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# *Gymnodinium nagasakiense*, a red-tide forming dinoflagellate, and its culture medium*

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**Abstract** Seven culture media were examined for investigating the morphological changes of a red-tide forming dinoflagellate *Gymnodinium nagasakiense*. The Wilson-Collier medium was concluded to be most suitable because relatively higher growth rate, longer stationary phase and smaller variations in cell length and width of this species were obtained in culture experiments.

## 1. Introduction

*Gymnodinium* (Dinoflagellata) has been known as red-tide forming and toxin-producing phytoplankton (SPECTOR, 1984). DAVIS (1984) described *G. brevis* collected from Mexico Bay. Its bloom has frequently occurred along the east coast of USA and has become an important problem for marine products. Many researches relating to the toxicity of dinoflagellates have been published (e.g. TAYLER and SELIGER, 1979; ANDERSON *et al.*, 1985). A big bloom of *Gymnodinium nagasakiense*, a species observed in 12 coastal areas or inland bays of the western part of Japan (TAKAYAMA and ADACHI, 1984), occurred at Kumano-nada, off the southern coast of Kii Peninsula in 1984. This bloom caused the second biggest damage to the fisheries ever reported in Japan, and now intensive researches on this species are urgently required. However, studies on this species from the growth physiological point of view are limited at present to those by IIZUKA (1976, 1979).

In this paper, we describe the results of the examination of several culture media for investigating morphological variations during the growth, which will provide a basic information to the successive experiments on morphological variations of this species under the different environmental stress.

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## 2. Materials and methods

*Gymnodinium nagasakiense* used for this experiment was kindly offered from Fisheries Experiment Station of Tokushima Prefecture by the courtesy of Mr. Masao YOSHIDA. This clone was isolated from Harima-nada in May 1980 when a dense bloom of this species developed and covered this sea area, and maintained at the station using the culture medium SWM-3 (CHEN *et al.*, 1970) at 23°C under fluorescent lamps of 4,000 lux. The stock culture in our laboratory was maintained axenically using the Wilson-Collier (WC) medium (WILSON and COLLIER, 1955) at 20°C under fluorescent lamps of 6,000 lux with a photoperiod of 12 hr-light and 12 hr-dark and used as inocula in culture experiments. The natural seawater taken from the offing of Owase Bay, Mie Prefecture, was used for making enriched culture medium. It had a considerably neritic nature with a salinity of 34.280 ‰ and was considered to be water of a branch of the *Kuroshio*. It was aged for more than half a year before use.

A 10 m*l* aliquot of 14-day-old mother culture at the logarithmic phase was inoculated to 200 m of the following culture media and was cultivated axenically at 20°C under fluorescent lamps of 6,000 lux with a photoperiod of 12 hr-light and 12 hr-dark. The culture media examined were WC (WILSON and COLLIER, 1955), SW II (IWASAKI, 1961) and f/2-Si (GUILLARD, 1975)

which were nutrient-enriched natural seawater and $ASP_2$, $ASP_2$ NTA (PROVASOLI *et al.*, 1957), $ASP_6$ (PROVASOLI, 1963) and $ASP_{12}$ NTA (PROVASOLI, 1963) which were nutrient-enriched artificial seawater.

The cell number was counted under the light microscope (Olympus, Tokyo) using a Sedwick-Rafter counting chamber. The cell length and width used as indexes of the cell size were measured under the light microscope using a stage micrometer (Olympus, Tokyo) and an eyepiece ruler (Olympus, Tokyo) equipped to the ocular. The cell growth rate ($\mu_2$) in each culture medium was calculated by the following equation:

$$\mu_2=\frac{\ln(N_2-N_1)}{t_2-t_1},$$

where $N_1$ and $N_2$ are the cell number at the time of $t_1$ and $t_2$, respectively, during the logarithmic growth phase. Measurements were made at 3-day intervals when the culture was at the first half of the logarithmic phase.

## 3. Results

The variations of cell number during the growth observed in three different enriched natural seawater are illustrated in Fig. 1. WC medium supported good growth with no induction phase. The growth rate observed in this medium was 0.63/day and the logarithmic phase continued for 1.5 weeks. The cell number reached the maximum (5,500 cells/m*l*) 2.5 weeks after inoculation and thereafter decreased gradually to 1,300 cells/m*l* 8 weeks after inoculation. The growth in SW II medium was good during the logarithmic phase similarly to that in WC medium, but a rapid decrease of cell number was observed during the later stage of culture. The growth rate in this medium was 0.33/day during the logarithmic phase which continued for 2.5 weeks. The cell number reached the maximum (5,650 cells/m*l*) 3 weeks after inoculation. There was a large difference in the growth curves at the latter half of death phase between WC and SW II media. Contrary to these two media, f/2-Si medium did not support the growth.

In Fig. 2, the growth curves obtained in four kinds of artificial seawater media were illustrated. Growth was observed only in $ASP_2$ NTA medium, and other three media did not support growth. The increase of cell number in $ASP_2$

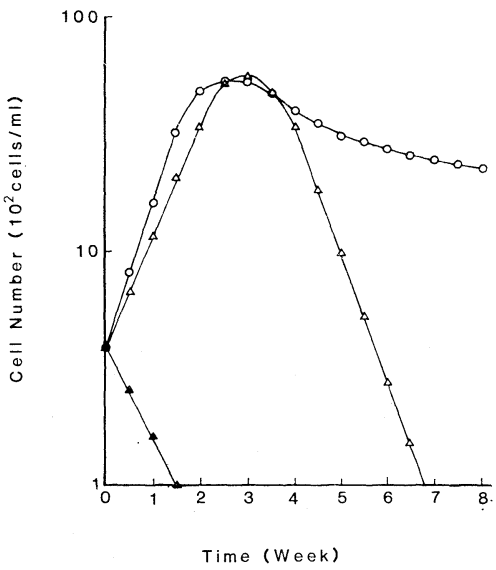


Fig. 1. The growth of *G. nagasakiense* in enriched natural seawater: Wilson-Collier medium (open circle), SW II medium (open triangle) and f/2-Si medium (solid triangle).

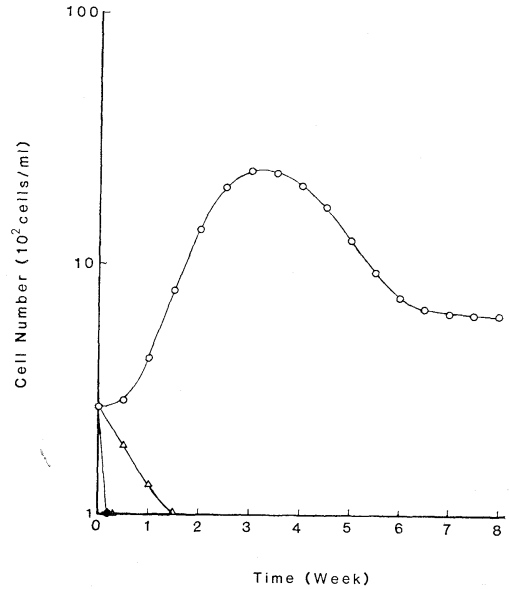


Fig. 2. The growth of *G. nagasakiense* in artificial seawater media: $ASP_2$ (solid circle), $ASP_2$ NTA (open circle), $ASP_6$ (open triangle) and $ASP_1$ (solid triangle).

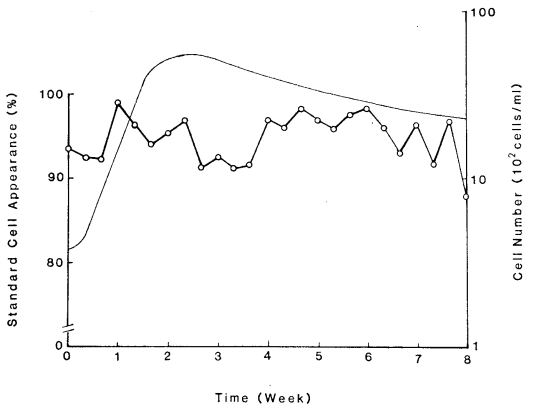


Fig. 3. Abundance of the standard cell (thick line) of *G. nagasakiense* in WC medium. Thin line indicates the cell number.

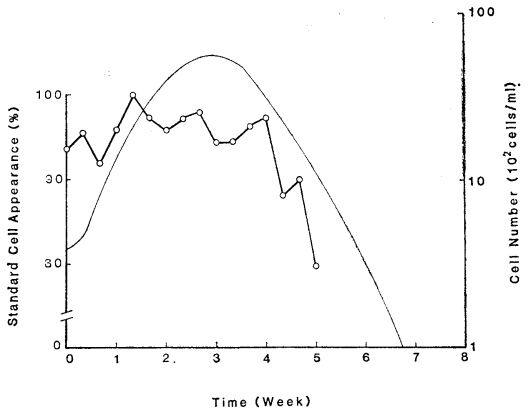


Fig. 4. Abundance of the standard cell (thick line) of *G. nagasakiense* in SW II medium. Thin line indicates the cell number.

NTA medium continued for 3 weeks after inoculation with an initial lag, and the growth rate during the logarithmic phase was 0.49/day which was considerably lower than the value in WC medium (Fig. 1). The maximum cell number (2,250 cells/m*l*) was observed 3 weeks after inoculation in $ASP_2$ NTA medium. This value is less than half that observed in WC and SW II media.

Abundance of the standard cell during the culture in WC medium is shown in Fig. 3. The standard cell in this paper was defined after the morphological description by TAKAYAMA and ADACHI (1984). In WC medium in which the present species of dinoflagellate was successfully cultured, the abundance of the standard cell from inoculation to the stationary phase was about 95 % (average value) with a little fluctuation. At the transition from the stationary to the death phase the abundance temporally decreased to around 92 %, but recovered to about 95 % during the later period of culture. In SW II medium (Fig. 4), a high abundance of the standard cell was observed from inoculation to the stationary phase, but the abundance decreased with aging of the culture.

The variations of cell size expressed by the cell length and width are shown in Fig. 5. The average cell length tended to increase until the middle of the logarithmic phase and then decreased until the end of that phase. A very slight decrease in the cell length continued until the end of the culture. The variations of the cell width showed the similar trend as observed in the cell length during the logarithmic phase.

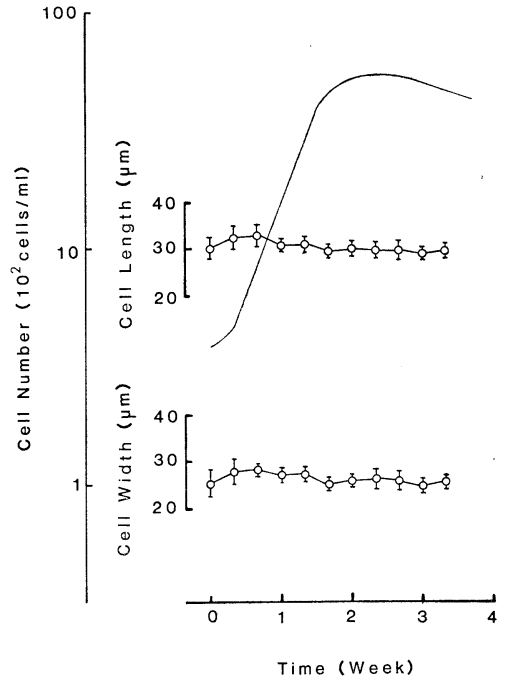


Fig. 5. Variations of the cell length and width of *G. nagasakiense* during the culture in WC medium. The vertical bar attached to the open circle indicates the standard deviation. Thin line shows the cell number.

## 4. Discussions

When cultured at 20°C under 6,000 lux with

a photoperiod of 12 hr-light and 12 hr-dark, the growth rate of *G. nagasakiense* in WC medium and SW II medium was 0.63/day and 0.33/day, respectively. The growth of this species was not complete in other media examined. The stationary phase continued until 8 weeks after inoculation in WC medium, whereas in SW II medium it ended 4.5 weeks after inoculation. IIZUKA and MINE (1983) reported the growth rate (0.64/day) of this species cultivated in Erd-Schreiber medium at 22°C under 0.05 ly/min, equal to 9,000 lux after WESTLAKE (1965). The present value (0.63/day) in WC medium was consistent with their value.

It is a well-known fact that morphology of the cell influentially changes with the physico-chemical environmental stress. In WC medium, the variations of the cell length and width were relatively small, and about 95 % of the cells were the standard cell during the culture period of 8 weeks. In SW II medium, on the other hand, a high percentage of the standard cell appeared from the logarithmic phase to the stationary phase, but the abundance of the standard cell decreased during the death phase.

The average cell length and width increased and fluctuated slightly during the logarithmic phase. As a whole, the range of variation of the cell length and width was within the values originally described by TAKAYAMA and ADACHI (1984).

Consequently, it is concluded that WC medium is one of the suitable media to observe the morphological change of the *G. nagasakiense* cells under different environmental conditions, because a relatively higher growth rate was obtained, a long period of the stationary phase was sustained and small variations of the cell length and width were observed in the medium.

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# 赤潮形成渦鞭毛虫類 *Gymnodinium nagasakiense* とその培養液

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**要旨**: 近年，西日本各地においては *Gymnodinium nagasakiense* の赤潮が発生し，水産上の被害が起こっている。本種の生物学的研究には適正な生育条件を満たす培養液が必要である。このために本報では2系列の7培養液（人工海水系—$ASP_2$，$ASP_2$-NTA，$ASP_6$，$ASP_{12}$-NTA，及び自然海水系—Wilson-Collier，SW II, f/2-Si）について検討した。培養液の評価にあたっては高増殖性，定常期の安定性，種の基本形態の出現の3点を重視した。イ）高増殖性についてはWilson-Collier（増殖率 0.63/day，以下同様），$ASP_2$-NTA（0.47/day）および SW II（0.33/day）の3者が優れており，最大密度が $5\times10^3$ cells/m*l* 以上に達したのは Wilson-Collier 及び SW II の2培養液であった。 ロ）定常期は SW II では短く，以後急速に減少するが，Wilson-Collier では長く，以後8週間目においても高密度で残存し，安定性があった。 ハ）基本形態の出現に関しては SW II より Wilson-Collier の方が優れ，高比率で安定した出現が得られた。以上の結果から *Gymnodinium nagasakiense* の培養には Wilson-Collier が最も適正な培養液であると推察された。