fluorescence measurements of a *Prorocentrum minimum* culture maintained in cyclostat with a LD cycle 12:12.

lag before the rate of nitrate absorption fails to achieve a maximum. Figure 6 shows the variation in *in vivo* fluorescence of a *Prorocentrum minimum* culture subject to a 12:12 h light:dark cycle. The *in vivo* fluorescence is seen to increase rapidly during the first hours of the dark phase.

Discussion

This culture system maintains phytoplankton cultures monospecific and healthy for several months

(Sciandra, 1991). It can be modified and used in order to optimize phytoplankton production in larger volumes for the feeding of breeding larvae.

In its actual configuration, it is a tool adapted for time dependent experiments on the physiology of phytoplankton. By maintaining the cultures during long times, it is possible to study the adaptation processes which are induced by successive perturbations in the milieu. The excretion of nitrite (and the subsequent reabsorption) which follows a pulse of nitrate added to nitrogen-limited cells of *Prorocentrum minimum* reflects their inability to assimilate nitrate at low growth rate (Collos, 1982). The importance of this phenomenon (i.e. rate of nitrite excreted versus nitrate absorbed), and the time necessary for the cells to recover their ability to assimilate external nitrate correctly depends on the degree of the cell limitation (Martinez, 1991).

Under severe nitrogen deprivation, uptake becomes non-linear (Harrison *et al.*, 1989) and cannot be described by Michaelis-Menten equation. Since the form of the non linearity (Fig. 5) depends on the physiological condition of the cells, pulse experiments have to be made with cells at different growth rate and having experienced various past histories of nitrogen feeding. Such experimental conditions can be obtained with our culture system, which can be programmed to perform pulses of the limiting factor at different frequencies and amplitudes. Then, it becomes possible to relate the instantaneous growth rate not only to the concentration of the limiting factor, but also to its mode of variation in the milieu (Sciandra, 1991). The difficulty in interpreting the non-linear variations of growth and nutrient uptake and the uncoupling between these two processes can be reduced by increasing the frequency of data acquisition, so that the measured signal becomes almost continuous. Figure 6 shows that the *in vivo* fluorescence measured by the automatic system reflect a cellular chromatic adaptation which is complex and rapid in *Prorocentrum minimum* (Harding *et al.*, 1983). During such transient changes induced by variable light regime, fluorescence cannot be used as a satisfactory indicator of the biomass when the cells are

nitrogen-limited (Fenaux *et al.*, 1985). Such data are that needed to provide useful support for modeling the complexity of the adaptive processes in phytoplankton. Moreover, automation in collecting standard data on variables such as nutrients, fluorescence and cell concentration, makes it easier for other measurements (internal quota, enzymatic activities) to be carried out at the same time.

The automatic culture system presented here is well adapted to support competition experiments, since it is able to discriminate between different cell sizes. Moreover, as underlined by Grover (1990), because competitive exclusion proceeds slowly when resource supply is variable, experiments must run for several weeks to allow competitive dynamics to be discerned, again suggesting the advantage of automation.

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