AN ABSTRACT OF THE THESIS OF

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Title: Influence of Ultraviolet-B Radiation on the Heterotrophic Activity of Estuarine Bacterioplankton

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Henry Van Dyke

The electromagnetic radiation absorption characteristics of nucleic acids and proteins are such that ultraviolet (UV) radiation is biologically important. While it is conceivable that UV radiation has been and may continue to be beneficial to some organisms, most experimentally observed responses are deleterious.

Ozone absorbs radiation in the UV-C (< 290 nm) and UV-B (290-320 nm) wavebands. Thus, the presence of an equilibrium amount of stratospheric ozone effectively shields the earth from this potentially detrimental radiation of solar origin. Concern about anthropogenic changes in the composition of the stratosphere, especially the possible depletion of ozone coincides with concern for the biological effect of the concomitantly enhanced UV-B transmission to the earth's surface.

To appropriately assess the potential ecological impact of such a change, it is desirable to understand the direct and indirect effects of UV-B radiation on the components of the biosphere. This study considered the estuarine system, and specifically the

contribution of heterotrophic bacterioplankton to its functioning. Continuously seeded estuarine microcosms were chronically irradiated with UV-B at absolute fluence levels of $2.21 \cdot 10^2$ to $7.06 \cdot 10^3$ $J \cdot m^{-2} \cdot d^{-1}$. When biologically weighted, these fluences range from effectively deficient to an enhancement of about 60% above current autumnal levels at the experimental latitude. The microcosms also received photosynthetically active radiation at normal levels.

Samples were drawn periodically, sieved to exclude eukaryotic components, and tested for glucose heterotrophic potential. This technique gives measures of velocity maximum for the uptake of the substrate under study (V_{max}), turnover time (T_t), and assemblage affinity for the substrate plus its natural concentraton ($K_t + S_n$). Because initial samples indicated a correlation between accumulated UV-B fluence and ($K_t + S_n$), a bioassay for S_n was performed which permitted further exploration of the nature of the combined response. Cell concentration, biomass, dissolved organic carbon, chlorophyll *a*, and temperature were also measured.

It was determined, within the fluence and time limits of this study, that the heterotrophic activity of the bacterioplankton assemblage changed. Such change was linked primarily to effects on other trophic levels, but limited evidence of direct effect was also noted.

One potentially useful measure of bacterial stress was $V_{max} \cdot cell^{-1}$ which was negatively correlated with UV-B fluence provided one or more additional environmental variables were

incorporated into the model. Resolution of $(K_t + S_n)$ into its components suggested that both were negatively correlated with UV-B fluence. It also permitted the comparison of actual and potential glucose uptake velocities. Reduction in actual velocity of glucose utilization with accumulated UV-B was accompanied by an increase toward the potential velocity of the system. If V_{max} represents a tolerance limit for the heterotrophic bacterial assemblage, the ecological implications of the measure are deserving of further study.

Influence of Ultraviolet-B Radiation on the Heterotrophic Activity of Estuarine Bacterioplankton

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Magnificat anima mea Dominum
Et exsultavit spiritus meus in Deo,
salutari meo.
Quia fecit mihi magna qui potens est,
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I. INTRODUCTION

The spectral distribution of solar electromagnetic radiation is altered by a number of components of the earth's atmosphere (see Gast 1965 for graphic presentation). These alterations, which result from resonant absorption, effectively reduce the fluence of those wavebands for which absorbers exist.

One such absorber, ozone (O_3) is formed in the stratosphere by the photolysis of oxygen (O_2):

$$O_2 + h\nu_1 \longrightarrow 2\ O$$

$$O + O_2 + M \longrightarrow O_3 + M$$

and is itself dissociated:

$$O_3 + h\nu_2 \longrightarrow O_2 + O$$

$$O + O_3 \longrightarrow 2\ O_2$$

in a series of reactions in which M is any background molecule and $h\nu$ is a photon of the appropriate energy: (1) $\lambda < 246$ nm; (2) $\lambda <$ 320 nm (Chapman 1930).

In its formation and destruction, ozone effectively shields the earth from solar electromagnetic radiation less than 320 nm in wavelength. This portion of the spectrum, known as ultraviolet (UV) -B (290-320 nm) and -C (40-290 nm), is included in the absorption bands of nucleic acids and proteins, which exhibit maxima at 260

and 278 nm, respectively (Giese 1968a). While some authors refer to this coincidence as fortuitous (Seliger 1977), others stress the coevolutionary forces of organisms and their environment (Berkner and Marshall 1967; Sagan 1973; Margulis et al. 1976; Caldwell 1979). Whatever the chronology of events, it seems apparent that, prior to the existence of phototrophy, the absence of oxygen, and therefore its allotrope ozone, would have permitted the transmission of ultraviolet radiation through the earth's atmosphere. This radiation may have supplied some of the energy necessary for the initial formation of complex molecules (Urey 1960), but would, under current conditions, be detrimental to the integrity of those molecules (Giese 1968b; Caldwell 1972, 1979; Pollard 1974; Murphy 1975).

It has been suggested that the rate of formation of the ozone shield over geologic time was such that successful organisms were those possessing avoidance, shielding, and/or repair mechanisms to mitigate UV damage (Caldwell 1972).

While several authors have noted organisms for which current levels of UV are deleterious (Calkins 1974, 1975b; Nachtwey 1975; Jokiel 1980), the assumption has been that stratospheric shielding is adequate to prevent UV from overwhelming the survival mechanisms of assemblages of organisms. This assumption seems to be justified for UV-C, but concern over UV-B penetration has grown throughout the 1970's.

In the unperturbed stratosphere, the Chapman reactions, plus

naturally occurring ozone destructive reactions involving NO_x, HO_x, and possibly other materials, result in equilibrium amounts of stratospheric ozone averaging 0.32 cm (0.24 - 0.46 cm) at standard temperature and pressure (Environmental Studies Board 1973). This concentration could be expected to change if either rates of formation or rates of destruction were altered.

The development of supersonic transports (SSTs) stimulated consideration of the potential for anthropogenic introduction of ozone-destructive materials into the stratosphere (Crutzen 1972). Although later refinements of the SSTs and of the models used to predict their effects on ozone showed the impact to be minimal (NAS 1979a), the concern generated extensive investigation of the problem, especially in the United States. The Climatic Impact Assessment Program (CIAP) of the Department of Transportation and the Climatic Impact Committee of the National Research Council alerted regulatory agencies to the existence of potentially serious problems due to a diminished ozone layer.

Other possible agents of stratospheric ozone depletion include chlorofluorocarbons (Lovelock 1971; Molina and Rowland 1974; Stolarski and Cicerone 1974b), carbon tetrachloride (Singh et al. 1976), methyl chloride, methylene chloride, and chloroform (Spence et al. 1976), nuclear weapons (Koslow 1977), methyl bromide (McElroy et al. 1977), and industrially-fixed nitrogen fertilizers (Liu et al. 1977). Of this group, chlorofluorocarbons have been studied most extensively and are felt to pose the greatest threat

(NAS 1979a, b).

In 1971, Lovelock reported the presence of atmospheric chlorofluorocarbons. Large quantities of these materials, especially CCl_2F_2 and CCl_3F which are commonly referred to by the trade name Freons, have been widely used as refrigerants, solvents, and propellants (Howard and Hanchett 1975; McCarthy et al. 1977). Their apparent inertness made them seem especially appropriate for these applications. Because Freons were assumed to maintain their inertness as they diffused, Lovelock viewed them as potential tracers of gaseous movement. Shortly thereafter, a catalytic cycle involving free chlorine atoms was described by Stolarski and Cicerone (1974a):

$$\begin{aligned} Cl + O_3 &\longrightarrow ClO + O_2 \\ O_3 + h\nu &\longrightarrow O_2 + O \\ ClO + O &\longrightarrow Cl + O_2 \\ \hline 2\,O_3 + h\nu &\longrightarrow 3\,O_2 \quad \text{(net)} \end{aligned}$$

The significance of this relationship became apparent through the hypothesis of Molina and Rowland (1974). Photolysis of chlorofluorocarbons in the stratosphere was seen to provide free chlorine for ozone catalysis until downward diffusion and tropospheric rainout removed the chlorine from the atmosphere. Reaction by the scientific community was rapid, controversial, and continuing. For an historical review, see Dotto and Schiff (1978).

In two recent reports (NAS 1979a,b), the U.S. National

Academy of Sciences affirmed earlier predictions (NASA 1976, NASA 1977) that chlorofluorocarbon release will result in a decrease in stratospheric ozone. Barring the discovery of currently unknown sinks or substantial changes in the rate constants for some of the 125 reactions apparently involved, the potential for that depletion is greater than had previously been predicted. Limits vary, since they are predicated on several chlorofluorocarbon release scenarios, but the most probable value is taken to be an eventual ozone reduction of 16.5 percent with 1977 release rates (NAS 1979b).

Such a depletion would shift the current short-wavelength limit of solar UV radiation, permitting shorter wavelengths to reach the earth (Environmental Studies Board 1973). Also there would be increased fluences of the longer UV-B wavelengths. With such spectral changes, the previously noted UV-B absorption spectra for nucleic acids and proteins focus the cause for concern (Setlow 1974).

The biological implications of increased UV-B radiation may be studied at the level of the individual organism, as they are in epidemiological work dealing with human skin cancer. Another valid consideration is the effect of UV-B and its potential enhancement on a functioning ecosystem. Here such effects can be viewed economically or ecologically, including the effect upon the general quality of human life.

In 1974, Calkins found that the majority of microorganisms in the fresh water habitat under study were exposed to near-tolerance

limits of UV-B. Calkins and Thordardottir (1980) concluded that marine diatoms were under considerable stress. Increase in UV-B fluence could be expected to adversely affect the overall microbial community or specific microbial populations within the community by overwhelming UV-B survival mechanisms (Nachtwey 1975). Either effect could cause disruptions in productivity and nutrient and element cycling. The consequences of such perturbations might pose a threat to the stability of the ecosystem (Caldwell 1972), and thus to the food webs involved (Smith 1972). The ecological implications are substantial (Provasoli et al. 1959; Hulburt 1970; Worrest et al. 1978).

Estuaries are accepted as among the most productive ecosystems (Odum 1972). UV-B absorption is significant in such waters since chlorophyll, dissolved organic material, and other dissolved and suspended materials increase attenuation, thus decreasing penetration (Jerlov 1950; Calkins 1975a; Smith and Baker 1979). Even with such absorption, though, organisms high in the water column could be stressed (Nachtwey 1975; Calkins et al. 1976; Worrest et al. 1978).

As noted previously, stress to one or more components of an ecosystem may have impact throughout the entire system. Therefore, this study was undertaken to elucidate the effect of UV-B on the bacterioplankton of an estuarine ecosystem, specifically on the response of the functional capabilities of the bacterial assemblage. Is the bacterioplankton assemblage functionally sensitive,

highly stable, or amenable to adaptive change when exposed to UV-B radiation? What is the response of the bacterioplankton to changes in other community components that may, in turn, have been UV-B induced?

In terrestrial ecosystems, bacteria have been viewed primarily in their role as decomposers. In aquatic systems, however, their role is more diverse. The importance of suspended heterotrophic bacteria in sea water has been recognized for some time. In 1933 Waksman et al. reported the decomposition of zooplankton and algae by marine bacteria. Krizencky and Podhradsky (1927, cited in ZoBell 1954) emphasized the potential importance of bacteria as a food source for aquatic animals. This has been substantiated in the work of Seki and Kennedy (1969), Reiswig (1975), Sieburth et al. (1977), and Porter et al. (1979) for zooplankton, sponges, suctorians, and protozoans, respectively. Through respiration and extrusion, bacteria contribute to the recycling of inorganic substances for the primary producers (Hoppe 1976). Bacteria have been shown to contribute vitamins, CO_2, and possibly nitrogen to primary producers (Burkholder 1959; Gorden et al. 1969). Another mechanism, long recognized, is the bacterial transformation of dissolved organic carbon (DOC) into bacterial biomass (ZoBell and Grant 1943). Since this latter function leads directly or indirectly to most of the other functions noted above, and since, further, it helps to moderate DOC levels, it is undoubtedly among the most important contributions of marine bacteria (Wright and

Hobbie 1966; Holmes et al. 1967; Sorokin 1971; Crawford et al. 1974; Hoppe 1976; Azam and Hodson 1977).

If then, one were to study the functioning bacterial assemblage, it would seem appropriate to measure its DOC utilization. Two questions must be posed before attempting the interpretation of such a measurement: is a substantial proportion of the bacteria within the system involved in this activity, making this a reasonable measure, and can DOC utilization per se be measured?

Prior to the development of epifluorescent enumeration techniques, bacterial concentrations were generally determined by counts of colonies on specified agar media (Rodina 1972). Such counts indicated a preponderance of bacilli whose population was known to vary directly with that of the phytoplankton (ZoBell 1946). It appeared to have been the general consensus of marine ecologists that such bacteria, normally utilizing detritus as a nutrient source, comprised the vast majority of bacteria in the sea (Parsons and Strickland 1962; Seki and Kennedy 1969).

The work of Wiebe and Pomeroy (1972) revolutionized this consideration. Not only did they note that more than 50% of the recognizable cells in freshly taken bacterial samples were coccoid, but they found that greater than 70% of the cells were unattached. Apparently the selective nature of plate counts had resulted in the erroneous underestimation of bacterial concentrations by as much as three orders of magnitude (Jannasch and Jones 1959; Francisco et al. 1973; Hoppe 1976, 1977, 1978; Zimmermann 1977;

Sieburth et al. 1978).

It is now known that 80 to 90% of the marine and estuarine pelagic bacteria can be considered to be unattached cells (bacterioplankton) and that it is these cells which are primarily responsible for the activities noted previously, with the possible exceptions of detrital decomposition and some animal feeding. See Sieburth (1976, 1979) for a review of evidence that bacterioplankton are viable, planktonic, dependent upon DOC for nutrients, and important in the maintenance of energy flow within the system.

While DOC represents the most abundant carbon source in most aquatic systems (Hanson and Snyder 1979), its chemical composition and biological availability are such that it is not currently adaptable to the development of a radiotechnique in which the investigator supplies appropriately labeled DOC for the study of its utilization. (See subsequent section for a further consideration of DOC).

For a simplified approximation of bacterial activity, investigators have utilized single nutrient molecules such as monosaccharides or amino acids or simple mixtures thereof in heterotrophic potential studies (Wright and Hobbie 1965, 1966; Hobbie and Crawford 1969a), tracer turnover studies (Williams and Askew 1968; Azam and Holm-Hansen 1973), uptake measurements (Griffiths et al. 1977), and autoradiography (Hoppe 1976; Meyer-Reil 1978).

These techniques permit prudent extrapolation to the natural system, providing possible measures of the heterotrophic activity

of the bacterial assemblage. When coupled with total cell counts, an activity index (Wright 1978) could permit comparison among systems. The suggestion that such indices reflect the response of the bacterioplankton to environmental variables should be evaluated.

In this study, estuarine microcosms were exposed to varying fluences of UV-B. Heterotrophic potential determinations were performed on samples drawn from these microcosms. These determinations provide three measures of activity: potential uptake velocity (V_{max}), turnover time (T_t), and transport constant plus natural substrate concentration ($K_t + S_n$). Each of the other methods noted previously gives only a single measure of heterotrophic activity. Since the nature of the bacterial activity response in the UV-B stressed microcosms was not known, it seemed appropriate to determine as many such evaluations as possible. Additionally, the heterotrophic potential technique yields respiration or mineralization data for the substrate utilized. Besides the activity measures, cell enumeration was also relevant to this study.

The data acquired were examined for patterns that might elucidate the nature of the bacterioplankton response to ultraviolet-B radiation. Because heterotrophic potential and direct cell count methods are laborious, time-consuming, and therefore difficult to perform on replicate systems, additional measures of biomass, chlorophyll _a_, and DOC were made to explore the possibility of a less complex measure correlated to bacterial activity, as well as to better describe the total system response to UV-B.

II. MATERIALS AND METHODS

In the study of a natural system samples may be removed to the laboratory where a single experimental variable is introduced. In such a procedure sampling difficulties are encountered and the subsystem is removed from the influence of numerous physical, chemical, and biotic variables.

The alternative approach, that of studying the entire ecosystem, in this case, the estuary, and particularly its bacterioplankton, would seem preferable, but the experimental logistics are impractical. Therefore, flow-through microcosms, described subsequently, were used in an attempt to define an experimental system and to expose that system to a measured stress (UV-B), while still subjecting it to most normal environmental variables (Cooper and Copeland 1973; Van Dyke and Thomson 1975; Worrest and Van Dyke 1978; Cairns 1979; Draggan and Van Voris 1979).

Continuously Seeded Microcosms

A small, Fiberglas greenhouse located on the property of the Oregon State University Marine Science Center, Newport, Oregon, was the site of sample exposure and collection. Estuarine water was pumped continuously into the common settling tank, from which the water passed into the greenhouse. At no point was this water filtered. Therefore its microbial community is considered to be representative of the Yaquina Bay estuarine community, minus

nekton, at a given time. Within the greenhouse, 15-l containers (microcosms) were immersed to within 4 cm of their tops in 800-l tanks (water baths), with four microcosms to a water bath (Figure 1). The microcosms were supported on rotating turntables (0.25 revolutions per minute). With each rotation, estuarine water entered the sidearm of the microcosm, was introduced into the microcosm proper approximately 3 cm from the bottom, and thus mixed substantially with the microcosm contents prior to overflow from the container. The inflow and overflow ports and the rate of revolution of the containers allowed approximately 15 liters of seawater to enter each container daily, but the system cannot be viewed as one of total replacement. The most obvious evidence that replacement was partial came through the establishment of diverse macroscopic communities among the radiation regimes, as well as measurable differences in diatom assemblage composition, chlorophyll _a_ concentration, and radiocarbon uptake (Worrest and Van Dyke 1978).

Electromagnetic Radiation: Fluences

Since the upper walls and roof of the greenhouse were constructed of Fiberglas, natural levels of solar radiation in the 380-800 nm waveband were transmitted to the microcosms, with only about 10% reduction in intensity (380 nm is the short-wavelength cutoff for Fiberglas). To compensate for this reduction, Westinghouse F40 Agro-Lites were included in the light bank over the

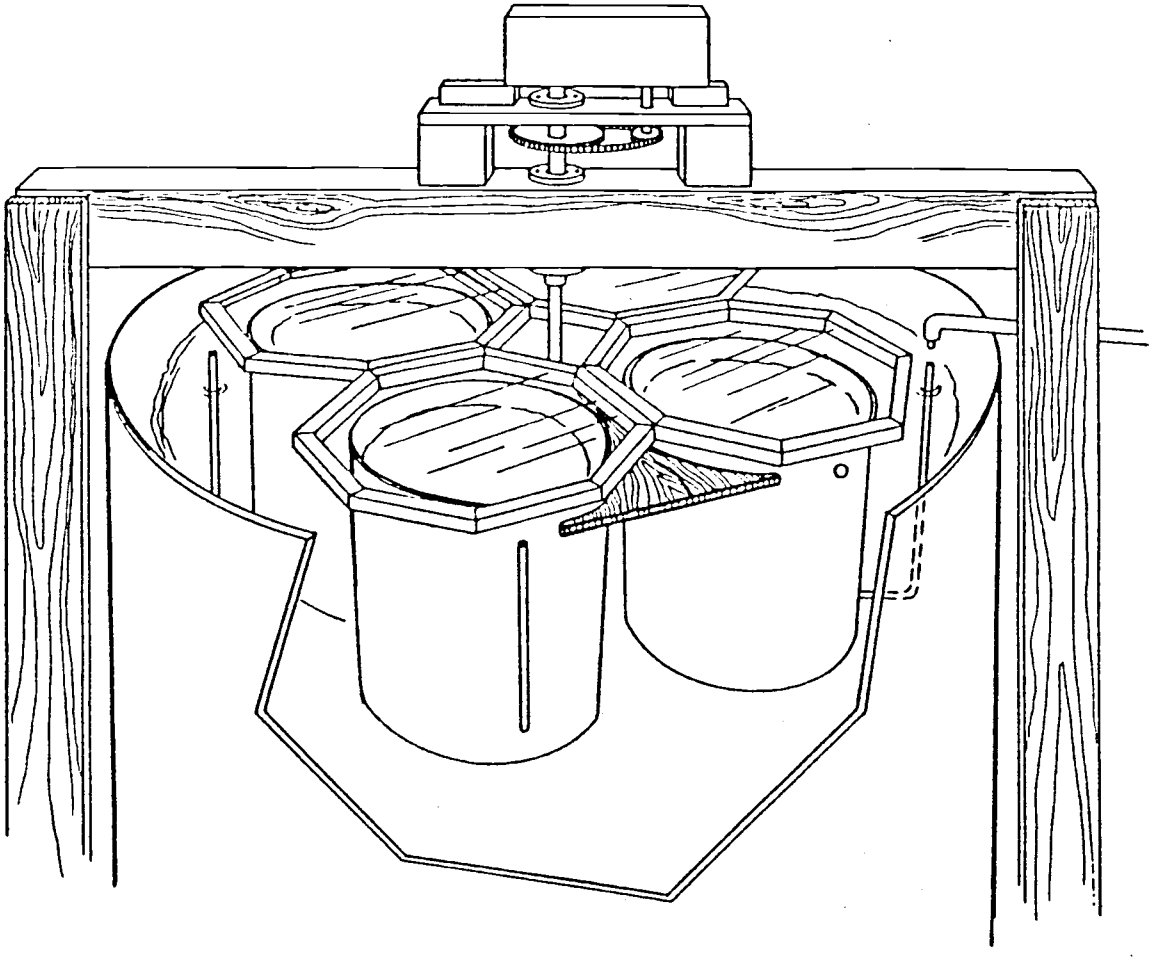


Figure 1. Four microcosms within a water bath.
See text for description of operational characteristics.

microcosms. The bank also included Westinghouse FS40 Sunlamps whose emission spectrum extends from approximately 275 to 380 nm (Karanas 1978), thus supplying radiation in the ultraviolet-B range (290-320 nm) (Figure 2). The level of photosynthetically active radiation was set to correspond with natural autumnal conditions at 45°N latitude, the approximate latitude of the Yaquina Estuary.

The interposition of filters between the radiation source and the microcosms, i.e. on top of the chambers, both shaped the spectra of the radiation reaching the surfaces of the microcosms and regulated the intensity of the UV-B. Each of the four containers studied was covered with a different filter: (A) a 0.18-mm (7 mil) thickness of Mylar 'D', (B) a 0.25-mm (10 mil) thickness of cellulose acetate (CA), (C) a 0.19-mm (7.5 mil) thickness of CA, and (D) a 0.13-mm (5 mil) thickness of CA (Hillcor Plastics, Santa Fe Springs, CA). The CA was partially photodegraded to a more stable transmission state prior to use, and all filters were changed weekly.

The daily "on" time for the light bank was chosen to provide radiation corresponding to (A) maximal natural UV-B exposure (about 54% of the total reaches the earth's surface within 4 hours around solar noon (Green and Mo 1975; Worrest 1978), (B) natural UV-B fluences (Table 1), and (C) predetermined fluences at the surfaces of the microcosms (Figure 3). The latter measurements were made by Worrest with a modified Gamma Scientific 2900SR Spectroradiometer

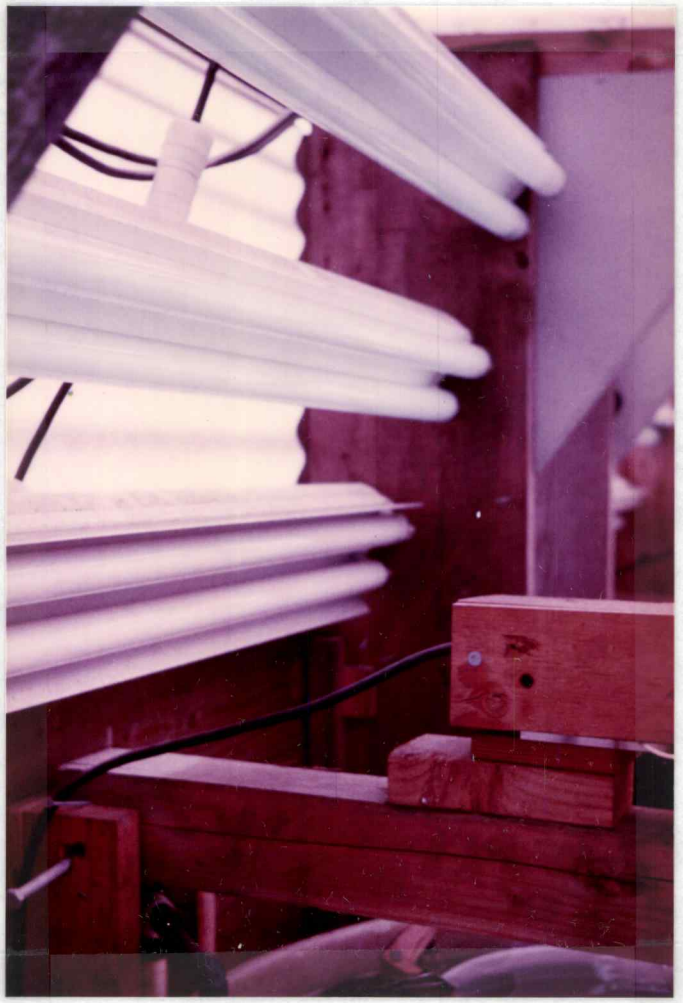


Figure 2. FS40 Sunlamps and F40 Agro-Lites positioned over microcosms.

Table 1. Current levels of solar UV-B radiation at the surface of the earth.

Latitude	Season*	Ozone Concentration (atm-cm)	Absolute Fluence (280 - 320 nm) ($J \cdot m^{-2} \cdot d^{-1}$)	Effective Fluence (Eff_{DNA} $J \cdot m^{-2} \cdot d^{-1}$)
40°N	Fall	0.281	81,915	53.2
	Winter	0.318	16,713	4.5
50°N	Fall	0.299	52,978	24.3
	Winter	0.357	4,214	0.8

*equinox or solstice initiating that season.

From Nachtwey (1979) and Green et al. (1980).

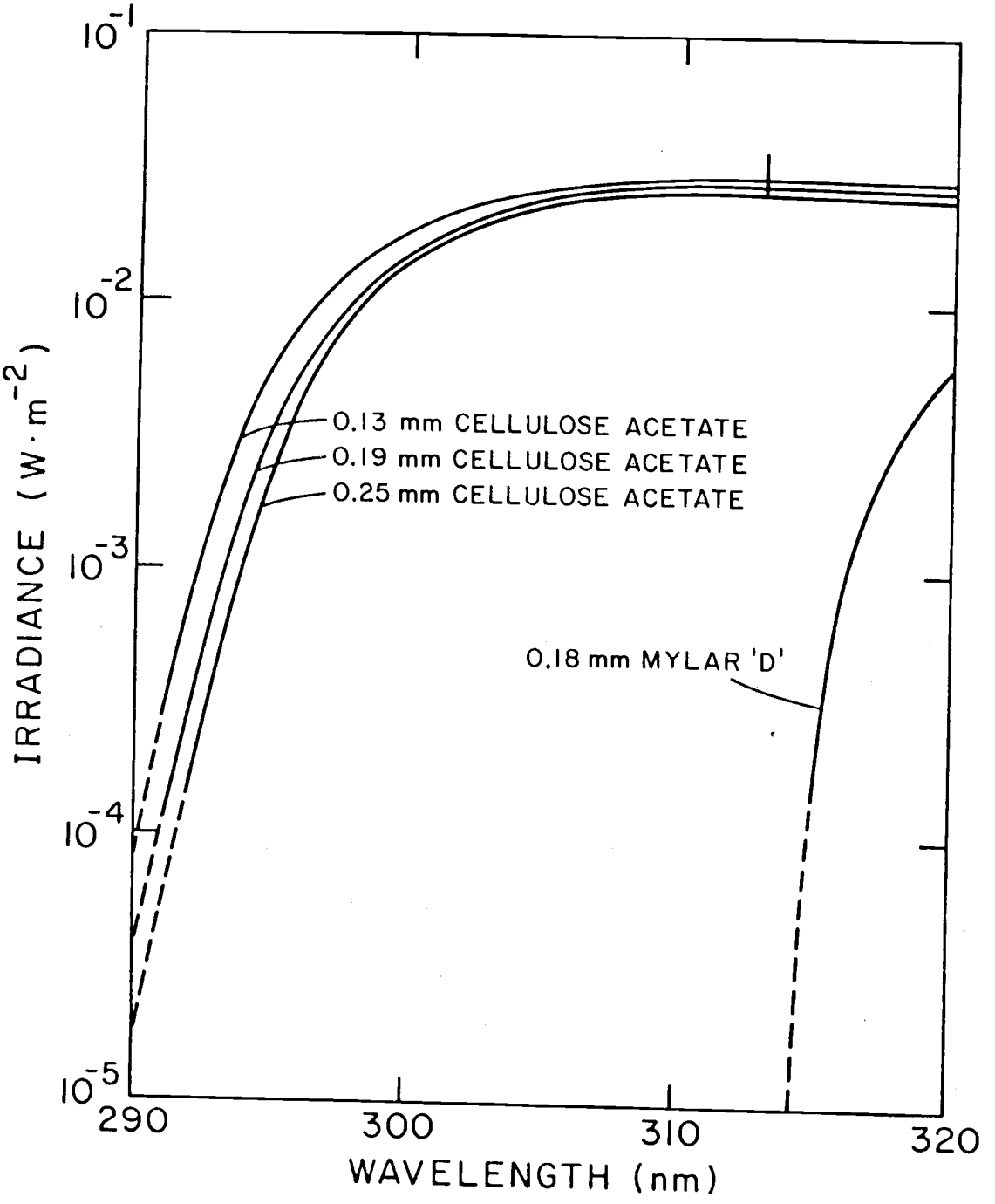


Figure 3. UV-B spectral irradiance at the surface of the four microcosms.

System which has been characterized by the U.S. National Bureau of Standards (Worrest et al. 1978). Corrections for stray light at shorter wavelengths were made by extrapolation.

The Setlow (1974) weighting function:

$$\varepsilon_{DNA}(\lambda) = \left\{ \exp \left[13.82 \quad \frac{1}{1 + \exp\left[(\lambda - 310)/9\right]} - 1 \right] \right\}$$

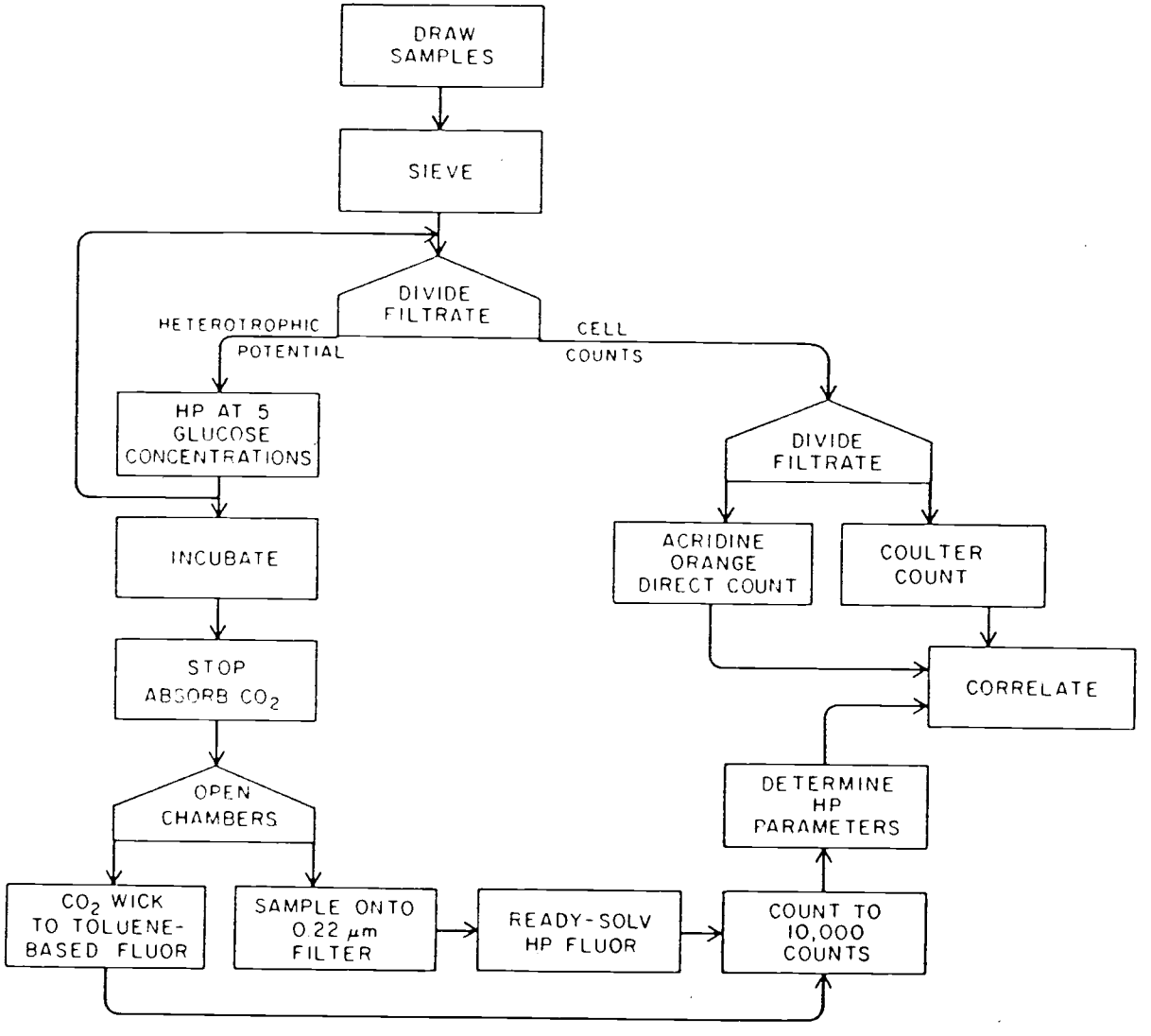
characterized the DNA absorption spectrum in the UV-B range (Green and Miller 1975). Summing the weighted fluence rates over the UV-B waveband permitted comparison among dissimilar radiation sources.

The three hour daily "on" time approximated normal autumnal DNA-weighted fluence levels in the 0.25-mm CA filtered microcosm, and provided enhancement of about 20% and 60% over current average autumnal fluences at 45°N latitude in the 0.19-mm and 0.13-mm CA filtered microcosms, respectively. That these enhancements deviated farther from the natural fluences as time progressed was recognized, and represented one of the time constraints on this research.

Sampling

The establishment and development of four microcosms, one under each of the radiation regimes described, was studied during November, 1979. During this time, the systems were sampled periodically for the determination of glucose heterotrophic potential, bacterial concentration, particle concentration, and dissolved organic carbon concentration (Figure 4). Salinity and water temperature were recorded. The complexity of the activity measures

Figure 4. Summary of the experimental protocol for the heterotrophic potential and cell count techniques.


DRAW SAMPLES
SIEVE
DIVIDE FILTRATE
HETEROTROPHIC POTENTIAL
CELL COUNTS
HP AT 5 GLUCOSE CONCENTRATIONS
INCUBATE
STOP ABSORB CO_2
OPEN CHAMBERS
CO_2 WICK TO TOLUENE-BASED FLUOR
SAMPLE ONTO 0.22 μm FILTER
READY-SOLV HP FLUOR
COUNT TO 10,000 COUNTS
DETERMINE HP PARAMETERS
DIVIDE FILTRATE
ACRIDINE ORANGE DIRECT COUNT
COULTER COUNT
CORRELATE

prevented replicate measures among the water baths, as did non-uniform flow into one of the water baths. Parallel determinations of biomass and chlorophyll *a* content were performed by another investigator (R. C. Worrest 1979).

Sampling at each test period was done at approximately the same time of day to eliminate possible variation due to diel metabolic activity (Hug et al. 1972; Jolley et al. 1976; Sieburth et al. 1977; Tilzer and Horne 1979).

For those measurements carried out by this investigator, sampling was done by means of a 50-ml sterile syringe, the opening of which was shielded to depth to minimize the inclusion of neuston in the sample (Parker 1967; Dietz et al. 1976; Sieburth 1976; Sieburth et al. 1976; Fehon and Oliver 1979). All samples were drawn from the approximate planar center of each microcosm at a depth of about 8 cm. Care was taken to avoid agitation of the contents of the microcosm which could have resulted in the inclusion of attached or benthic forms in the sample. Through repeated sampling, a total volume of 800 to 1000 ml was drawn from each microcosm for each of the periodic tests.

The samples were then transported to the laboratory, where they were divided for the treatments described subsequently. All such handling was done in sequential, random order, and was completed within two hours to minimize sample variation due to physical change and biological activity.

Sieving

Since the primary purpose of this study was to measure the response of one component of the estuarine ecosystem, the bacterioplankton, to UV-B, a sieving technique was utilized to exclude the eukaryotic and large detrital components from the samples prior to determination of activity and numbers (Williams 1970; Wiebe and Pomeroy 1972; Azam and Holm-Hansen 1973; Derenbach and Williams 1974; Berman 1975; Azam and Hodson 1977; Hanson and Wiebe 1977; Hanson and Snyder 1979). Following the work of Salonen (1974) and Burnison (1975), Nuclepore filters (Nuclepore Corp., Pleasanton, CA) were employed. The production of pores in these polycarbonate filters is so accomplished that actual filter retention corresponds closely to their stated porosity, whereas cellulose filters retain particles somewhat smaller than their stated porosity (Sheldon and Sutcliffe 1969; Sheldon 1972). Preliminary tests by the investigator suggested that either a 1-μm or a 2-μm pore diameter Nuclepore filter would separate effectively the prokaryotic component, especially the non-attached forms (Figure 5). Although it was recognized that the largest prokaryotes might be excluded from the sample by the 1-μm filter, it was more efficient than the 2-μm filter in the removal of unwanted material while still permitting the passage of sizable concentrations (10^5 or more cells·ml^{-1} in the samples used) of bacteria (Azam and Hodson 1977; Sieburth 1979). Therefore the 1-μm filter was selected. Additionally, sieving facilitated Coulter counting and assured activity and particulate

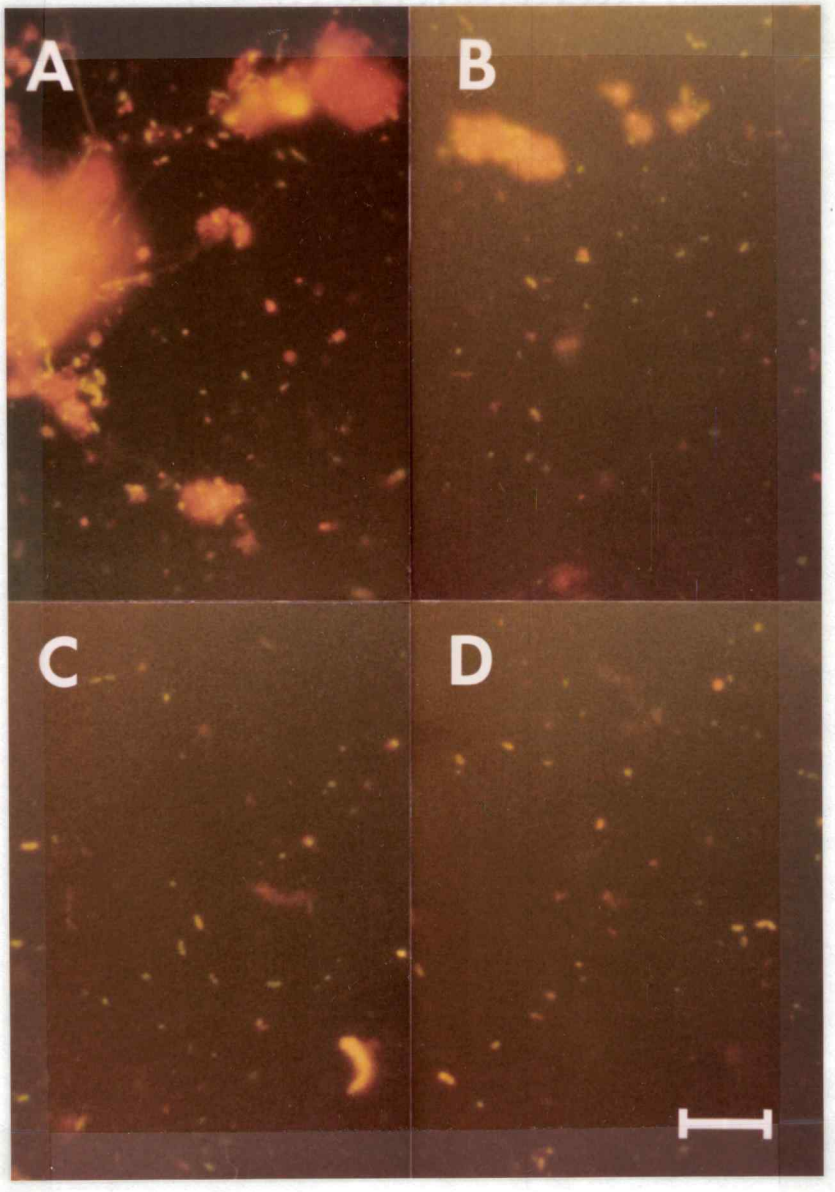


Figure 5. Selective filtration of a 0.18-mm Mylar 'D' sample through Nuclepore polycarbonate filters: (A) Unsieved; (B) 5-µm pore size; (C) 2-µm pore size; (D) 1-µm pore size. Acridine orange stained preparation photographed with epifluorescent illumination. ⊢ = 10 µm.

measures of the same assemblage (Gordon 1970).

Sieving was carried out on a sterile Millipore fritted glass base with a stated porosity of 60 μm (Kopek 1979) which had been thoroughly rinsed with particle-free (0.22-μm Millipore GS filtered), sterile, glass-distilled water. The vacuum was maintained at 20 cm_{Hg} or less to minimize cell damage (Allen 1971; Derenbach and Williams 1974; Berman 1975; Azam and Hodson 1977). Subsamples of the sieved material were preserved for later enumeration, as described subsequently.

Heterotrophic Potential

In situ determinations of total heterotrophic bacterial activity are not possible. However, a number of parameters can be measured which permit cautious extrapolation to the natural system. One of the more frequently used methods, based on the work of Parsons and Strickland (1962) as later modified by Wright and Hobbie (1965, 1966) and Hobbie and Crawford (1969b), is the heterotrophic potential determination. Parsons and Strickland studied the heterotrophic uptake of ^{14}C-labeled glucose and acetate by marine planktonic microorganisms. Their data showed that uptake, when related to substrate concentration, followed the Michaelis-Menten first-order kinetics of the enzyme-substrate relationship (Cohen and Monod 1957; Kepes 1963). In the method which evolved from their work, a ^{14}C-labeled organic compound at several final concentrations in the microgram per liter range is added to

natural samples. Incubation in a closed system permits recovery of $^{14}CO_2$ from microbial respiration, as well as measurement of assimilated ^{14}C. Liquid scintillation counts of samples yield data which can be analyzed by means of the modified Lineweaver-Burk transformation to give measures of potential uptake velocity (V_{max}), natural turnover time (T_t), and the sum of the bacterial assemblage transport constant and the natural substrate concentration ($K_t + S_n$) (Lineweaver and Burk 1934; Wright and Hobbie 1965, 1966; Hobbie and Crawford 1969a). All such measures are limited to the substrate selected for study, and caution is required in interpretation (Wright 1973).

Since the natural concentration of glucose in estuarine waters is in the microgram per liter range (Hicks and Carey 1968; Vaccaro et al. 1968; Andrews and Williams 1971; Moshiri et al. 1979), the use of high specific activity ^{14}C-glucose is required. This permits the addition of amounts comparable to natural concentrations which still contain sufficient ^{14}C to facilitate adequate uptake during incubation for detection by the radionuclide counting methodology. The labeled substrate utilized in this study, D-[U-^{14}C] glucose (190 $\mu Ci \cdot ml^{-1}$) was obtained from Amersham/Searle Corporation, Arlington Heights, Illinois. It was diluted to 1 $\mu Ci \cdot ml^{-1}$ by standard volumetric technique. The diluent, sterile, particle-free distilled water, had been acidified with HCl to pH 3 to rid the solution of ^{14}C-bicarbonates that might be present as contaminants (Williams and Askew 1968; Wright 1975b; Sepers and

Van Es 1979). The solution was dispensed and sealed into 5-ml glass ampules, autoclaved at 15 lbs pressure for 30 minutes, cooled, checked for leakage, and frozen until used.

Sieved samples from each of the microcosms were tested at nine day intervals for bacterial heterotrophic activity. Ten-ml aliquots of each sample were dispensed into 25 60-ml serum bottles which had been previously acid washed and pressure sterilized. The bottles were divided into groups of five, and blanks were prepared by adding 0.2 ml of 50% phosphoric acid to two bottles from each group (Williams and Askew 1968; Wright 1975b; Albright and Wentworth 1976). The resulting reduction in pH to < 2 stopped bacterial activity prior to the addition of labeled substrate. These blanks were used to correct for substrate adsorption onto sample particulates, including bacteria, and for dissolved radioactive contaminants in the substrate. The bottles in which the heterotrophic activity was not stopped at this point in the procedure are referred to subsequently as 'actives'.

Aliquots of the ^{14}C-glucose solution ranging from 20 µl to 400 µl were dispensed into the bottles to give triplicate actives and duplicate blanks at final concentrations of 1.70, 8.48, 16.95, 25.42, and 33.90 $\mu g_{glucose} \cdot l^{-1}$. This range corresponds well with the studies of Vaccaro and Jannasch (1966), Vaccaro (1969), Wright (1978), Hanson and Snyder (1979) and others, all of whom worked with estuarine or marine systems. The dispensing was facilitated by the use of a Drummond Dialamatic Microdispenser (Drummond

Scientific Co., Broomall, PA), which had been shown previously to give excellent reproducibility (Appendix I).

Bottles were stoppered immediately with serum cap - plastic cup - filter paper assemblies (Figure 6), placed in handling trays, and mixed briefly by gentle rotation on a laboratory rotator (New Brunswick Scientific Co., Model G2).

Temperature during incubation was maintained at 15 ± 1°C by means of a circulating, thermostatically controlled water bath. This temperature was chosen because it approximated in situ conditions at the beginning of the study, and was continued because of ease of maintenance during handling and incubation procedures. It was recognized that such maintenance represented a greater change from in situ to incubation temperature for later studies, and that this must be taken into account when interpreting results.

The subdued light of the laboratory was further restricted by the opaque handling trays. Therefore photosynthetic activity of the samples was presumed to have been blocked.

Incubation time was from one to five hours, depending upon the presumed activity of the samples. It is required that the time be sufficiently long to permit significant uptake, but that uptake must be restricted to < 5% of the glucose added. Should larger quantities be taken up, the substrate becomes limiting, and Michaelis-Menten kinetics no longer apply (Wright and Hobbie 1965; Giese 1968a; Wright 1973; Dietz et al. 1977; Delattre et al. 1979). Isotopic preference and soluble ^{14}C excretions were presumed to

Figure 6. Serum bottles (60-ml) and handling tray. An accordian-folded piece of Whatman #1 chromatography paper (2.6 x 4 cm) is contained in the plastic cup assembly (Kontes Glass Co., Vineland, NJ: K-882320) suspended in the air space over each water sample.

remain insignificant during the incubation.

Metabolic activity of the actives was terminated by the injection through the spectum of 0.2 ml of 50% phosphoric acid into the filtrate. While the resulting pH reduction promotes CO_2 release from solution, some investigators suspect that it is responsible for cell lysis and concomitant loss of assimilated ^{14}C (Griffiths et al. 1974; Baross et al. 1975). To limit such loss, the samples were placed in an ice bath following acidification, and kept at 0 °C until filtration (Ramsay 1976).

One hour after acid fixation, 0.15 ml of β-phenylethylamine (Sigma Chemical Co., St. Louis, MO) was injected through the septum onto the filter paper in the cup. This chemical has been the CO_2-absorber of choice for liquid scintillation counting since Hobbie and Crawford introduced the technique in 1969(b). Approximately one hour after the addition of the CO_2 absorbant the bottles were placed on the laboratory rotator for 15 minutes to further promote CO_2 absorption by the treated filter paper.

The serum cap assemblies were removed, and the filter papers placed in coded liquid scintillation vials containing 10 ml of cocktail [0.4% 2,5-diphenyloxazole (PPO) and 0.01% 1,4 bis- 2-(4-methyl-5-phenoxazolyl)-benzene (dimethyl POPOP) in toluene] (Wright 1975b). Polyvinyl chloride cap liners for the vials alleviated the problem of activity loss by diffusion.

The fluid content of each serum bottle was filtered, using a Millipore manifold assembly, through a 25-mm diameter 0.22-μm

Millipore GS filter to retain assimilated and adsorbed ^{14}C. Bottles and filters were rinsed with two 10-ml aliquots of chilled, sterile, particle-free seawater to insure transfer and removal of non-assimilated ^{14}C (Baross et al. 1975; Albright and Wentworth 1976). The filters were drawn to apparent dryness, and then transferred to coded scintillation vials. Ten ml of Beckman Ready-Solv HP (Beckman Instruments, Inc., Fullerton, CA) fluor was added to each vial. This scintillation cocktail was chosen to cause membrane solubilization of the wet filters.

All vials were counted for $1 \cdot 10^4$ counts or 10 minutes in a Beckman LS 8000 Liquid Scintillation System with Texas Instruments Silent 700 Electronic Data Terminal. Liquid Scintillation Library Program #3 was modified to repeat the counting sequence and to record only channel 2 data. In this system, channel 2 includes discriminators 397 through 655 of 1000, and is especially suited for ^{14}C detection (Beckman Instruments 1978). Vial positioning in the counter resulted in random wick orientation for the CO_2 absorbers, and no further correction was deemed necessary (Wang and Jones 1959; Hobbie and Crawford 1969b).

The printout included counts per minute (cpm) and H-number. The latter is a quench monitoring technique which utilizes a ^{137}Cs source as an external standard, and analyzes the shift in the cesium Compton distribution (Wang et al. 1975; Beckman Instruments 1978). Data were corrected for background and converted to disintegrations per minute (dpm) using the quench curve given in

Appendix II.

Data were manipulated as illustrated in Appendix III. The two blanks for each concentration and each exposure regime were averaged, but the actives were considered individually. Only when outliers could be traced to experimental errors were they deleted.

The plot of $T \cdot F^{-1}$ versus A gave the modified Lineweaver-Burk transformation:

$$T \cdot F^{-1} = A \cdot V_{max}^{-1} + (K_t + S_n) \cdot V_{max}^{-1}$$

of the Michaelis-Menten enzyme kinetics equation:

$$v = V_{max} \cdot S \cdot (K_t + S)^{-1}$$

where: A = added substrate concentration ($\mu g \cdot l^{-1}$)

F = fraction of the available substrate taken up

K_t = substrate concentration at which $v = V_{max} \cdot 2^{-1}$ (transport constant when applied to uptake)

S = substrate concentration ($\mu g \cdot l^{-1}$)

S_n = natural substrate concentration ($\mu g \cdot l^{-1}$

T = incubation time (h)

v = uptake velocity ($\mu g \cdot h^{-1}$)

V_{max} = theoretical maximum uptake velocity which presumably applies when all uptake sites are saturated (Wright and Hobbie 1965; Lehninger 1975; Wright 1975b)

Least squares regression was used to determine each line (Neter and Wasserman 1974). Values thus made available for

analysis were V_{max}, the inverse of the slope of the modified Lineweaver-Burk transformation; T_t, the Y-intercept; and $-(K_t+S_n)$, the X-intercept.

Bioassay

Because the $K_t + S_n$ value appeared to be most sensitive to accumulated fluence through the first three heterotrophic potential experiments, a bioassay was introduced. This procedure was designed to permit the independent determination of S_n, the natural glucose concentration in each of the microcosms (Hobbie and Wright 1965). It was then possible to calculate the bacterial assemblage transport constants as well.

A glucose-utilizing bacterium, YB II, was isolated from Yaquina Bay and was maintained in the laboratory on Difco 2216 Marine Agar (Difco Laboratories, Detroit, MI). Prior to use, the bacterium was grown in modified Hamilton and Austin (1967) liquid medium (100 $mg \cdot l^{-1}$ glucose, 10 $mg \cdot l^{-1}$ KH_2PO_4, 1 $g \cdot l^{-1}$ Na_2SO_4, 1 $g \cdot l^{-1}$ $NH_4H_2PO_4$ in artificial seawater [Instant Ocean Synthetic Sea Salts, Aquarium Systems, Inc., Eastlake, OH]) at 15°C. Stationary phase organisms were separated from this culture medium by centrifugation 12 hours prior to use, then washed and resuspended in Instant Ocean to assure nutrient depletion (ZoBell and Grant 1943; Hobbie and Wright 1965; Hamilton et al. 1966; Vaccaro and Hannasch 1966). Before use, the YB II washed cell concentration was determined by Petroff-Hauser count, and adjusted to approximately 4.10^7 $cells \cdot ml^{-1}$ in Instant Ocean.

Waters to be tested were drawn from the microcosms at the time of the fourth heterotrophic potential determination. They were Millipore filtered (0.22-μm GS) within one hour of collection, then frozen and held for bioassay. An Instant Ocean sample, in which manufacturer's specifications indicate no glucose, was also frozen. On the day of the bioassay, the water samples were thawed and warmed to incubation temperature, approximately 18°C.

The heterotrophic potential procedures described previously were used. The 20 μl ^{14}C-glucose addition was eliminated, and a series of serum bottles for the testing of Instant Ocean was added.

Some procedural changes were necessary due to the high K_t of YB II. Phosphoric acid was predispensed to the blanks, and labeled glucose was predispensed to all bottles. The test organisms were mixed with the sample waters to give a final concentration of approximately $3.2 \cdot 10^5$ cells·ml^{-1}. This mixture was then dispensed into the serum bottles in 10 ml aliquots, and the bottles were capped immediately. The incubation was accomplished at room temperature (18°C) and acid stopped at 0.5 h. Further manipulation and counting were as described previously.

Because of the relatively large cell size of the assay organism, cell surface adsorption of glucose gave high and irregular blank counts. Blanks for further calculation were determined, where possible, by the method of least squares with the elimination of outliers. If more than one linear regression seemed possible, that which gave the most consistent ratio of respired ^{14}C to total

^{14}C in subsequent calculations was the line of choice.

Among the final data, those with disparate respiratory ratios were eliminated. Usually such a high ratio indicated the partial loss of an assimilation sample in filtering. Final data maximized R^2.

The X-intercept for the Instant Ocean sample gave the K_t in $\mu g \cdot l^{-1}$ for YB II (Vaccaro 1969). This value was assumed to have remained constant in all chambers during the limited incubation period (Vaccaro and Jannasch 1966). Therefore, S_n values for the microcosms could be obtained by subtraction: $(K_t + S_n) - K_t$. The resulting S_n values were compared with the day 29 heterotrophic potential determinations which had measured the same water samples with their natural bacterial assemblanges. The transport constants for those assemblages were determined by subtraction: $(K_t + S_n) - S_n$.

These results completed the information necessary to compute v, the actual velocity of glucose utilization:

$$v = S_n \cdot T_t^{-1}$$

(Vaccaro and Jannasch 1966; Hobbie 1967)

Acridine Orange Direct Count

The occurence in seawater of filterable bacteria, i.e. bacteria small enough to pass through a membrane with a pore diameter of approximately 0.4 μm, was reported by Oppenheimer (1952). Subsequent studies emphasized the importance of these picoplankton (0.2 - 2.0 μm) (Anderson and Heffernan 1965; Williams 1970; Azam

and Holm-Hansen 1973; Ferguson and Rublee 1976; Azam and Hodson 1977; Sieburth et al. 1978). To enumerate such bacteria the direct-count method using a fluorescent dye and the epifluorescent microscope has been widely adopted (Francisco et al. 1973; Zimmerman and Meyer-Reil 1974; Daley and Hobbie 1975). Jones and Simon (1975) and Bowden (1977) were among those identifying errors associated with certain membranes and methodology. The technique of Hobbie et al. (1977) which alleviated problems of filter fluorescence, excessive preparation time, and optical sectioning or specimen loss within the membrane was the method utilized in this work.

Nuclepore filters (0.2-µm pore size, 25-mm diameter) (Zimmermann 1977) were stained before use by soaking for 12 to 72 hours in a solution of 2 g of irgalan black in 1 l of 2% acetic acid. The dye (Chemical Index, acid black 107) was obtained from Ciba-Geigy Corp., Dyestuffs and Chemicals Division, Greensboro, NC. The filters were rinsed individually in particle-free distilled water immediately before use.

Microcosm filtrate subsamples had been preserved in particle-free containers with filtered Formalin (0.1 ml stock · 10 ml sample^{-1}) when drawn and were refrigerated for no more than three days prior to the direct count procedure.

The samples to be stained, usually 10 ml, were placed in particle-free test tubes. One ml of freshly filtered 0.1% acridine orange stain in distilled water was added to each tube, the contents

were mixed by vortex action, and then incubated at room temperature for 20 minutes (Meyer-Reil 1978).

Sample size was dictated both by the cell concentration required for accuracy in counting and by the tendency for nonrandom distribution of the cells on the filter which was especially pronounced when < 6 ml were filtered (Jones and Simon 1975).

To give better distribution of the vacuum, a cellulose filter was placed under the Nuclepore filter on the Millipore filter tower. The sample was filtered and then rinsed with 5 ml particle-free distilled water (Daley and Hobbie 1975). The moist Nuclepore filter was transferred to a microscope slide, and immersion oil (Cargille A), a cover slip, and oil were placed on top.

Slides were viewed with a Zeiss Photomicroscope III equipped with an epifluorescent illumination system. The light source was a 200 W (HBO 200) ultrahigh pressure mercury lamp. A Zeiss filter set 48 77 16 was used. It consisted of a fluoroscein isothiocyanate (FITC) excitation-barrier filter and reflector combination (reflector 510, excitation 485/20 nm band) and a barrier filter at 520. The image was viewed through barrier filter 41 (410 nm cut off). A 100 x Ph 3 Neofluar (1.30 Oel) oil-immersion objective was used for viewing at a total magnification of 1,600 x.

A Whipple eyepiece micrometer disc (7 · 7 grid; 2177.78 μm^2 field area at 1600 x) was used to delineate a portion of the field for actual counting. Only bodies with distinct fluorescence, clear outline, and recognizable bacterial shape were regarded as

bacterial cells (Watson et al. 1977). At least 500 bacteria were counted per sample by examining representative fields extending from the membrane center to the edge (approximately 15 fields per sample) (Meynell and Meynell 1970; Sorokin and Overbeck 1972; Meyer-Reil 1978). Orange (yellow) and green fluorescing cells were differentiated in the count (Rigler 1966; Yamabe 1973; Goulder 1976; Hobbie et al. 1977), and were initially assumed to represent active and inactive cells respectively.

All samples were examined, counted, and photographed within three hours of preparation. Microphotographs were taken with the built-in camera, using Ektachrome, Fujichrome, or Fujicolor ASA 400 film exposed automatically at a light-lens combination corresponding to ASA 800. Film was processed commercially.

Blanks of acridine orange were run, as were unstained samples. Background counts were negligible. Autofluorescent cells comprised an insignificant proportion of the samples (Johnson and Sieburth 1979).

Bacterial size distributions were determined from enlarged photographs by comparison with similar projections of 0.481-μm diameter latex spheres (Ted Pella Inc., Tustin, CA).

Electronic Counting

Coulter counting is based on the simultaneous passage of a conducting fluid and current through a small aperture. The aperture serves as a resistance element in a measuring circuit; the passage

of a suspended particle momentarily blocks a portion of the aperture and changes its resistance. The magnitude of the resistance pulse is a measure of the volume of the particle. Under suitable conditions, the pulse height is proportional to the volume of the particle (Kubitschek 1958, 1969; Lark and Lark 1960). Thus an instrument equipped with a multichannel pulse-height analyser can be used both for concentration and for particle size distribution determination (Harvey and Marr 1966; Drake and Tsuchiya 1973).

Counting was carried out with a Coulter Counter Model TA_{II} Multichannel Particle Counter with a 30-μm orifice. To adequately resolve the small cells in the samples with this aperture, channels 14 to 3 were active, and the aperture matching switch was set at 16-80 K, with a corresponding current of 800 μA. Size calibration was empirically set at 330 to give a maximum signal-to-noise ratio, while shifting the distribution of latex spheres (0.481-μm diameter) to channels 6 and 7.

The backing electrolyte was 1000 mOsmole NaCl (0.22 μm Millipore GS prefiltered). Since this approximated the density and conductivity of seawater, schlieren patterns were minimized, and counts were thus more consistent (Martignoni 1978). Careful rinsing of both receiving and storage flasks gave saline with a total background of less than 3000 counts per 0.05 ml using the instrument settings noted.

All sieved samples were refrigerated to minimize change prior to counting. A delay was necessitated since the heterotrophic

potential test had to be carried out first. It was necessary to allow the samples to return to room temperature for the actual count, however. Otherwise condensation interfered with the visualization of the aperture.

Sieved samples gave counts of $2.0 \cdot 10^4$ to $1.3 \cdot 10^5$ in a counted volume of 0.05 ml. The 95% confidence limits for the total particle count are 2 to 5% of the mean of four counts of a single sample for counts in this range (Sheldon and Parsons 1967; Drake and Tsuchiya 1973). Therefore, dilution was not deemed necessary. Each sample was counted until four reproducible values, two at each polarity, were achieved. These were corrected for background and coincidence, were averaged, and were converted to counts per ml.

Population by channel and percentage total volume by channel were recorded.

Supplementary Data

Water temperature was monitored throughout the course of this study.

Parallel determinations of chlorophyll *a* concentration, community biomass, and dissolved organic carbon concentration were performed, and the data supplied to this investigator.

Split samples were utilized for the chlorophyll and biomass analyses, with one-half of each sample being utilized for pigment extraction and the other half being used for biomass determination. Chlorophyll *a* concentrations were determined by the SCOR/UNESCO

technique (Strickland and Parsons 1972a). The samples were processed in subdued light. They were filtered through 4.5-cm Whatman GF/C glass filters which had been coated with magnesium carbonate. The samples were then homogenized in a Brinkman/Polytron Homogenizer, Model PT20ST, for < 30 seconds, extracted in the dark in fresh 90% aqueous spectrophotometric grade acetone for 20 minutes with regular mixing, centrifuged in swing-out tubes, and measured immediately by spectrophotometry at 750, 663, 645, and 630 nm against a 90% acetone blank. A Coleman Model 124D Double Beam Spectrophotometer with 1.0 nm bandwidth and 10 cm cell pathlength was utilized (Worrest et al. 1978).

Biomass determinations were calculated from the other half of the split samples utilizing dry weight and organic weight (ash-free dry weight) measurements (Strickland and Parsons 1972b; Soeder et al. 1974). Dry weight determinations were made after desiccation on standard GF/C glass filters over a drying agent. Then the samples were ashed at 475°C to complete the organic weight determinations (Worrest et al. 1978).

Dissolved organic carbon (DOC) measurements utilized the wet oxidation method of Menzel and Vaccaro (1964) as further described in Strickland and Parsons (1972c). Samples drawn concurrently with those for other studies were filtered (prerinsed Millipore GS, 0.22-μm) (Parker 1967), preserved with $HgCl_3$ (40 $mg \cdot l^{-1}$ final concentration), and stored in acid-washed, particle-free glass bottles. Phosphoric acid and potassium persulphate were added

to an aliquot of sample in a glass ampule. All inorganic carbonate was removed by passing nitrogen gas through the sample, and organic carbon in the water was oxidized to carbon dioxide by heating the sealed ampule to 130°C for 40 minutes. This carbon dioxide was then estimated by dispelling it from the ampule in a stream of nitrogen gas which was passed through a non-disruptive infrared absorption gas analyzer. Samples were run in triplicate, giving a precision at the 1.0 $mg \cdot l^{-1}$ level of the mean ± 0.035 $mg \cdot l^{-1}$ (Kristaponis 1980).

Statistical Evaluation

In addition to those methods noted previously, a number of statistical techniques, primarily computerized, were utilized in the evaluation of the data. Measurements throughout the course of the study were compared, each with all others, by means of the Statistical Package for the Social Sciences (SPSS) (Version 8.0, June 18, 1979. Vogelback Computing Center, Northwestern University). SPSS is a computer package which generates a correlation matrix giving R values for linear relationships, the number of data points involved in each correlation, and a t test on the significance of the correlation coefficient. All relationships having an R greater than ± 0.7 and a probability less than or equal to 0.1 were examined in more detail, especially by means of scattergrams and data transformation.

Since it had been hypothesized that a 'specific activity

index' such as $V_{max} \cdot cell^{-1}$ would be an appropriate measure of bacterial activity under experimental conditions, various permutations of this index were examined: $V_{max} \cdot cell^{-1}_{AODC}$; $V_{max} \cdot active\ cell^{-1}_{AODC}$; $V_{max} \cdot particle^{-1}_{Coulter\ count}$; $V_{max} \cdot active\ particle^{-1}_{Coulter\ count}$ (Wright 1978). Each was regressed with all other variables in the study.

Forward and backward step-wise multiple regression was used with the non-bacterial parameters (accumulated fluence, fluence rate, temperature, day, DOC, chlorophyll *a*, and biomass) entered as independent variables, in an effort to derive a model indicative of UV-B effect on some bacterial or dependent variable. That model which maximized R^2 while incorporating only significant variables was utilized. While such a model cannot be assumed to be of predictive value, the signs of the fitted parameters may be suggestive of the nature of the bacterial response.

Although it usually was not necessary to go to the $P = 0.2$ level of significance, it was recognized that such a level is acceptable in bacterial studies (P. Wood 1956).

III. RESULTS AND A CONSIDERATION OF THEIR VALIDITY

Accumulated Fluence

As noted in the Introduction, changes in UV-B fluence rates correlated with ozone depletion would result in chronic increases in ecosystem exposure. The exposure history of such a system might be important to the identification of stress-related variations (Table 2).

Heterotrophic Potential

The heterotrophic potential technique was repeated periodically as one possible measure of the influence of ultraviolet-B radiation on the establishment and development of the microcosms (Table 3). Such development may be expected to reflect successional changes in the bacterioplankton, as well as in the planktonic diatom populations (Worrest 1979; Martin and Bianchi 1980), as these were affected by the experimental variable, UV-B fluence.

The V_{max} gives a measure of potential heterotrophy, that is, the velocity of uptake of the given substrate when transport systems are saturated. If the bacteria reproduce or more uptake sites are induced, the V_{max} will increase proportionately. While it is not a measure of the actual velocity of uptake (Crawford et al. 1974), V_{max} approximates the kinetic capabilities of the bacterial assemblage (Hobbie 1969). Therefore it offers a parameter for the comparison of such assemblages (Hobbie and Crawford 1969a).

Table 2. Accumulated UV-B fluence: $J \cdot m^{-2}$.

Filter	Day			
	2	11	20	29
0.18-mm Mylar 'D'	$3.50 \cdot 10^{2}$	$1.93 \cdot 10^{3}$	$3.50 \cdot 10^{3}$	$5.08 \cdot 10^{3}$
0.25-mm CA	$1.20 \cdot 10^{4}$	$6.59 \cdot 10^{4}$	$1.20 \cdot 10^{5}$	$1.74 \cdot 10^{5}$
0.19-mm CA	$1.28 \cdot 10^{4}$	$7.02 \cdot 10^{4}$	$1.28 \cdot 10^{5}$	$1.85 \cdot 10^{5}$
0.13-mm CA	$1.41 \cdot 10^{4}$	$7.76 \cdot 10^{4}$	$1.41 \cdot 10^{5}$	$2.05 \cdot 10^{5}$

Table 3. Comparison of heterotrophic activity among microcosms.

Filter	Day	V_{max} ($\mu g \cdot l^{-1} \cdot h^{-1}$)	T_t (h)	($K_t + S_n$) ($\mu g . l^{-1}$)	R [a]
0.18-mm Mylar 'D'	2	0.447	21.34	9.54	0.9831
	11	0.355	9.77	3.46	0.9703
	20	0.269	8.61	2.32	0.9966
	29	3.085 [b]	9.03	27.85	0.9051
0.25-mm CA	2	0.035	68.53	2.39	0.9391
	11	0.499	2.59	1.30	0.9963
	20	0.203	10.36	2.10	0.9954
	29	0.965	3.78	3.65	0.9922

Continued.

Table 3. Continued.

Filter	Day	V_{max}	T_t	$(K_t + S_n)$	R
0.19-mm CA	2	0.094 [b]	61.24	5.77	0.8940
	11	0.336	10.72	3.60	0.9984
	20	0.738	2.38	1.76	0.9776
	29	0.281	5.00	1.40	0.9982
0.13-mm CA	2	0.116	11.92	1.38	0.9703
	11	0.300	9.68	2.91	0.9914
	20	0.279	8.88	2.48	0.9948
	29	0.244	6.35	1.55	0.9994

[a] Correlation coefficient for linearity of V_{max} calculation.

[b] When the linear regression was not significant over the entire range of concentrations, the initial, linear portion alone was considered.

V_{max} as measured in this study was comparable to the results of other estuarine studies utilizing glucose, especially those carried out during less productive seasons or at colder temperatures (Vaccaro and Jannasch 1966; Crawford et al. 1974; Goulder 1976; Hanson and Snyder 1979).

Cell counts permitted the determination of $V_{max} \cdot cell^{-1}$ which has been viewed by some as a more appropriate measure for comparison (Table 4) (Hamilton et al. 1966; Goulder 1976; Palumbo and Ferguson 1978; Wright 1978).

Of the three parameters determined in heterotrophic potential measurements, only turnover time (T_t) is a simple measurement of in situ bacterial activity. T_t has been found to vary inversely with temperature, biomass, cell concentration (Allen 1969), V_{max} (Hanson and Snyder 1979), and nutrient availability (Hobbie 1967; Morgan and Kalff 1972). It is also apparent that deviations from the relationships noted could indicate variations in bacterial numbers or activity or in the concentration of naturally available substrate (Vaccaro and Jannasch 1966; Albright and Wentworth 1973; Goulder 1976). Thus, T_t could be a valuable ecological measure. It must be used cautiously, however, since its magnitude is likely to be overestimated in the heterotrophic potential technique (Williams 1973; Griffiths et al. 1977; Barvenik and Malloy 1979). The comparison of T_t values is complicated by the variables noted previously, as well as the incomplete literature descriptions of methodology. Generally, however, the values in the present study

Table 4. $V_{max} \cdot cell^{-1}$.

Filter	Day	$V_{max} \cdot cell^{-1}$ ($\mu g \cdot h^{-1} \cdot cell^{-1}$)
0.18-mm Mylar 'D'	2	$6.03 \cdot 10^{-10}$
	11	$5.30 \cdot 10^{-10}$
	20	$3.93 \cdot 10^{-10}$
	29	$6.89 \cdot 10^{-9}$
0.25-mm CA	2	$3.76 \cdot 10^{-11}$
	11	$8.41 \cdot 10^{-10}$
	20	$4.09 \cdot 10^{-10}$
	29	$2.31 \cdot 10^{-9}$
0.19-mm CA	2	$9.96 \cdot 10^{-11}$
	11	$7.23 \cdot 10^{-10}$
	20	$7.94 \cdot 10^{-10}$
	29	$6.84 \cdot 10^{-10}$
0.13-mm CA	2	$2.02 \cdot 10^{-10}$
	11	$6.44 \cdot 10^{-10}$
	20	$3.88 \cdot 10^{-10}$
	29	$1.53 \cdot 10^{-9}$

seemed comparable to literature values (Crawford et al. 1974).

The term ($K_t + S_n$) sets upper limits on both the assemblage transport constant and the natural substrate concentration, in this work, glucose. Values reported in the literature correspond well with those of this study (Vaccaro and Jannasch 1966; Gocke 1975; Hanson and Snyder 1979).

Some investigators have reported little annual variation in ($K_t + S_n$), attributing this to a stable system for glucose regeneration or to the "natural" K_t for bacterial populations (Allen 1969). Others found variations as great as three orders of magnitude (Hanson and Snyder 1979). Since variation did emerge in the present work, with a range of 1.30 to 27.85 $\mu g \cdot l^{-1}$, it seemed appropriate to devise a test for S_n, and thus permit separate consideration of K_t and S_n.

DOC measurements, described subsequently, did not suffice for this purpose. Although some marine waters may have relatively large total DOC concentrations, labile and rapidly metabolized compounds probably represent only a small fraction of the total (Plunkett and Rakestraw 1955; Vallentyne and Whittaker 1956; Allen 1971). Because glucose has been estimated repeatedly in the $\mu g \cdot l^{-1}$ range (Degens et al. 1964; Vaccaro and Jannasch 1966, 1967; Vaccaro et al. 1968; Hamilton and Preslan 1970; Andrews and Williams 1971), changes in its concentration are unlikely to be reflected in DOC measurements which are accurate only to 0.09 $mg \cdot l^{-1}$ (Menzel and Vaccaro 1964).

Bioassay

The bioassay method of Hobbie and Wright (1965) offers a technique for combining separate measurements of heterotrophy for a test organism and a natural assemblage. It was adapted to estuarine studies and performed in conjunction with Day 29 measurements (Table 5).

For valid results, it is imperative that the uptake constant (K_t) of the test organism remains unchanged during the test period (Vaccaro and Jannasch 1966). One way analysis of variance on respiratory ratios indicating no difference among the water samples ($P < 0.1$) was suggestive of such stability (Snedecor and Cochran 1967).

When the concentration of naturally occurring glucose had been determined, it was possible to calculate the velocity of utilization:

$$v = S_n \cdot T_t^{-1}$$

The relationship between actual and potential heterotrophic velocities seemed to offer another measure for the assessment of stress on the system (Table 6).

Respiratory Ratio

As performed in this study, the heterotrophic potential technique permitted the concomitant determination of the glucose respiration ratio (See Appendix III).

Table 5. Separation of S_n and K_t by bioassay: Day 29.

Filter	S_n ($\mu g \cdot l^{-1}$)	K_t: natural assemblage ($\mu g \cdot l^{-1}$)	$K_t \cdot cell^{-1}$ ($\mu g \cdot cell^{-1}$)
0.18-mm Mylar 'D'	5.41	22.44	$5.01 \cdot 10^{-8}$
0.25-mm CA	1.67	1.79	$4.29 \cdot 10^{-9}$
0.19-mm CA	1.03	0.37	$8.97 \cdot 10^{-10}$
0.13-mm CA	0.94	0.30	$1.90 \cdot 10^{-9}$

Table 6. Actual glucose utilization: Day 29.

Filter	v ($\mu g \cdot l^{-1} \cdot h^{-1}$)	v·cell^{-1} ($\mu g \cdot cell^{-1} \cdot h^{-1}$)	% V_{max}
0.18-mm Mylar 'D'	0.60	$1.43 \cdot 10^{-9}$	19.4
0.25-mm CA	0.46	$1.10 \cdot 10^{-9}$	48.2
0.19-mm CA	0.21	$5.03 \cdot 10^{-10}$	73.7
0.13-mm CA	0.18	$1.14 \cdot 10^{-9}$	75.7

Respiratory ratios reported in estuarine studies range from 8-17% (Crawford et al. 1974) to 23-49% (Williams 1970). The results of this study were generally within this range (Table 7).

Several investigators have considered this measure indicative of other variables within the system. Williams and Gray (1970) noted that estuarine organisms responded to an increased concentration of easily assimilated substrate by an increased rate of oxidation. The duration of the increased substrate concentration was inversely related to the capacity of the bacterial assemblage to increase uptake. Andrews and Williams (1971) summarized this response by concluding that the simplest level of control was by the law of mass action, modified by rate limiting enzyme kinetics. Consequently, the increase in the rate of oxidation is an hyperbolic function of the increase in substrate concentration. In addition to this immediate response, the bacteria present are capable of enhanced activity, presumably by population increase.

L. Wood (1973), Wood and Chua (1973), and Harrison (1976) correlated the increased respiratory ratio with pollution stress, noting that as pollution increases, a greater proportion of substrate taken up is catabolized and less is assimilated into bacterial biomass. The two interpretations are not necessarily mutually exclusive if the stress results in increased algal excretion (Hellebust 1965; Sharp 1977) without inhibiting bacterial activity (Iturriaga and Hoppe 1977; Goulder et al. 1979a,b).

Table 7. Respiration ratios. Glucose respired as percentage of uptake.

Filter	Day	% Respiration*	No. of samples
0.18-mm Mylar 'D'	2	20.66 ± 2.51	15
	11	29.47 ± 0.88	12
	20	30.35 ± 3.08	14
	29	50.06 ± 2.47	7
0.25-mm CA	2	25.38 ± 3.02	14
	11	29.67 ± 3.33	14
	20	26.75 ± 2.82	15
	29	34.81 ± 4.19	14
0.19-mm CA	2	23.74 ± 1.93	8
	11	27.09 ± 3.67	14
	20	34.67 ± 3.10	15
	29	31.41 ± 5.54	14
0.13-mm CA	2	18.23 ± 3.37	14
	11	28.60 ± 3.96	13
	20	27.99 ± 2.61	15
	29	32.21 ± 4.54	11

* Mean ± 1 SD.

Acridine Orange Direct Count and Coulter Count

Significant correlations between the total number of bacteria and V_{max} have been recorded by a number of authors, including Wright and Hobbie (1966), Allen (1969), and Morgan and Kalff (1972). Albright and Wentworth (1973) concluded that this may reflect the ability of heterotrophic bacterial populations to maximize activity and cell numbers at each unique physico-chemical condition. Although it was recognized that the microcosms in this work were subjected to a stress that might alter the heterotrophic potential of the bacteria, the correlation between numbers and activity was among the factors considered in evaluating the relative accuracy of the cell count methods used.

Acridine orange direct counts (AODC) were done on each heterotrophic potential sieved sample, as were Coulter counts. The former technique has been widely recommended as the method of choice (see Materials and Methods). Because AODC is dependent upon subjective judgment of the nature of a particle (Wiebe and Pomeroy 1972; Bowden 1977), as well as on other experimental difficulties noted, its accuracy should be checked when possible. AODC did not correlate with V_{max} ($R = -0.122$), but $V_{max} \cdot cell^{-1}$ did fit a UV-B stress model described subsequently.

AODC permits an approximation of cell activity, based on fluorescence color (Rigler 1966; Yamabe 1973). Zimmermann et al. (1978) reported that yellow fluorescence was somewhat higher among respiring bacteria than nonrespiring, 93% to 76%. However, others

have limited the discrimination to cells involved in protein synthesis as compared to those not so involved (Hobbie et al. 1977). Since several investigators have noted experimental factors which pose difficulties for either distinction: dye concentration and/or contact time (Jones 1974); fluorescence fading (personal observation); general nonspecificity (Wright 1978), and because the AODC measure was not directly related to glucose metabolism, fluorescence color was not pursued (Table 8).

Coulter particle counts showed the expected correlation with V_{max} ($R = 0.8511$), but were examined more closely because of the nature of the sample. In bacterial samples from marine and estuarine habitats, the size distribution of the cells imposes special limitations on various enumeration techniques, especially on the use of electronic counting. Direct microscopic measurements reveal the minuteness of the assemblage members: approximately 95% of the cells are cocci (Oppenheimer and Jannasch 1962) with 70-80% < 0.06 µm in diameter (< 0.11 µm^3) (Ferguson and Rublee 1976; Ferguson and Palumbo 1979). Measurements of typical cell size distributions in this work corresponded well with published values (Figure 7), but the experimental weighted cell volume, 0.017 µm$^3 \cdot$cell^{-1}, was somewhat less than that found by Bowden (1977) and others. The discrepancy may reflect differences in both the assemblages and the techniques used to measure them. The former is suspected to figure prominently as Bowden's samples contained 43% bacilli, whereas bacilli represented less than 20% of the assemblages

Table 8. Acridine orange direct counts of bacterial assemblages.

Filter	Day	Cells·ml^{-1}[a]	Yellow fluorescence: %
0.18-mm Mylar 'D'	2	$7.41 \cdot 10^5$	98.3
	11	$6.70 \cdot 10^5$	96.6
	20	$6.84 \cdot 10^5$	97.4
	29	$4.48 \cdot 10^5$	97.1
0.25-mm CA	2	$9.32 \cdot 10^5$	98.9
	11	$5.93 \cdot 10^5$	96.6
	20	$4.96 \cdot 10^5$	97.6
	29	$4.17 \cdot 10^5$	96.2
0.19-mm CA	2	$9.44 \cdot 10^5$	97.8
	11	$4.65 \cdot 10^5$	96.4
	20	$9.29 \cdot 10^5$ [b]	96.6
	29	$4.11 \cdot 10^5$	95.7
0.13-mm CA	2	$5.73 \cdot 10^5$	92.1
	11	$4.66 \cdot 10^5$	82.4
	20	$7.19 \cdot 10^5$ [b]	96.5
	29	$1.59 \cdot 10^5$	87.3

a ± 10% : 90% CI with homogeneous cell distribution on filter (Sorokin and Overbeck 1972). Cell concentrations were converted to cells·l^{-1} in subsequent comparisons involving other volume measures.

b Elimated from data analysis. Count reflects clumping of sample.

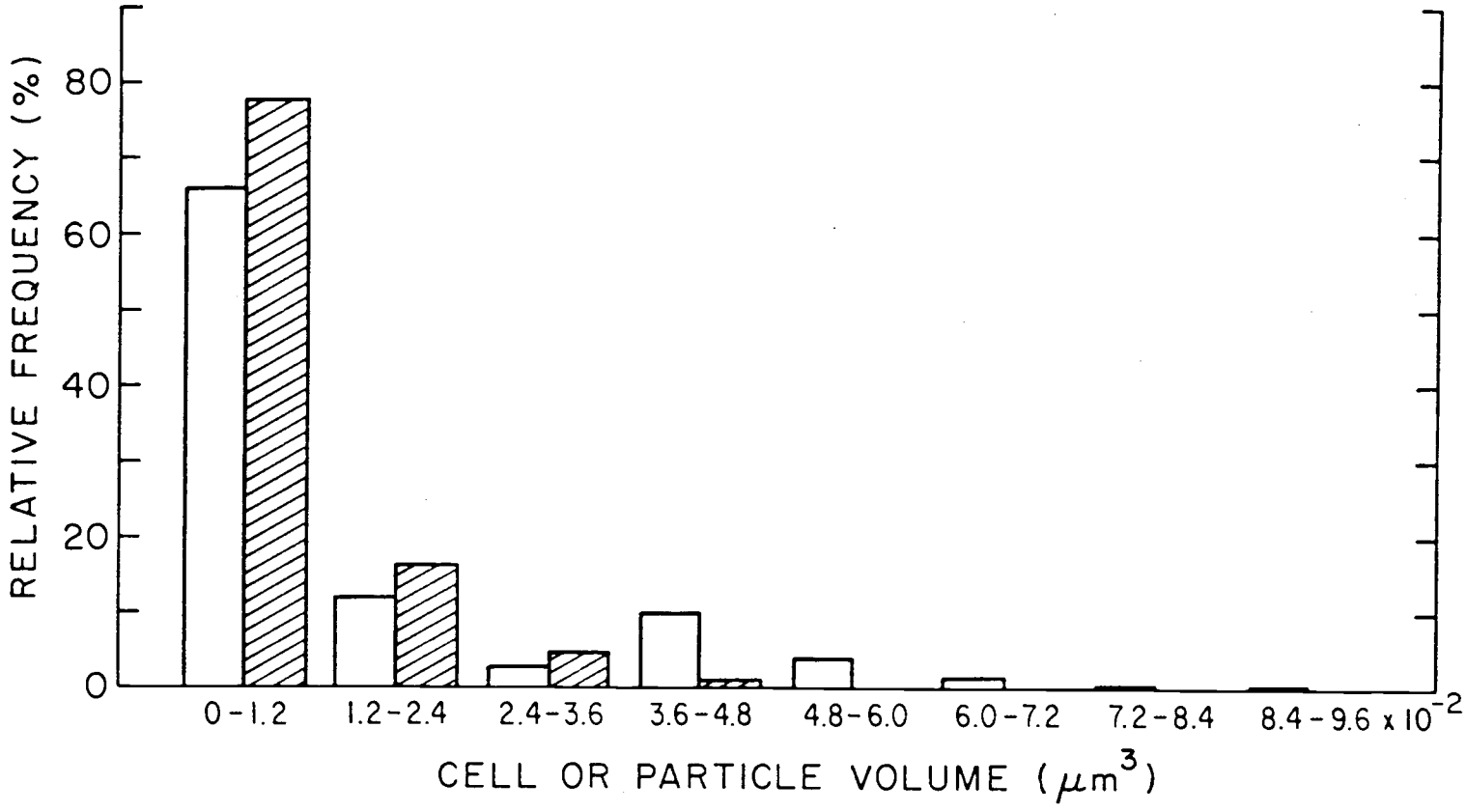


Figure 7. Comparison of microscropic and Coulter volume frequency distributions. Cell volumes (open bars) were calculated from photographic dimension measurements. Particle volumes (hatched bars) were approximated by comparison with the Coulter distribution of latex spheres of known diameter.

in this work.

It has been suggested that bacteria with cell volumes of about 0.25 μm^3 approach the limits of resolution of a 30-µm Coulter aperture (Drake and Tsuchiya 1973). Sheldon and Parsons (1967) extend that limit to 0.12 μm^3. Preliminary calibration studies in this work indicated the possibility of extending that limit further, to about 0.06 μm^3, with appropriate precautions (Kubitschek 1969; Martignoni 1978). Still, a sizable proportion of estuarine bacterial assemblages would be expected to be indistinguishable from electronic noise at the lower end of the Coulter multichannel analyzer range. Further, the Coulter counter does not distinguish bacterial from other similarly sized particles such as clay and detritus (Sheldon and Parsons 1967; Wiebe and Pomeroy 1972). Finally, multiple regression of several possible interfering variables suggested that the lack of correlation between the Coulter counts and the AODC ($P = 0.570$) was due primarily to biomass, presumably flocs, flakes, and detritus (Wiebe and Pomeroy 1972). Therefore, interpretation based on AODC cell concentrations only was utilized throughout this work.

Supplementary Data

One basic assumption underlying this study was that the bacterial activity and numbers could be expected to vary with nutrient availability (Wright and Hobbie 1966; Hobbie 1967, 1969; Morgan and Kalff 1972; Albright and Wentworth 1973; Hoppe 1978).

Since bacteria are known to be opportunistic, their greatest activity is expected in areas of organic enrichment (Bell et al. 1974).

Nutrient availability, and thus marine bacterial activity, may be closely associated with the activity of the primary producers (ZoBell 1946; Sieburth 1968). Many workers have reported that bacterial numbers (E. Wood 1963; ZoBell 1963; Parsons et al. 1969; Takahashi et al. 1973; Bell et al. 1974; Straskrabova and Komarkova 1979) as well as microbial heterotrophic activity (Hobbie 1967; Vaccaro et al. 1968; Morgan and Kalff 1972; Crawford et al. 1974) are enhanced by increases in phytoplankton production. While the cause - effect relationship appears to be neither simple nor well-understood, it is generally accepted that a dynamic equilibrium exists between bacterioplankton and phytoplankton (Ferguson and Palumbo 1979; Martin and Bianchi 1980).

Since biomass (Hamilton and Austin 1967; Wright 1970; Larsson and Hagström 1979), chlorophyll _a_ (Bell and Mitchell 1972; Palumbo and Ferguson 1978; Ferguson and Palumbo 1979), and DOC (Andrews and Williams 1971; Albright and Wentworth 1973; Sieburth 1976; Albright 1977; Geesey and Costerton 1979; Martin and Bianchi 1980) have been implicated as influencing bacterial heterotrophic activity and numbers, all three were monitored throughout this study (Tables 9 and 10). It was assumed that some combination of these, under varying UV-B fluence regimes, might change in such a way as to suggest an explanation for the bacterial changes (Worrest et al. 1978).

Table 9. Supplementary data: Biomass and chlorophyll *a*.

Filter	Day	Biomass ($mg \cdot m^{-3}$)	Chlorophyll *a* ($mg \cdot m^{-3}$)
0.18-mm Mylar 'D'	5	719	1.11
	12	829	2.15
	20	620	*
	27	747	2.59
0.25-mm CA	5	726	1.27
	12	851	1.79
	20	489	*
	27	553	1.93
0.19-mm CA	5	672	1.34
	12	587	1.91
	20	425	*
	27	409	0.89
0.13-mm CA	5	723	1.39
	12	619	1.65
	20	321	*
	27	315	1.09

* not available.

Table 10. Supplementary data: Temperature and DOC.

Filter	Day	Temperature (°C)	DOC ($mg \cdot l^{-1}$)
0.18-mm Mylar 'D'	2	13.5	0.854
	11	11.7	0.539
	20	10.5	0.680
	29	9.5	0.561
0.25-mm CA	2	13.5	0.681
	11	11.7	0.576
	20	10.5	0.396
	29	9.5	0.569
0.19-mm CA	2	13.5	0.707
	11	11.7	0.402
	20	10.5	0.430
	29	9.5	0.426
0.13-mm CA	2	13.5	1.069
	11	11.7	0.473
	20	10.5	1.304
	29	9.5	1.243

Biomass accumulation may give an indication of the reproductive viability of the organisms within a system and of the maturation of that system (Gorden et al. 1969).

If changes in chlorophyll a with time are accepted as an index of the phytoplankton standing crops, it is possible to document general changes in the phytoplankton by means of its measurement. Further, Vaccaro et al. (1968) reported a positive correlation of phytoplankton densities with glucose concentration. This, in turn, would be expected to influence the heterotrophic potential parameters. The extent of this influence was considered in more detail in that section devoted to bioassay results.

While DOC represents the most abundant carbon source in most natural aquatic systems (Hanson and Snyder 1979), its chemical composition and microbial utilization remain poorly defined. Not all DOC is equally available to bacterial heterotrophs. One fraction, perhaps as much as 60% of the total, consists of compounds or complexes of compounds relatively resistant to biochemical oxidation (Barber 1966). As little as 10 to 20% can be utilized easily by bacteria (Plunkett and Rakestraw 1955; Vallentyne and Whittaker 1956; Ogura 1972). The simple organic molecules utilized in heterotrophic potential methodology are a minor component of this latter category (Jørgensen 1976). Therefore direct correlations between DOC and bacteria may not be evident.

Of more importance to this study is the suggestion that the release of DOC may reflect the general condition of the

phytoplankton in the system. Photooxidative cell damage (Hellebust 1965; Ignatiades and Fogg 1973), nutrient depletion (Thomas 1964; Hellebust 1965), cell senescence and lysis (Duursma 1963; Fogg 1963; Marker 1965; Nalewajko and Lean 1972; Sharp 1977), cultural shock (Duursma 1963; Williams and Yentsch 1976; Sharp 1977), and heavy metal contamination (Vaccaro et al. 1977) have been implicated in elevated DOC levels. Ultraviolet-B radiation may cause a similar response.

IV. INDICATIONS OF UV-B IMPACT ON THE ESTUARINE BACTERIOPLANKTON

Considering the nature of ecosystem balance and energy flow, it seemed appropriate to assume the likelihood of two types of bacterial response to UV-B stress. In one, the bacteria would be the stress target, while in the second, the bacteria would be responding to stress-caused environmental changes.

Some bacterioplankton have been shown to exist sufficiently high in the water column to receive a significant UV-B irradiance, even in estuarine systems (Calkins 1975a). Because of their small size, microorganisms are especially subject to nucleic acid absorption of UV-B (Giese 1968b). Whether or not a cell could survive such irradiation depends in large part on its ability to repair the damage produced. Living things occur, therefore, in a balance between the photochemical destruction of cellular components and their biochemical repair (Smith 1972). When this balance is upset, as might be expected at higher fluence rates, growth delay, mutations, and/or death may result (Jagger 1976). It is not a question of whether solar UV can be harmful to organisms, but at what level this damage becomes apparent (Caldwell 1972). Such damage has been measured routinely, utilizing pure bacterial cultures. The interpretation of measured changes in a natural system is complicated by the existence of varied species of bacterioplankton, the more resistant of which might be expected to assume more prominent positions in the functioning ecosystem under the

selective pressure of UV-B stress. Previous work has shown such to occur (Thomson 1975). In this study, no attempt was made to monitor such shifts in species composition. It was accepted that the capabilities of the bacterioplankton taken as a functioning assemblage would be of ecological relevance (see Introduction). Such an emphasis requires cautious interpretations of changes in bacterial function, as these may, in fact, reflect stress at other trophic levels.

Cell Concentration

That such stress did occur is seen in the decrease in biomass with accumulated UV-B fluence (Figure 8). What are not decipherable here are the relative amounts of viable cells and/or multicellular organisms and detritus. However, a parallel drop in the bacterial population (Figure 9) and the trend toward lower chlorophyll *a* values with increased accumulated stress suggest a decreased viability of the systems under stress. Similar results were obtained by Worrest et al. (1978).

While bacterial cell concentration decreased with accumulated UV-B stress, the likelihood that this was not a simple cause-effect relationship, i.e., the result of direct cell damage only, is seen in a multiple regression model for cell concentration.

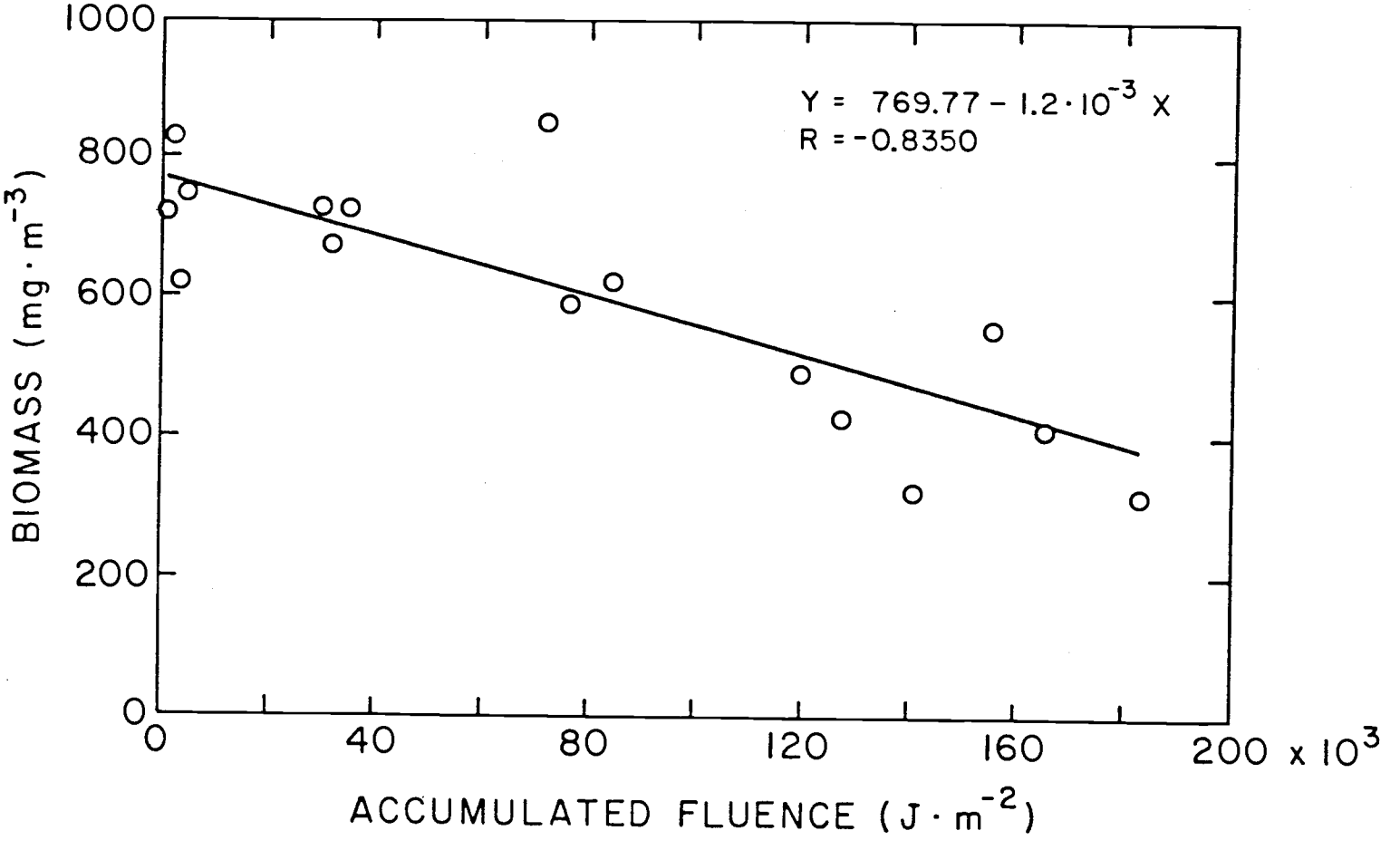


Figure 8. Biomass with accumulated UV-B fluence.

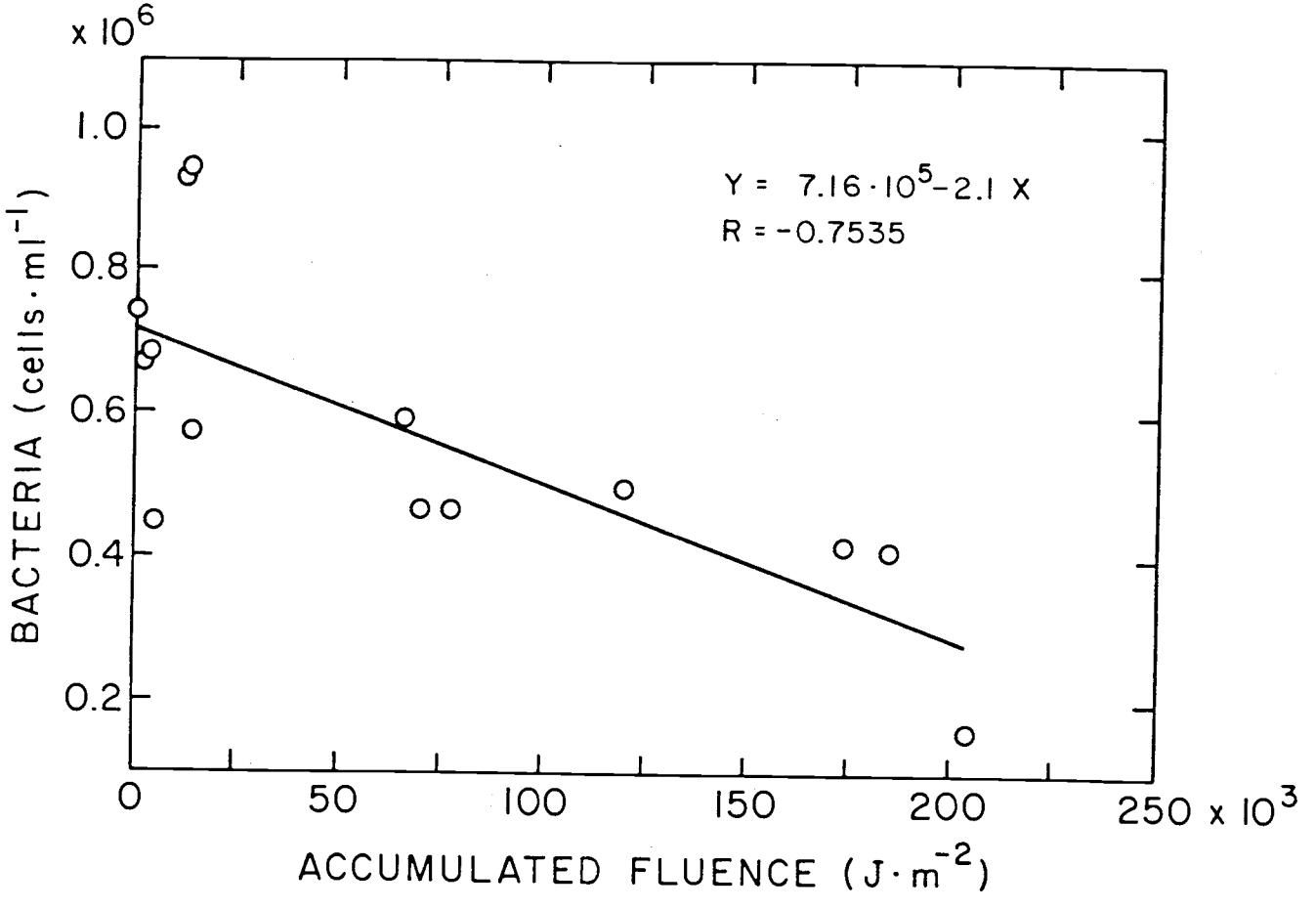


Figure 9. Bacterial concentration with accumulated UV-B fluence.

$$\text{Cells}\cdot ml^{-1} = -6.21 \cdot 10^{6} - 4.19 \cdot 10^{5}\ X_1\ mg\cdot l^{-1}_{DOC} + 5.36 \cdot 10^{5}\ X_2\ °C + 6.12 \cdot 10^{4}\ X_3\ \text{days}$$

R^2 for this model is 0.8465 and all independent variables within the fitted model are significant at the level defined for this work: DOC: $P = 0.020$; °C: $P = 0.020$; Day: $P = 0.051$.

When Day, a measure which seemed appropriate to microcosm studies was not included in the model, fluence rate was marginally significant ($P < 0.2$). Thus it appeared that UV-B may have contributed to diminution of the bacterial population, but that environmental temperature and nutrient availability were more important in the determination of assemblage concentrations. (The latter may, itself, be a function of UV-B fluence. Refer to bioassay sections.)

Heterotrophic Potential Activity Index

The relation of other measures of bacterial response to UV-B was likewise an involved one. Especially noteworthy in this regard was the complexity of the $V_{max}\cdot cell^{-1}$ 'activity index' suggested by Wright (1978) (Figure 10). Statistical tests for differences in slope were limited by the degrees of freedom available, and only the two extremes were shown to differ ($P < 0.1$). However, apparent differences here, coupled with actual respiration differences (considered subsequently) implied the existence of

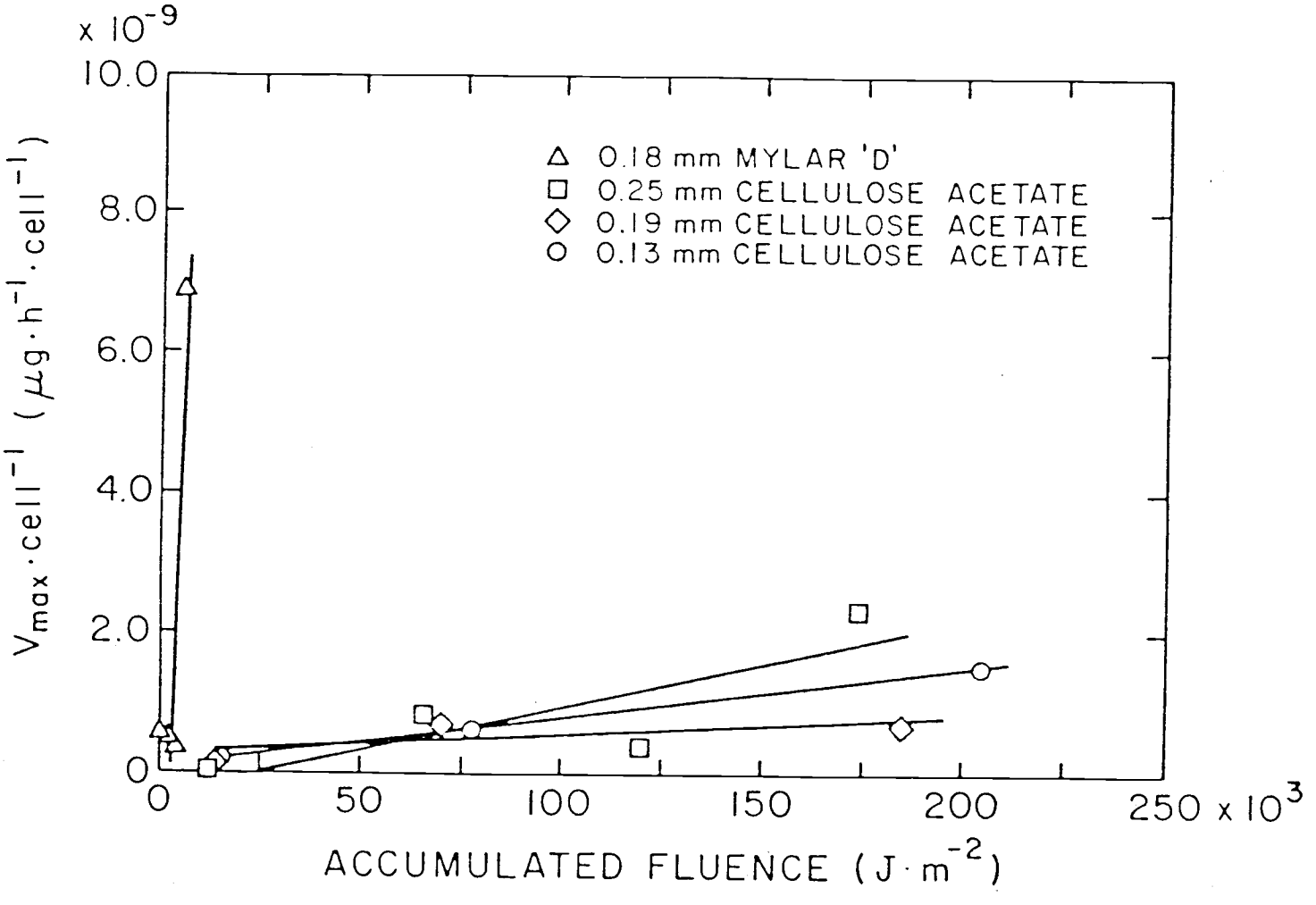


Figure 10. $V_{max} \cdot cell^{-1}$ within each microcosm with accumulated UV-B fluence.

variables, in addition to accumulated fluence, which contributed to the observed activity response.

Multiple regression equations were computed to relate $V_{max} \cdot cell^{-1}$ to observed environmental factors. Two acceptable models were generated:

$$V_{max} \cdot cell^{-1} = - 3.52 \cdot 10^{-8} \qquad (P = 0.1532)$$

$$- 1.66 \cdot 10^{-14} X_1 \ J \cdot m^{-2}$$

accumulated UV-B fluence $(P = 0.0724)$

$$+ 1.24 \cdot 10^{-9} X_2 \ mg \cdot m^{-3}$$

chlorophyll a $(P = 0.2322)$

$$+ 5.24 \cdot 10^{-10} X_3 \ days \qquad (P = 0.0332)$$

$$+ 2.44 \cdot 10^{-9} X_4 \ °C \qquad (P = 0.1570)$$

$$R^2 = 0.9052$$

$$V_{max} \cdot cell^{-1} = - 1.46 \cdot 10^{-10} \qquad (P = 0.6836)$$

$$- 2.53 \cdot 10^{-14} X_1 \ J \cdot m^{-2}$$

accumulated UV-B fluence $(P = 0.0002)$

$$+ 2.24 \cdot 10^{-10} X_2 \ days \qquad (P = 0.0000)$$

$$R^2 = 0.8702$$

Although the effect of temperatue has been referenced extensively (Takahashi and Ichimura 1971; Wright 1973, 1978; Sayler et al. 1979), and phytoplankton are obviously the ultimate nutrient source for heterotrophic bacteria (Hobbie 1969; Wright and Shah 1975; Moshiri et al. 1979), the second of the two models seems preferable statistically. Note P values for the fitted coefficients.

The inverse relationship between $V_{max} \cdot cell^{-1}$ and accumulated UV-B fluence could not have been due to increased cell concentration with increased fluence (noted previously). Thus there may have been an inhibition of bacterial activity (Goulder et al. 1979a). This concept, important to this study, was considered in greater detail, as described in a subsequent section.

Respiratory Ratios

This study revealed an apparent contradiction; both the microcosm that was UV-B deficient and the microcosm that received maximal UV-B enhancement responded with significantly elevated respiratory ratios ($P < 0.0005$) (Figure 11).

Several suggestions of cause-effect relationships are available in the literature. ZoBell and Grant (1943) noted an increased respiration ratio with higher glucose concentration in bacterial suspensions. Wright (1975a) confirmed that nutrient enrichment resulted in increased mineralization as measured in the heterotrophic potential technique. Thomas (1964) suggested that a possible source of elevated nutrients was the environmental stress of autotrophs. That Thomas' statement cannot be generalized to include all environmental stresses has been shown in the conflicting results of recent works. Goulder et al. (1979b) and Sayler et al. (1979) found no significant correlation between stress and the proportion of CO_2 evolved, while L. Wood (1973) and Thomson (1975) found respiration ratio to be indicative of stress.

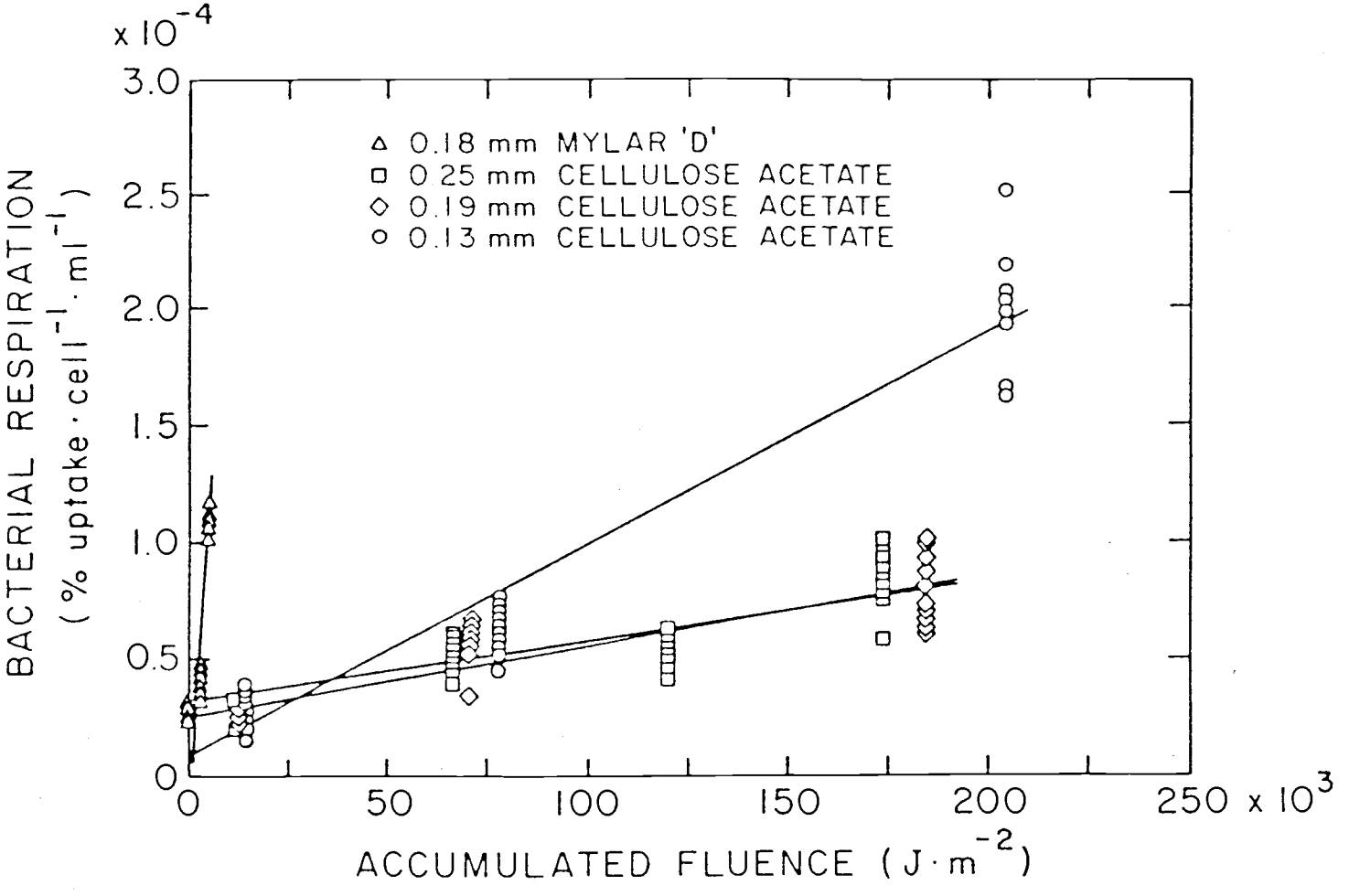


Figure 11. Bacterial respiration per cell within each microcosm with accumulated UV-B fluence.

Harrison (1976) in a review of bacterial respiration studies, noted that respiration rate is a function of substrate uptake rate. (Also see bioassay section.) He cautioned, however, that superimposed on this regulation must be other regulatory mechanisms which would allow for modification of respiration in response to environmental factors.

Multiple regression manipulation resulted in the following equation:

$$\begin{aligned}
\text{Average \% respiration} \cdot \text{cell}^{-1} = \; & 1.33 \cdot 10^{-3} && (P = 0.0162) \\
& - 2.80 \cdot 10^{-10} X_1 \; J \cdot m^{-2} \text{ accumulated UV-B fluence} && (P = 0.1082) \\
& - 1.47 \cdot 10^{-6} X_2 \; mg \cdot m^{-3} \text{ biomass} && (P = 0.0568) \\
& + 1.12 \cdot 10^{-4} X_3 \; mg \cdot l^{-1} \text{ DOC} && (P = 0.0020) \\
& - 9.81 \cdot 10^{-6} X_4 \text{ days} && (P = 0.0528) \\
& - 9.41 \cdot 10^{-5} X_5 \; ^{\circ}C && (P = 0.0166) \\
& && R^2 = 0.9512
\end{aligned}$$

Of particular interest in this work were the inclusions of fluence, DOC, and temperature. The sign of each supported theoretical considerations, while the complexity of the overall model allowed for the apparent contradiction noted previously.

Caution must be recommended in using respiration or respiration per cell as an indicator of stress. Not only do many environmental variables affect this response, but the complexity of the

bacterial assemblage permits the presence of both rapid functional fluctuation and relatively slower response by less metabolically active species and/or mutant selecton which might also be expected to vary the response (Harrison 1976).

Other Indices

It was hypothesized that the turnover capabilities of a bacterial assemblage would give an indication of the glucose concentration in the system and/or of the functional capabilities of the bacterial assemblage. Some investigators have recommended, therefore, that T_t would give an appropriate comparative measure of bacterial function (Williams and Askew 1968; Azam and Holm-Hansen 1973; Griffiths et al. 1977).

It proved impossible to generate a satisfactory multiple regression model for T_t with the variables utilized in the other sections of this study. However, a high correlation between T_t and cell concentration ($P < 0.0012$) seemed to support Wright's (1978) suggestion that $T_t \cdot cell^{-1}$ might be an appropriate comparative index.

The highest coefficient of multiple determination (R^2) that could be generated with nonbacterial measured parameters was 0.5982. The inclusion of bacterial measures, especially V_{max} and respiration, increased R^2 to 0.9912 while generating a model in which nine parameters were significant, including both fluence and fluence rate. However, functional correspondence between T_t and

the bacterial parameters might invalidate the model. Further, the techniques involved were not simplified by this method if V_{max} and respiration data were required. Therefore, $T_t \cdot cell^{-1}$ would not seem an appropriate measure in UV-B studies.

A statistically acceptable model for observed variation in $(K_t + S_n)$ was found.

$$K_t + S_n = 3.08 \qquad (P = 0.0944)$$

$$- 1.25 \cdot 10^{-4} X_1 \ J \cdot m^{-2} \qquad \text{accumulated UV-B fluence} \quad (P = 0.0002)$$

$$- 7.76 \cdot 10^{-1} X_2 \text{ days} \qquad (P = 0.0004)$$

$$R^2 = 0.8134$$

The addition of temperature to the model increased R^2 to 0.8562 without affecting the significance of accumulated fluence. The trend toward a decrease in $(K_t + S_n)$ with accumulated fluence had been noted throughout this study. To clarify the decrease, a bioassay for S_n was introduced, which seemed to demonstrate that the $(K_t + S_n)$ decrease was the result of a UV-B mediated decrease in both the transport parameter and the dissolved glucose concentration. This is considered in more detail in the subsequent section.

The experimental complexity of the $(K_t + S_n)$ determination coupled with the uncertainty of the relative contribution of each parameter to the total mediate against its utilization as a routine assemblage measure.

Transport Parameters and Natural Substrate Concentrations

A further suggestion of the involved nature of the assemblage response to UV-B was obtained by bioassays for glucose which were done in conjunction with the Day 29 heterotrophic potential study of the natural assemblages. This permitted the separate consideration of K_t and S_n. Their respective changes with accumulated fluence (Figure 12) cannot be confirmed within this study. However, they do provide the basis for speculation on the nature of UV-B influence.

As the system was subjected to increased UV-B, both K_t and S_n decreased. Lower S_n requires the concurrent appearance of lower K_t. Should K_t fail to follow S_n, i.e., decrease proportionately, the bacterial assemblage could no longer utilize that particular nutrient. Depending upon the importance of that nutrient to bacterial metabolism, such a change might reduce the viability of the assemblage or cause bacteria less dependent upon that substrate to become relatively more prominent (Harrison 1976; Harder et al. 1977). Should K_t or $K_t \cdot cell^{-1}$ be the sensitive moiety, a lower K_t would result in the more efficient utilization of S_n (Hobbie 1973) and conceivably in the establishment of the lower equilibrium concentration of S_n. Which of the two changes initiated the overall decrease is uncertain. Initial decrease in S_n would seem to agree with other models that have suggested the importance of other trophic levels in the bacterial response. However, observed changes in heterotrophic potential and respiration should

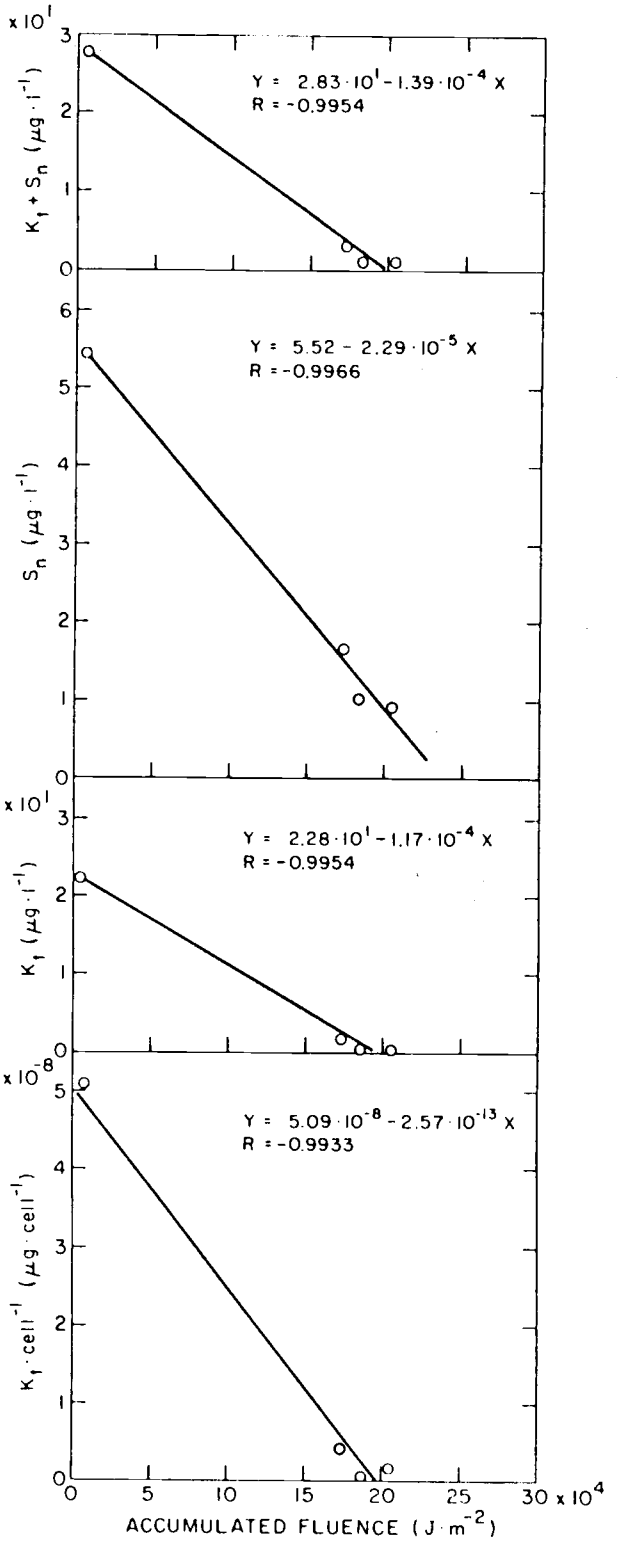


Figure 12. $(K_t + S_n)$ and its component parts with accumulated UV-B fluence. Data derived from Day 29 samples.

be viewed as indicative of the complexity of the interaction. The nature of this interaction remains speculative and open to further experimentation.

Velocity of Glucose Utilization

The actual velocity of glucose utilization dropped with increased UV-B (Figure 13). This drop corresponded with decreases in both the transport parameter (K_t) and glucose concentration (S_n). The latter would seem to limit the velocity, as a low K_t indicates high utilization efficiency, whereas transport follows first order kinetics when substrate limited (i.e., below enzyme saturation) (Lehninger 1975).

In itself, such decreased utilization of one substrate does not necessarily indicate potentially irreversible change in bacterioplankton activity. A comparison of v with V_{max}, however, suggests a tolerance limit.

Wright (1970) reported that the actual velocity of utilization of glucose was about 31% of V_{max}. Those microcosms receiving UV-B fluences in excess of current natural levels showed actual utilizations of more than 70% of V_{max}. Low K_t would facilitate this, but the assemblage *per se* could not compensate for much further stress, at least in regard to glucose utilization.

If V_{max} is a tolerance limit for the bacterial assemblage, and if UV-B causes that limit to be approached, UV-B is implicated as a significant ecological factor. In their work with diatoms,

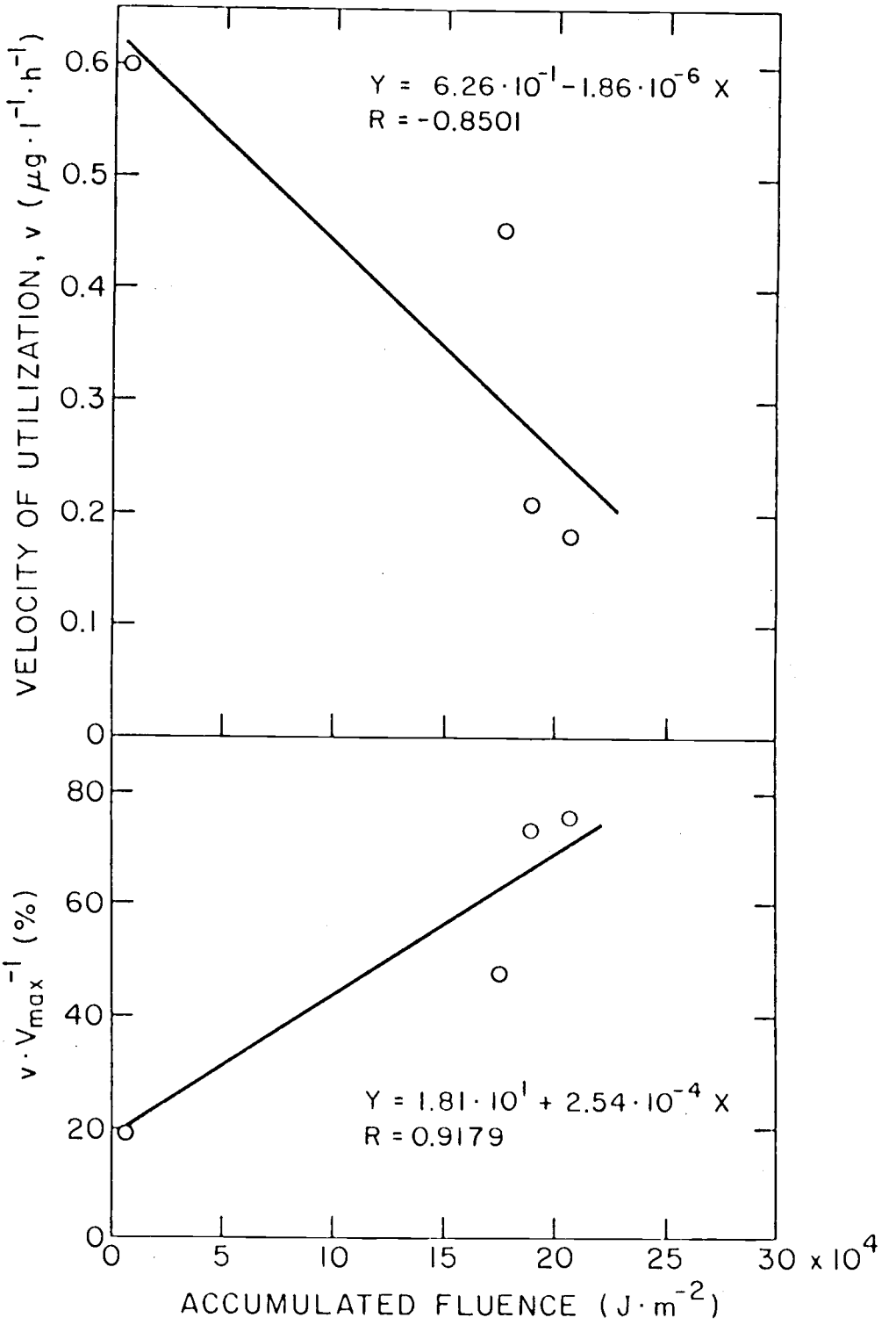


Figure 13. Heterotrophic velocity (v and $v \cdot V_{max}^{-1}$) with accumulated UV-B fluence. Data derived from Day 29 samples.

Calkins and Thordardottir (1980) found that tolerance and exposure to solar UV were approximately equal. It follows that there may be no large reserve of resistance which could cope with altered solar UV without physiological or behavioral adaptation or genetic selection. While such adaptation is possible, it would tend to limit adaptation and repair capabilities for other purposes.

It seems reasonable to extrapolate this rationale to bacterioplankton. Therefore, it is recommended that the concept of V_{max} as a tolerance limit be pursued.

V. CONCLUSIONS

Within the fluence and time limits of this study, the heterotrophic activity of the bacterioplankton changed. Such change was linked primarily to effects on other trophic levels, although limited evidence of direct effect was also noted.

One potentially useful measure of bacterial stress was $V_{max} \cdot cell^{-1}$. This was shown to be negatively correlated to accumulated UV-B fluence. It must, however, be incorporated in a model which includes one or more additional environmental variables if it is to be of predictive or comparative value.

The complexity of interacting variables mediated against the use of cell concentration or respiration ratio as stress indicators. T_t and $T_t \cdot cell^{-1}$ also proved to be inappropriate for this study.

Variation in $(K_t + S_n)$ was negatively correlated to accumulated UV-B fluence. An attempt to resolve K_t from S_n raised the question: which of the two was the sensitive moiety? If K_t or $K_t \cdot cell^{-1}$ was reduced by UV-B, the effect may be direct; if S_n was reduced, other trophic levels were implicated as well.

Reduction in actual velocity of glucose utilization with accumulated UV-B was accompanied by an increase toward the V_{max} of the system. If V_{max} represents a tolerance limit for the heterotrophic bacterial assemblage, the ecological implications of the measure are deserving of further study.

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APPENDICES

Appendix I: Accuracy of ^{14}C-Glucose Solution Dispensing

The addition of known, reproducible amounts of radioactively labeled substrate to equivalent subsamples of test waters is a requirement of the heterotrophic potential technique. To test the accuracy of a Drummond Dialamatic Microdispenser, ^{14}C-glucose solutions were dispensed into Beckman Ready-Solv HP scintillation fluor. Each of the volumes utilized in the experimental protocol was delivered in triplicate and counted according to standard procedure (Figure 14). Both the reproducibility of a given volume and the linear relationship among the volumes (R^2 = 1.0000) were indicative of the accuracy of the instrument and the investigator's technique.

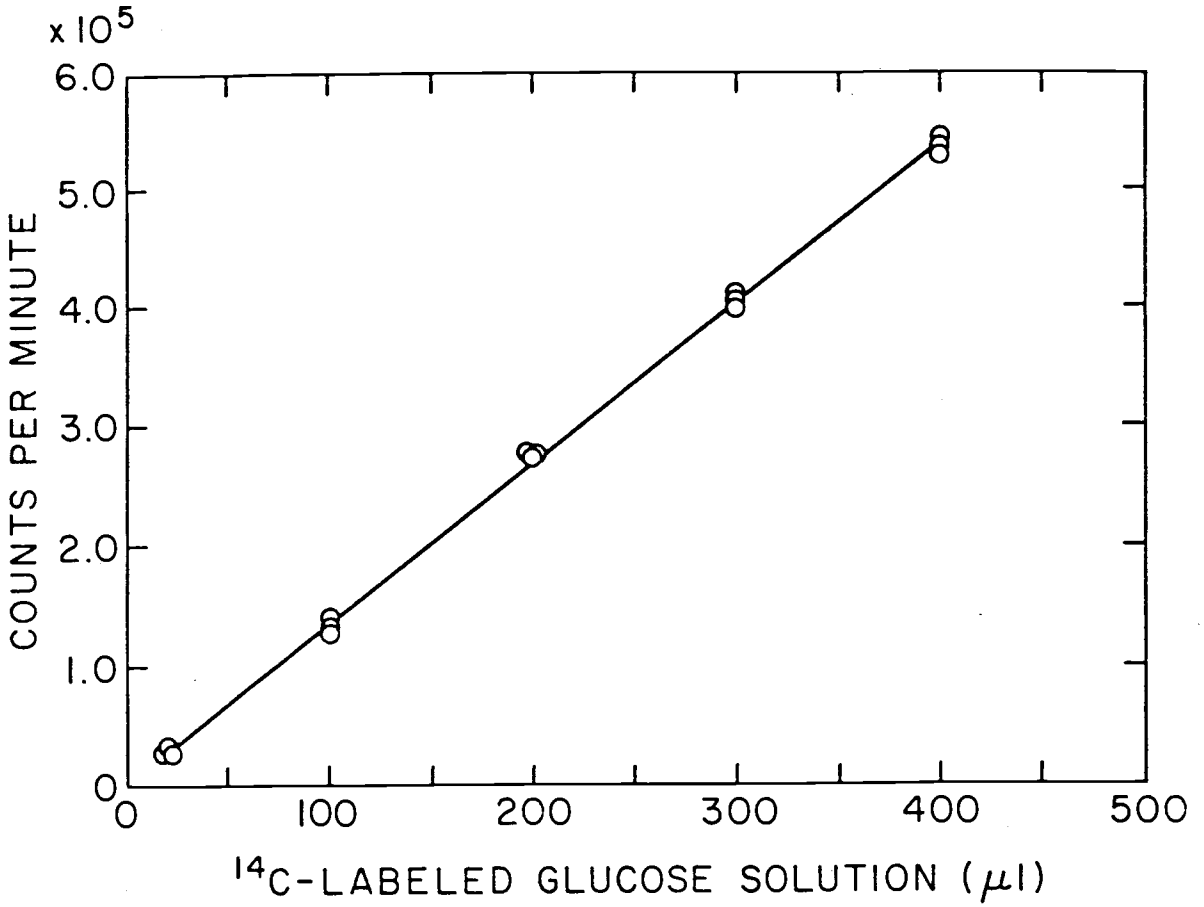

Figure 14. Accuracy and reproducibility of ^{14}C-glucose solution delivery by Drummond Dialamatic Microdispenser.

Appendix II: Quench Correction

In scintillation methods for the detection of radionuclides, a variety of different mechanisms, including the presence of non-fluorescent dissolved molecules, of colored sample materials, or of nonhomogeneity within the scintillation system, can result in reduced efficiency in the energy transfer process. This reduced efficiency is known as quenching. Quenching decreases light output per β-particle, reducing detection efficiency. Because quenching can vary considerably among samples, it is necessary to quantify the counting efficiency of each sample before comparison of activity is possible (Wang et al. 1975).

The detection instrument used in this study, a Beckman LS 8000 Liquid Scintillation System, is equipped with an automatic measure of quench, the H-number. This is calculated in a modified external standard technique. During part of the counting cycle for each sample a ^{137}Cs external standard is automatically positioned near the scintillation vial, exposing the sample to gamma radiation. Compton electrons produced by the gamma radiation excite molecules within the system, producing photons. The energy distribution of these photons is measured in a multichannel analyzer, and its Compton edge (the maximum possible energy that the Compton electron can acquire in a single gamma encounter) is determined. In a quenched sample, the Compton edge is shifted toward channels indicating less energetic light output. The channel difference in

inflection points of the Compton edge between unquenched and quenched samples is the H-number (Beckman Instruments 1978).

To use H-number to correct for quench, standards with known disintegration rates are measured. Their counts per minute and H-numbers are recorded automatically. Counting efficiencies are then calculated:

$$\text{Counting efficiency (\%)} = \frac{\text{cpm}}{\text{dpm}} \cdot 100\%$$

Counting efficiency is related to H-number graphically (Figure 15).

In subsequent sample counting, H-number is converted to counting efficiency, and DPM for the unquenched sample is calculated:

$$\text{dpm} = \frac{\text{cpm}}{\text{counting efficiency}} \cdot 100\%$$

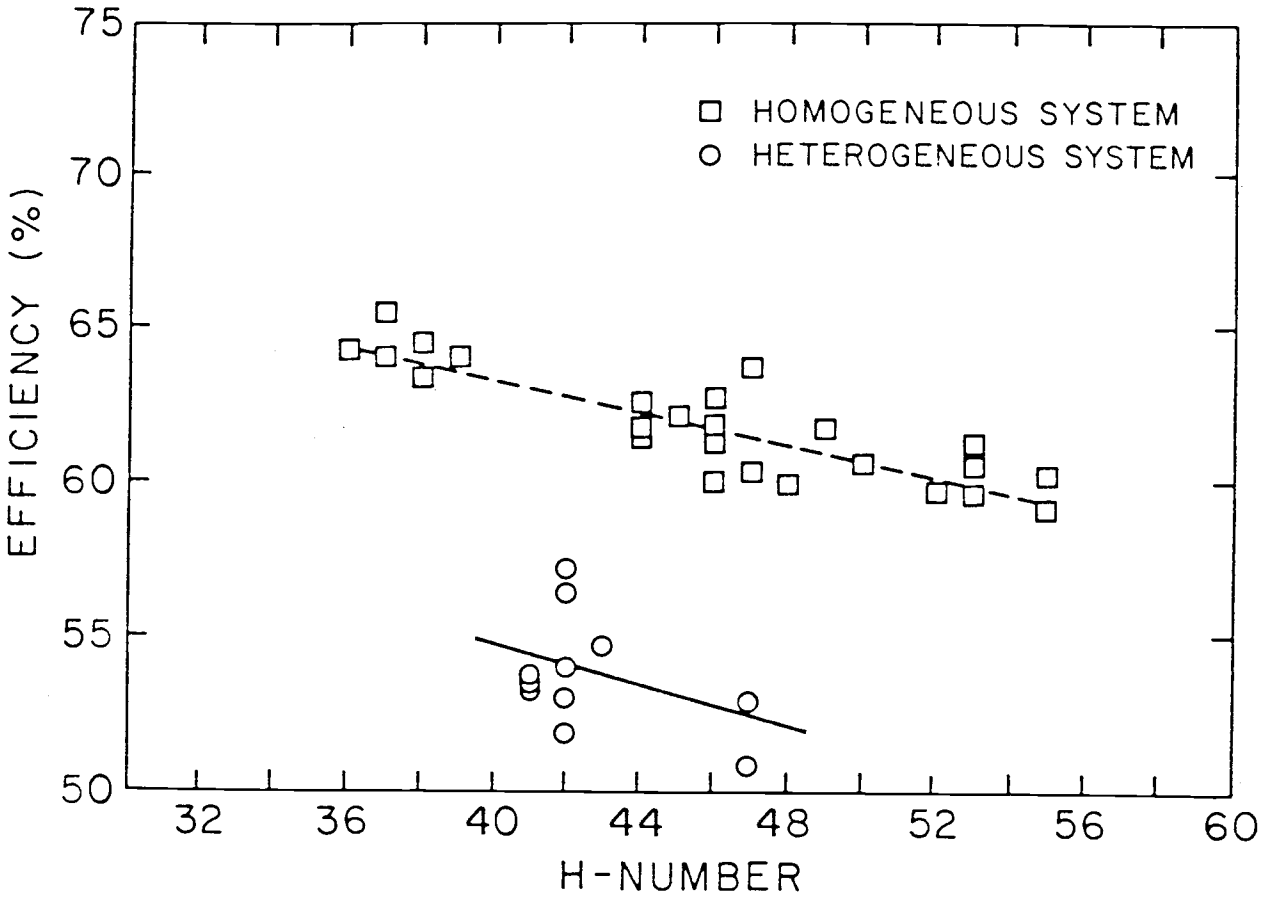

Figure 15. Correction for loss in detection efficiency due to quenching as measured by H-number. Note that the presence of a membrane in the scintillation fluor (heterogeneous system) reduces the efficiency by about 10%.

Appendix III: Data Manipulation in Heterotrophic Potential Technique

In order to better visualize the data handling in the heterotrophic potential technique and to judge the validity of its use with this experimental system, a representative data set (Day 2, 0.18-mm Mylar 'D' microcosm) is presented in Table II and graphed in Figures 16 and 17.

Table 11. Manipulation of scintillation data: Day 2, 0.18-mm Mylar 'D' microcosm.

	20-1[a]	20-2	20-3	100-1	100-2	100-3	200-1	200-2
dpm assimilated[b]	7685.8	8091.7	7652.6	22202.7	20652.9	19964.2	30823.2	30793.1
dpm assimilated, blank corrected[c]	7531.2	7937.1	7498.0	21821.8	20272.0	19583.3	30254.2	30215.1
dpm respired[d]	1888.5	2072.8	1724.1	4855.3	4690.1	4332.2	7506.4	7880.3
dpm respired, blank corrected	1867.6	2051.9	1703.2	4819.6	4654.4	4296.5	7436.4	7810.3
total dpm	9398.8	9989.0	9201.2	26641.4	24926.0	23880.0	37681.6	38025.0
ratio: respiration·total^{-1}	0.199	0.205	0.185	0.187	0.180	0.197	0.205	0.187
F: total dpm·dpm added^{-1}	0.212	0.225	0.207	0.120	0.112	0.108	0.085	0.087
T: h[e]	5	5	5	5	5	5	5	5
T·F^{-1}	23.62	22.22	24.13	41.66	44.53	46.48	58.92	58.38

Continued.

Table 11. Continued.

	200-3	300-1	300-2	300-3	400-1	400-2	400-3
dpm assimilated	29840.6	36349.9	37205.8	38118.0	36335.6	34047.0	31502.2
dpm assimilated, blank corrected	29262.6	35750.1	36606.0	37518.2	35652.0	33363.4	30818.6
dpm respired	6791.4	10073.1	9707.9	9389.8	12623.0	10205.0	10726.0
dpm respired, blank corrected	6721.4	9963.1	9597.9	9279.8	12482.0	10064.0	10585.0
total dpm	35984.0	45713.2	46204.0	46798.0	48134.0	43428.0	41404.0
ratio: respiration·total^{-1}	0.187	0.218	0.208	0.198	0.259	0.232	0.256
F: total dpm·dpm added^{-1}	0.081	0.069	0.069	0.070	0.054	0.049	0.047
T: h	5	5	5	5	5	5	5
T·F^{-1}	61.69	72.85	72.07	71.16	92.24	102.24	107.24

a volume of labeled glucose added - replicate (converted to µg glucose·l^{-1} for graphs).

b activity associated with membrane filter, in cells or macromolecules.

c nonviable sample activity subtracted to correct for adsorption.

d activity on β-phenylethylamine impregnated wick, respired CO_2.

e incubation time.

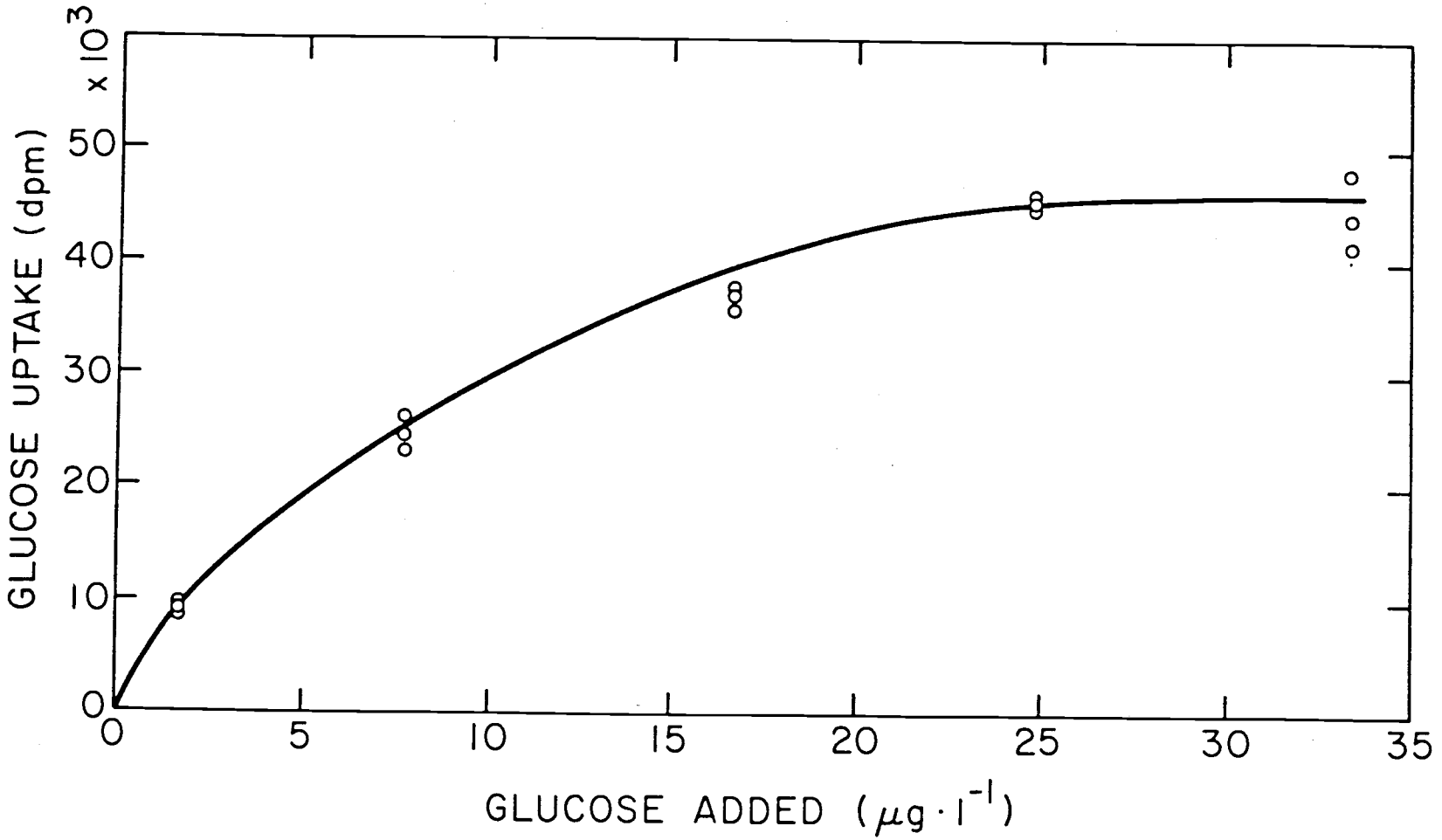

Figure 16. ^{14}C-glucose uptake (assimilation plus respiration) at increasing concentrations of added glucose: Day 2, 0.18-mm Mylar 'D' microcosm.

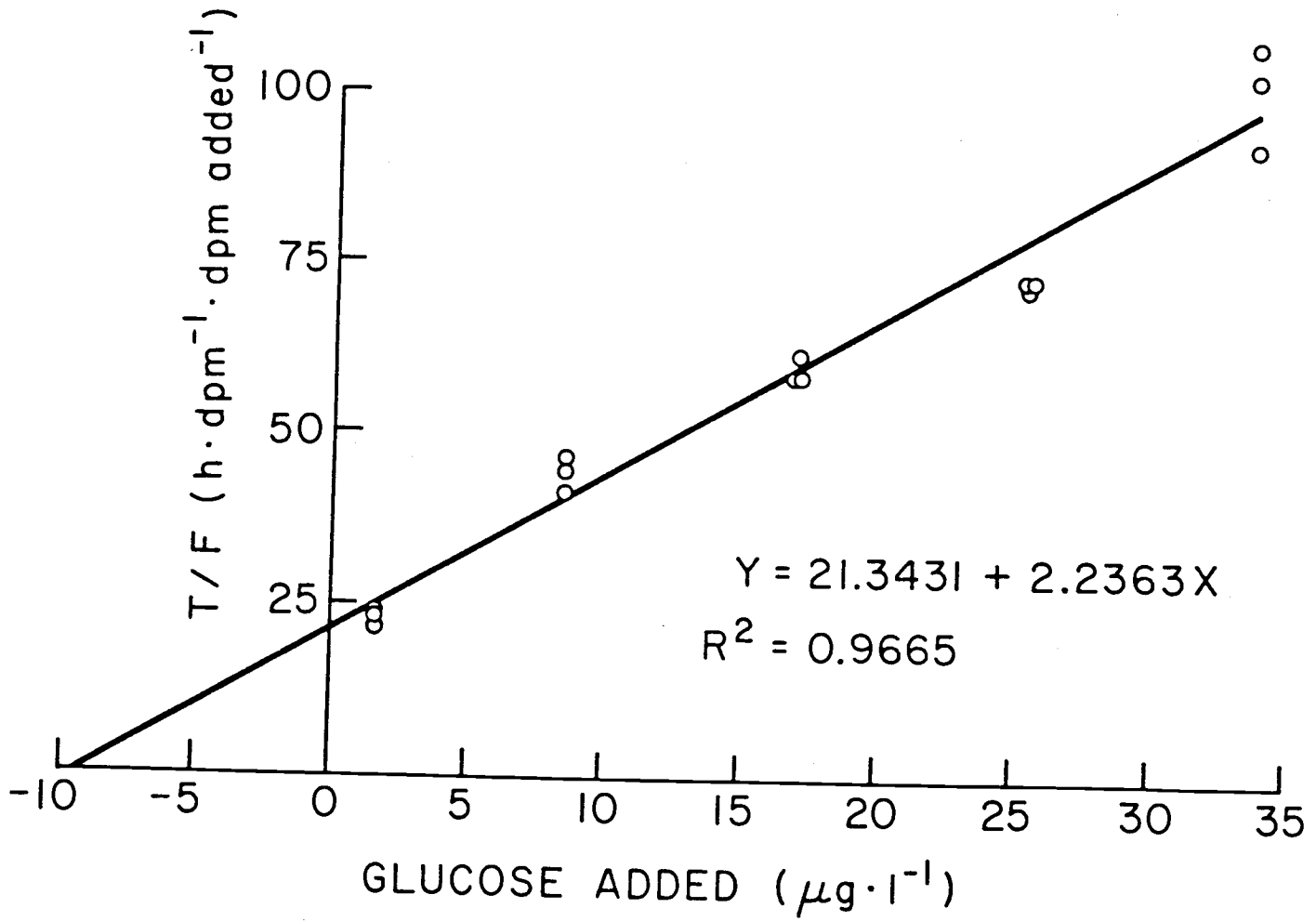

Figure 17. Modified Lineweaver-Burk transformation of ^{14}C-glucose uptake at increasing concentrations of added glucose: Day 2, 0.18-mm Mylar 'D' microcosm. $V_{max} = 1 \cdot slope^{-1}$; T_t = Y intercept; $-(K_t + S_n)$ = X intercept.