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NOTE

Phytoplankton potential growth rate versus increase in cell numbers: estimation of cell lysis

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ABSTRACT: The *in situ* growth rate of phytoplankton is defined as the potential growth rate without adjustment for losses due to cell mortality when it is measured by the cell cycle approach. In contrast, the method that assesses the cell number variation of the population in diffusion chambers takes cell mortality into account. We compare the 2 methods for natural blooms of dinoflagellates (*Alexandrium minutum*, *Alexandrium taylori*, *Ceratium furca*, *Dinophysis sacculus*, *Gymnodinium pulchellum* and *Gyrodinium corsicum*) grown in various situations and locations and data reported elsewhere. The *in situ* potential growth rates showed great intraspecific variability. The use of the differences between the 2 methods as a measure of *in situ* cell lysis is discussed.

KEY WORDS: Dinoflagellate · HABs · Growth rate · Loss rate

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Efforts in modeling harmful algal bloom (HAB) dynamics focus on the interactions between biological and physical processes. When testing a model against field data, the latter has many limitations, such as lack of spatial and temporal resolution and inappropriate criteria for the selection of the variables to measure (Franks 1997). Few studies report *in situ* specific growth rates during major events such as dinoflagellate proliferation (Chang & Carpenter 1985, Reguera et al. 1996), since spatial and temporal monitoring of such events is extremely difficult. A lack of *in situ* growth and loss rates hinders modeling and thus the prediction of HABs.

Two methods are currently used to calculate growth rates: the cell cycle method (the mitotic index) (McDuff & Chisholm 1982, Carpenter & Chang 1988) and the cell number method, where the increase in cell number either in diffusion chambers (Furnas 1982) or in cultures is followed.

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The cell cycle method (the mitotic index) defines the phytoplankton growth rate as potential growth rate without adjustment for losses due to cell mortality (Chang & Carpenter 1988, 1990), whereas the changes in cell numbers experienced by a population, either in diffusion chambers or in cultures, take into account losses of population as cell mortality. The 2 methodologies (potential growth rate and changes in cell numbers) have seldom been compared. Moreover, most data are from culture experiments (Olson & Chisholm 1986, Chang & Carpenter 1988, 1991, Antia et al. 1990, Pan & Cembella 1998, Peperzak et al. 1998). The aim of the present paper is to review previous data and add new results from *in situ* measurements. We would like to point out that we contributed more than 80% of the data available from *in situ* studies.

Material and methods. To measure the *in situ* growth rate by cell cycle and changes in cell numbers (diffusion chamber method), experiments were carried out on several dinoflagellate proliferations, mainly in Alfacs Bay (Ebro Delta, NW Mediterranean Sea) and on the Catalan Coast (Table 1; Garcés et al. 1997, 1998, 1999, Garcés 1998).

The *in situ* potential growth rates were measured by taking phytoplankton samples of 2.5 l every 2 to 3 h for 24 h. Microfluorometry was then applied to measure the relative nucleic acid content of cells or to monitor the phase frequency of the cell cycle (as cytokinesis or recently divided cells as in the case of *Dinophysis*). The S and G_2M phases were used as the terminal event with the equation:

$$\mu = \frac{1}{n(T_S + T_{G2M})} \sum_{i=1}^{n} (t_S) \ln[1 + f_S(t_i) + f_{G2M}(t_i)] \quad (1)$$

where μ is an estimate of the *in situ* growth rate from DNA synthesis on Day 1, $f_S(t_i)$ and $f_{G2M}(t_i)$ are the fractions of cells in phases S and G2M at time t_i, respectively, n is the number of samples collected during 1

Table 1. Locations and times of the year for which the cell cycle method was applied to the different species in the Mediterranean area

	Location	Time of the year	Photoperiod (h)	Surface water temperature (°C)	μ (d^{-1})	Source
Alexandrium minutum	Arenys Harbour	Feb	10:14	15	0.25–0.42	Garcés (1998)
Alexandrium taylori	La Fosca	Aug	14:10	27	0.4–0.5	Garcés et al. (1998)
Dinophysis sacculus	Delta Ebro	May–Jun	15:9	20	0.13–0.24	Garcés et al. (1997)
		Oct	11:13	17	0.11	Garcés et al. (1997)
Ceratium furca	Delta Ebro	Dec	9:15	12	0.14	Garcés (1998)
		Jan	10:14	8	0.13	Garcés (1998)
		Feb	10:14	13	0.2–0.37	Garcés (1998)
Gymnodinium pulchellum	Delta Ebro	Jan	10:14	8	0.36	Garcés (1998)
Gyrodinium corsicum	Delta Ebro	Dec	9:15	12	0.94	Garcés et al. (1999)
		Jan	10:14	8	0.6	Garcés et al. (1999)
		Feb	10:14	13	0.39–0.59	Garcés et al. (1999)

daily cycle, T_S and T_{G2M} are the durations of the S and G2M phases, respectively, t_S is the interval in hours and t_1 and t_2 correspond to the peak values in the number of cells in the S and G2M phases, respectively. The duration term (T) was calculated by the following equation:

$$(T_S + T_{G2M}) = 2(t_2 - t_1) \quad (2)$$

where t_1 and t_2 were determined from the time between the peaks of the S and G2M phases, estimated by fitting fourth-degree polynomial curves to the data (Carpenter & Chang 1988, 1990).

To measure the changes in cell numbers (diffusion chamber method), water samples were incubated using 500 ml diffusion cages with a permeable surface of 50 cm^2 (10 µm mesh). The samples were pre-filtrated with a 150 µm mesh to exclude grazers. The chambers were incubated at 0.5 m depth, and dinoflagellates were exposed to natural irradiance, temperature and photoperiod for 24 h. Initial and final samples were removed from the cages. Accurate counts of the incubation samples were performed and changes in cell number were measured (Guillard 1973):

$$\mu = \frac{1}{(t_2 - t_1)} \ln \frac{N_2}{N_1} \quad (3)$$

where μ is the net growth rate in d^{-1} and N_2 and N_1 are the cell concentration at t_2 and t_1 respectively.

Results and discussion. Our results show that the intraspecific spatial-temporal variability of *in situ* potential growth rates was highly significant (Table 1); for example, the potential growth rate of *Gyrodinium corsicum* ranged from 0.3 to 0.9 d^{-1}. Moreover, the growth rates measured at different locations at the same time varied by a factor of 1.5, showing that the growth rate reflected the response of organisms to the environment (Garcés et al. 1999). In the same *in situ* conditions, the potential growth rate of a co-occurring dinoflagellate, *Ceratium furca*, was between 0.1 and 0.37 d^{-1}.

Some dinoflagellates do not grow well in enclosures, which leads to an underestimation of the growth rate calculated from changes in cell number. It is out of the scope of the present paper to discuss these limitations—that has been done before (Chang & Carpenter 1988, 1990, 1991)—but some specifications are necessary. The natural population may divide actively, but the enclosure is not a suitable environment for measuring growth rate. For example, incubations of high cell densities (as in the case of huge dinoflagellate proliferations) directly influence growth conditions and can produce an effect of no growth (Agustí & Kalff 1989), while the natural population would be dividing actively. This is what happens with *G. corsicum*, which produces a high amount of mucus. Thus, the growth rate measured in incubation cages is not appropriate in these cases, and so it will not be considered in the following discussion.

The relationship between potential growth rate by cell cycle and growth rate by cell increase, in either cultures or diffusion chambers, is shown in Fig. 1, where the 2 lines show no losses (1:1) and maximum loss rate found (0.4 d^{-1}). The concept of phytoplankton death or mortality is understood to encompass all phytoplankton losses, which includes all the processes that remove phytoplankton cells from the system (grazing, cell lysis, dispersion, sinking). We can assume that the difference (loss rate) between potential growth and cell increase is only due to physiological death and subsequent cell lysis, since other sources of loss (like grazing) have been avoided. In addition, it can be considered that loss due to sinking is negligible, because we are dealing with dinoflagellates.

Cell lysis may result from various factors in natural populations, including attack by parasites (Noren et al.

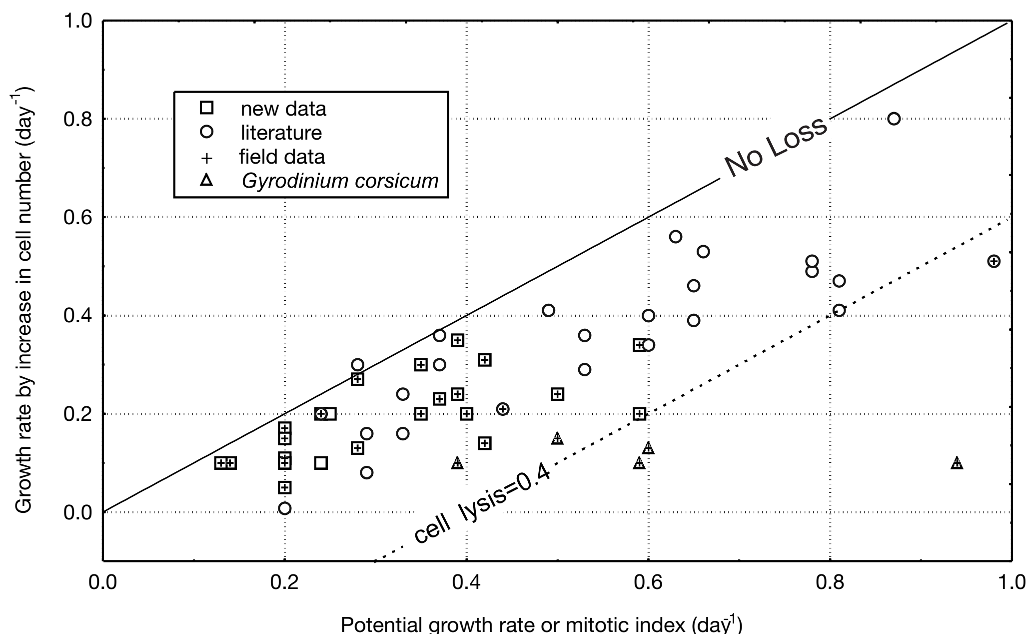

Fig. 1. Association between potential growth rate by cell cycle (mitotic index) and growth rate by increase in cell number. Lines have been added to show the bounds of no losses and cell lysis of 0.4 d^{-1}

1999, Erard Le Denn et al. 2000), viruses (Suttle 1994), bacteria (Doucette 1995) and fungi. Phytoplankton cell lysis results in the release of the cell contents and therefore in an increase in nutrient-rich dissolved organic components. Furthermore, cell lysis appeared to be the third major loss factor determining algal bloom dynamics (Brussaard et al. 1995). Despite the potential importance of cell lysis, field studies on cell lysis rates are scarce (van Boekel et al. 1992, Agustí et al. 1998). Agustí et al. (1992) found ranges from 0.026 to 1.9 d^{-1} in the Mediterranean phytoplankton community; cell lysis rates were higher in surface waters, where gross growth rates were also higher and biomass lower than in the deep chlorophyll maximum that characterize the area.

Not only are there very few field studies on cell lysis rates, but very little is known about *in situ* growth and loss rates of phytoplankton at the species level (e.g., Furnas 1990). The problems in quantifying species-specific growth and loss rates in a natural plankton assemblage are in part due to the multitude of processes involved. Various techniques have been used to measure phytoplankton cell lysis (dissolved esterase activity assay, direct cell counts and viability assays), but at present there is still a lack of reliable methods to quantify the process. One of the major problems is confusion about the definition of cell death. For instance the significance of internally driven mortality (apoptosis) is still unclear. An approach that can provide the complete set of growth and loss parameters for a particular phytoplankton species is to combine the cell-cycle analysis (potential growth rate) (Carpenter & Chang 1988) in field with the actual changes in cell numbers.

Our compilation of the 2 methods to measure growth rate, both in culture and in the field, suggest a wide range (0.01 to 0.4 d^{-1}) of cell lysis rates (Fig. 1). The fact that differences between growth rates are significant and can reach 50% indicated that this loss rate is significant in nature. Concurrent measurements of growth rates and realised increases in cell numbers may provide useful constraints for HAB models.

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