A Mechanism of 'Red Tide' Formation.
I. Growth Rate Estimate by DCMU-Induced Fluorescence Increase

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ABSTRACT: Growth experiments with unialgal cultures of 'red tide' flagellates Prorocentrum triestinum and Olisthodiscus luteus showed that relative growth rates of phytoplankton can be estimated from DCMU-induced fluorescence increase (DCMU-FI). This method was applied to the estimation of relative growth rate of a natural population in Tanigawa Harbor (Japan) where O. luteus was dominant throughout the experiment. Measurements were made three times a day over a week. A steady increase of photosynthetic capacity indicated by DCMU-FI was followed by a red tide of O. luteus. Such increase of photosynthetic capacity was associated with accumulation of ammonia and low dissolved oxygen levels in the water column near the bottom.

INTRODUCTION

'Red tide' is a massive accumulation of one or a few phytoplankton species in water. Such accumulation is believed to be associated with biological, chemical and physical processes (Yanagita, 1976). Two types of mechanisms are important: endogenous processes and interaction between cells, including different species and prey-predator relations.

Endogenous processes involve: (1) conditions allowing germination of cysts or resting cells; (2) growth controlling parameters of vegetative cells; and (3) conditions facilitating forming cysts or resting cells. Among these points, growth-controlling parameters under natural conditions are considered in the present study.

In laboratory experiments, growth rates can be followed in the same phytoplankton population throughout the experiment, even over a long period of time. However, it is impractical to follow a given phytoplankton population under natural conditions because of water movement, grazing, sinking and plankton mobility . It is necessary, therefore, to establish a suitable approach for estimating growth rate of natural phytoplankton populations, such that a result can be obtained within a short period of time.

The radioisotope technique, while promising, is now prohibited for use in the field in many countries because of potential radiation hazards. Samuelsson et al. (1978) found a positive relation between photosynthetic activity of phytoplankton and the increase in chlorophyll fluorescence following addition of DCMU [3-(3,4-dichlorophenyl)-1,1-dimethyl-urea]. DCMU is known to inhibit specifically the reoxidation of reduced Q, the primary electron acceptor of Photosynthetic Reaction Center II (Duysens and Sweers, 1963). While Q can quench the chlorophyll fluorescence, reduced Q does not. Thus chlorophyll fluorescence of phytoplankton cells increases when DCMU is added. In this paper, we term such increase of chlorophyll fluorescence DCMU photosynthetic capacity, because the increase tends to correspond to the photosynthetic capacity of phytoplankton cells (Samuelsson and Öquist, 1977; Samuelsson et al., 1978). A technique for measuring DCMU photosynthetic capacity was recently improved and applied to natural phytoplankton populations by Fujita and Ishimaru (1980). In the present study, the DCMU photosynthetic capacity was examined as a possible way for estimating growth rate of cultured red tide flagellates. Application of the DCMU photosynthetic capacity was further evaluated for estimating growth rates of natural phytoplankton populations. Growth-rate changes in natural phytoplanktion populations have also been examined in terms of corresponding changes in some environmental parameters.

MATERIALS AND METHODS

Two red tide flagellates, *Prorocentrum triestinum* (isolated from Tokyo Bay by T. Ishimaru in 1978) and *Olisthodiscus luteus* (isolated from Tanigawa Harbor by S.Yamochi in 1979), were studied in laboratory experiments in order to evaluate the feasibility of employing the DCMU photosynthetic capacity for estimating phytoplankton growth rates. Phytoplankton cells were inoculated into the f/2 medium (control, McLachlan, 1973) at the initial cell concentration of 10^2 cells ml^{-1}, and incubated at 25 °C under day-light type fluorescent lamps (6,000 lux). *P. triestinum* was exposed to continuous illumination, *O. luteus* to a L/D = 14/10 cycle. A medium in which the N-source was reduced to $^1/_8$ of the control value served as N-limit, the other medium of $^1/_2$ P as P-limit. *P. triestinum* grown in the f/2 medium was directly inoculated in three different media while *O. luteus* was precultured for 4 d in different media prior to the experiments.

Field observations were carried out in Tanigawa Harbor, Osaka Bay, where red tides caused by *Olisthodiscus* sp., *Skeletonema costatum*, *Eutreptiella* sp. and some dinoflagelltates had been reported (Yamochi et al., 1979).

DCMU was added to phytoplankton suspension to make up a final concentration of 10^{-5} mol l^{-1} and left in the dark for 30 min. Chlorophyll fluorescence was determined by a Turner 111–003 fluorometer equipped with a 2 cm (light path) flow cell. Energy distribution of the source lamp of the fluorometer was centered at 400–520 nm (Turner 110–853), and light passed through a filter (Corning 5–60) was used for excitation. Fluorescence was measured after passing through a filter (Corning 2–64); a flow-cell system was used. In the flow-cell system, a slit (5 mm in y axis) was inserted between flow cell and detector, so that each algal cell was exposed to the light for less than 0.2 s; this provided stable fluorescence during the measurement. Fluorescence of phytoplankton suspension without adding DCMU was also measured and served as control.

The following formula was used for DCMU photosynthetic capacity:

$$DCMU \text{ photosynthetic capacity} = \frac{F_d - F_o}{F_o} \quad (1)$$

where F_o and F_d = steady-state fluorescence intensity with and without the addition of DCMU, respectively.

DCMU photosynthetic capacity of natural populations collected from 0.5, 1.2, 2.5 and 3.5 m were measured three times (at sun-rise, noon and sun-set) each day for 1 week. Water samples were filtered through Whatman GF/C glass fiber filters and chlorophyll determined by the procedure recommended by SCOR-UNESCO (Strickland and Parsons, 1972). Phytoplank-

ton cell counts were made in samples fixed with glutaraldehyde (1 %) employing the inverted microscopic counting procedure (Utermöhl, 1958). Diel increasing rates of cells (μ_2) were estimated from cell counts by using the following formula:

$$\mu_2 = \frac{lnC_1 - lnC_o}{(T_1 - T_o) \, ln2} \quad (2)$$

where C_o and C_1 = cell number at times T_o and T_1, respectively.

RESULTS

Growth Rate Estimate by DCMU Photosynthetic Capacity

Both *Prorocentrum triestinum* and *Olisthodiscus luteus* grew logarithmically in all three media. There was not much difference in the growth at the log-phase, but the growth curve obtained under N-limit levelled off faster than the other two (Fig. 1). Cell yield was highest in the control and lowest in the N-limited medium with both phytoplankton species.
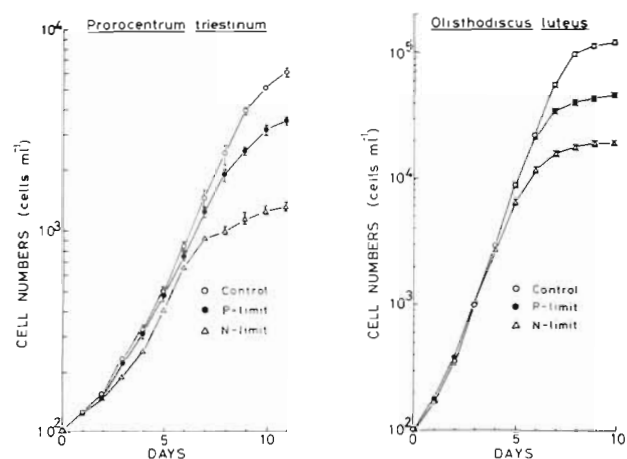

Fig. 1. *Prorocentrum triestinum* and *Olisthodiscus luteus*. Increase in cell numbers in three different media

Diel growth rates (μ_2) were determined based on day-to-day cell counts (Fig. 2). A similar continuous increase was observed in the three different media during the first 6 d for *Prorocentrum triestinum* and 3 d for *Olisthodiscus luteus*. Later, the growth rate of N-limited cells decreased rapidly.

A similar but less pronounced decrease occurred in P-limited cells. There was no significant difference in maximum growth rate in the three different nutrient regimes, but the control maintained the highest rate for a longer period of time, compared to the other two. There were slight reductions in growth rate, on the 2nd and 4th days, of *P. triestinum*. Such reductions were not observed in *O. luteus*.
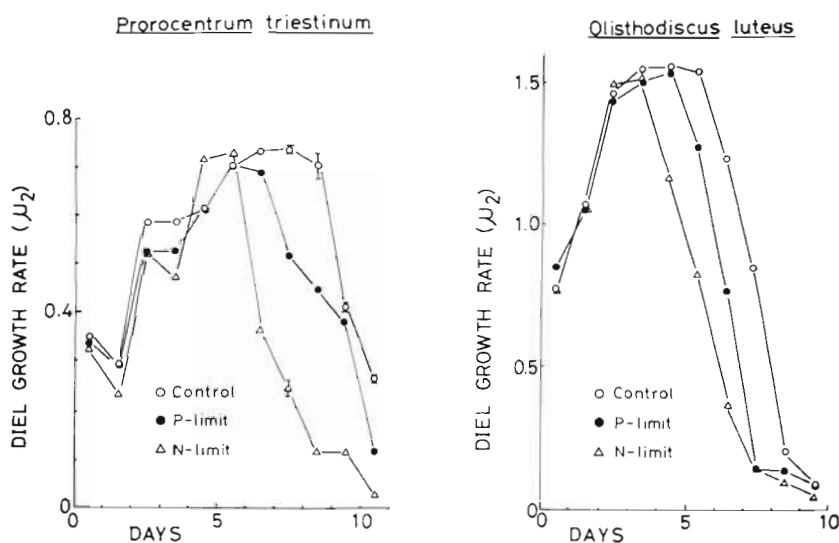
Fig. 2. *Prorocentrum triestinum* and *Olisthodiscus luteus*. Changes in diel growth rate (μ_2) in three different media
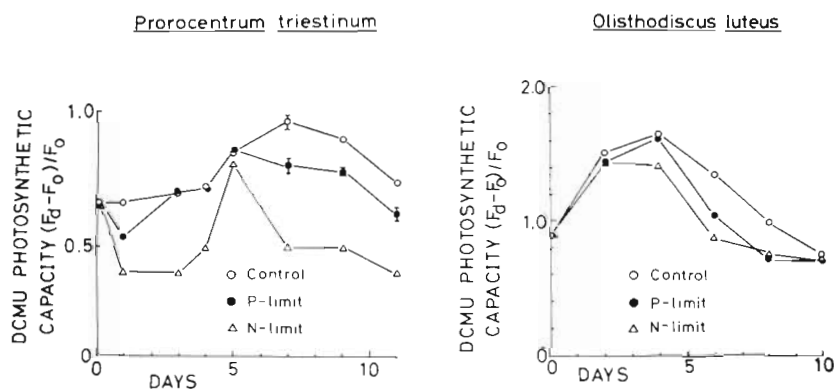

Fig. 3. *Prorocentrum triestinum* and *Olisthodiscus luteus*. Changes in DCMU photosynthetic capacity (F_d-F_o)/F_o in three different media

DCMU photosynthetic capacities showed changes similar to the diel growth rates (Fig. 3); this was particularly obvious in the controls. In the latter, DCMU photosynthetic capacities showed a continuous increase over the first 7 d in *Prorocentrum triestinum* and over the first 4 d in *O. luteus*; thereafter they decreased gradually. DCMU photosynthetic capacities initially dropped in N- and P-limited *P. triestinum*; the drop was particularly pronounced and prolonged in the N-limited medium. The drop observed in P-limited cells recovered quickly. There was no such initial drop in *O. luteus* when the cells were precultured for 4 d in each experimental medium. The DCMU photosynthetic capacity seems to respond more sensitively to environmental changes than the apparent growth rate.

There was a strong linear relation between diel growth rate and the DCMU photosynthetic capacity both in *Prorocentrum triestinum* and *Olithodiscus luteus* in the three different nutrient regimes, although during the first few days the data for *P. triestinum*

deviated somewhat, particularly in the N-limited medium (Fig. 4). When such deviations are excluded (possible effects of sudden environmental changes), there is no significant difference between curves in the three different media. Regression curves for *P. triestinum* and *O. luteus* are $y = 0.62x + 0.44$ ($r = 0.891$) and $y = 0.58x + 0.69$ ($r = 0.937$) respectively, where y = DCMU photosynthetic capacity and x = diel growth rate (μ_2).

Two differences were observed between the equations of the two phytoplankton species; they concerned the slope of the curve and the y-intercept. *Prorocentrum triestinum* showed a little steeper slope and a smaller y-intercept than *Olithodiscus luteus*. The potential significance of these differences must be evaluated in the furture. They may be related to differences in species or intracellular conditions.

Our results suggest that the diel growth rates of the phytoplankters tested can be estimated from their DCMU photosynthetic capacities. However, absolute
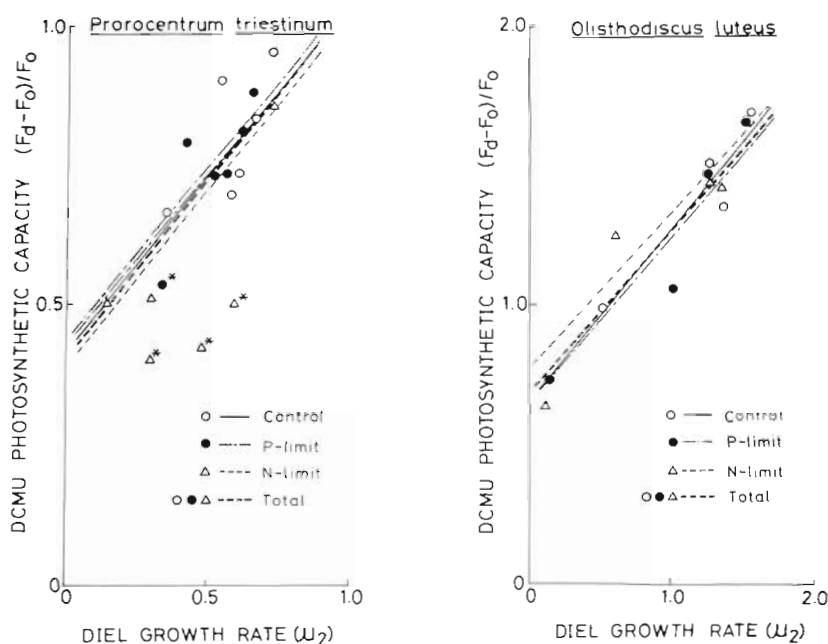
Fig. 4. *Prorocentrum triestinum* and *Olisthodiscus luteus*. Relations between DCMU photosynthetic capacity and diel growth rate. Data with asterisk were excluded for the regression analysis (see text). Regression curves for each medium are as follows: *P. triestinum*: Control, $y = 0.62x + 0.42$ ($r = 0.853$); N-limit, $y = 0.64x + 0.39$ ($r = 0.787$; P-limit, $y = 0.62x + 0.44$ ($r = 8.91$); total, $y = 0.64x + 0.41$ ($r = 0.825$). *O. luteus*: control, $y = 0.60x \times 0.66$ ($r = 0.932$); N-limit, $y = 0.54x + 0.78$ ($r = 0.937$); P-limit, $y = 0.59x + 0.65$ ($r = 0.950$); total, $y = 0.58x + 0.69$ ($r = 0.937$)

values of growth rates can hardly be obtained from DCMU photosynthetic capacities, and estimations may also be difficult when the dominance of the phytoplankton species tested vary during the experiment. However, as long as the phytoplankton populations are dominated by a single species, relative growth rates can be estimated on the basis of DCMU photosynthetic capacities.

Growth Rate Changes of Natural Populations Estimated by the DCMU Photosynthetic Capacity

Data on DCMU photosynthetic capacity, chlorophyll a and phytoplankton cell counts were obtained in Tanigawa Harbor on August 22 and 27, 1979. *Olisthodiscus luteus* was the dominant phytoplankter throughout the measurements although cell concentrations were too low (a few hundred cells ml^{-1}) to form a 'red tide' at the beginning of the experiment (Table 1). Visually obvious red tide appeared on August 25 with *O. luteus* attaining values over 6,000 cells ml^{-1} at the surface during the night.

Average chlorophyll concentrations in the water column slowly increased at a rate of $\mu_2 = 1.51$ d^{-1} from August 23 to 25; however the increase was not logarithmic but linear during that period (Fig. 5). A great increase was noticed on August 25; the rate $\mu_2 =$
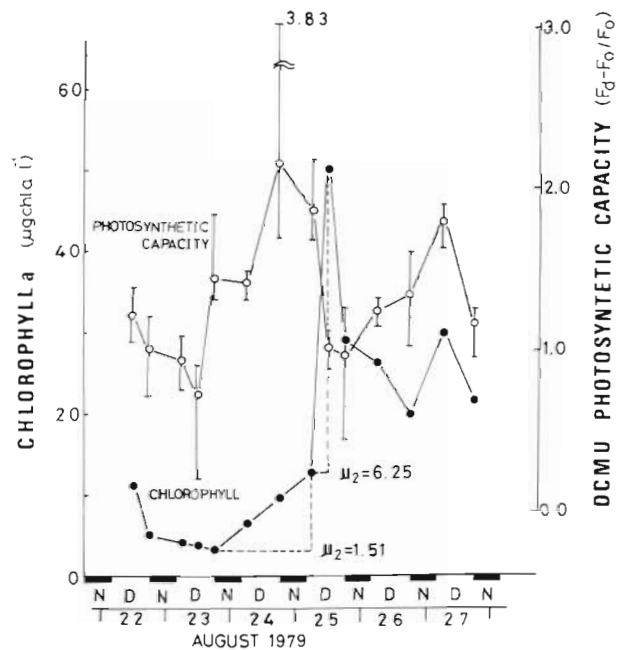

Fig. 5. Changes in DCMU photosynthetic capacity and chlorophyll a of natural phytoplankton in Tanigawa Harbor. Averages at four depths: 0.5, 1.5, 2.5 and 3.5 m. Vertical bars: minimum-maximum range of the four measurements. N night, D day

6.25 d^{-1} DCMU. Photosynthetic capacity started to increase in the afternoon of August 23 and reached a maximum in the evening of August 24. Thereafter, a

Table 1. *Olisthodiscus luteus.* Changes in cell numbers (cells ml⁻¹) in Tanigawa Harbor as a function of date, time and depth

Depth (m)	August 22 15:30	August 22 19:30	August 23 15:30	August 25 8:30	August 26 19:30
0.5	1310 (95)	130 (35)	540 (85)	2260 (85)	270 (30)
1.5	560 (45)	310 (50)	270 (30)	1430 (65)	1310 (80)
2.5	260 (10)	450 (60)	160 (25)	410 (40)	2310 (85)
3.5	190 (44)	550 (65)	100 (30)	210 (25)	2740 (50)
Average	580 (44)	360 (50)	270 (45)	1078 (53)	1658 (60)

Numbers in brackets: total cell numbers of phytoplankters species other than *O. luteus*
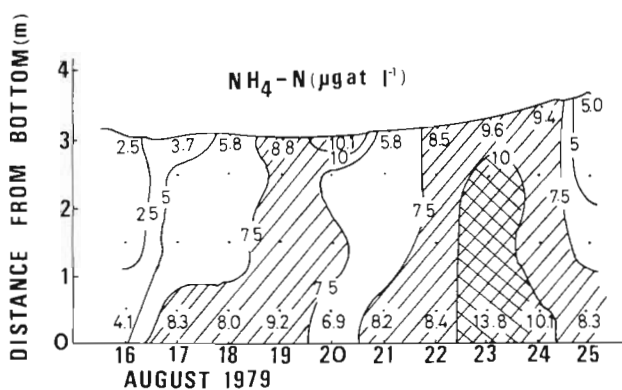
Fig. 6. Changes in ammonia in Tanigawa Harbor water. Dots indicate sampling depths. Ammonia concentrations shown for surface and near bottom. Isopleths of 2.5 μg at l⁻¹ intervals

rapid decrease occurred. Thus, a rapid increase of DCMU photosynthetic capacity occurred 2 d prior to red-tide formation; the capacity almost doubled within these two days.

DISCUSSION

Our laboratory experiments suggest that changes in DCMU photosynthetic capacity are strongly related to those of diel growth rate. The energy-yielding metabolism of microorganisms varies as a function of environmental conditions usually attaining maximum values in environments which fully satisfy their requirements for maximum growth. Photosynthetic activities of phytoplankters reveal a comparable trend (Ebata and Fujita, 1971). Since the DCMU photosynthetic capacity can be measured within an hour with a minimum amount of effort, this method is very suitable for estimating growth rates of natural populations. However, there are two restrictions: the method yields relative, not absolute growth rates and it specific or at least to be dominated by a single species. Possibly, these restrictions can be overcome in the future.

Since the phytoplankton in Tanigawa Harbor was clearly dominated by *Olisthodiscus luteus* throughout the experiment, the change in DCMU photosynthetic capacity is expected to indicate possible changes in relative growth rate. We observed an increase in DCMU photosynthetic capacity during August 23 and 24. At the same time chlorophyll a as well as cell numbers of *O. luteus* increased. This suggests that the relationship between the photosynthetic activity and the growth rate established in laboratory experiments can also be in the study of natural populations forming a red tide and that the variation in DCMU photosynthetic capacity can be used as indicator of the physiological state of such phytoplankters.

Yamochi et al. (1980) carried out extensive daily field observations between August 16 and 25 at the same station as that used for the present study. Day-to-day changes of various environmental parameters – such as temperature, salinity, dissolved oxygen, phosphate, nitrate and nitrite, ammonia and precipitation – obtained by Yamochi et al. (1980), reveal marked changes in ammonia (Fig. 6) and dissolved oxygen in the water column on August 23. Furthermore, an increase in ammonia towards the sea bottom and an opposite vertical profile for dissolved oxygen are obvious. Phosphate increased 1.5 times near the bottom. These environmental changes suggest suddenly increased decomposition or release of sediment substances into the water column. Under both conditions various kinds of metals including Mn and Fe would be released into the water as well as ammonia and phosphate (Hoshika et al. 1978). Abundant in bottom sediments of highly eutrophic waters Vitamin B_{12} (Kurata, 1969; Ohwada and Taga, 1969) would be released into the water column together with other nutrients. Organic chelators released from the bottom could significantly reduce possible heavy metal stress on red tide flagellate(s) (Anderson and Morel, 1978).

It is known from laboratory culture experiments that the growth of red-tide flagellates is specifically stimulated by minor nutrients such as metals, vitamins and some other organic compounds (Iwasaki, 1973, 1979).

These compounds generally exert no significant stimulative effect on phytoplankters other than red-tide flagellates. Uyeno and Nagai (1973) found a significant stimulation of cold water extract of bottom sediments on the growth of *Heterosigma (Chattonella) inlandica*, which is a red-tide flagellate similar to *O. luteus*; the effect was most pronounced during the low-oxygen period at the bottom. Uyeno and Nagai further mention that hot water extract (100 °C) had no stimulative effect. Iwasaki (1979) pointed out that all 3 species of *Chattonella* tested required vitamin B_{12} for growth; in some species growth was promoted by metals, nucleotides and plant hormones.

Our experiments and the information available from literature suggest that the growth of *Olisthodiscus luteus* in Tanigawa Harbor is selectively stimulated by substance(s) released from the bottom sediments under low-oxygen conditions. Red-tide occurrence is forecasted by changes in DCMU photosynthetic capacity. We were in fact able to forecast the occurrence of 'red-tide' 1 d before it actually appeared. DCMU photosynthetic capacity should be given more attention as a new criterion for forecasting 'red-tide'.

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