Sensitivity of Estuarine Phytoplankton to Hexavalent Chromium[1]

by

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ABSTRACT

Experiments were conducted in which we tested the effects of hexavalent choromium on both natural assemblages of phytoplankton and cultures of Thalassiosira pseudonana clone 3H. Water was collected from various locations in Yaquina Bay, Oregon, with salinities ranging from 32.5‰ to 0.03‰. The water was filtered, and a variety of nutrient regimes were tested by adding major nutrients and micro-nutrients, and/or stripping micro-nutrients with activated carbon. Inoculation of natural population experiments was with unfiltered water collected from the same location, added at 1:1000 dilution. In one high salinity experiment, chromium was stimulatory, as were micro-nutrient additions. In this experiment, two species and Nitzchia were stimulated. In other high and medium salinity experiments, chromium was neither stimulatory nor inhibitory, at levels up to 0.1 mg/L. However, in one high salinity experiment, where we added 1 mg/L chromium, there was some inhibition of growth due specifically to inhibition of Skeletonema costatum. In our low salinity experiments, chromium was very inhibitory at 0.1 mg/L, and slightly inhibitory at 0.01 mg/L. Species inhibited by chromium were Surirella ovata, Detonnal conferencia, and Cycletella sp. Nutrient regimes appeared to have no effect on chromium toxicity. Two experiments were conducted with T. pseudonana grown over a wide range of salinities. Chromium was found to be a very inhibitory at 0.03‰ and became progressively less toxic as the salinity increased. Most inhibition was neutralized by a salinity of 2.1‰.

INTRODUCTION

Chromium is an important trace constituent of industrial and domestic waste, yet there exists little information regarding its effects on natural freshwater, estuarine, or marine phytoplankton. Of the commonly occuring forms of chromium (III and VI), the hexavalent form seems to be the most toxic (Towill, et. al., 1978).

Almost all the work dealing with the effects of hexavalent chromium on algae has been conducted in freshwater. Patrick et al. (1975) found in freshwater that chromium levels of about 0.1 ppm inhibited diatom growth in a mixed population, and at about 0.4 ppm, diatoms were completely replaced by blue-green algae. In cultures of the freshwater algal species Nitzchia palea and Chlorella pyrenoidosa, Wium-Andersen (1974) found severe inhibition of growth at 0.3 ppm, moderate inhibition at 0.15 ppm, and slight inhibition at 0.05 ppm. Nollendorf et al. (1972) found inhibition of the growth of Chlorella sp. at chromium concentrations greater than 0.5 ppm. Selenastrum capricornutum was severly inhibited by chromium concentrations of 1.4 ppm or higher, was slightly inhibited at 0.14 ppm, and not inhibited at 0.014 ppm (Garton 1973). North et al. (1973) found that Scenedesmus was inhibited at 0.7 ppm, Navicula seminulum at 0.2 ppm, and Macrocystis pyrifera at 1.0 ppm. Clearly, hexavalent chromium is toxic at certain levels in freshwater, and the toxicity varies from species to species.

Little work has been performed on the effects of hexavalent chromium on marine algae. Hollibaugh et al. (1978) reported that chromium levels of 0.05 ppm were not toxic to natural populations of marine phytoplankton or

unialgal cultures of the marine species Thalassiosira aestivalis.

Toxicity of hexavalent chromium (and most other toxic substances) to algae has not been examined in the estuarine environment. In light of the possibly divergent results obtained from work in freshwater and seawater, the effects of chromium on natural phytoplankton populations in estuarine waters appears of particular interest.

MATERIALS AND METHODS

Seven long-term experiments using natural phytoplankton populations, and two unialgal culture experiments using Thalassiosira pseudonana clone 3H, were conducted.

For the natural population experiments, water was collected from three stations on Yaquina Bay, Oregon, at seven times during the years 1977-79. The water was pumped from a depth of 0.5 meter and passed through a spun polypropylene Framweb® pre-filter and a Pall DE® 3 µm pore-size cartridge filter. Filter housings were of polypropylene with ethylene-propylene "O" rings. All tubing used was clean Tygon®. Water thus filtered was pumped into two 150-gallon polyethylene tanks. To strip out some trace organics and trace metals, Darco G-60 activated carbon (1 gram/liter) was added to one of the tanks. The water was stored for two days, during which period it was vigorously stirred several times.

At the end of the two-day period, all water, both carbon-treated and non-treated, was filtered directly into new, rinsed, 10-liter polyethylene Cubitainer® growth containers through a Framweb® pre-filter and a Pall Ultipore® 0.45 µm pore-size cartridge filter. Treatment additions consisted of combinations of chromium (added as sodium dichromate at 0.1, 0.01, and 0.001 mg Cr/l), the major nutrients (N, P, Si), trace metals (Fe, Cu, Zn, Co, Mn, Mo), and vitamins (thiamin, biotin, B_{12}). Nutrients were added as 1/10 strength medium f (Guillard and Ryther, 1962).

Incubation was carried out by suspending the growth containers in a large, outdoor temperature-controlled water bath, kept within 1°C of the collection temperature under the natural diel light-dark cycle. Once the growth containers had equilibrated to the temperature of the water bath (about two hours), they

were inoculated with natural phytoplankton from Yaquina Bay. Water for the inoculum was collected at the same location and depth as the experimental water, during the high tide preceding inoculation. Ten milliliters of inoculum were added by repipet to each growth container, giving a ratio of inoculum to treated water of 1:1000. There were three replicates of each treatement, except where noted in the results.

Samples were collected daily between 1030 and 1200 hours from each growth container. They were kept at the temperature of the incubation tank until analyzed. Growth was monitored by measuring *in vivo* fluorescence, extracted chlorophyll *a*, and disappearance of major nutrients through time. Fluorescence measurements were carried out with a Turner Designs® fluorometer, equipped with a blue fluorescent lamp, Corning® color specification 2-64 emission filter, and Corning® color specification 5-60 excitation filter. To optimize the precision of fluorescence measurements, samples for *in vivo* fluorescence determinations were kept in the dark at the incubation temperature for an hour prior to measurement.

Nutrient samples were analyzed initially and during the course of each experiment on a Technicon® Auto-Analyzer. Nitrate plus nitrite, reactive phosphate, and reactive silicate were analyzed according to the methods described by Atlas *et al.* (1971). Extracted chlorophyll *a* measurements were made according to the fluorescence technique of Yentsch and Menzel (1963). At the peak of phytoplankton growth, samples were taken for microscopic enumeration. These samples were preserved in Lugo's solution, and at least 200 cells were counted in each sample.

The unialgal culture experiments with Thalassiosira pseudonana cone 3H were conducted to test in detail the effect of salinity on the toxicity of hexavalent chromium to phytoplankton. T. pseudonana clone 3H was selected for this study because it grows well over the full range of salinities to be tested (Guillard and Ryther, 1962). Cultures were grown in 500 ml polycarbonate Erlenmeyer flasks at 20°C, with 250 micro-einsteins continuous fluorescent illumination.

Water for the T. pseudonana experiments was collected from the low-salinity end of Yaquina Bay (less than 0.01°/$_{oo}$) and from the high-salinity end of the bay (32.5 °/$_{oo}$). All water was filtered (0.45 mμ pore-size) and autoclaved. Major nutrients (nitrate, phosphate, and silicate) were added at 1/10 strength medium f concentrations. A range of salinities was developed by mixing low-and high-salinity water. In Experiment I, the toxicity of three concentrations of chromium to T. pseudonana (zero, 0.01, and 0.10 mg/l chromium, added as sodium dichromate) was examined over a range of salinities from <0.01 °/$_{oo}$ to 32.5 °/$_{oo}$. In Experiment II, chromium concentrations of zero, 0.02 and 0.20 mg/l were used, over a greatly reduced salinity range of 0.03 °/$_{oo}$ to 2.11 °/$_{oo}$. Cultures were monitored daily with measurements of in vivo fluorescence.

In addition to the bioassays, we performed experiments to determine if the hexavalent chromium additions remained in solution and if the additions remained hexavalent. The first of these experiments was designed to determine if adsorption of chromium VI to the growth container walls might present a problem. Four polyethylene cubitainers were filled with one liter each of filtered (0.45 mμ) sea water. One μCi chromium-51 (added

as sodium dichromate) was added to each container. Five-ml aliquots were collected over a 33-day period, and were analyzed from chromium-51 on a Nuclear Data ND 13 OAT multi-channel analyzer coupled to a sodium-iodide detector. Maximum loss of chromium to the walls was 2% after 33 days.

Chromium VI additions remaining in both fresh water and sea water after two-month incubations in 10-liter cubitainers was determined. Sodium dichromate (0.1 mg Cr/l) was added to filtered (0.45 mμ) water. Chromium VI was concentrated and separated from chromium III by use of an anion exchange resin (AG1-X4) and reactive ion exchange elution (Pankow and Janauer, 1974). Analysis was carried out on a Varian AA-5 atomic absorption spectrophotometer, using a neutral flame to avoid potential interferences. We were able to recover nearly all the additions as chromium VI after two months. The nominal initial addition was 0.10 μg Cr VI/l, and we recovered 0.096 ± 0.010 μg Cr VI/l seawater (3 replicates), and 0.085 ± 0.011μg Cr VI/l in freshwater (4 replicates). Thus there was little, if any, reduction of chromium VI to chromium III in our experimental water. These results are consistant with the findings of Cutshall, et al. (1966), in which chromium VI introduced into the ocean was found to remain in the hexavalent state.

RESULTS AND DISCUSSION

Among the seven natural population experiments, four were conducted with high-salinity water (about 32.5 °/$_{oo}$), two with low-salinity water (0.03 and 0.04 °/$_{oo}$), and one with intermediate-salinity water (20.4 °/$_{oo}$). Results from these experiments are shown in Figs. 1-9. While *in vivo* fluorescence results are shown in the figures, extracted chlorophyll *a* showed the same results.

In the first experiment (November, 1977) one which was evidently micro-nutrient-limited, the addition of chromium gave a marked enchancement of growth identical to or greater than the enhancement caused by the addition of iron, other trace metals, and vitamins. Enhancement was greatest at the 0.001 mg/l chromium concentration, and somewhat less at higher chromium concentrations. A similar pattern was shown in the more severely micro-nutrient-limited (carbon-treated) water (Fig. 1). The stimulation of growth was characterized by increased yield of two species of the diatom *Nitzchia*. Among the other high-salinity experiments, there was no observable inhibitory or stimulatory effect of added chromium on the developing phytoplankton populations (Fig. 2) nor was there any effect on the species composition, at concentrations up to 0.1 mg Cr/l. However, in March, 1978, the only time that an unrealistically high 1.0 mg Cr/l was tested, some lag at the 1.0 mg Cr/l level in growth was observed. Growth of the diatom *Skeletonema costatum* was inhibited at this concentration. In the medium salinity experiment (August, Fig. 2), chromium had little or no effect relative to the control. In the low-salinity experiments, the 0.1 mg/l chromium additions either completely eliminated measurable growth of phytoplankton (January, 1979, Fig. 3), or greatly reduced the

rate of growth (October, 1979, Fig. 3) relative to controls. The 0.01 mg/l additions caused an apparent lag in growth relative to the controls in both of these low-salinity experiments. Species inhibited by the chromium additions were Surirella ovata, Cyclotella sp., and Detonula confervacea. Similar results were found when nutrients were added in these experiments (Fig. 3).

In Experiment I with T. psuedonana, at the lowest salinity 0.1 mg Cr/l was moderately inhibitory to growth (not shown). At all higher salinities (4.0 to 32.5 °/$_{oo}$) there was no effect at either level of chromium addition.

In Experiment II with T. pseudonana, a detailed examination of chromium toxicity over a range of low salinities was made. At the lowest salinity (0.03 °/$_{oo}$), growth in the control was healthy, but both 0.20 and 0.02 mg Cr/l additions inhibited all growth (Fig. 4). At the next higher salinity (0.09 °/$_{oo}$), the highest chromium addition still suppressed all growth; however, there was slow growth at 0.02 mg Cr/l. At 0.29 °/$_{oo}$ salinity, there was still no growth with the highest chromium addition, but there was only slight inhibition of growth with the 0.02 mg Cr/l addition. At 1.10 °/$_{oo}$ salinity, the low-chromium addition caused no inhibition of growth, and there was slow growth with the 0.20 mg Cr/l addition. At the highest salinity of this experiment (2.11 °/$_{oo}$) there was no inhibition of growth caused by the low-chromium addition, and relatively slight inhbition from the high-chromium addition.

It is clear from both the natural population experiments and the T. pseudonana experiments that salinity exerts a strong effect on the toxicity of hexavalent chromium. That sea water can neutralize toxic effects of

hexavalent chromium on phytoplankton has obvious implications for the disposal of chromium in natural waters. The mechanism of this detoxification is not yet clear.

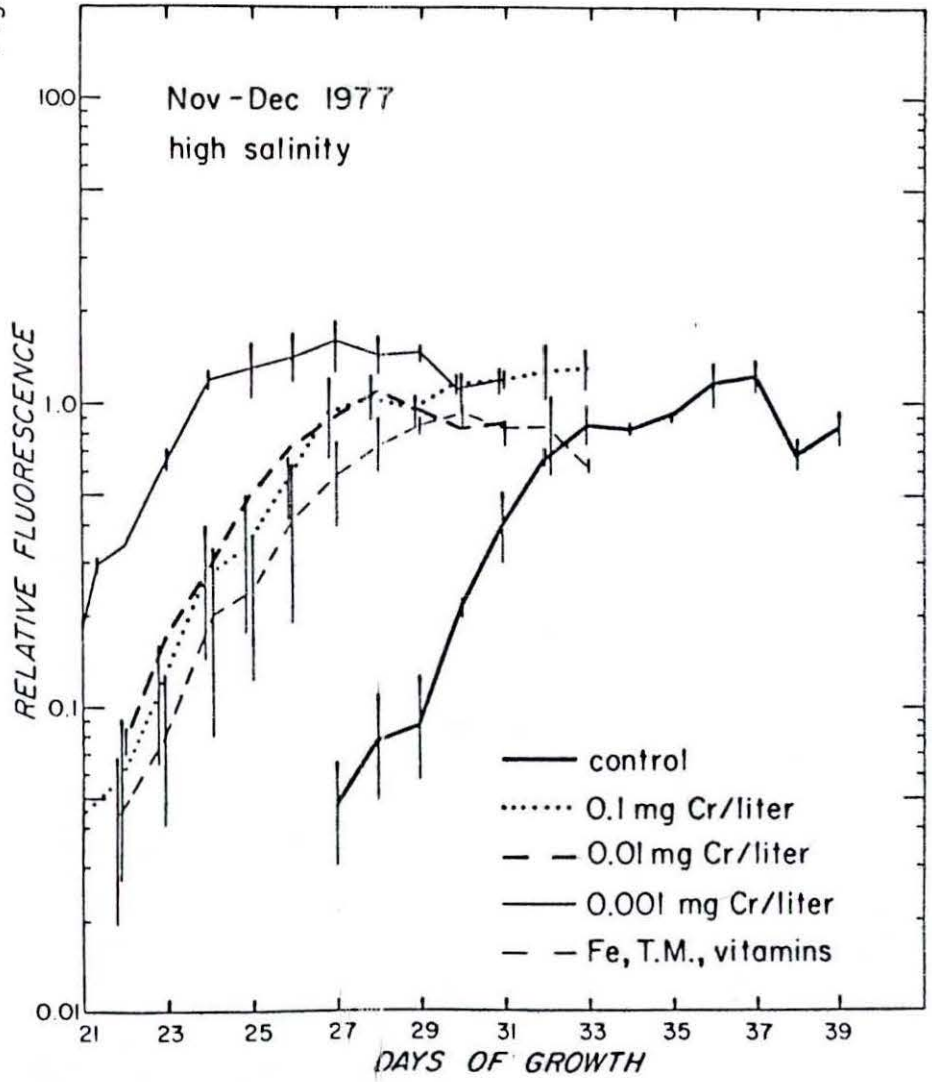
Fig. 1. Effects of chromium additions and micro-nutrient additions on phytoplankton growth in high salinity water collected in November, 1977. Results in both non-carbon-treated and carbon-treated water are shown. Lines join means of two replicates. Vertical bars show ranges.

Fig. 2. Effects of chromium additions on phytoplankton growth in four high and medium salinity experiments. Lines join medians of three replicates. Vertical bars show ranges.

Fig. 3. Effects of chromium additions on phytoplankton growth in two low salinity experiments. Results with and without nutrient additions are shown. Lines join medians of three replicates. Vertical bars show ranges.

Fig. 4. Effects of chromium additions on growth of Thalassiosira pseudonana over a range of low salinities.

Fig. 1


Nov-Dec 1977
high salinity
RELATIVE FLUORESCENCE
100
1.0
0.1
0.01
21
23
25
27
29
31
33
35
37
39
DAYS OF GROWTH
control
0.1 mg Cr/liter
0.01 mg Cr/liter
0.001 mg Cr/liter
Fe, T.M., vitamins

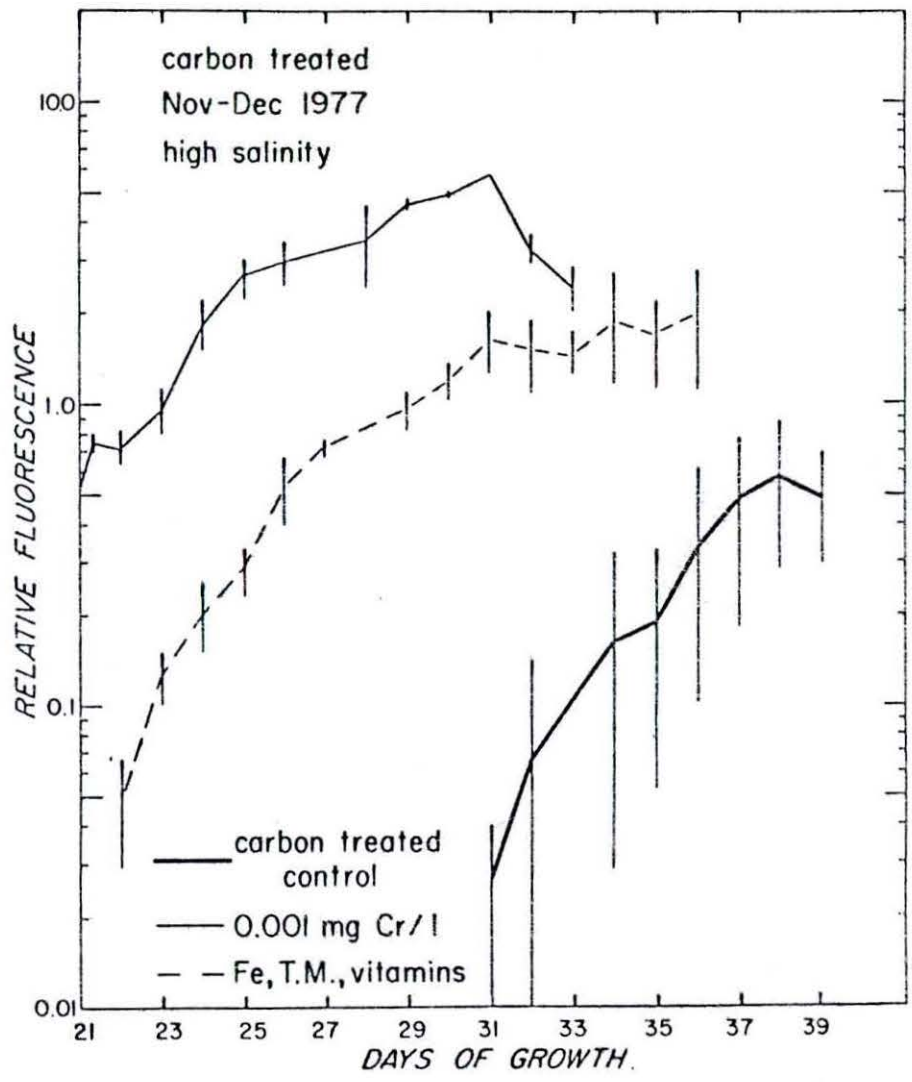
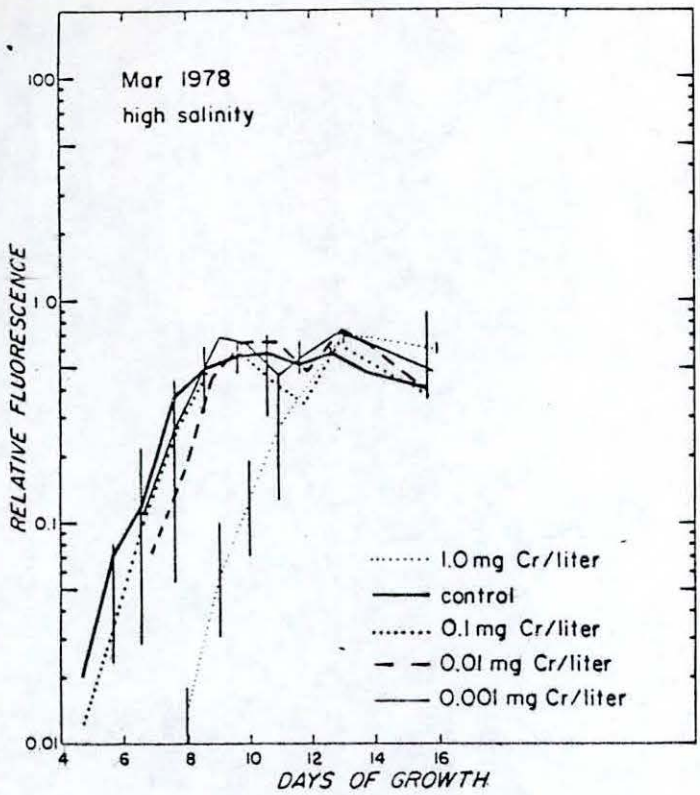
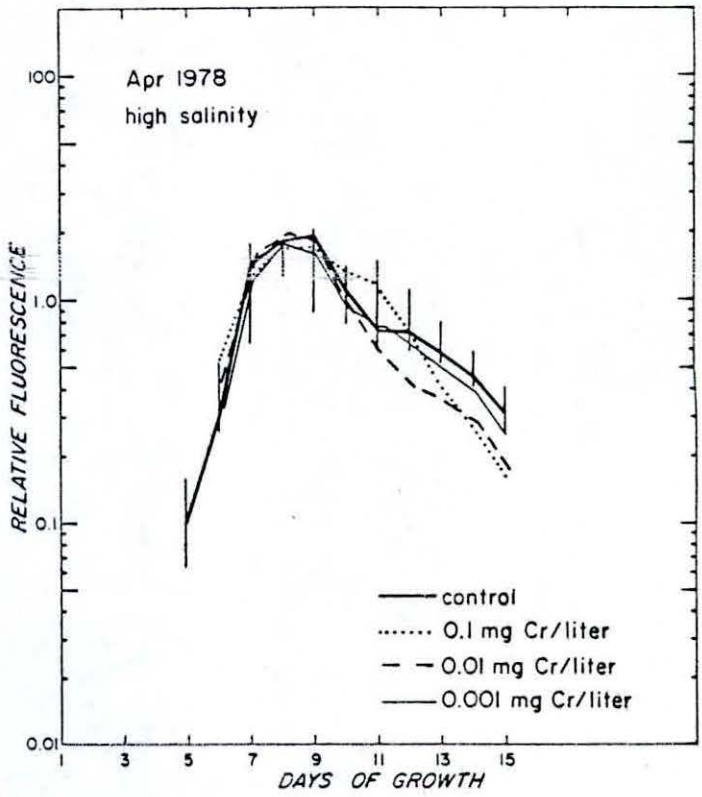
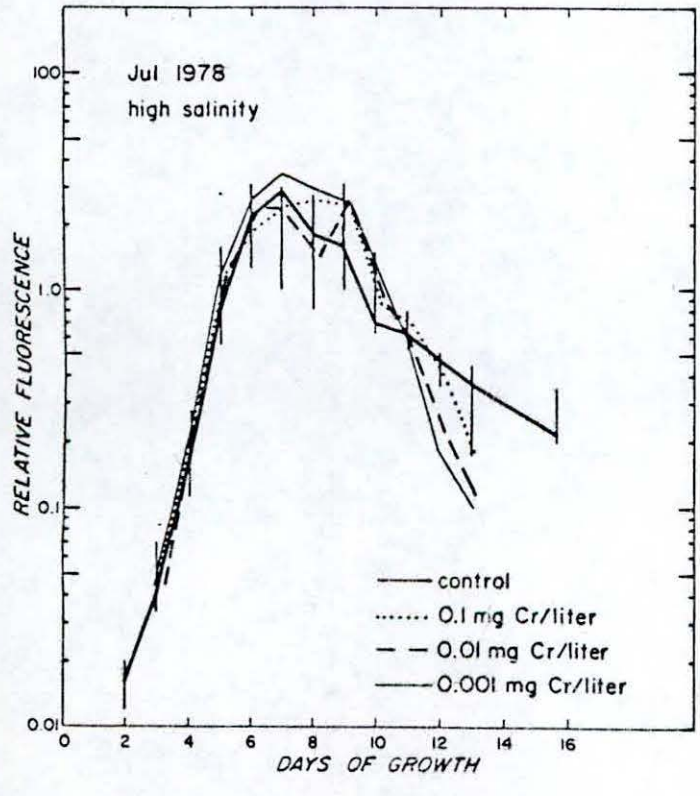
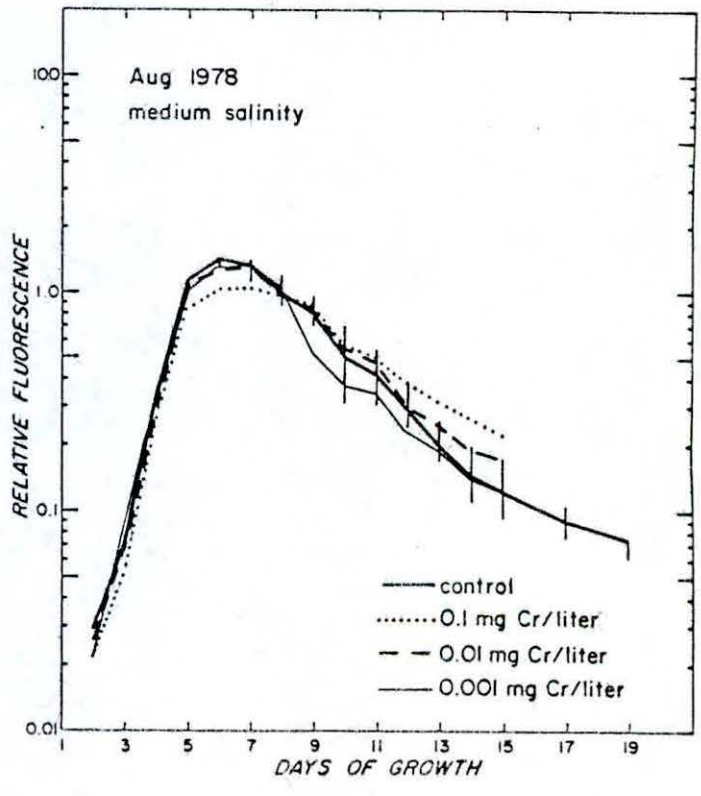

carbon treated
Nov-Dec 1977
high salinity
RELATIVE FLUORESCENCE
100
1.0
0.1
0.01
21
23
25
27
29
31
33
35
37
39
DAYS OF GROWTH
carbon treated
control
0.001 mg Cr/l
Fe, T.M., vitamins

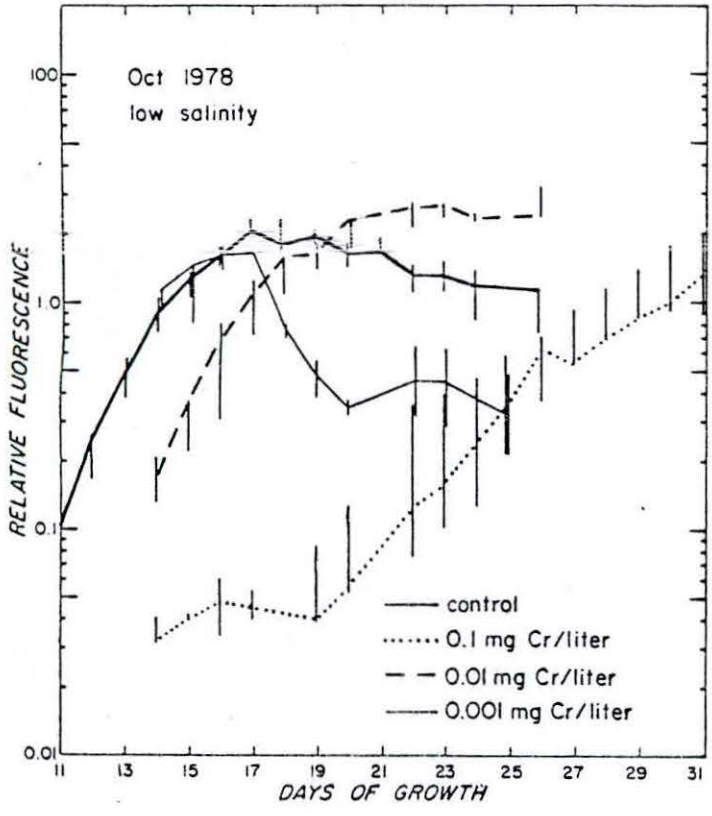
Fig. 2

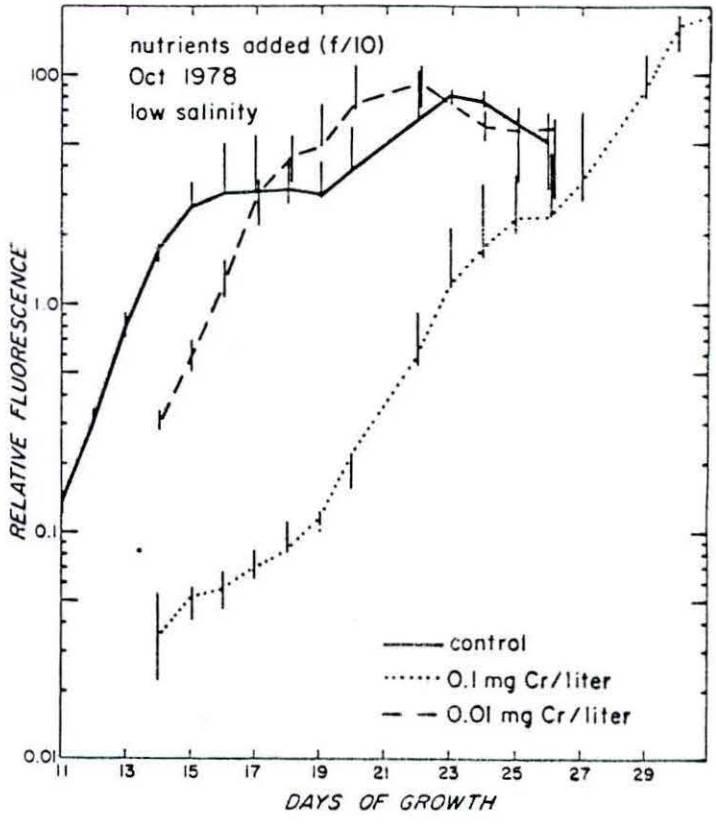

Mar 1978
high salinity
RELATIVE FLUORESCENCE
DAYS OF GROWTH
1.0 mg Cr/liter
control
0.1 mg Cr/liter
0.01 mg Cr/liter
0.001 mg Cr/liter

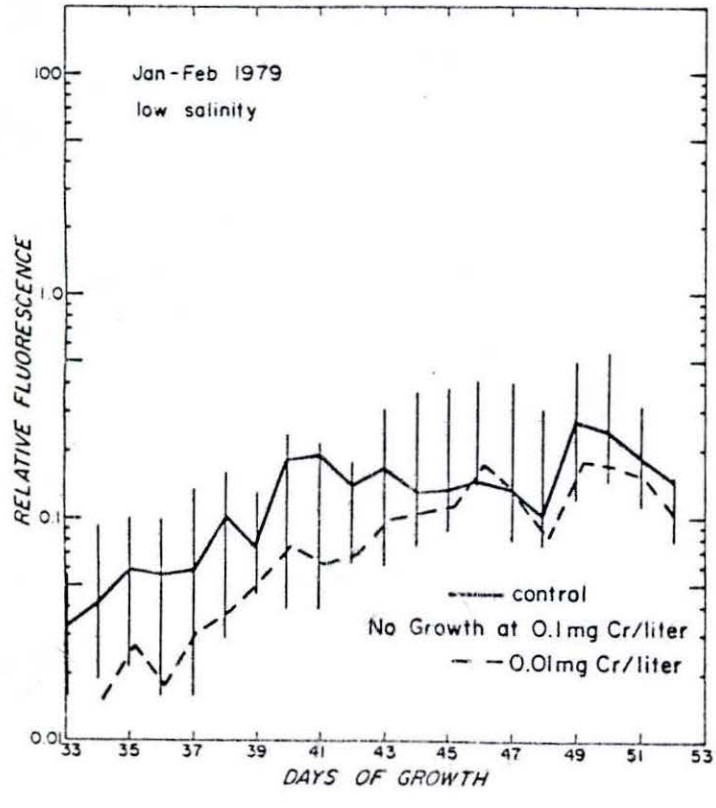

Apr 1978
high salinity
RELATIVE FLUORESCENCE
DAYS OF GROWTH
control
0.1 mg Cr/liter
0.01 mg Cr/liter
0.001 mg Cr/liter

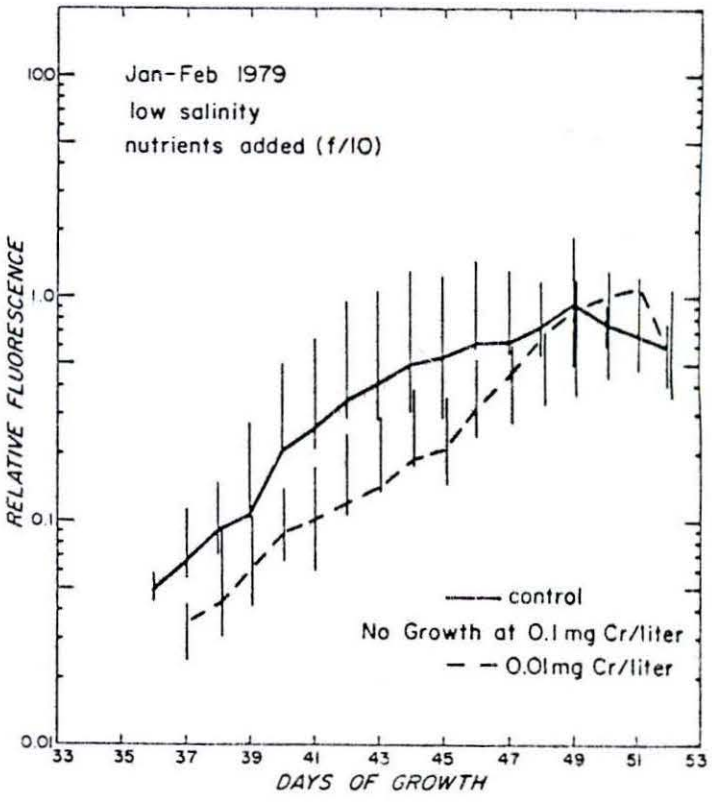

Jul 1978
high salinity
RELATIVE FLUORESCENCE
DAYS OF GROWTH
control
0.1 mg Cr/liter
0.01 mg Cr/liter
0.001 mg Cr/liter

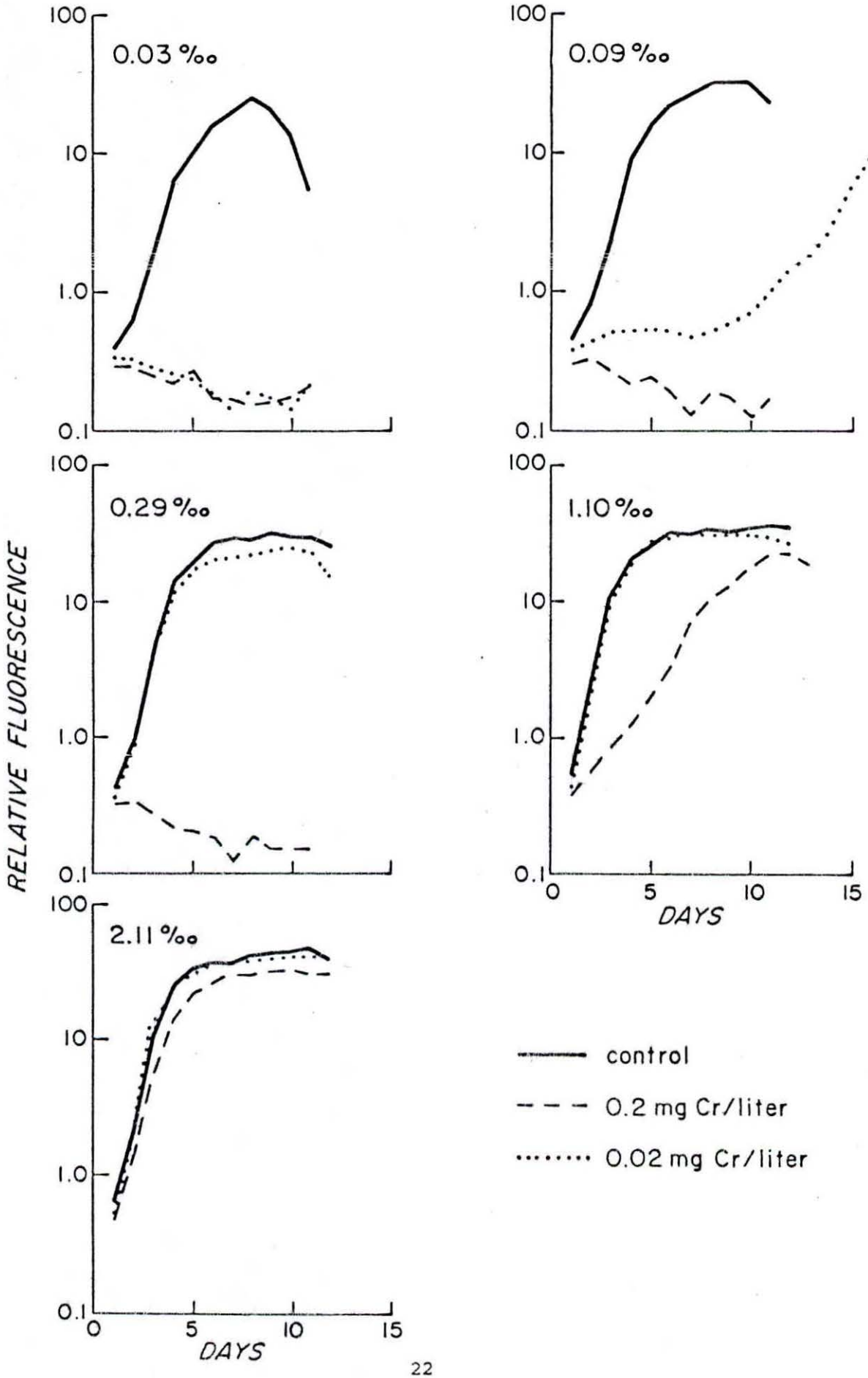

Aug 1978
medium salinity
RELATIVE FLUORESCENCE
DAYS OF GROWTH
control
0.1 mg Cr/liter
0.01 mg Cr/liter
0.001 mg Cr/liter


Oct 1978
low salinity
RELATIVE FLUORESCENCE
DAYS OF GROWTH
control
0.1 mg Cr/liter
0.01 mg Cr/liter
0.001 mg Cr/liter


nutrients added (f/10)
Oct 1978
low salinity
RELATIVE FLUORESCENCE
DAYS OF GROWTH
control
0.1 mg Cr/liter
0.01 mg Cr/liter


Jan-Feb 1979
low salinity
RELATIVE FLUORESCENCE
DAYS OF GROWTH
control
No Growth at 0.1 mg Cr/liter
0.01 mg Cr/liter


Jan-Feb 1979
low salinity
nutrients added (f/10)
RELATIVE FLUORESCENCE
DAYS OF GROWTH
control
No Growth at 0.1 mg Cr/liter
0.01 mg Cr/liter


0.03 ‰
0.09 ‰
0.29 ‰
1.10 ‰
2.11 ‰
100
10
1.0
0.1
0
5
10
15
DAYS
RELATIVE FLUORESCENCE
control
0.2 mg Cr/liter
0.02 mg Cr/liter

DCMU-ENHANCED *IN VIVO* FLUORESCENCE AS A MEASURE OF THE STATE OF HEALTH AND POTENTIAL GROWTH CAPACITY OF NATURAL PHYTOPLANKTON POPULATIONS[1]

by

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Running title: DCMU-enhanced fluorescence

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ABSTRACT

The relationship between *in vivo* fluorescence, DCMU-enhanced *in vivo* fluorescence, and extracted chlorophyll *a* were examined in large cultures of natural phytoplankton populations. A variety of nutrient conditions and species assemblages were tested. DCMU-ratios were calculated by dividing *in vivo* fluorescence after DCMU addition, by *in vivo* fluorescence without DCMU. DCMU-ratios of 2.5 or more were indicative of healthy, exponentially growing populations, while DCMU-ratios of 2.0 or less were indicative of senescent populations. DCMU-ratios were correlated with incremental exponential growth rates. The correlation coefficient was 0.83, and growth rates could be approximated by the DCMU-ratio minus 2. The DCMU-ratio likely reflects potential growth capacity, rather than actual growth rates. *In vivo* fluorescence estimates of chlorophyll *a* were not much improved through the use of DCMU.

In vivo fluorescence has come into increasing use among marine and aquatic scientists as a rapid technique to estimate concentrations of chlorophyll *a*, which in turn are used to estimate the amount of plant material present in the water. It has the great advantage of being simple and rapid, and can be used with a continuous-flow system (Lorenzen, 1966). *In vivo* fluorescence, however, has proved to be a mercurial parameter, having a quite variable relationship to chlorophyll *a* concentration (Strickland, 1963; Loftus and Seliger, 1975). Part of the variability results from a variable fraction of the energy from an excited chlorophyll *a* molecule going into chemosynthesis, and a variable fraction going into fluorescence. Other sources of variation include variable geometric arrangements of chloroplasts within the cells, and structural changes within the chloroplasts (Kiefer, 1973; Murakami and Packer, 1970).

Slovacek and Hannan (1977) showed that the electron-transport inhibitor DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) could be used to maximize the *in vivo* fluorescence of several phytoplankton species, resulting in an improved correspondence between *in vivo* fluorescence and extracted chlorophyll *a*. Much of the variability caused by differing nutrient conditions as well as differences among species appeared to be removed by maximizing fluorescence with DCMU. However, Esaias (1978), in waters off Peru and New York, and McMurray (1978), in San Francisco Bay, found no improved correlation between *in vivo* fluorescence and extracted chlorophyll *a* with the addition of DCMU.

Samuelsson and Oquist (1977) showed that in pure cultures of *Chlorella*, *Selenastrum*, and *Ankistrodesmus*, concentrated by centrifugation to a constant cell density, the amount of increase of *in vivo* fluorescence induced by DCMU was closely related to the amount of carbon-14 uptake. Both the DCMU-induced

fluorescence increase and carbon-14 uptake were high during exponential growth, and lower during senescence.

With the aim of assessing the suitability of DCMU as a tool to assess the state of growth of natural phytoplankton populations in the field, I grew large batch cultures of natural phytoplankton populations under simulated field conditions and monitored the populations through the various phases of growth. *In vivo* fluorescence with and without DCMU, and extracted chlorophyll *a* were measured. To test the effects of different nutrient limitations on these measurements, several nutrient combinations were tested. I analyzed the results to determine if DCMU-enhanced fluorescence could be used as an indicator of the state of health of our natural phytoplankton cultures, as an estimator of potential growth rates, or as an improved estimator of chlorophyll *a* concentrations.

I thank A. E. Bass for help with the collection and analysis of samples. S. Moore, F. Riedel, S. Case, and L. Helgeson also assisted with the collection and analysis of samples. T. Nagel and T. Mape assisted with the data analysis. I also thank L. F. Small and D. Nelson for discussions and their review of this manuscript.

Our experiments were carried out with natural phytoplankton populations collected from Yaquina Bay, Oregon. Cultures were grown in 10-liter polyethylene Cubitainers® immersed in a large, outdoor temperature-controlled water bath. Temperatures were held within one degree Centigrade of the collection temperature, and incubation was under natural sunlight. Water was collected from Yaquina Bay, Oregon, and filtered through a .45 μm filter. After addition of nutrients (see below), a 10 ml aliquot of unfiltered water (collected from the same location as the filtered water) was added to each container, giving a 1/1000 dilution of the inoculum.

Two experiments were conducted, one with high-salinity water (32.7 °/₀₀) collected near the mouth of the bay at high tide, and one with medium-salinity water (20.8 °/₀₀) collected at mid-bay at low tide. This gave us the opportunity to test the consistency of response with quite different species assemblages and water chemistry. The first experiment was performed in July, 1978. Treatments consisted of 1) no additions; 2) complete nutrient additions [1/10 strength medium f of Guillard and Ryther, 1962]; 3) complete nutrients minus nitrate; and (4) complete nutrients minus phosphate. There were two replicates for each treatment. The second experiment was performed in August, 1978. Treatments consisted of 1) no additions; 2) complete nutrient additions (f/10 medium); 3) complete nutrients minus nitrate; 4) complete nutrients minus phosphate; and 5) complete nutrients minus silicate. There were three replicates for treatments 1 and 2 and two replicates for each of the remaining treatments.

Samples were taken from the culture containers daily between 1030 and 1200 noon. They were kept at the same temperature as the incubation tank until

analyzed. Extracted chlorophyll a measurements were made according to the fluorescence technique of Yentsch and Menzel (1963). At the peak of phytoplankton biomass, samples were taken for microscopic enumeration. These samples were preserved in Lugol's solution and at least 200 cells were counted in each sample.

Fluorescence measurements were carried out with a Turner Designs® fluorometer, equipped with a blue fluorescent lamp, Corning® color specification 2-64 emission filter, and Corning® color specification 5-60 excitation filter. To standardize the light history, samples for in vivo fluorescence determinations were kept in the dark at the incubation temperature for an hour prior to measurement. Values were read about 15 seconds after the sample was placed in the fluorometer. For DCMU-enhanced measurements, two drops from a saturated aqueous solution of DCMU were added to the 10 ml fluorometer cuvette, to give a final DCMU concentration of 0.4 mg/liter. The sample was then poured into the cuvette and fluorescence measured. The DCMU acts within seconds and no waiting period is necessary. No further enhancement of fluorescence is obtained with DCMU concentrations higher than 0.4 mg/liter.

Cultures with no nutrient additions became nitrogen limited in the first experiment, and nitrogen and phosphorus limited in the second experiment. Cultures with complete f/10 additions became silicon limited. Cultures with a single nutrient deletion became limited by the deleted nutrient.

The "DCMU-ratio" for each pair of fluorescence values was calculated by dividing the DCMU-enhanced fluorescence by the *in vivo* fluorescence without DCMU. This procedure gave ratios ranging from over 3.0 to values approaching 1.0. Daily exponential growth rates (day^{-1}) were calculated on a fluorescence basis by calculating the successive slopes from day n-1 to day n, through the total experimental period, using the equation $y = ae^{bx}$, where y is *in vivo* fluorescence, x is time in days, a is the y intercept, and b is the slope. Using biomass estimates other than *in vivo* fluorescence gave similar results to incremental growth rates based on *in vivo* fluorescence. The DCMU ratios and incremental growth rates for each treatement were plotted against time on a linear scale (Figures 5 and 6). Lines join the means of two replicates or the medians of three replicates, with ranges indicated by vertical bars. Cumulative population growth, as indicated by *in vivo* fluorescence through the experimental period, is also shown on a logarithmic scale for each treatment, with means of two replicates or medians of three replicates shown.

During exponential growth, the incremental growth rates were high, and the DCMU-ratios were likewise high, with values ranging from about 2.5 to 3.0 (Figures 5 and 6). As the populations became nutrient-limited and incremental growth rates abruptly dropped, the DCMU-ratios invariably showed a similar rapid decline. Where cultures declined precipitously immediately following

exponential growth, giving a negative growth rate, DCMU-ratios tended to drop to values of about 1.5, often approaching 1.0. Both nitrogen and phosphorus limitation resulted in this growth pattern. When cultures maintained a plateau after exponential phase, giving an incremental growth rate of about zero, the DCMU-ratio tended to maintain a value close to 2.0. This pattern was principally seen with silica limitation. In cultures where secondary growth took place, a secondary rise in both the DCMU-ratio and the incremental growth rate occurred.

The DCMU-ratios and the incremental growth rates from both experiments were pooled, and a linear regression was calculated (Figure 7). The correlation coefficient was 0.83 and the dependent variable, growth rate, was estimated by the equation: Growth Rate = 1.0 x (DCMU-Ratio) - 1.9.

Linear regressions were calculated on pooled data from both experiments to determine if DCMU-enhanced *in vivo* fluorescence gave a better estimate of extracted chlorophyll *a* than *in vivo* fluorescence without DCMU. *In vivo* fluorescence without DCMU yielded a correlation coefficient of 0.87 and DCMU-enhanced *in vivo* fluorescence gave a correlation coefficient of 0.89.

The DCMU-ratio was a reliable indicator of the general state of health of the phytoplankton populations. Healthy, growing phytoplankton populations were characterized by a DCMU-ratio of 2.5 or higher, while senescent phytoplankton populations were characterized by a DCMU-ratio of 2.0 or less. We found a 1-hour dark incubation of samples at *in situ* temperatures to be essential in obtaining consistent results.

The DCMU-ratio reflects the chemosynthetic capacity of the chlorophyll *a* present in the sample. A high level of chlorophyll *a* not associated with a chemosynthetic system, as in dissolved chlorophyll *a*, can mask low levels of healthy cells. For this reason, blanking with filtered sea water is important at low cell densities. At high detrital chlorophyll *a* concentrations, the DCMU-ratio will correctly show that much of the chlorophyll *a* is not chemosynthetically active, though low densities of healthy cells may be masked by the detritus.

In my experiments, the DCMU-ratio was a good estimator of the exponential growth rates. Despite a diversity of nutrient conditions and species assemblages, the correlation was high, and potential growth rate could be approximated by the DCMU-ratio minus 2. Physiologically healthy cells in a sub-saturation light field (as at night or at depth) will doubtless give a high DCMU-ratio though growing at a reduced rate. For this reason the DCMU-ratio must be considered to reflect growth capacity or potential, rather than the actual rate of growth. Although our phytoplankton populations included a range of species, it is possible that healthy cells of a given species with a relatively low maximum growth rate would still show a high DCMU-ratio, because the lower growth rate would not reflect a less-than-optimal state of health.

McMurray (1978), using a flow-through system with parallel *in vivo* and DCMU-enhanced *in vivo* fluorescence measurements in transects of San Francisco Bay, found the DCMU-ratio to be consistently highest in the denser areas of phytoplankton patches, with the ratio dropping off toward the fringes of the patch. This finding is consistent with the view that a high proportion of chlorophyll *a* associated with exponentially growing cells results in a high DCMU-ratio, while outside the patches, a large portion of the chlorophyll *a* associated with senescent cells or detritus gives a low DCMU-ratio, with a gradation of proportions as the relative density of exponentially growing cells increases.

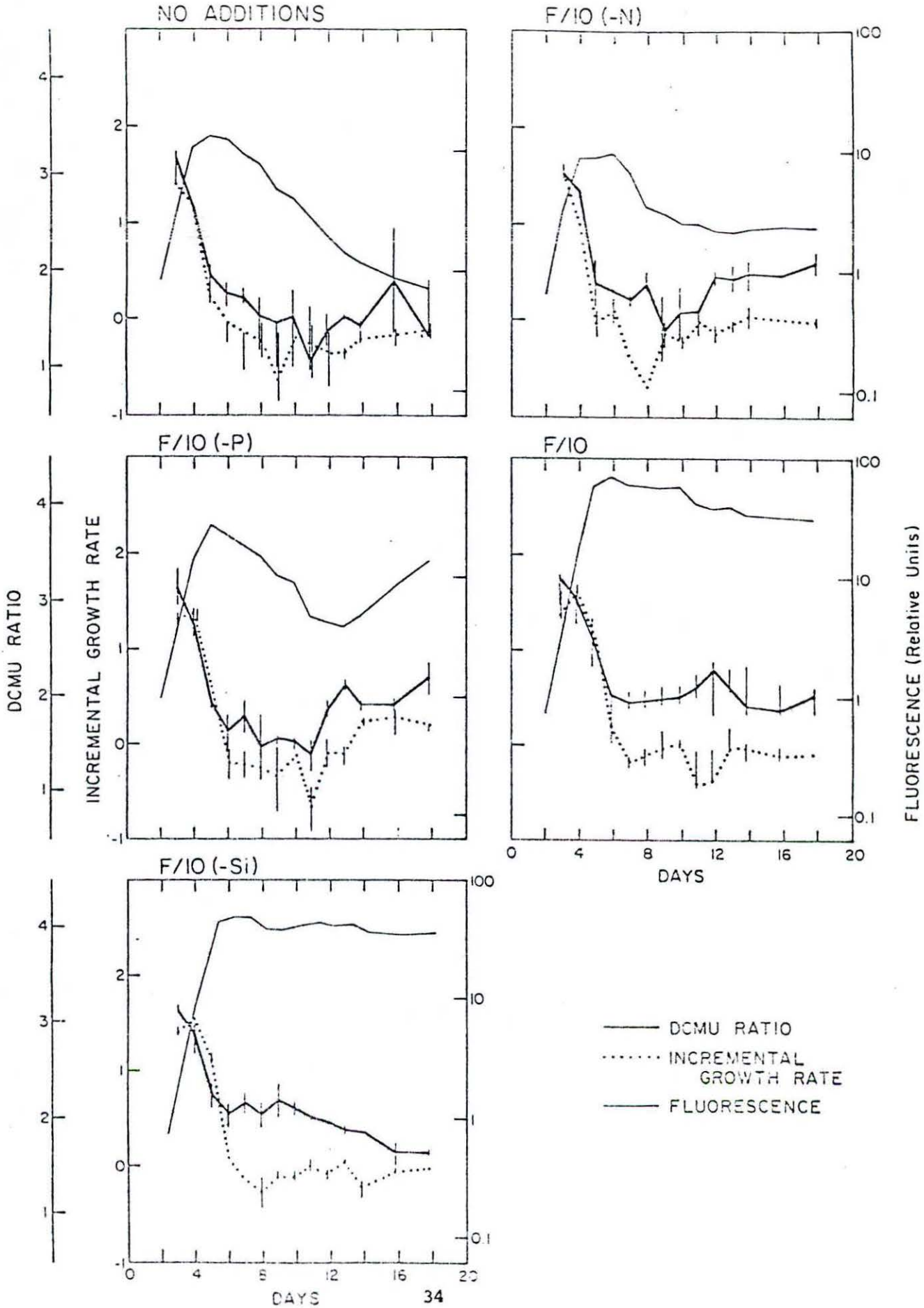
The technique used in obtaining the DCMU-ratio is fast and easy. Since it can be easily used in the field and can be adapted for use with a continuous-flow system, the DCMU-ratio seems to be an ideal technique for determining the state of health of phytoplankton populations in the field, as well as in the laboratory. It is well suited for looking at vertical and horizontal patterns of phytoplankton physiological states in the field. It can be used in examining critical mixing depths or the viability of phytoplankton patches.

The variability between extracted chlorophyll *a* and in vivo fluorescence was not substantially reduced through the use of DCMU in my experiments. This agrees with the findings of other studies involving field populations (Esaias, 1978; McMurray, 1978). Slovacek and Hannan (1977), however, using pure phytoplankton cultures, found that DCMU removed much of the variability in the relationship between *in vivo* fluorescence and chlorophyll *a*. It is not clear why DCMU does not appear to improve *in vivo* estimates of chlorophyll *a* in natural phytoplankton populations.

Fig. 5. Incremental growth rates (day^{-1}) and DCMU-ratios from medium salinity experiment. Lines join medians of three replicates or means of two replicates. Vertical bars show ranges.

Fig. 6. Incremental growth rates (day^{-1}) and DCMU-ratios from high salinity experiment. Lines join medians of three replicates or means of two replicates. Vertical bars show ranges. Key is the same as in Fig. 1.

Fig. 7. Scatter plot and linear regression of incremental growth rates versus DCMU-ratios from medium salinity and high salinity experiments.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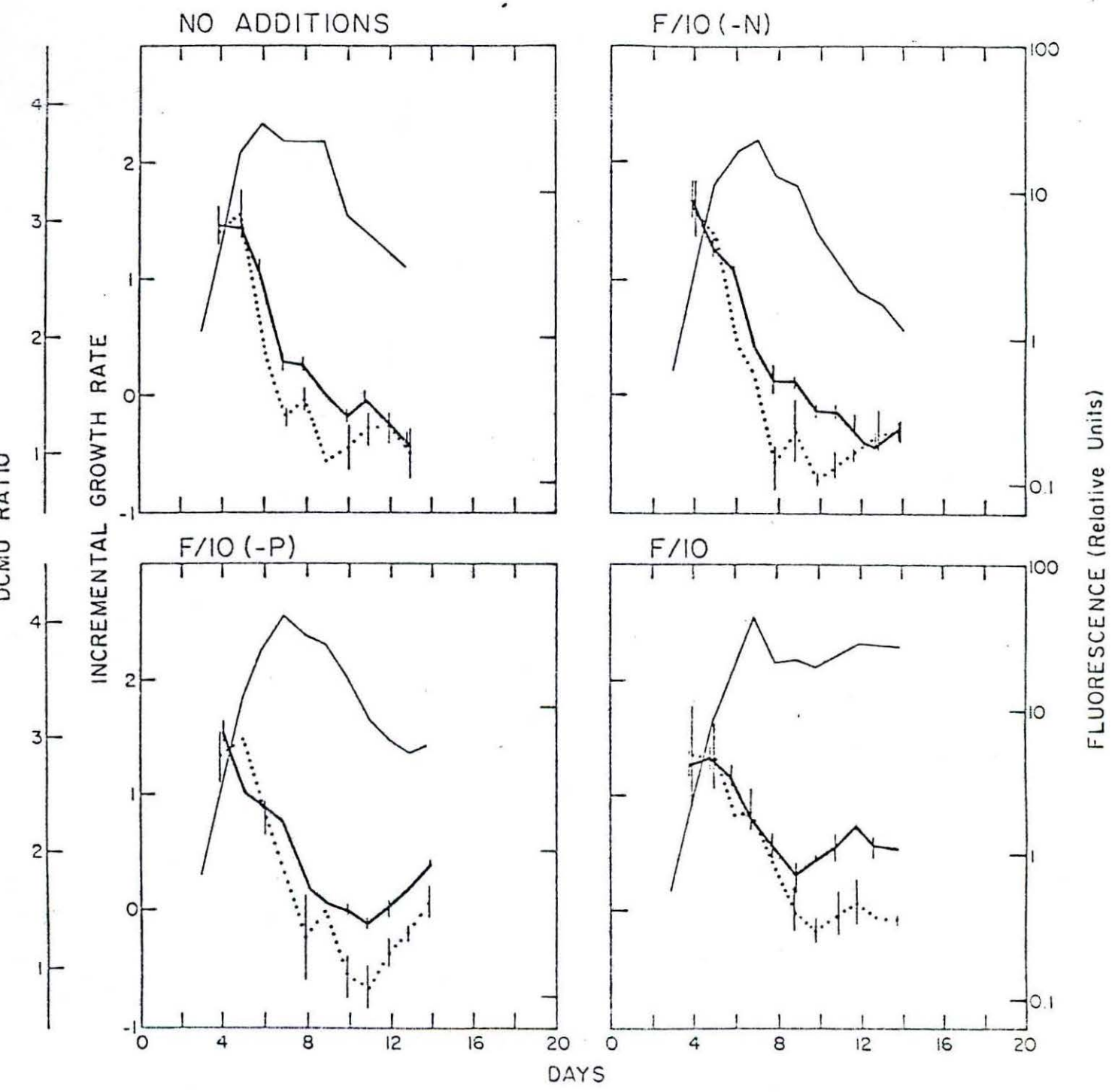
F/10 (-P)
F/10
F/10 (-Si)
DCMU RATIO
INCREMENTAL GROWTH RATE
FLUORESCENCE (Relative Units)
DAYS
DCMU RATIO
INCREMENTAL GROWTH RATE
FLUORESCENCE

Fig. 6


NO ADDITIONS
F/10 (-N)
F/10 (-P)
F/10
INCREMENTAL GROWTH RATE
FLUORESCENCE (Relative Units)
DCMU RATIO
DAYS
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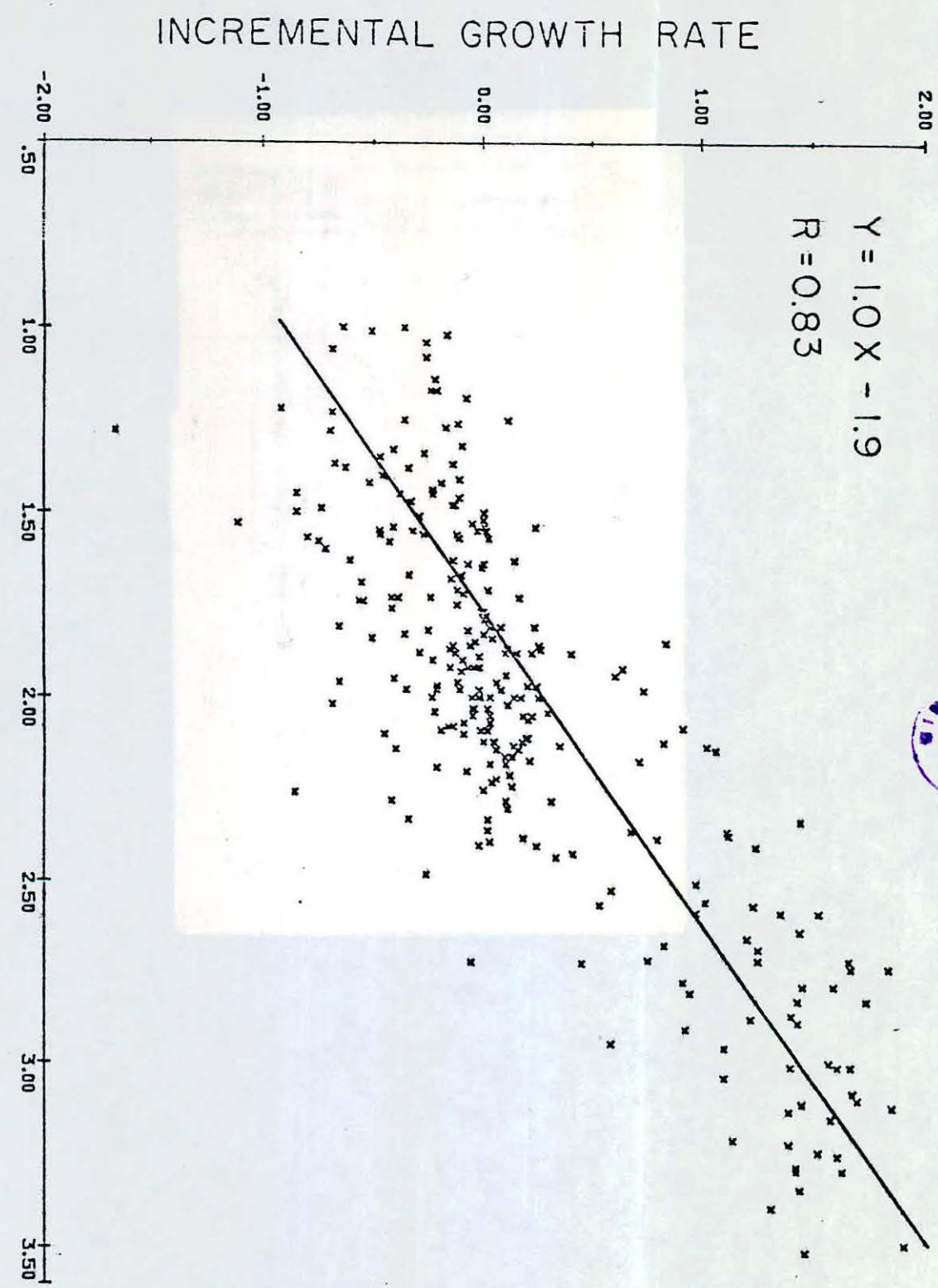
8
12
16
20
-1
0
1
2
100
10
1
0.1

Fig. 7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1.00
2.00
Y = 1.0 X - 1.9
R = 0.83
DCMU RATIO
.50
1.00
1.50
2.00
2.50
3.00
3.50