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CR 151201

ANNUAL PROGRESS REPORT

To: NASA Lyndon B. Johnson Space Center
Houston, TX 77058

Reporting Period: October 8, 1975, to October 8, 1976

Project: Assessment of the Impact of Increased Solar
Ultraviolet Radiation upon Marine Ecosystems

Contract Number: NAS 9--14860

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(NASA-CR-151201) ASSESSMENT OF THE IMPACT
OF INCREASED SOLAR ULTRAVIOLET RADIATION
UPON MARINE ECOSYSTEMS Annual Progress
Report, 8 Oct. 1975 - 8 Oct. 1976 (Oregon
State Univ.) 40 P HC A03/MF A01 CSCI 06C G3/51

N77-17695

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ASSESSMENT OF THE IMPACT OF INCREASED SOLAR
ULTRAVIOLET RADIATION UPON MARINE ECOSYSTEMS

Space Shuttle operations through the stratosphere may lead to a reduction of the Earth's ozone layer. Increases in solar ultraviolet radiation in the 290-315 nm waveband (UV-B) can be expected to result from such a depletion of ozone in the stratosphere. Studies initiated under the Climatic Impact Assessment Program (CIAP) of the U. S. Department of Transportation indicate that simulated solar UV-B radiation can, under experimental conditions, detrimentally affect marine organisms (algae, protozoans, small invertebrates) that form the base of the food web of oceanic and estuarine ecosystems. These organisms survive in nature by a combination of mechanisms for tolerating the detrimental effects of UV-B radiation. The key question is whether a small increase in UV-B radiation might overwhelm these mechanisms and produce changes that will have damaging consequences to the biosphere.

The objective of the present study is to provide data to assess the potential impact upon marine ecosystems if Space Shuttle operations contribute to a reduction of the stratospheric ozone layer.

Specifically, the study to date has addressed the following two questions:

1. Is there potential for irreversible damage to the productivity, structure and/or functioning of a model estuarine ecosystem by increased UV-B radiation or are these ecosystems highly stable?
2. What is the sensitivity of key community components (the primary producers) to increased UV-B radiation?

The methods by which these questions were experimentally examined consisted of irradiating model estuarine ecosystems with simulated solar UV-B radiation at various dosages and dose rates and comparatively analyzing the following community attributes:

- a. Concentration of photosynthetic pigments - via standard spectrophotometric procedures (Tett et al., 1975).
- b. Community biomass - determination of dry weight and organic weight (ash-free dry weight) (Soeder et al., 1974; Strickland and Parsons, 1972).
- c. Population density and community composition - an enumeration of the diatoms providing an estimator of the common Information index (Shannon and Weaver, 1949), a measure of Redundancy (McIntire and Overton, 1971), and a Difference measure (MacArthur, 1965).

Two seminatural ecosystem chambers (SNEC) of continuous flow-through design, each with a capacity of 720 liters were located at the Oregon State University Marine Science Center, Newport, Oregon (Fig. 1). The bottom of each chamber was graduated in a "stair-step" manner to provide a series of seven steps with step 7 being the uppermost and located at a depth of 10 cm below the water surface, step 5 at a depth of 26 cm and step 3 at a depth of 41 cm (Fig. 2). Seawater flowed through each of the chambers at a rate of approximately 6 liters per minute. The seawater, drawn from the lower Yaquina Estuary, was monitored for nutrient levels. Both chambers were supplied with visible radiation from a combination of "cool-white" fluorescent lamps and 60 W incandescent bulbs at an intensity of about 13,000 lumens/m² at the water surface. UV-B radiation was supplied by banks of fluorescent sunlamps (Westinghouse FS40) filtered by a 0.13 mm thickness of Kodacel

plastic film. UV-B intensities were measured by a submersible CIAP model UV-B Meter and/or an SUV model UV-B Meter (Solar Light Co., Philadelphia) (Berger, 1976). Samples of attached algae were collected from assemblages that developed on acrylic plates located on the steps of the chambers.

Studies utilizing experimentally constructed assemblages of diatoms were carried out via the microcosm approach in a controlled temperature room (15.3 ± 2.2 °C). These batch culture experiments were established with species of marine algae that had been cultured in our laboratory in 5-liter plastic containers containing Guillard's f/2 or f/4 enriched seawater medium. Stock cultures of these algae were reared under visible radiation supplied by Vita-Lite fluorescent lamps (5,000 lumens/m²). UV-B radiation at 14.3 SU/d (SUV model UV-B Meter) was provided by an FS40 sunlamp filtered by a 0.13 mm thickness of Kodacel. Each species of alga was reared as a unialgal culture, with populations of agnotobiotic bacteria present in each stock culture. Species of diatoms utilized in different combinations in these studies were: Chaetoceros septentrionalis, C. didymus, C. socialis, Thalassiosira pacifica, T. sp. 6, T. aestivalis, and Skeleronema costatum. One species of a green flagellate, Platymonas suerica, was also included.

A series of studies utilizing the seminatural ecosystem chambers demonstrated a depression in the synthesis of chlorophyll a in diatoms when exposed to enhanced levels of UV-B radiation (6-44% enhancement of surface irradiance). One study employing a 25% enhancement of UV-B radiation exhibited altered chlorophyll a values in samples collected from a depth of 26 cm. These results are presented in Figure 3. The simulated natural level of UV-B radiation during this study was approximately 15 SU/d (CIAP model UV-B Meter) at the surface. A stepped

24-hour photocycle was utilized with incandescent lights "on" from 0500 h to 2100 h, the "white-light" fluorescent lamps "on" from 0600 h to 2000 h, one bank of FS40 sunlamps "on" from 0800 h to 1800 h, and the other bank of sunlamps "on" from 0900 h to 1700 h. The maximum UV-B dose rate was 2.2 SU/h.

Figures 4 and 5 show the results of weekly samples taken at depths of 41 cm and 26 cm during a subsequent study. The acrylic plates were cleaned and replaced after each set of samples was collected. The photoregimen was changed to the following: incandescent lights (0800-2000 h), fluorescent lamps (0900-1900 h), one bank of FS40s (1000-1800 h), and the second bank of FS40s (1100-1700 h). The simulated natural level of UV-B radiation during this study was approximately 10.7 SU/d (CIAP model UV-B Meter) and the surface of other chamber received an 18% enhancement. A problem with fluctuating water-flow into the chambers was corrected during week #4. After this time there was a significant decrease in the weekly chlorophyll a concentrations in samples from the chamber exposed to the enhanced levels of UV-B radiation.

Organic weight determinations (Table 1) gave results paralleling chlorophyll a concentrations and an analysis comparing concentrations during the seven week period demonstrated that the ratios of chlorophyll a concentrations to organic weights at comparable depths were significantly reduced by exposure to the enhanced UV-B levels (Fig. 6).

An apparent reduction of chlorophyll a concentrations was also noted when batch cultures of algae were analyzed. As an example, the study represented in Figure 7 was initiated with the inoculation of equal amounts (10^6 cells) of each of four diatoms (Thalassiosira aestivalis, Skeletonema costatum, Chaetoceros didymus, and C. socialis) into twelve

5-liter containers. Each assemblage of organisms was reared in Guillard's f/2 enriched seawater. The containers were exposed on a daily L:D photoperiod of 9:15 at a peak illumination of 5,000 lumens/m². Groups of four containers were exposed for seven hours, centered within the light cycle, to each of three different levels of UV-B radiation (20.3, 21.7, and 23.1 SU/d - SUV model UV-B Meter) emitted by Kodacel-filtered FS40 sunlamps. Midway through the experiment a small number of Platymonas suecica cells was introduced into each of the containers. Although the rates of increase were not significantly different, a one-way analysis of variance of the chlorophyll concentrations from the three populations on the last day of the experiment indicated an apparent depression of the chlorophyll a concentration by enhanced levels of UV-B radiation ($0.25 > p > 0.10$).

A non-linear least squares fit analysis of the growth curves for the densities of the assemblages in the preceding batch culture experiment is illustrated in Figure 8. A one-way analysis of variance demonstrated that extremely significant differences did exist on the last day of the experiment between the three experimental conditions ($p < 0.001$).

The growth curves in Figures 9 and 10 for Thalassiosira sp. 6 and Platymonas suecica, respectively, further illustrate the effect of UV-B enhancement on population density. This experiment was initiated with the inoculation of equal amounts (5×10^4 cells) of each of three algae (Chaetoceros septentrionalis, T. sp. 6, and P. suecica) into each of twelve 5-liter containers. The cultures were reared in Guillard's f/4 enriched seawater. The containers were exposed on a daily L:D photoperiod of 16:8 at a peak illumination of 5,000 lumens/m². Groups of three containers were exposed for eight hours, centered within the light

cycle, to each of four different levels of UV-B radiation. The Chaetoceros never developed in any of the experimental conditions, whereas it did develop in assemblages not exposed to UV-B radiation.

The penetration of UV-B radiation through the water column of the two flow-through chambers was determined by utilizing a submersible CIAP model UV-B Meter. An increase in the absorption coefficients (Table 2) derived from readings taken at depths of 0, 33, 41 and 49 cm in the chamber exposed to 10.7 SU/d as contrasted with those obtained from the chamber exposed to 12.6 SU/d indicated an inhibition of planktonic development at the enhanced level of UV-B radiation.

For the analysis of community composition, a computer program was utilized which is capable of providing two indices of diversity: an estimator of the common Information measure (H'') and a measure of Redundancy (R'). Pielou (1966a, 1966b), McIntosh (1967) and Lloyd et al. (1968) have discussed the advantages, disadvantages, uses and misuses of the Information index of diversity. The estimator for the common Information measure was derived from

$$H'' = - \sum_{i=1}^G (n_i/N) \log_e (n_i/N) ,$$

where n_i is the number of diatoms in the i-th taxon, N is the number of diatoms in the sample, and G is the number of genera represented in the sample (Table 3). H'' ranges from 0 ($\log_e 1$), if all of the diatoms in the assemblage are of one taxon, to $\log_e N$, if the number of taxa equals the number of diatoms. The more taxa there are and the more nearly equal their proportions, the greater the uncertainty of predicting the taxon of the next diatom to be observed and, therefore, the greater the diversity.

Pielou (1966b) points out that H'' is subject to sampling error, with a sample variance of approximately

$$\frac{1}{N} \sum_{i=1}^G \frac{n_i}{N} \left(\log_e \frac{n_i}{N} \right)^2 - (H'')^2$$

To calculate a Redundancy index for a specific assemblage of diatoms, a maximum and minimum diversity for that assemblage must be determined. As explained by McIntire and Overton (1971), a conditional maximum and minimum diversity based on the observed number of species in a sample can be derived from

$$H''_{\text{CMAX}} = \log_e G ,$$

and

$$H''_{\text{CMIN}} = - \frac{G-1}{N} \log_e \left(\frac{1}{N} \right) + \left(\frac{N-G+1}{N} \right) \log_e \left(\frac{N-G+1}{N} \right) .$$

The results of these calculations were used to determine the Redundancy index R' (Table 3), where

$$R' = \frac{H''_{\text{CMAX}} - H''}{H''_{\text{CMAX}} - H''_{\text{CMIN}}} .$$

The Redundancy index expresses the degree of dominance in a given sample relative to the partitioning of diatoms among the taxa. Values of R' range from 0, when the diatoms are equally distributed among the taxa, to 1, when all but one taxon are represented by a single individual.

A comparison of two diatom assemblages was determined by utilizing the Difference measure of MacArthur (1965) (Table 3),

$$D_{hk} = \exp (H''_T - \bar{H}'') ,$$

where H''_T is the common Information measure for the combined h-th and k-th assemblages treated as one assemblage, and \bar{H}'' is the mean diversity for the two individual assemblages. These are determined, utilizing a computer program, from

$$H''_T = - \sum_{i=1}^G \frac{(n_{ih}/N_h + n_{ik}/N_k)}{2} \log_e \frac{(n_{ih}/N_h + n_{ik}/N_k)}{2} ,$$

and

$$\bar{H}'' = \frac{(H''_h + H''_k)}{2} .$$

If the taxa composition and relative abundances are the same, D_{hk} has the minimum value of 1, and if the assemblages possess no taxa in common, D_{hk} has the maximum value of 2. The Difference measure for samples from comparable depths exhibited an increase as the study progressed.

The sensitivity of three diatom species to radiation emitted by FS20 fluorescent sunlamps was determined. The radiation was filtered by a 0.13 mm thickness of Kodacel. Except where noted in Table 4, the diatoms were irradiated on seawater agar plates. All diatoms were cultured on seawater agar plates during the post-irradiation period.

Based on the work accomplished to date, it appears that chlorophyll a concentrations of marine algae in model estuarine ecosystems illuminated

by artificial light, are depressed by enhanced levels of UV-B radiation, as are the ratios of chlorophyll a concentration to organic weight. Enhanced levels of UV-B radiation also resulted in a shift in the community structure which may create significant alterations in the food web of the ecosystem. More work is underway to include an assessment of the impact of enhanced UV-B on planktonic algae and primary consumers (e.g. copepods and rotifers). Also, a study is being initiated to determine the impact of enhanced levels of UV-B radiation upon a model estuarine ecosystem utilizing natural solar radiation enhanced by radiation from fluorescent sunlamps. The sunlight will provide the model ecosystem with natural levels of photoreactivating radiation.

An enlargement of our survival study has been initiated. Isolates from the model ecosystem will be tested routinely as to their sensitivity to UV-B radiation. An assessment of the effect on both growth rate and survival will be made. As more data accumulates, it may be possible to determine which characteristics of organisms and/or ecosystems can most easily be employed to predict the marine ecosystem's sensitivity on a global scale for an assessment of the biospheric impact of Shuttle operations.

The model ecosystems that have been established, or others that are in the process of being established by our laboratory, are excellent facilities for future enlargement of the scope of the present study. With additional personnel, an investigation into the effect of enhanced levels of UV-B radiation on marine bacteria could be carried out. It has been found that enhanced levels of UV-B radiation increases the relative incidence of pigmented bacteria. These pigmented forms may be pathogenic to economically and/or ecologically important marine

organisms (e.g. oysters). The equipment and facilities are available for the isolation and culture of the bacteria and a bioassay technique involving the oyster as the host organism has been developed.

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Table 1. Organic weight determinations from weekly samples of comparable steps of chambers A and B. The surface irradiance of chamber A was 10.7 SU/d and for chamber B, 12.6 SU/d. Step 3 has a depth of 41 cm and step 5 has a depth of 26 cm. A fluctuating difference in water flow between the two chambers was corrected during week #4.

| CHAMBER-STEP | TIME (WEEKS) | | | | | | |
|--------------|---------------|----------------|----------------|----------------|----------------|----------------|----------------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| A-3 | .434 ±.048 | .439 ±.029 | 2.099 ±.180 | 1.125 ±.113 | 1.407 ±.240 | 1.006 ±.300 | .673 ±.115 |
| B-3 | .421 ±.085 | .986 ±.076 | 2.147 ±.573 | 1.762 ±.090 | .461 ±.011 | .518 ±.041 | .540 ±.034 |
| A-5 | .311 ±.017 | .848 ±.031 | 2.166 ±.257 | 1.335 ±.280 | 1.404 ±.090 | 1.140 ±.310 | 1.134 ±.052 |
| B-5 | .531 ±.065 | 1.382 ±.119 | 2.708 ±.499 | 2.207 ±.082 | .502 ±.071 | .446 ±.032 | .609 ±.003 |

Table 2. Apparent absorption coefficients in the UV-B region of the seawater in the two flow-through chambers were determined at four time intervals during the experiment. A submersible UV-B Meter (CIAP model) was used to determine irradiance.

| TIME (WEEKS) | CHAMBER | APPARENT ABSORPTION COEFFICIENT (cm ⁻¹) | CALCULATED PERCENT SURFACE IRRADIANCE AT 1 AND 2 METERS |
|-----------------|---------|--|---|
| 2 | A | .020 ±.002 | 14 2 |
| | B | .018 ±.003 | 17 3 |
| 3 | A | .019 ±.003 | 15 2 |
| | B | .016 ±.002 | 20 4 |
| 5 | A | .034 ±.003 | 3 0.1 |
| | B | .024 ±.002 | 10 0.9 |
| 7 | A | .028 ±.005 | 6 0.4 |
| | B | .019 ±.004 | 15 2 |

Table 3. Analysis of community composition. Irradiance level in chamber A, 12.1 SU/d (CIAP model UV-B Meter) and in chamber B, 15.6 SU/d. N is the number of diatoms in the sample, G is the number of taxa represented in the sample, H'' is the estimator for the common Information index, R' is a measure of Redundancy, and D is MacArthur's Difference measure. Step 4 has a depth of 34 cm and step 6 has a depth of 18 cm. Steps 4T and 6T indicate assemblages allowed to develop for a five week period.

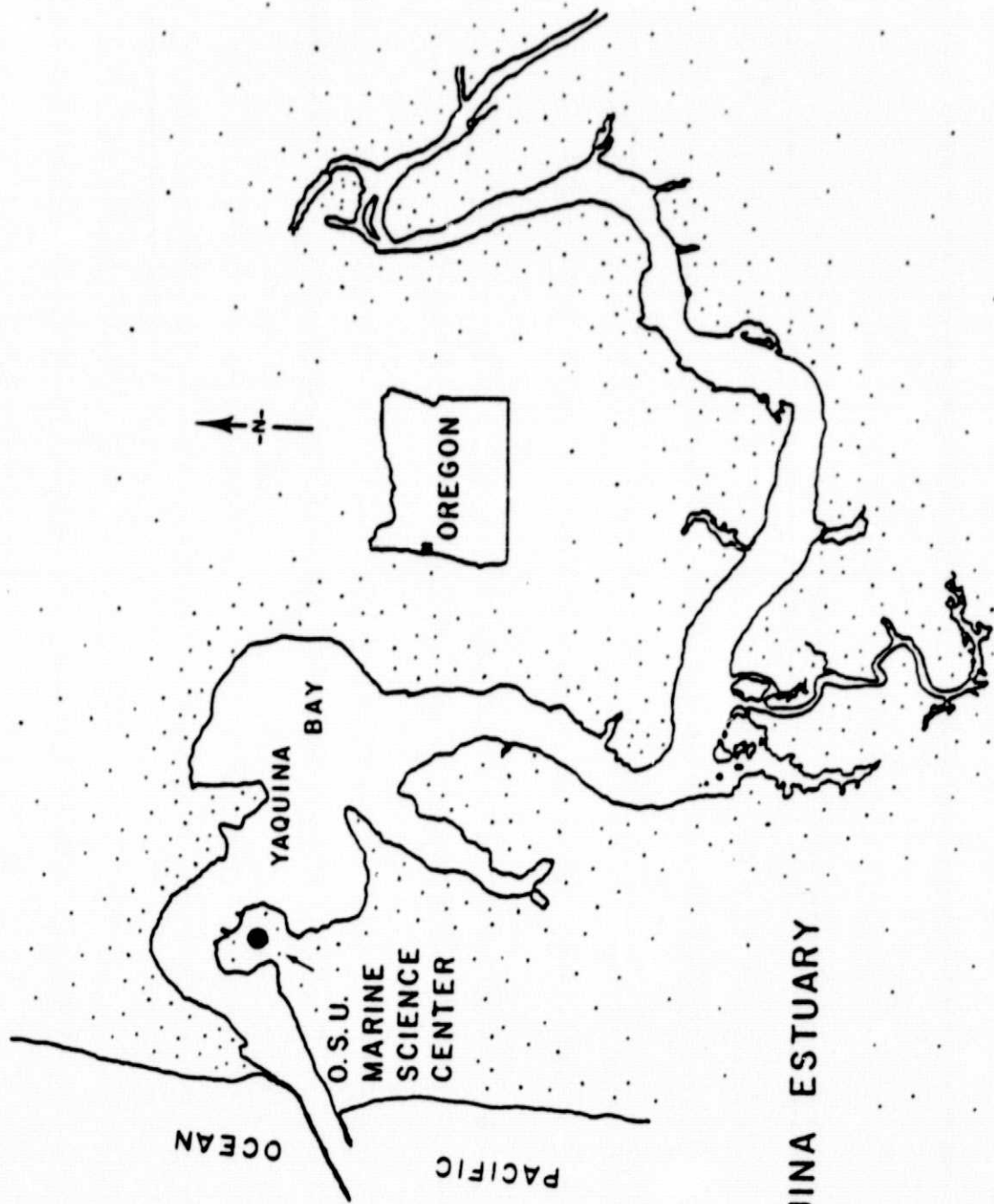
| <u>WEEK-CHAMBER-STEP</u> | <u>N</u> | <u>G</u> | <u>H''(nat)</u> | <u>R'</u> | <u>D</u> |
|--------------------------|----------|----------|-----------------|-----------|----------|
| 2-A-4 | 581 | 11 | 1.620 | 0.342 | |
| 2-B-4 | 547 | 14 | 1.615 | 0.415 | 1.022 |
| 4-A-4 | 558 | 18 | 1.930 | 0.360 | |
| 4-B-4 | 555 | 16 | 1.948 | 0.320 | 1.102 |
| 5-A-4 | 552 | 18 | 2.022 | 0.326 | |
| 5-B-4 | 559 | 16 | 1.882 | 0.346 | 1.169 |
| 2-A-6 | 565 | 16 | 1.587 | 0.460 | |
| 2-B-6 | 533 | 15 | 1.851 | 0.340 | 1.039 |
| 4-A-6 | 595 | 18 | 2.038 | 0.318 | |
| 4-B-6 | 561 | 17 | 2.112 | 0.275 | 1.083 |
| 5-A-6 | 575 | 16 | 2.049 | 0.280 | |
| 5-B-6 | 618 | 17 | 1.801 | 0.391 | 1.150 |
| 5-A-4T | 532 | 14 | 2.010 | 0.256 | |
| 5-B-4T | 591 | 14 | 1.975 | 0.268 | 1.113 |
| 5-A-6T | 554 | 16 | 2.013 | 0.295 | |
| 5-B-6T | 604 | 14 | 1.958 | 0.275 | 1.131 |

Table 4. Sensitivity of three species of diatoms to UV-B radiation.

| <u>Diatom</u> | <u>Exposure Rate (SU·h⁻¹)</u> | <u>LD₅₀</u> |
|--|--|------------------------|
| <i>Nitzschia</i> sp. 2 | 2.85 | 3 SU* |
| <i>Thalassiosira</i> sp. 6 | 2.5 | 2.7 SU* |
| <i>Thalassiosira pacifica</i> | 2.5 | 1.8 SU* |
| <i>Thalassiosira pacifica</i> (exposed in 1 cm of liquid culture medium) | 2.5 | 5.0 SU* 5.9 SU** |

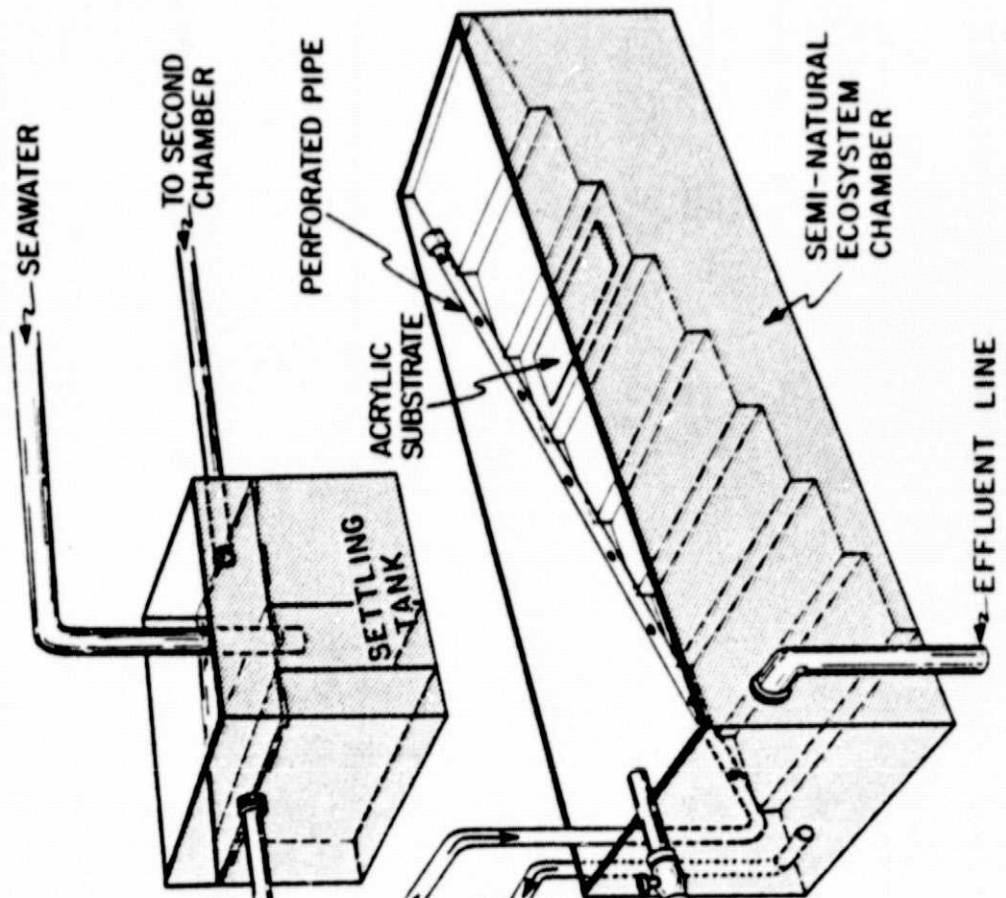
*Not exposed to photoreactivating light for 24 h post-irradiation.
 **Exposed to photoreactivating light immediately after irradiation
 (6000 lux Vita-Lite).

Fig. 1. Yaquina Bay and Estuary, Oregon.



YAQUINA ESTUARY

Fig. 2. Diagram of seminatural ecosystem chamber (continuous flow-through design) and associated water supply system. Arrangement of radiation sources is illustrated.



CHAMBER DIMENSIONS
 INSIDE WIDTH - 76 cm
 INSIDE LENGTH - 3 m
 MAXIMUM INSIDE DEPTH - 65 cm

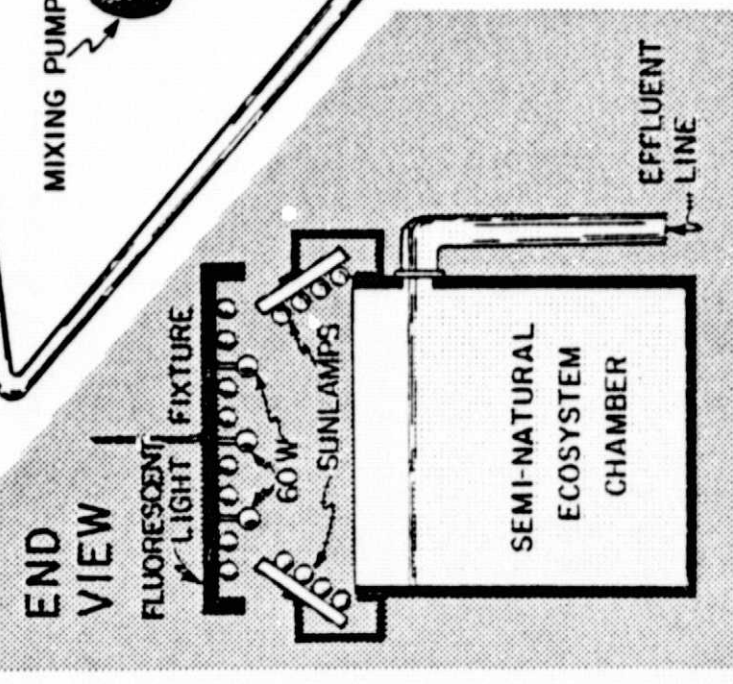


Fig. 3. Chlorophyll a concentrations from samples collected at a depth of 26 cm from the two flow-through ecosystems. The simulated natural level of UV-B radiation was approximately 15 SU/d (CIAP model UV-B Meter) at the surface and the other chamber received a 25% enhancement. Error bars = +1 St.Dev.

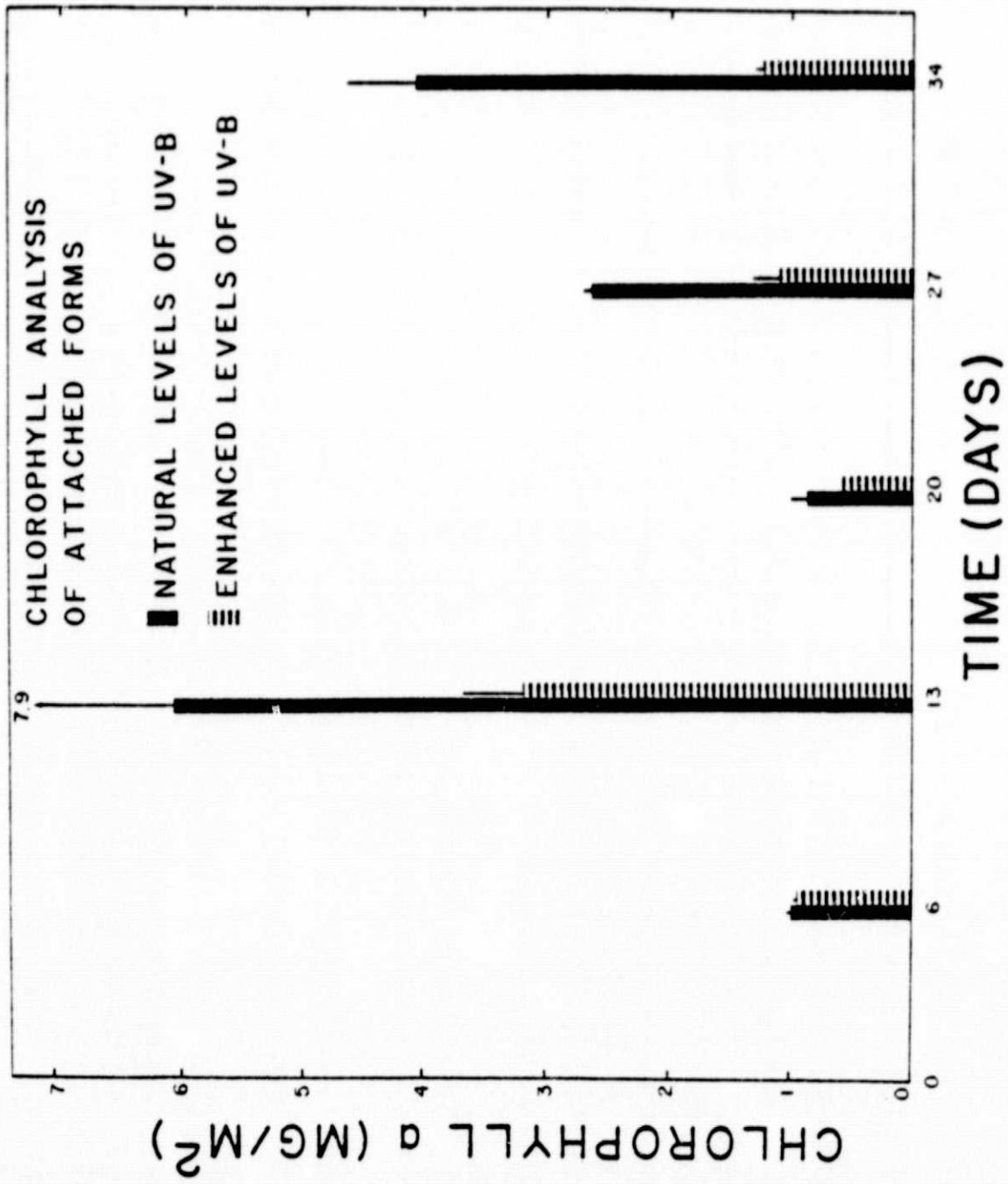


Fig. 4. Chlorophyll a concentrations from samples collected at a depth of 41 cm from the two flow-through ecosystems. Solid bars represent samples from the chamber receiving a surface irradiance of 10.7 SU/d (CIAP model UV-B Meter), shaded bars represent samples from the chamber receiving a surface irradiance of 12.6 SU/d. Error bars = +1 St.Dev.
* = $p < 0.05$ ** = $p < 0.01$

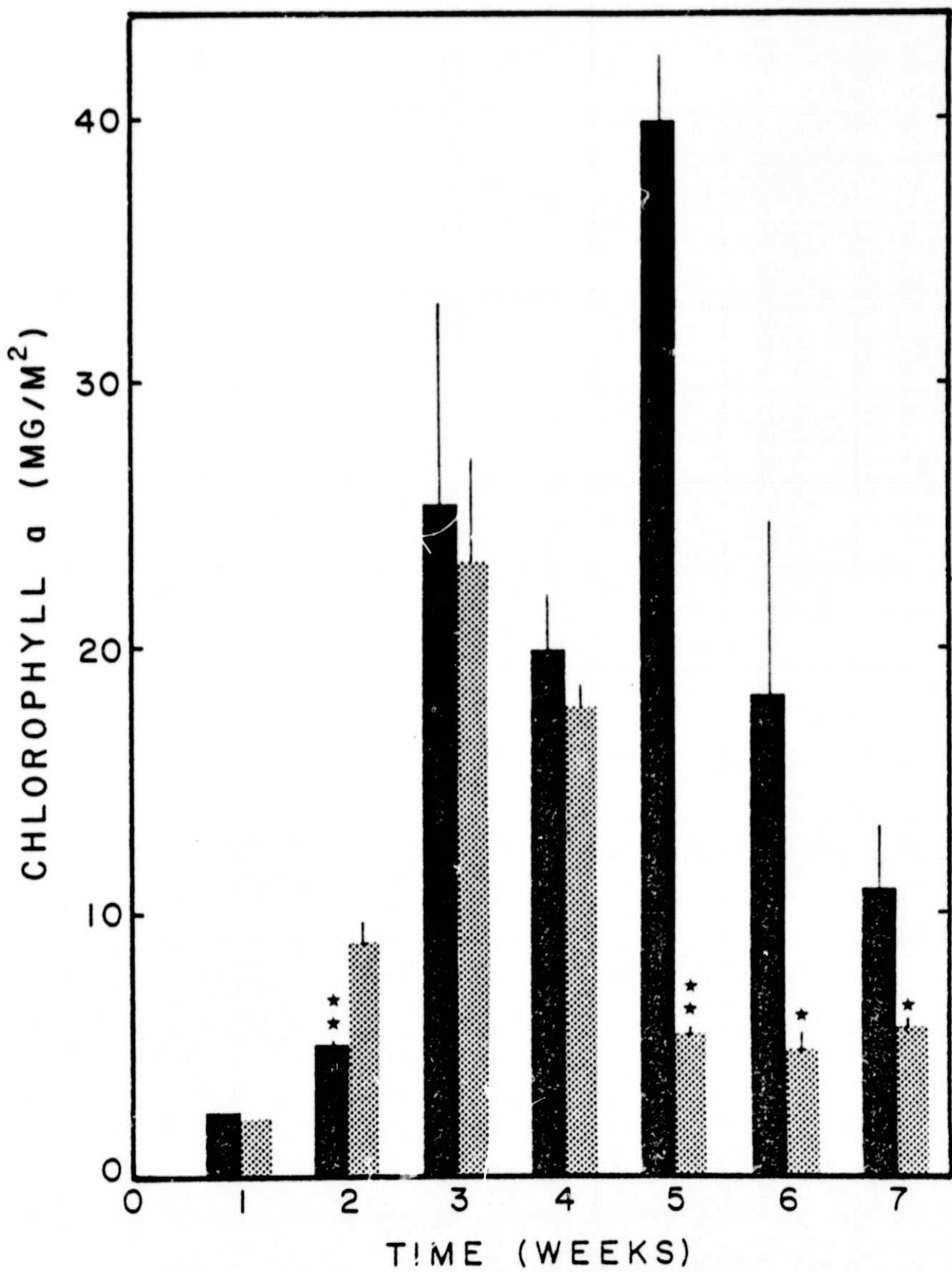


Fig. 5. Chlorophyll a concentrations from samples collected at a depth of 26 cm from the two flow-through ecosystems. Solid bars represent samples from the chamber receiving a surface irradiance of 10.7 SU/d (CIAP model UV-B Meter), shaded bars represent samples from the chamber receiving a surface irradiance of 12.6 SU/d. Error bars = +1 St.Dev.
* = $p < 0.05$ ** = $p < 0.01$

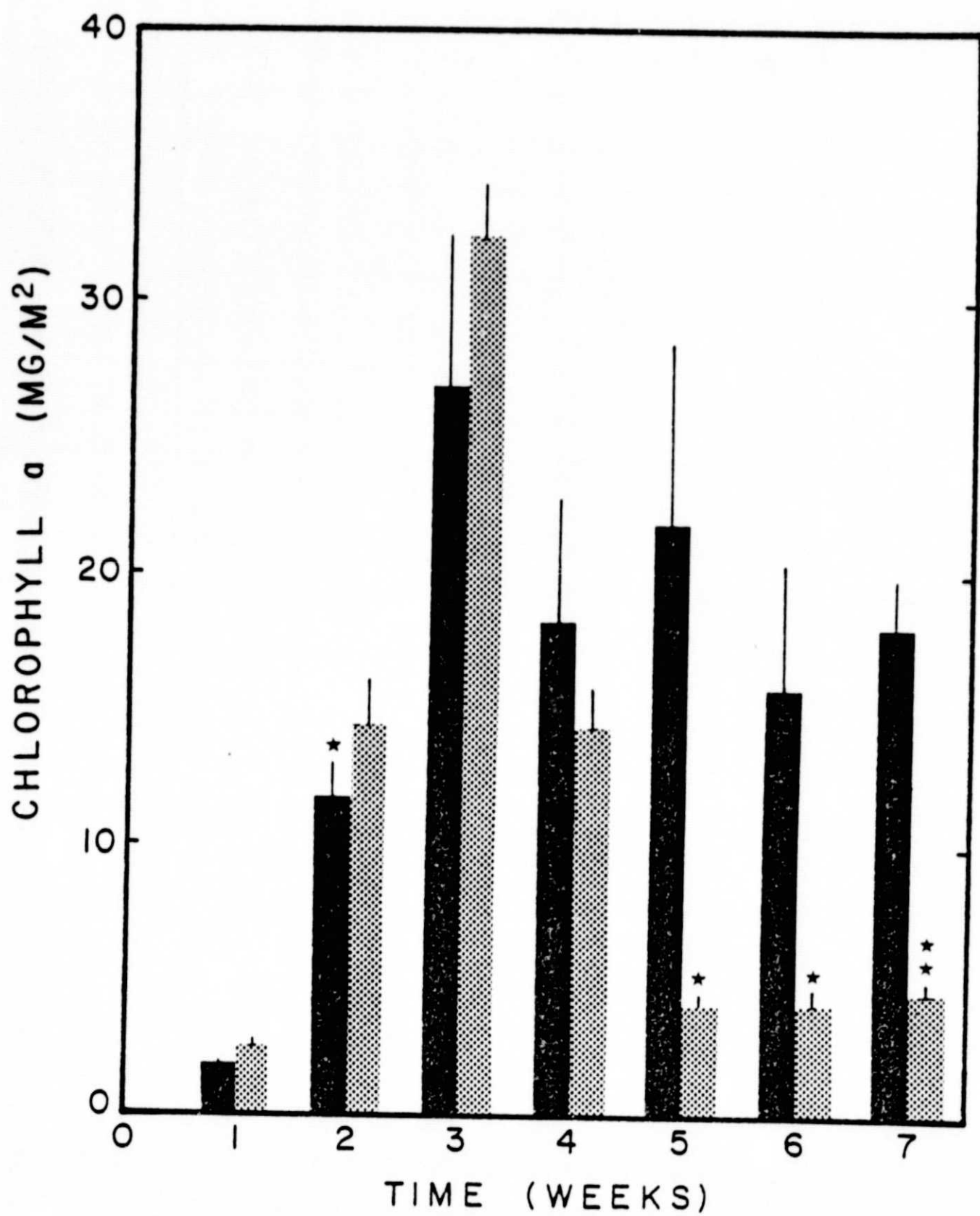


Fig. 6. Linear regression lines of chlorophyll a concentrations on organic weight of samples collected during a seven week period from the two flow-through ecosystems. A₃ = chamber A (10.7 SU/d - CIAP model UV-B Meter), step 3 (depth, 41 cm); A₅ = chamber A, step 5 (depth, 26 cm); B₃ = chamber B (12.6 SU/d), step 3; B₅ = chamber B, step 5. R = correlation coefficient.

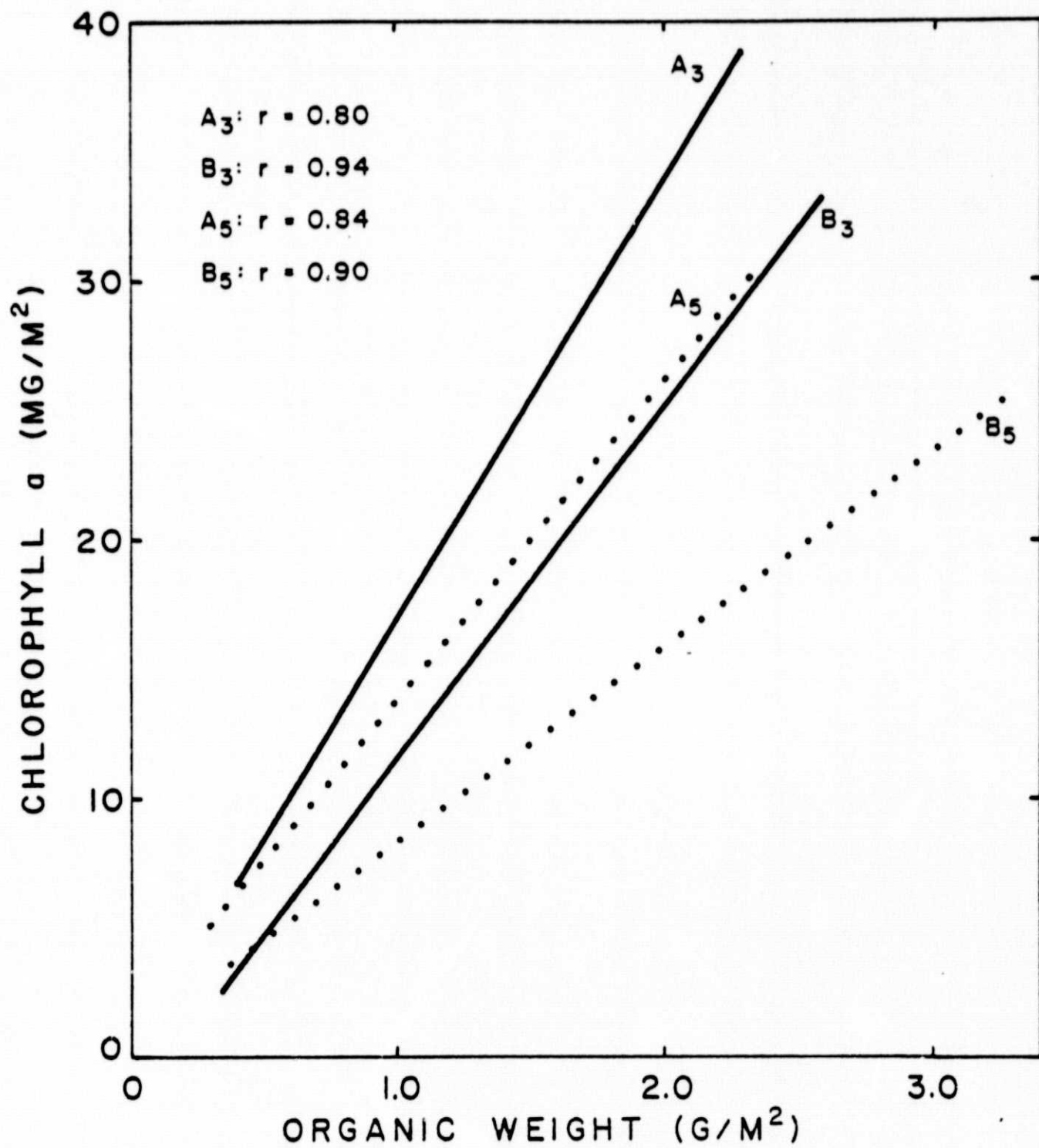


Fig. 7. Non-linear least squares fit analysis of increase in chlorophyll a concentrations in three batch cultures of algae exposed to three different levels of UV-B radiation. R = correlation coefficient.

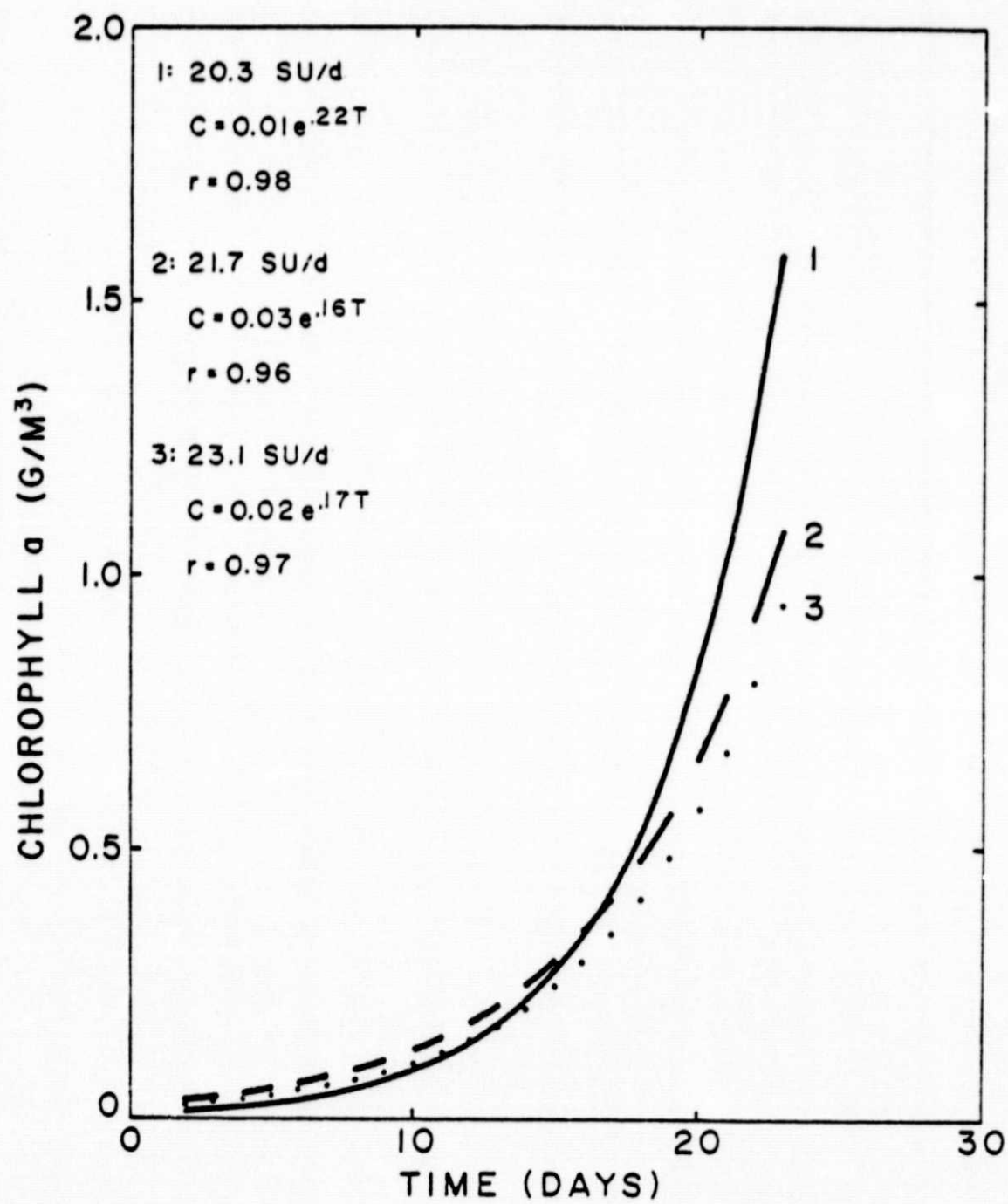


Fig. 8. Non-linear least squares fit analysis of increase in population density of three batch cultures of algae exposed to three different levels of UV-B radiation. R = correlation coefficient.

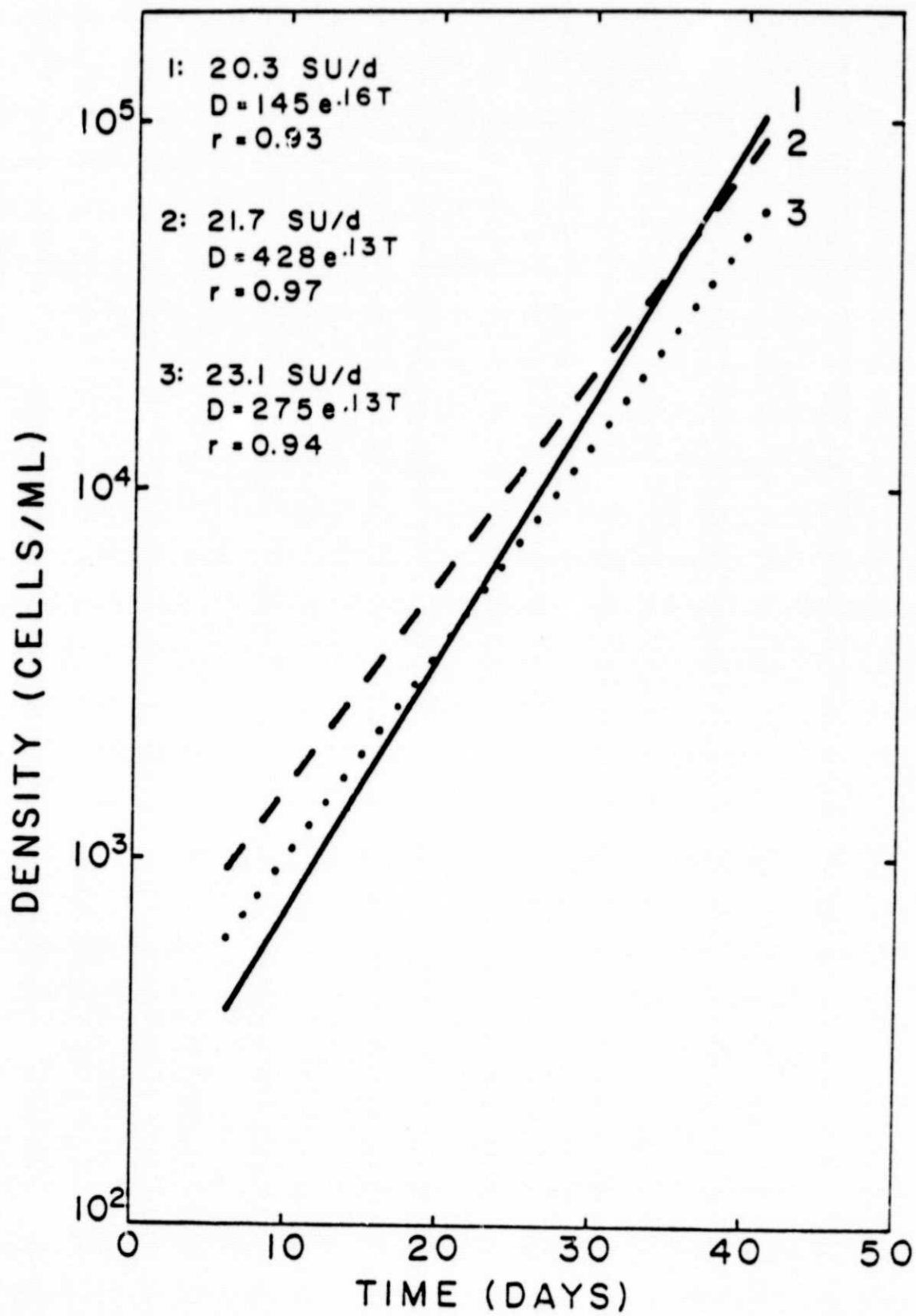


Fig. 9. Growth curves for Thalassiosira sp. 6 in batch cultures exposed to four different levels of UV-B radiation.

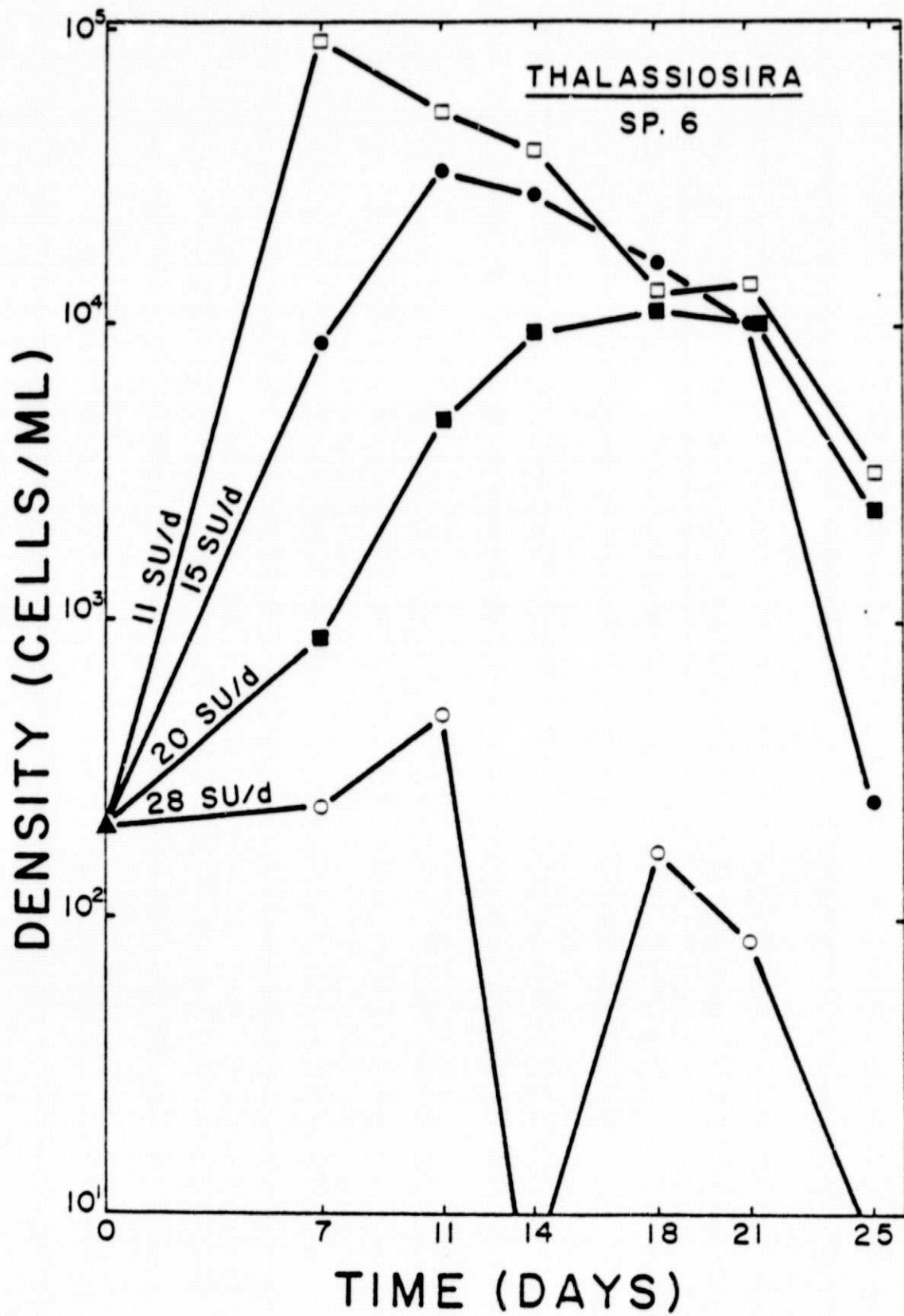


Fig. 10. Growth curves for Platymonas suecica in batch cultures exposed to four different levels of UV-B radiation.

