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EFFECT OF TEMPERATURE, SALINITY, LIGHT AND SELECTED NUTRIENTS ON THE GROWTH OF THE DINOFLAGELLATE GYMNODINIUM CATENATUM (GRAHAM) UNDER CULTURAL CONDITIONS

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Key Words: *Gymnodinium catenatum*, **cultural conditions**

ABSTRACT

*The chain-forming dinoflagellate, **Gymnodinium catenatum**, was cultured from vegetative cells isolated from Mex Bay, west of Alexandria (Egypt). Its optimal growth occurred at a temperature of 25°C, 30 salinity and light intensity of 500 $\mu E\ m^{-2} s^{-1}$ (12:12 LD cycle). The growth rate (μ_2) during the exponential phase varied between 0.19 and 0.57 d^{-1}, mean doubling time 2.8 - 3.8 days. Limited growth was observed at the low temperature 15°C and the high salinity of 35. Low light illumination in the dark-light cycle caused higher growth than the continuous high light. This dinoflagellate grew best at a combination of ammonia and phosphate concentrations (doubling time 0.97 - 1.10 days). Implication for the bloom dynamics of G. catenatum in the neritic waters of Alexandria was discussed. The results of the present cultural experiment coincided well with the field observations.*

INTRODUCTION

The first reported outbreaks of paralytic shellfish poisoning attributed to the unarmoured, chain-forming dinoflagellate, ***Gymnodinium catenatum*** occurred in 1976 in Spain (Estrada ***et al.***, 1984), in 1979 in Mexico (Morey-Gaines

1982), in 1986 in Portugal (Franca and Almeida 1989), Japan (Ikeda ***et al.***, 1989), and Tasmania, Australia (Hallegraeff and Sumner 1986).

A few years ago, ***Gymnodinium catenatum***, was first recorded from the coastal waters of Alexandria (Egypt), where its occurrence and ambient environmental conditions were followed during the 4 years survey 1993 - 1996 (Labib 1998). This study showed ***Gymnodinium catenatum*** to achieve its massive red tide blooms during summer-early autumn, with low salinity, density stratified water column and wide range of nutrient concentrations. Apparently no cases of toxicity have accompanied its blooms in Alexandria waters.

I report here about the effect of temperature, salinity gradients and light intensities on the growth of ***Gymnodinium catenatum*** under cultural conditions, as well as its growth stimulation by selected nutrients, in order to determine the relative importance of these factors for its population dynamics in Alexandria waters.

MATERIAL AND METHODS

Mex Bay, west of Alexandria, is subjected to daily land-runoff from different sources, mainly agricultural waste waters (6.5×10^6 m^3 d^{-1}). Discharges into the bay are largely the cause of eutrophication (Labib 1997).

Water samples (2 L) were collected at 50 cm below the surface from Mex Bay during May 1996. Cells of ***Gymnodinium catenatum*** were isolated and clonal cultures were grown on f/2 medium (Guillard and Ryther 1962), at a temperature of 20°C, salinity of 25 PSU, with overhead illumination of 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ from white fluorescent lights in a daily cycle of alternated 12:12h light/dark period. Then the filtered (through a Whatman type C glass fiber filter) - autoclaved Mex Bay seawater samples was used for the cultural experiments, with nutrients added according to the medium f/2. These experiments were carried out to determine the growth of ***Gymnodinium catenatum*** at temperature of 15, 20, 25 and 30°C, salinity of 20, 25, 30 and 35 PSU and irradiance of 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ (continuous light), and 12:12 LD cycle of 200 and 500 $\mu E \cdot m^{-2} \cdot s^{-1}$. Triplicate 60ml cultures were prepared and the initial cell density was counted. Subsamples of 2 ml were fixed in Lugol's solution

and counted every two days from the start of each experiment in a counting chamber. Each subsample was counted in triplicate. A counting error of <12 was calculated.

On the other hand, the effect of selective nutrient concentrations on the stimulation of the growth of ***Gymnodinium catenatum*** was tested when nutrient concentrations were added to the filtered Mex Bay seawater to the final concentrations given in Table1. The filtered seawater was also used as a control. The initial concentrations of nitrate, ammonia and phosphate in the filtered seawater were determined according to Strickland and Parsons (1972). These cultures were grown at 25°C, salinity of 30 PSU and 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ (12:12LD cycle).

Table 1. Nutrient concentrations for enriched cultural experiments

Substance	Nutrient symbol	Final concentration (μg-at. l^{-1})
$NaNO_3$		
	$NO_3(1)$	5
	$NO_3(2)$	10
NH_4CL		
	$NH_4(1)$	10
	$NH_4(2)$	20
	$NH_4(3)$	50
$NaH_2PO_4.H_2O$		
	$PO_4(1)$	5
	$PO_4(2)$	10
	$NO_3(1), PO_4(1)$	
	$NO_3(2), PO_4(2)$	
	$NH_4(1), PO_4(1)$	
	$NH_4(2), PO_4(2)$	

Growth rate (μ_2) and doubling time (D.T) of *G. catenatum* were estimated from the following equation (Weiler & Eppley 1979):

$$\mu_2 = 1/t \text{ Ln } (N_1/N_0); \quad \text{D.T } d^{-1} = \text{Ln2} / \mu_2$$

where N_1 and N_0 were the cell counts at times t_1 and t_0, respectively.

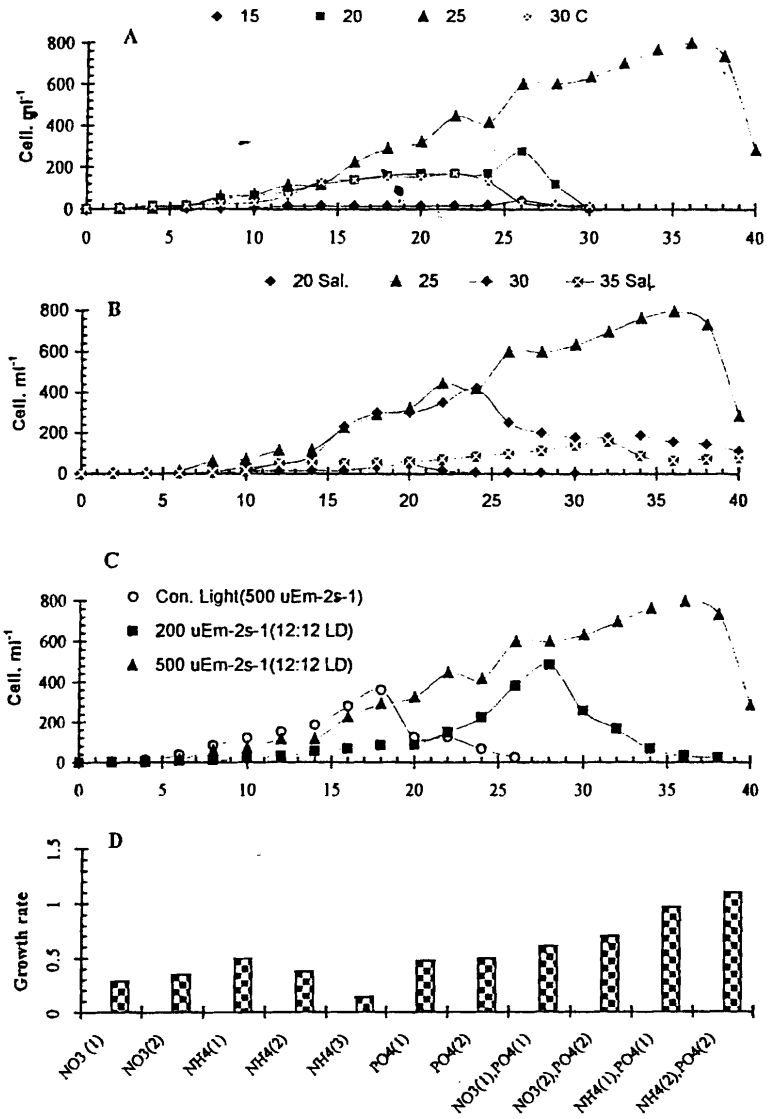

Figure 1. Growth responses of *Gymnodinium catenatum* to temperature (A), salinity (B), light (C) and nutrients (D)

Growth as a function of nutrients

The effect of selective nutrient concentrations on the growth of *G. catenatum* is shown in Fig. 1 D.

The initial nutrient concentrations in the filtered seawater were 1.5 µg-at. l^{-1} for nitrate-N, 2.5 µg-at. l^{-1} for ammonia-N and 2 µg-at. l^{-1} for phosphate-P.

The growth rate of *G. catenatum* in the control was very limited (0.15 d^{-1}, doubling time 4.6 days, final cell concentration of 51 cell. ml^{-1}). It was enhanced by the individual addition of NO_3 (1) and NO_3 (2), >2 times greater than the control, 3.3 times by NH_4 (1), 2.5 times by NH_4 (2), 3.2-3.3 times by PO_4 (1) and PO_4 (2), respectively. The growth was stimulated by the addition of combined nitrate and phosphate (0.61-0.70 d^{-1}, doubling time 0.9-1.03 days), but remarkably by ammonia and phosphate (0.97-1.1 d^{-1}), final cell concentration of 810 cell. ml^{-1}).

DISCUSSION

Cells of ***Gymnodinium catenatum*** in the present study are more elongate (40-50µm) than those of wild Spanish population (31-39 µm, Fraga and Sanchez 1985), but smaller than those of Japanese cultures (Yuki and Yoshimatsu 1987), as well as those from estuarine waters of Australia (Blackburn ***et al.***, 1989). This strain produces short-chains (up to 4 cells long) during those experiments. Fraga *et al.* (1989) reported those long chains of *G. catenatum* and ***Alexandrium affine*** swam faster than short chains and speculated that chain formation might be a mechanism for producing dense local concentrations of cells.

Gymnodinium catenatum showed characteristic growth responses under the tested cultural conditions, using f/2 medium. The optimum growth occurred at a temperature of 25°C, 30 salinity and light intensity of 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ (12:12 LD cycle). The growth was poor at 15 and 30°C (5 and 21.5% to the maximum growth, respectively), salinity of 20 and 35 (39.4 and 28.9% to the maximum, respectively). The division rate at the continuous light was about 61% of the maximum growth occurred with 12:12LD cycle. Meanwhile, low light illumination in the light-dark cycle caused higher growth rate than the continuous high light. However, the growth was faster at the continuous light. Comparing the present data with others, Bravo (1986) demonstrated doubling

time of 2.5 days for ***G. catenatum*** of Spanish strain in a medium including soil extract. Blackburn ***et al.*** (1989) reported that the growth of ***G. catenatum***, of estuarine strains cultured on GPM medium of Loeblich (1975), was optimal (mean doubling time 3 - 4 days) at temperature from 14.5-20°C, salinity of 23-34 and light irradiances of 50-300 $\mu E\ m^{-2} s^{-1}$. On the other hand, the toxic dinoflagellate, ***Alexandrium tamarense***, also forming chains, showed a temperature tolerance of 13-23°C (Watras ***et al.***, 1982), and different salinity optima (15-23, Prakash 1967; 20-40 salinity, White 1978). These differences presumably reflect existence of different physiological races.

The growth rate of ***G. catenatum*** was less affected by the single addition of nitrate, ammonia and phosphate than their combination. The growth stimulation by nitrate was less than that by the addition of ammonia. Yet, 50 $\mu g\text{-at. } l^{-1}$ of ammonia depressed the growth to its minimum ($0.14\ d^{-1}$). This agrees with the finding of Takahashi and Fukazawa (1982) for the growth of a ***Gymnodinium*** sp.

The ecological study of the massive occurrence of ***G. catenatum*** in the neritic waters of Alexandria during 1993-1996 (Labib 1998) declared its bloom peaks to take place at a narrow temperature range of 25-26.8°C and low salinity of 22-26, while the ambient nutrient concentrations, particularly ammonia exhibited large fluctuations. Nitrate fluctuated between 3.5-7.4 $\mu g\text{-at. } l^{-1}$, ammonia 4.2-20.3 $\mu g\text{-at. } l^{-1}$ and phosphate 3.8-8.2 $\mu g\text{-at. } l^{-1}$. This study failed to determine factor/s triggering its blooms, the coastal waters of Alexandria are subjected to daily discharge of different amounts of waste waters. However, the present cultural experiments showed coincidence with the previous ecological study. Hallegraeff ***et al.*** (1989) reported the occurrence of ***G. catenatum*** to be closely linked with nutrients input from land-runoff, forming a bloom in southern Tasmanian waters (Australia) at a temperature range 12-18°C and salinity of 28-38. This species disappeared from the water column when temperature fell below 11-12°C. A very low growth was observed at 15°C during the present study.

Since there are instances in which the effect of one factor may be modified by the presence of another factors, the effect of combination of factors will be considered in future studies.

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REFERENCES

Blackburn, S. I., Hallegraeff, G. M. and Bolch, C.J., 1989. Vegetative reproduction and sexual life cycle of the toxic dinoflagellate ***Gymnodinium catenatum*** from Tasmanian, Australia. *J. Phcol.*, **25**: 577-90.

Bravo, I., 1986. Germinacion de quistes, clutivo y enquistamient ***Gymnodinium catenatum*** Graham. *Invest. Pre.* **50**: 313-21.

Estrada, M, Sanchez, F. J. and Fraga, S., 1984. ***Gymnodinium catenatum*** (Graham) en Las rias gallegas (NO de Espana). *Invest. Pesq.* **48**: 31-40.

Fraga, S., Sanchez, F. J., 1985. Toxic and potentially toxic dinoflagellates found in Galician Rias (N.W. Spain). In: *Toxic Dinoflagellates*, Anderson, D. M., A.W. White and D. G. Baden (eds.), Elsevier, Amsterdam, 51-54.

Fraga, S.; Gallager, S.M.; and Anderson, D. M., 1989. Chain-forming dinoflagellates: an adaptation to red tides. In: *Red Tides: Biology, Environmental Science and Technology*. Okaishi, T., D. M. Anderson and T. Nemoto (eds.), Elsevier, New York, 281-84.

Franca, S., Almeida, J.F., 1989. Paralytic shellfish poisons in bivalve molluscs on the Portugese coast caused by a bloom of the dinoflagellate ***Gymnodinium catenatum***. In: *Red Tides: Biology, Environmental Science and Technology*. Okaishi, T., D. M. Anderson and T. Nemoto (eds.), Elsevier, New York, 93-96.

Guillard R. R. and Ryther, J. H., 1962. Studies on marine planktonic diatoms.I. ***Cyclotella nana*** Husted and ***Detonula confervaceae*** (Cleve) Gran. *Can. J. microbio.* **8**: 229-39.

Hallegraeff, G. M., Sumner, C., 1986. Toxic plankton blooms affect shellfish farms. *Aust. Fish.* **45**: 18-28.

Hallegraeff, G. M., S. O. Stanley, C. J. Bolch, S. I. Blackburn, (1989). ***Gymnodinium catenatum*** blooms and shellfish toxicity in southern Tasmanian, Australia. In: *Red Tides: Biology, Environmental Science and*

Technology. Okaishi, T., D. M. Anderson and T. Nemoto (eds.), Elsevier, New York, 77-80.

Ikeda, T., Matsuno, S.; Sato, S.; Ogata, T.; Kodama, M.; Fukuyo, Y. and Takayama, H., 1989. First report on paralytic shellfish poisoning caused by ***Gymnodinium catenatum*** (Dinophyceae) in Japān. In: *Red Tides: Biology, Environmental Science and Technology*. Okaishi, T., D. M. Anderson and T. Nemoto (eds.), Elsevier, New York, 411-414.

Labib, W., 1997. Eutrophication in Mex Bay, Alexandria (Egypt). Environmental studies and statistical approach. *Bull. Nat. Inst. of Oceanogr. & Fish.*, A.R.E., **23**: 49-68.

Labib, W., 1998. Occurrence of the dinoflagellate ***Gymnodinium catenatum*** (Graham) along the Mediterranean coast of Alexandria (Egypt). *Chemistry and Ecology*. **14**: 133-41.

Loeblich, A. R., 1975. A seawater medium for dinoflagellates and the nutrition of ***Cachonina niei***. *J. Phycol.*, **11**: 80-86.

Morey-Gaines, G., 1982. ***Gymnodinium catenatum*** (Dinophyceae): morphology and affinities with armored forms. *Phycologia*, **21**: 154-63.

Prakash, A., 1967. Growth and toxicity of a marine dinoflagellate, ***Gonyaulax tamarensis***. *J. Fish.Res. Bd.Can.*, **24**: 1593-1604.

Strickland, J. D. and Parsons, T.R., 1972. A practical handbook of sea water analysis. 2nd *Bull. Fish. Res. Bd. Canada*, **167**, pp 310.

Takahashi, M. and Fukazawa, N., 1982. A Mechanism of Red Tide Formation. II. Effect of selective nutrient stimulation on the growth of different phytoplankton species in natural water. *Mar. Biol.*, **70**: 267-73.

Watras C. J.; Chisholm, S. W. and Anderson, D. M.; 1982. Regulation of growth in an estuarine clone of ***Gonyaulax tamarensis*** Lebour: salinity-dependent temperature responses. *J. Exp. Mar. Biol. Ecol.*, **62**: 25-37.

Weiler, C. S. and Eppley, R. W., 1979. Temporal pattern of division in the dinoflagellate genus *Ceratium* and its application to the determination of growth rate. *J. Exp. Mar. Biol. Ecol.*, **39**: 1-24.

White, A.W., 1978. Salinity effects on growth and toxin content of ***Gonyaulax excavata***, a marine dinoflagellate causing paralytic shellfish poisoning. *J. Phycol.* **14**: 475-79.

Yuki, K. and Yoshimatsu, S., 1987. Morphology of a thecate dinoflagellate ***Gymnodinium catenatum*** in culture. *Bull. Plankton Soc.* Japan, **34**: 109-17.