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Ozone, present in the earth's atmosphere, filters incident solar ultraviolet radiation such that only a portion of the total ultraviolet spectrum emitted by the sun reaches the planet's surface. Violation of the integrity of this filter could mean an alteration in the solar ultraviolet radiation penetrating the atmosphere. Atmospheric increases in oxides of nitrogen and chlorofluoromethanes represent a threat to this shield. Among the known sources of these substances are the emissions from the engines currently used in the supersonic transport aircraft, fuels which are to be used for the space shuttle aircraft, propellants in aerosol spray cans, refrigerants, and the heat generated by nuclear explosions. It is not known how much perturbation can be tolerated by the ozone system. Nor is it well established how alterations of the incident solar radiation will be tolerated by the organisms residing at or near the earth's surface. The purpose of this investigation was to detect possible

effects of increased solar ultraviolet irradiation on a particular portion of the biosphere.

Samples of estuarine water were cultured in the laboratory in five liter rectangular containers which provided a surface area to volume ratio of 0.1. Autotrophic growth was encouraged by enrichment with inorganic nutrients and exposure to cool-white fluorescent lamps for 14 hours a day. Community photosynthesis and respiration were monitored over a six-week period and demonstrated oscillations suggesting that succession was occurring and that the microcosms were able to maintain themselves. Replicate microcosms were exposed to increased ultraviolet radiation (above 290 nm) for ten of the fourteen hours of exposure to cool-white radiation (310-750 nm) during the second through the fifth week of the experimental period.

The algal blooms which resulted supported the growth of the heterotrophic portion of the community. Changes in the natural bacterial populations were followed through enumeration studies and through an examination of a number of selected heterotrophic metabolic activities. Dilution plate counts, three-tube most probable number (MPN) determinations, and the utilization of a uniformly ^{14}C labelled organic compound were the three methods used to characterize the heterotrophic populations.

The differences observed in the bacterial populations of the two groups of microcosms indicate that increased ultraviolet irradiation does have a measurable effect. Among the effects of exposure to UV radiation were: a selection against non-pigmented cells; a selection

against certain specific metabolic types of organisms, such as cellulose degrading heterotrophs, and an increase in heterotrophic respiration.

Response of Natural Microbial Populations to Ultraviolet Irradiation in a Simulated Estuarine Microcosm

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES (Continued)

RESPONSE OF NATURAL MICROBIAL POPULATIONS TO ULTRAVIOLET IRRADIATION IN A SIMULATED ESTUARINE MICROCOSM

INTRODUCTION

Experimental System

The ecosystem is the basic functional unit in nature since it includes both living organisms and abiotic substances each influencing the properties of the other and both necessary for the maintenance of life as we know of it on the earth (Odum, 1964). Marine heterotrophic bacteria are important in the cycling of nutrients throughout the marine ecosystem. Marine heterotrophs present in the intestinal tracts of carnivorous and herbaceous grazers; epizooic and epiphytic bacteria; and bacteria found on rocks, sand, and mud contribute significantly to the processes resulting in the breakdown and conversion of organic material into nutrients and growth factors utilizable by the autotrophic members of the community.

The marine community viewed in this way can be regarded as an ecosystem. It is a closed system in that the internal cycling and "feedback" mechanisms predominate over any external inputs or outflows of energy. Also it is considered to be a stable system which is a function of it being closed. How much abuse will be tolerated by the oceans is not clearly known, but within man's temporally limited ability to study the ocean it is considered to be stable and closed. A third component of the marine ecosystem is its built-in redundancy of functional components; its variety of organisms providing functional overlap.

The approach taken in this investigation was to isolate a segment of the marine ecosystem, to supply this micro-community with inorganic nutrients, and to allow the community to maintain and develop through the recycling of the nutrients by the indigenous heterotrophs.

The estuarine community was the segment of the marine ecosystem chosen for study in this investigation. Although the transmission of UV-irradiation may be limited to the upper water column, UV may still have readily discernible effects on a community subject to tidal action. Diurnal or semi-diurnal tidal action provides energy for mixing of the water column in an estuary; the extent of mixing being a function of the fresh-water input, the physical parameters determining the shape and depth of the estuary, and the tidal range. As a result of tidal action organisms normally found in the water column as well as benthic organisms may be exposed to increased ultraviolet radiation as they are displaced upwards. These organisms may also be exposed as the tide recedes from both the shore and the rocks around the estuary. Estuaries are of significance not only in providing nutritional and spatial needs of commercially important fish and shellfish, but also the estuaries support a wide variety of organisms which are of aesthetic value to some portions of human society. A further consideration in choosing the estuary as an area of study, and a consideration of no minor importance, was the ease with which specimens could be obtained; thereby avoiding dependence on ship-time which is not only difficult to obtain, but also is costly, both financially and temporally.

Solar Electromagnetic Spectrum

The sun is the principal source of electromagnetic radiation penetrating to the surface of the earth. The energy spectrum of the radiation emitted by the sun is a function of the temperature and wavelength according to Kirchoff's law,

$$\frac{\text{energy emitted } (E_m)}{\text{energy absorbed } (E_b)} = f(\lambda, T).$$

A body which completely absorbs all incident radiation, i.e., E_b equal to one, is also the most efficient emitter of energy and is referred to as a black body. The total energy emitted per unit area per second is given by the Stephan-Boltzman law,

$$\text{energy emitted } (E_m) = \sigma T^4,$$

where σ is the Stephan-Boltzman constant, 5.73×10^{-5} erg cm^{-2} sec^{-1}.

When the energy emitted is lower by the same factor at all wavelengths than the predicted black body energy, then the body is referred to as grey. The sun does not emit energy either as a perfect black body or as a uniform grey body. According to Robinson (1966), the sun radiates as a grey body at 6×10^3 °K in the wavelength region near 2,400 nm; as a black body at 4.5×10^3 °K at 100 to 200 nm; as a black body at 5×10^5 °K at 4 to 10 nm; and as a black body at 4×10^6 °K at approximately 0.3 nm, the wavelength of energy emitted by solar flares. The variation in the wavelength of energy emitted by the sun is due to simultaneous emission of radiation from various depths of the sun which possess varying physical properties. The variation of

temperature across the solar disc is also a factor determining the spectrum of electromagnetic energy emitted by the sun.

The electromagnetic spectrum radiated by the sun is considered to approximate the energy distribution of a black body at 6×10^3 °K, with nearly 98% of the total energy in the region of 250 to 3,000 nm. Table I provides a classification scheme of solar radiation according to wavelength. Another scheme which is used to describe a portion of the spectrum in the ultraviolet region is shown in Table II.

Alteration of the Solar Spectrum by the Earth's Atmosphere

The geometrical configurations of the earth-sun system which affect seasonal and diurnal variations in the solar radiation reaching the outer regions of the earth's atmosphere are not of primary concern to this particular investigation and so will not be discussed. Rather, attention will be given to the alterations the solar electromagnetic energy spectrum undergoes as it penetrates the earth's atmosphere. The depletion of energy as the incident solar radiation passes through the atmosphere may be characterized by a coefficient of extinction. The extinction coefficient is a function of wavelength and represents both scattering and absorption.

Scattering is a continuous function of wavelength (Table III) and is due to refraction, reflection, and diffraction, or a combination of these three phenomena. In being dispersed throughout a field the radiant energy travels in all directions and becomes, to an extent, a new source of light referred to as "sky shine."

TABLE I. CLASSIFICATION OF SOLAR RADIATION ACCORDING TO WAVELENGTH (After Robinson, 1966)

Wavelength in nanometers (nm)	Energy Category
Less than 0.1	X-rays and gamma rays
1 to 200	Far ultraviolet radiation
200 to 315	Mid-ultraviolet radiation
315 to 380	Near ultraviolet radiation
380 to 720	Visible light
720 to 1500	Near infrared radiation
1500 to 5600	Mid-infrared radiation
5600 to 10,000	Far infrared radiation
Greater than 10,000	Radio and micro-waves

TABLE II. CLASSIFICATION OF ULTRAVIOLET RADIATION (Schaffer, 1969)

Wavelength (nm)	Category
Less than 280	Ultraviolet C (UV-C)
280 to 315	Ultraviolet B (UV-B)
315 to 400	Ultraviolet A (UV-A)

TABLE III. ALTERATION OF RADIANT ENERGY AS A FUNCTION OF WAVELENGTH (After Robinson, 1966)

Wavelength (NM)	Scattering Coefficient	Transmissivity*
200	954 x 10^{-8}	0.0005
250	338 x 10^{-8}	0.0669
300	152 x 10^{-8}	0.295
350	79 x 10^{-8}	0.53
400	45 x 10^{-8}	0.696
450	28 x 10^{-8}	0.800
500	18 x 10^{-8}	0.865
600	8.6 x 10^{-8}	0.933
700	4.6 x 10^{-8}	0.964
800	2.7 x 10^{-8}	0.979
900	1.7 x 10^{-8}	0.987
1000	1.1 x 10^{-8}	0.991
1100	0.74 x 10^{-8}	0.994
1200	0.53 x 10^{-8}	0.996
1500	0.21 x 10^{-8}	0.998
2000	0.068 x 10^{-8}	0.999
4000	0.042 x 10^{-8}	1.000

*Proportion of radiant energy reaching the earth's surface which is associated with wavelengths less than the given wavelength.

Absorption may be considered to be that energy which is not accounted for by summing the scattered and transmitted energies integrated over all angles in the sky. Whereas scattering is a continuous function of wavelength, absorption is generally a more selective function of wavelength (Table III). The chemical species responsible for much of the absorption of radiant energy are: molecular and atomic oxygen; molecular and atomic nitrogen; oxides of nitrogen; ozone; and other polyatomic molecules such as carbon monoxide, carbon dioxide, and methane.

In comparing Table I with Table III it is apparent that only a portion of the total solar electromagnetic spectrum reaches the surface of the earth. Very little energy below 200 nm or above 4000 nm penetrates the atmosphere. Indeed even the energy within this wavelength region does not pass through the earth's atmosphere without being altered. Absorption in the ultraviolet region results chiefly in electronic configurational transitions of ozone, and atomic and molecular oxygen and nitrogen. There is minimal alteration of the spectral energy in the visible region. In the infrared region absorption results primarily in vibrational and rotational changes in polyatomic molecules such as water, ozone and carbon dioxide.

Of primary concern to this investigation is the modification of the spectral energy in the ultraviolet region as a result of presence of ozone in the earth's atmosphere. The absorption of solar radiation by other gaseous molecules present in the atmosphere will be mentioned only in passing.

Water vapor and, to a lesser extent, carbon dioxide are responsible for the absorption of radiant energy in the infrared region. Absorption of energy in this wavelength region results chiefly in the heating of the lower atmosphere due to changes in molecular vibrational frequencies.

Ozone, molecular oxygen, and water vapor are responsible for the relatively limited amount of alteration of the solar radiation in the visible region.

Due to strong absorption in the ultraviolet region, up to 300 nm, by ozone, and by molecular and atomic oxygen and nitrogen, less than 30% of the solar energy spectrum below 300 nm reaches the earth's surface. In the process of absorbing energy in this wavelength region molecular oxygen and molecular nitrogen are dissociated to their atomic species which absorb electromagnetic energy of even shorter wavelengths. The result of the radiochemical dissociation of molecular species is the formation of ionized layers in the upper atmosphere.

Figure 1 depicts the alteration of the electromagnetic energy spectrum radiated by the sun as it penetrates the earth's atmosphere.

The atmospheric molecular species of primary concern to this investigation is ozone. It is present in trace amounts diffused throughout the atmosphere at an average concentration of three parts per million (ppm). At standard temperature and pressure this would represent a layer of three millimeters thickness compared to the thickness of the entire atmosphere under the same conditions of temperature and pressure of eight kilometers thickness. The natural production of

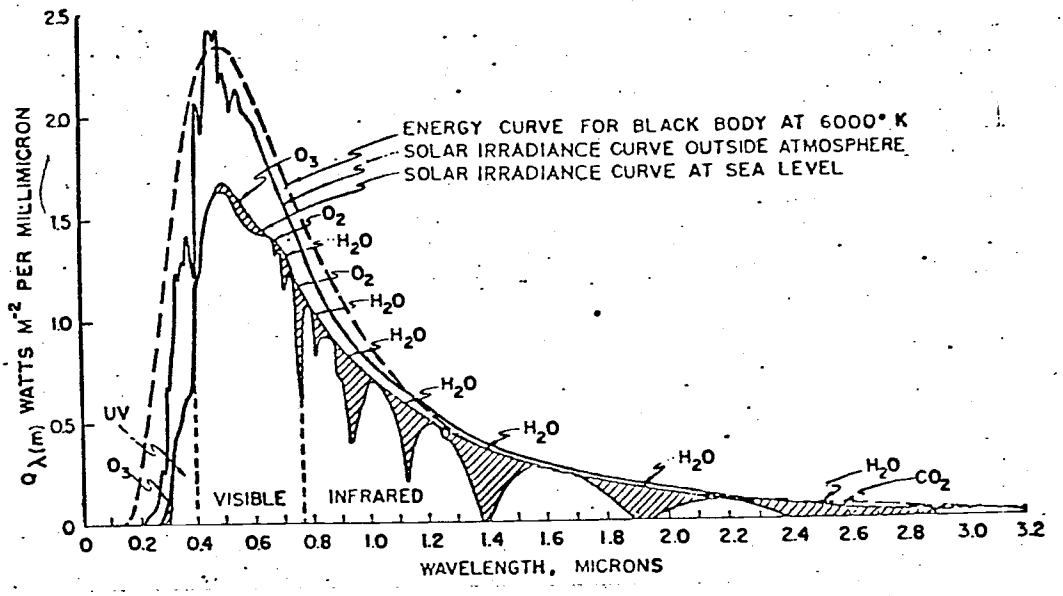

Figure 1. Spectral energy curves related to the sun (Handbook of Geophysics, 1960).

ozone occurs in the ionization layer between 60 and 90 kilometers above sea level. Radiolysis of oxygen by solar energy of wavelengths shorter than 242 nm followed by the reaction of free oxygen with molecular oxygen on the surface of airborne particulates accounts for the major portion of the natural production of ozone. Figure 2 depicts the absorption of radiant energy in the ultraviolet region by various atmospheric gases at given altitudes in the earth's atmosphere.

The natural production of ozone is considered to be in steady state equilibrium with the natural destruction of the molecule. According to Hammond and Maugh (1974) approximately 18% of the natural destruction of ozone results from the combination of free atomic oxygen with ozone thereby forming two oxygen molesules; 11% of the natural destruction is through combination with a hydroxide radical; 50% to 70% destruction by naturally occurring oxides of nitrogen; and up to 20% of the natural destruction of ozone cannot be accounted for at this time.

Naturally occurring oxides of nitrogen serve as catalytic agents and account for the major portion of the breakdown of ozone. If the natural destruction of ozone is indeed in equilibrium with the natural production of this molecule, then an introduction of additional oxides of nitrogen into the upper atmosphere could result in a distrubance of this equilibrium. One of the potential sources for the injection of these substances into the upper atmosphere is the emission from the engines of supersonic transport aircraft. Another source is the formation of oxides of nitrogen from the heat generated in thermo-

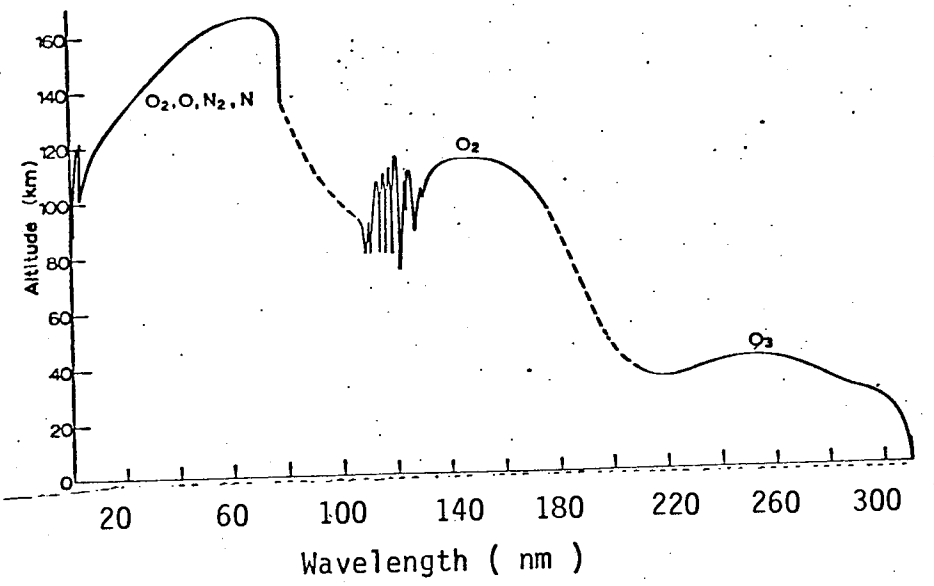

Figure 2. Penetration of solar radiation into the atmosphere. The curve indicates the altitude at which the intensity is reduced to e^{-1} (37%). (Friedman, (1960).

nuclear explosions. The heat which is generated in the explosion functions to dissociate molecular oxygen and molecular nitrogen with the subsequent recombination of oxygen with nitrogen. Oxides of nitrogen introduced in the lower atmosphere are not of primary concern since these molecules would be washed out of the atmosphere by rain.

Recently the role of chlorine compounds in the atmosphere has been investigated. Three groups of researchers working independently concluded that chlorine is a more efficient catalyst in the destruction of ozone. However these investigators, Cicerone and Stolarski (1974), Wofsy and McElroy (1973), and Crutzen (1974), did not consider their conclusions of importance since there was no known major source of chlorine. Molina and Rowland (1974) realized the threat posed by the presence of chlorine compounds in the atmosphere. The major sources of this efficient catalyst are the widely used aerosol propellants and refrigerants, namely those utilizing chlorofluoromethanes and di-chlorofluoromethanes. Hammond and Maugh (1974) stated that there have been sufficient quantities of these halomethanes bottled into aerosol cans to cause a 5% reduction in atmospheric ozone by the year 1990. At the current annual rate of increase on production of these substances, about 20% per year, a 30% reduction of ozone could occur by 1994. Another potential source of these halomethanes is the chlorinated fuels, ammonium perchlorates, which are scheduled for use in the space shuttle craft.

Figure 3 summarizes the reactions involved in the catalytic

3.a.

$$NO + O_3 = NO_2 + O_2$$

$$O_3 + h\nu = O_2 + O$$

$$NO_2 + O + NO + O_2$$

Net result $2O_3 + h\nu = 3O_2$

3.b.

$$Cl + O_3 = ClO + O_2$$

$$O_3 + h\nu = O_2 + O$$

$$ClO + O = Cl + O_2$$

Net result $2O_3 + h\nu = 3O_2$ (six times more efficient than 3.a.)

3.c.

$$ClO + NO = Cl + NO_2$$

$$NO_2 + O = NO + O_2$$

$$Cl + O_3 = ClO + O_2$$

Net result $O + O_3 = 2O_2$

Figure 3. Catalytic destruction of ozone (After Hammond and Maugh, 1974).

destruction of ozone by oxides of nitrogen and by halomethanes. The two processes undergo similar sets of reactions and can interact to provide a third set of reactions catalyzing the breakdown of ozone.

Penetration of UV in Natural Waters

Water is not transparent to visible light or ultraviolet radiation, the limits of penetration being determined by the amount of material either dissolved or suspended in the water column. Table IV describes the transmission of various wavelengths in distilled water.

Jerlov (1968) has classified oceanic water and coastal water into several subgroups according to their transmissivity to incident solar radiation, as shown in Table V.

Jerlov (1968) also presents data describing the transmission of specific wavelengths of the incident solar spectrum through the various characteristic water types, as shown in Table VI.

Using Jerlov's (1968) data the transmission of 310 nm wavelength of radiant energy can be calculated for the various water types, as shown in Table VII.

The calculations given in Table VII indicate that the penetrability, even within the first meter, varies greatly among the different water types, being maximum in the clearer oceanic waters. Ultraviolet radiation in the 310 nm region may be limited in its transmission to the upper 15 meters of oceanic water and perhaps to the upper two meters in coastal water.

Photosynthetic organisms, as well as other microscopic particles

TABLE IV. TRANSMISSION OF RADIANT ENERGY THROUGH DISTILLED WATER (After Koller, 1965).

Wavelength (nm)	Percent transmitted through water to 2.5 cm	21.2 cm	30.5 cm
400	--	99	98
380	--	98	96
360	--	97	94
340	--	96	92
320	99	94	88
300	98	90	83
280	98	89	79
260	98	87	76
240	97	81	61
220	92	61	36

TABLE V. PERCENT OF TOTAL INCIDENT IRRADIANCE AT DEPTH. (After Jerlov, 1968)

Depth	Oceanic Water				Coastal Water				
meters	IA	IB	II	III	1	3	5	7	9
0	100	100	100	100	100	100	100	100	100
1	44.1	42.9	42	39.4	36.9	33	27.8	22.6	17.6
2	37.9	36	34.7	30.3	27.1	22.5	16.4	11.3	7.5
5	29	25.8	23.4	16.8	14.2	9.3	4.6	2.1	1
10	20.8	16.9	14.2	7.6	5.9	2.7	0.69	0.17	0.052

TABLE VI. IRRADIANCE TRANSMISSION AS A FUNCTION OF WAVELENGTH THROUGH DIFFERENT WATER TYPES. (After Jerlov, 1968).

Wavelength (nm)	Irradiance transmitted (%/meter) in Water Type								
	IA	IB	II	III	1	3	5	7	9
310	86	80	69	50	16	9	3	--	--
350	92.5	90.5	84	71	32	19	10	5	1.5
400	96.3	95.5	92	84	69	53	36	22	9
450	97.4	96.7	94	88.5	84	75	60	42	21
500	96.6	96	93.5	89	88.8	82	71	56	37
550	93.6	93	90.5	86.5	86.6	81	72	63	53
600	84	83	80	75	75	71	67	62	55

TABLE VII. PERCENT SURFACE IRRADIANCE AS A FUNCTION OF DEPTH AND WATER TYPE FOR 310 nm WAVELENGTH

Depth Meters	IA	IB	II	III	1	3	5
0	100	100	100	100	100	100	100
1	83	80	69	50	16	9	3
2	69	64	48	25	2.6	0.8	0.1
3	57	51	33	13	0.4	0.1	---
4	47	41	23	6.3	0.1	---	---
5	39	33	16	3.1	---	---	---
6	33	26	11	1.6	---	---	---
8	23	17	5.1	0.4	---	---	---
10	16	11	2.4	0.1	---	---	---
%T in 1 m	83	80	69	50	16	9	3
%T in 10 m	16	11	2	0.1	---	---	---
Depth (m) radiant energy 10% surface value	12.1	10.5	6.2	3.3	1.26	0.96	0.66

both living and detrital, are important components of the water column which absorb radiant energy. This is especially true of coastal waters and other highly productive areas of the oceans and as a general rule the clarity of water is a function of the distance from shore. Jerlov's (1968) classification scheme supports this general rule as can be seen from Figure 4. The photic zone (i.e., the depth at which the radiant energy has decreased to 1% of its surface value) becomes more shallow as the coast is approached and materials dissolved or suspended in the water column increase. One factor contributing to the increase in particulates, and of special importance to the Oregon coastal waters, is the phenomenon of coastal upwelling. The upwelling of water is the result of two processes occurring simultaneously, namely the Coriolis acceleration and Eckman transport.

A simple explanation of how these two processes interact is the purpose of the following brief discussion. In the Northern hemisphere the Coriolis acceleration results in the net displacement of a mass to the right of the horizontal component of any force acting upon that mass to cause it to accelerate. An automobile traveling in the north-south direction would have a tendency to be displaced somewhat to the west of its intended end point due to the effect of the Coriolis acceleration. That this net displacement is not observed is the result of the friction of the automobile's tires on the road surface overcoming the effect of the Coriolis force. However, the effect of the Coriolis force can be observed and is responsible for the clock-

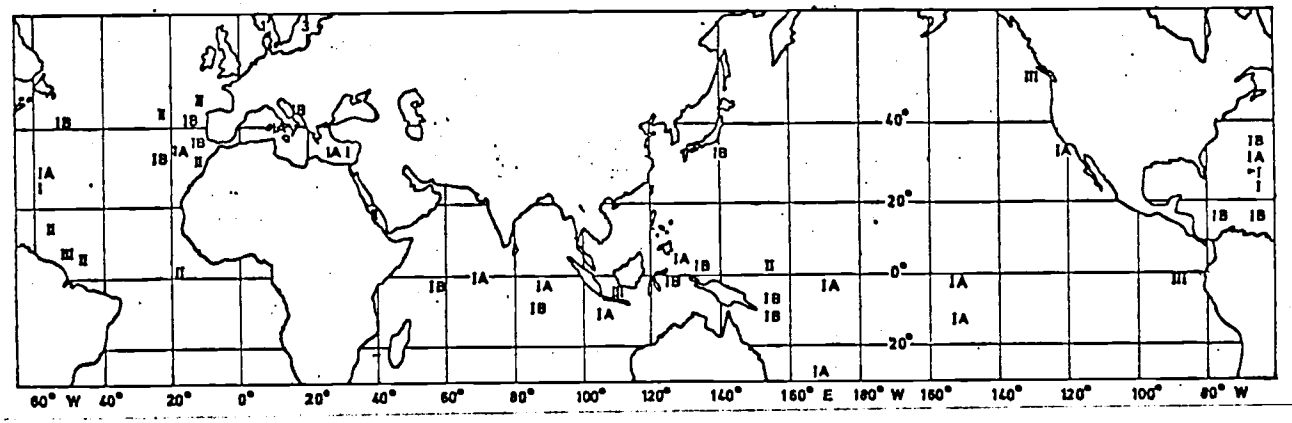

Figure 4. Regional distribution of oceanic optical water types. (Jerlov, 1968).

wise motion, in the Northern hemisphere, of a body of water as it drains out of a sink. In the Southern hemisphere the water draining out of a sink would have a counterclockwise motion. At the Equator the Coriolis acceleration is nullified and at the Poles it is maximal.

A column of water can be considered to be composed of a series of slabs of water one on top of the other. If a horizontal force were applied to the topmost slab it would move in the direction of the force (with a displacement to the right in the Northern hemisphere), until the friction of the slab of water underneath it caused its motion to cease. In this frictional process the upper slab would impart a somewhat diminished force to the lower slab and the motion would again be to the right of the direction of force. This second slab would then impart a force and direction of motion to a third and lower slab until overcome by friction. And so on down through the column of water until the frictional component nullified the horizontal component of the force and there would be no net movement of a deeper slab of water. The result of this dimunition of the initial force as it spirals to the right through the water column is the net movement of the column of water at some angle to the right of the initial force. This result is called Eckman transport.

One of the climatic changes which occurs along the Oregon coast in the late spring and early summer results in a prevailing north or north-westerly wind. This wind force acting on the surface of the coastal water results, through Eckman transport, in an offshore displacement of coastal surface water. As the surface water is displaced

offshore nutrient-rich deeper water is upwelled near the coast. With this input of nutrients to the photic zone an algal bloom results as the phytoplankton optimize on the new conditions of nutrient in the photic zone. As the bloom continues high productivity may influence the penetration of light and may indeed become light limiting. The increase in productivity provides nutritive support for other members of the marine food web. As the water column stabilizes during the late summer months and as the phytoplanktonic community becomes subjected to heavy grazing pressures by members of the zooplanktonic community and nektonic organisms, productivity decreases. In the early fall as weather conditions change once again the stability of the water column is disrupted and there may be a second bloom situation, although generally not as intense as the spring bloom. During the winter months although there is much surface mixing the water column once again becomes stable. The stability of the water column along with the shorter daylight period and the decreased intensity of the winter solar irradiance results in a period of relatively low productivity.

Ryther (1969) has estimated that although the coastal waters and other regions of upwelling account for only 10% of the surface area of the world's oceans, they are responsible for 90% of the total productivity of the world's oceans expressed in terms of grams of carbon fixed per unit of surface area. Table VIII summarizes his estimates.

TABLE VIII. DIVISION OF THE OCEAN INTO PROVINCES ACCORDING TO THEIR LEVEL OF PRIMARY ORGANIC PRODUCTIVITY. (After Ryther, 1969).

Province	% of ocean surface	Mean productivity gm $C/m^2/yr$	Total productivity 10^9 tons C/yr	Fish Production tons (fresh wt)
Open ocean	90	50	16.3	16×10^5
Coastal zone	9.9	100	3.6	12×10^7
Upwelling areas	0.1	300	0.1	12×10^7
Total	100	450	20	24×10^7

MATERIALS AND METHODS

Environmental Simulation in the Laboratory

The environmental conditions necessary for the maintenance of specimens required the construction of an apparatus which would allow for the control of radiant energy input and for the control of water temperature. A circulating water bath system was devised to maintain constant water temperature. The system consisted of a cooling unit, a reservoir, a series of water baths, garden hose, and two circulating pumps. The cooling device was a refrigeration unit similar to those used in soda-pop vending machines. Distilled water was circulated by a model 2E-N submersible pump (Little Giant Pump Company, Oklahoma City, Oklahoma), from the reservoir through the refrigeration unit and back into the reservoir. A second submersible pump circulated the cooled water to the water baths via one-half inch diameter garden hose. The water baths were constructed of fiberglass-coated plywood and were large enough to hold four 5-liter plastic containers, which measured 28 cm long x 18 cm wide x 15 cm deep (Maryland Plastics Company, Scientific Division, New York, New York), without restricting the flow of circulating cooling water. The rate of flow of the cooling water was regulated by an adjustable clamp at the inlet of the water baths. Overflow from the water baths returned via gravity through the garden hose at the outlet of the water baths and back to the reservoir. Coarse control of temperature was obtained by adjusting the thermostatic control on the refrigeration unit. Careful adjustment of the

clamps at the inlet of the water baths permitted a finer control of the temperature.

Radiant energy was supplied by four-foot fluorescent tubes suspended from sawhorses arranged over the water baths. The fluorescent fixtures were suspended on a pulley system which permitted adjustment in the intensity of the radiant energy by raising and lowering the fluorescent tubes. Cool-white fluorescent fixtures (F-40 CW, General Electric Company) supplied the energy required in the wavelength region of approximately 310 nm to 750 nm (Figure 5). The photoperiodicity of the source of radiant energy was controlled by a Model A221-4 Intermatic Appliance Timer (International Register Company, Chicago, Illinois).

Two such systems were constructed each with its own cooling system and source of radiant energy. Each complete system was enclosed within a tent of 4 mil black polyethylene sheeting (Mobil Chemical, Woodland, California). This was done to minimize the introduction of room contaminants into the cultures and to allow for the establishment of photoperiods which would not interfere with laboratory working hours. One of these isolation chambers was used for the exposure of microcosms to cool-white light only. The other chamber was used for the exposure of microcosms to both cool-white light and ultraviolet radiation.

The source of the UV irradiation was 48-inch fluorescent sunlamp tubes (FS-40 T 12, Westinghouse Electric Corporation, Bloomfield, New Jersey) which provided an emission spectrum from approximately 275 nm

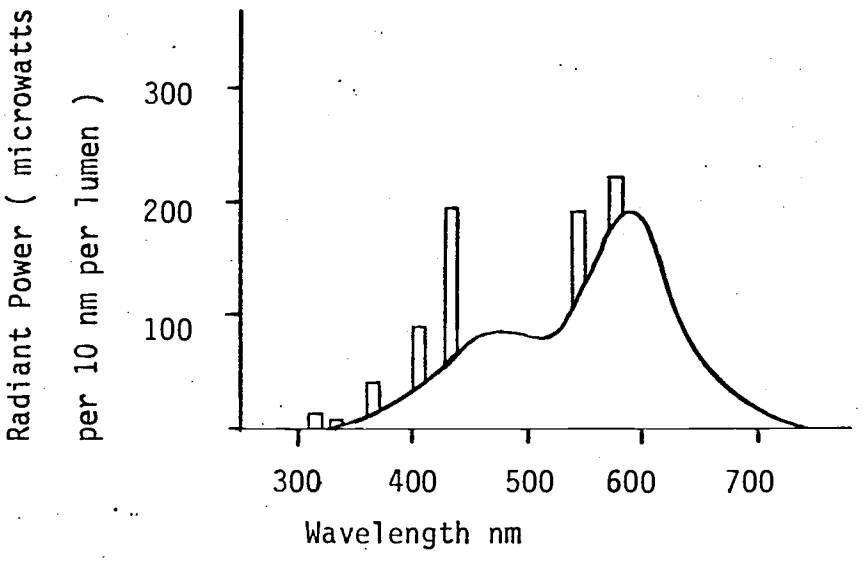

Figure 5. Energy spectrum of F-40 CW fluorescent tubes. (General Electric Data Sheet TP-111, Nela Park, Cleveland, Ohio).

to 380 nm with a peak emission of energy at 310 nm (Figure 6). Since the power output of new sunlamps declined rapidly before attaining a slower rate of change in the output (Figure 7) the tubes were "aged" prior to use in this investigation. Furthermore, a thin sheet of clear cellulose tri-acetate plastic (5 mil Kodacel TA401, Hillcor Plastics, Los Angeles, California) opaque to wavelengths less than 290 nm was used to filter undesired wavelengths. This filter, however, was radio-oxidized by the sunlamps and its transmission spectrum was altered over time. The rate of change of its transmission was rapid in the first ten hours but then the rate of change was much slower (Figure 8). The Kodacel therefore was aged for ten hours prior to use. Since the transmission spectrum of the Kodacel filter continued to be altered by further radio-oxidation over time (Figure 9), each sheet was used only for a designated length of time, i.e., 70 to 80 hours beyond the initial ten-hour aging period.

The FS-40 lamps were suspended along with the F-40 CW lamps above the water baths. The radiant energy supplied by the sunlamps was measured with a Robertson-Berger Sunburning Ultraviolet Meter (supplied by the Department of Transportation) or simply called a UV-B meter. This metering device consists of a sensor and an integrator unit. The components of the sensor include a filter plate coated with a uniform layer of a magnesium tungstate phosphor. On exposure to suitable radiation, i.e., wavelengths less than 330 nm, the phosphor fluoresces. Part of the fluorescence is absorbed by a green filter and part of the emission is transmitted to a photo-vacuum tube located below the green

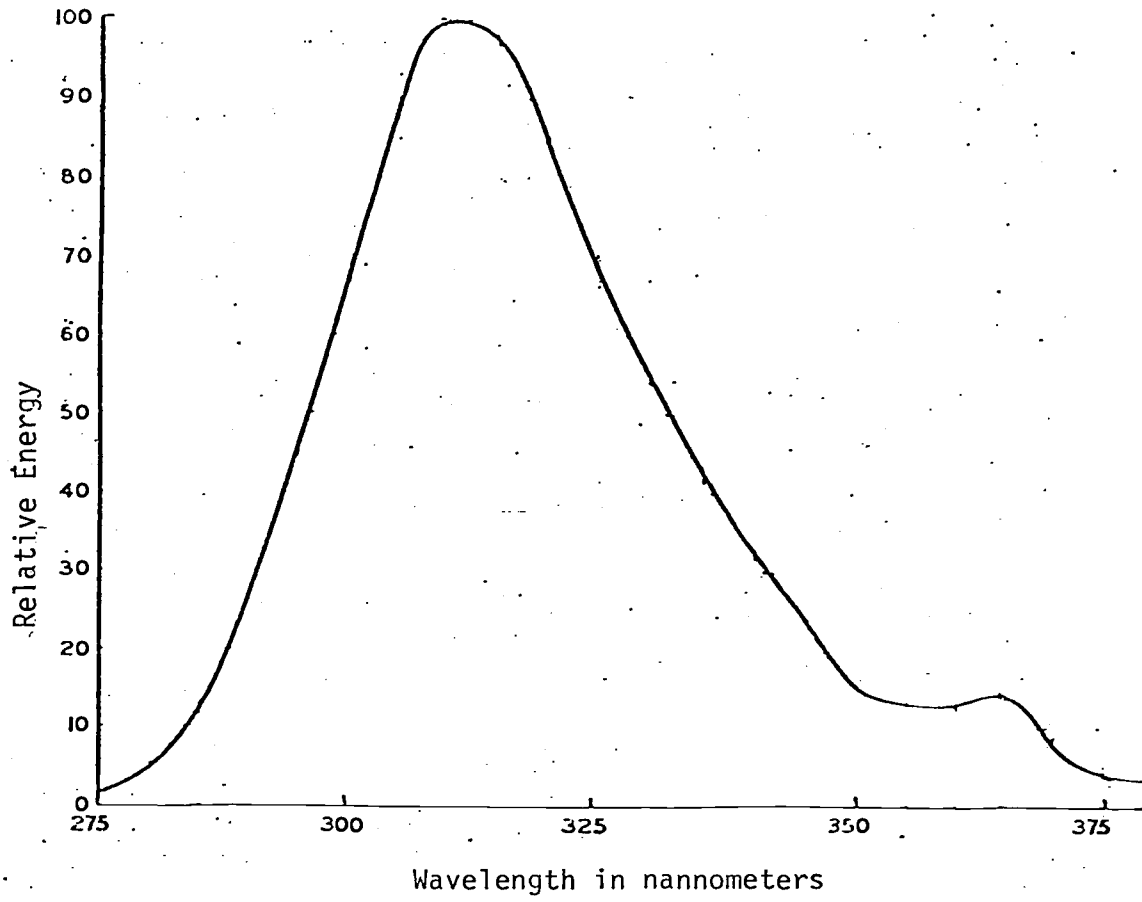

Figure 6. Relative energy distribution of Westinghouse fluorescent sunlamp tubes. (Westinghouse Corp., Data Sheet ASC-504).

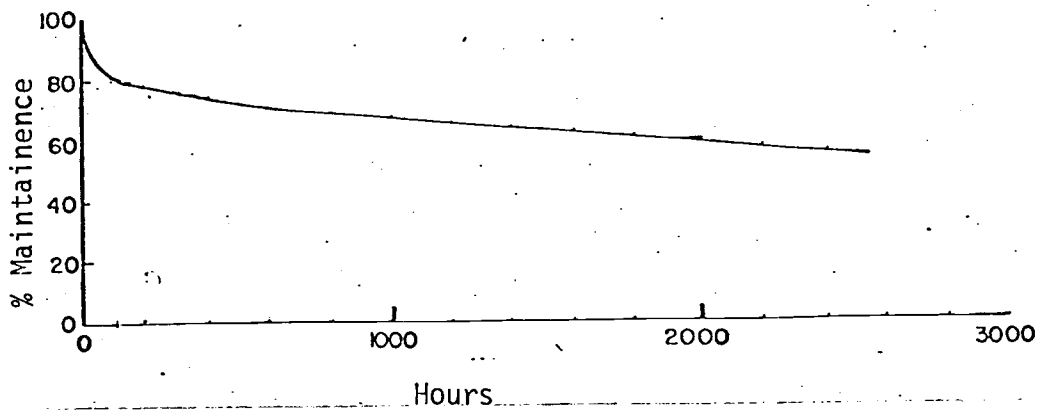

Figure 7. Output throughout life of Westinghouse fluorescent sunlamp tubes. (Westinghouse Corp., Data Sheet ASC-504).

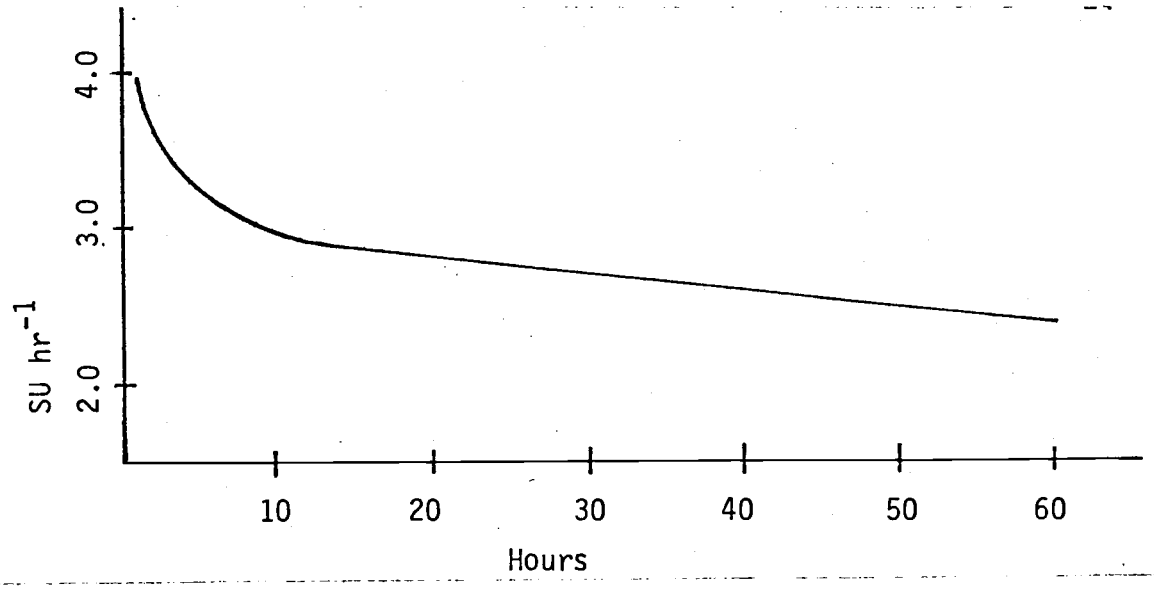

Figure 8. Radio-oxidation of Kodacel by FS-40 sunlamps.

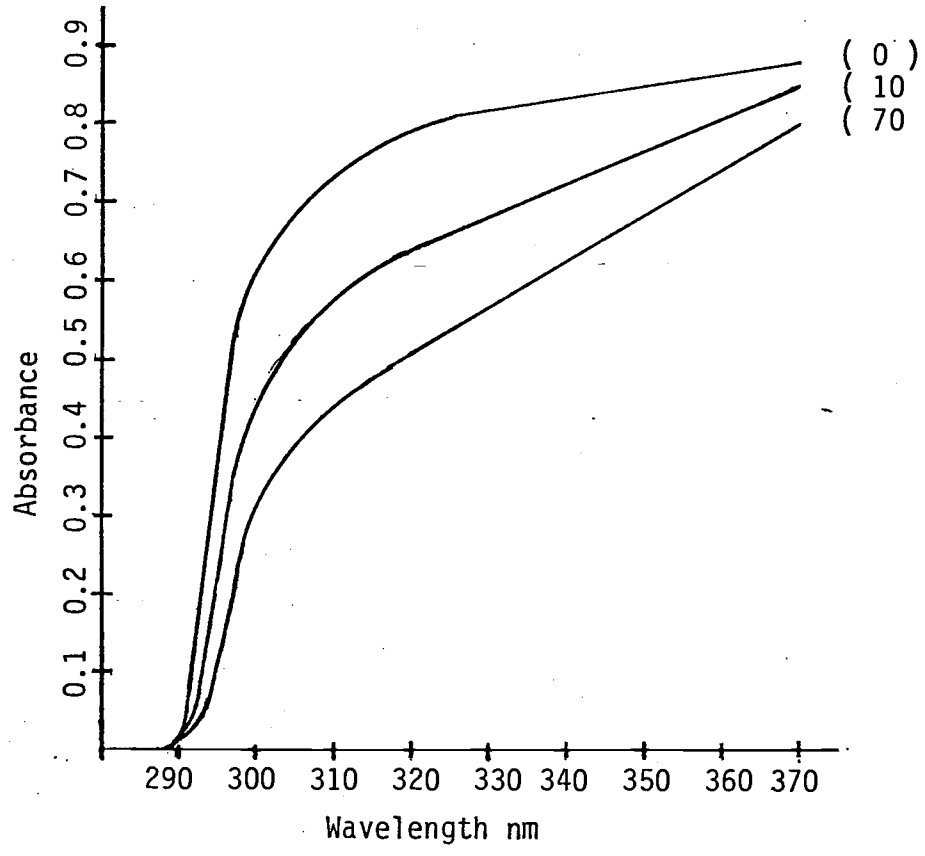

Figure 9. Alteration of transmission spectrum of Kodacel by radio-oxidation by FS-40 sunlamps. ( ) indicates hours of aging.

filter. The photo-current produced is proportional to the combined luminescent effect of all the photons reaching the phosphor. The phosphor is protected by 3 mm of ultraviolet transmitting, but visible light absorbing, "black" glass. Any UV irradiation that passes the black filter will be absorbed by the green filter. Without the magnesium tungstate phosphor there is no photo-tube response. Since there is no terrestrial solar radiation less than 290 nm the sensor response is limited to 290 to 330 nm wavelengths. A schematic representation of the components can be found in Figure 10.

The sensor response is integrated by a separate unit which is calibrated to convert the photo-current to "clicks" such that an accumulation of 400 clicks is equal to one sunburn unit. A sunburn unit of polychromatic radiation will produce the same erythemal effect as 200 J m^{-2} of 296.5 nm UV. Stated more generally one sunburn unit is the amount of ultraviolet radiation which will produce a minimal erythema in untanned skin. It is, therefore, a measure of relative erythemal effectiveness.

An ammeter may also be used in conjunction with the sensor unit to measure the photo-current directly and thereby give an instantaneous rate of exposure.

Collection and Maintenance of Specimens

Prior to the collection of samples a number of procedures were performed to assure optimum conditions for the maintenance of the estuarine micro-ecosystems. The plastic rectangular containers were

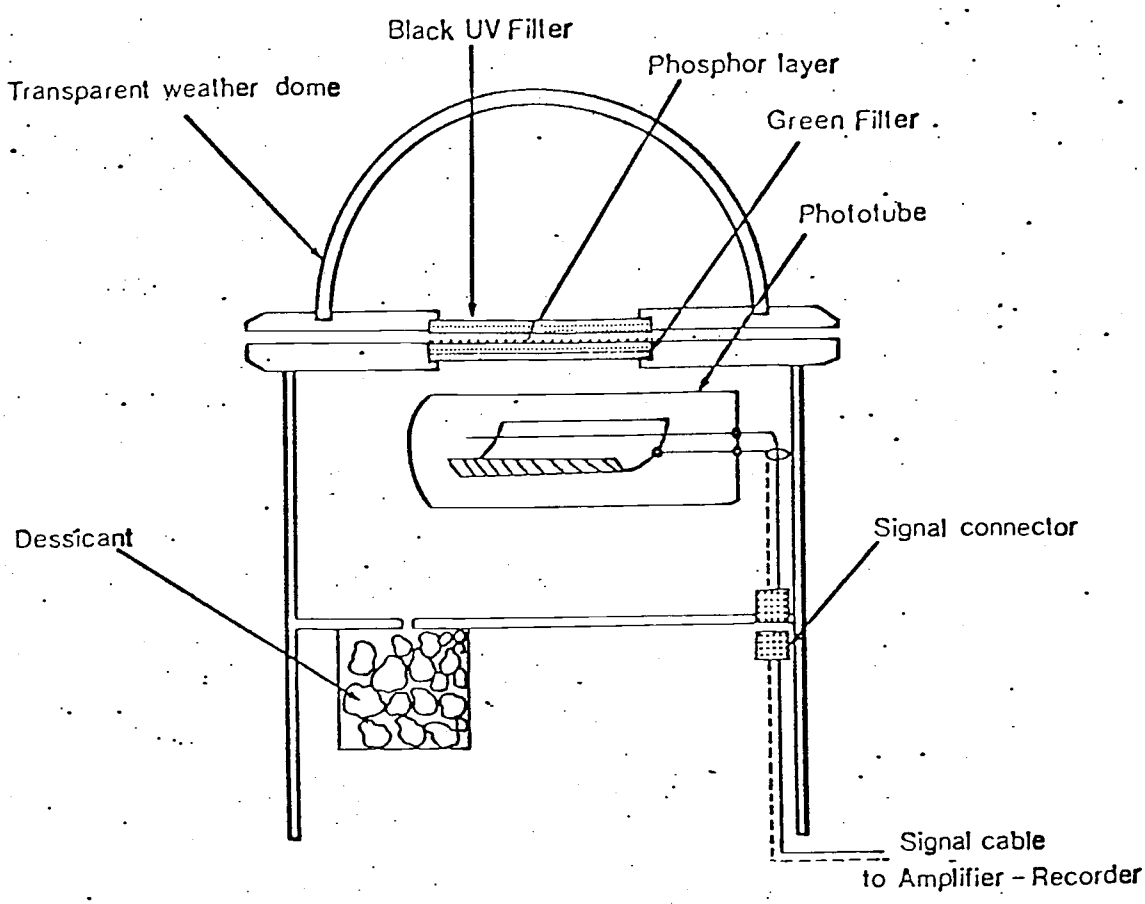

Figure 10. Schematic representation of the Robertson-Berger Sunburning Ultraviolet Meter. (Berger, et al., (1975).

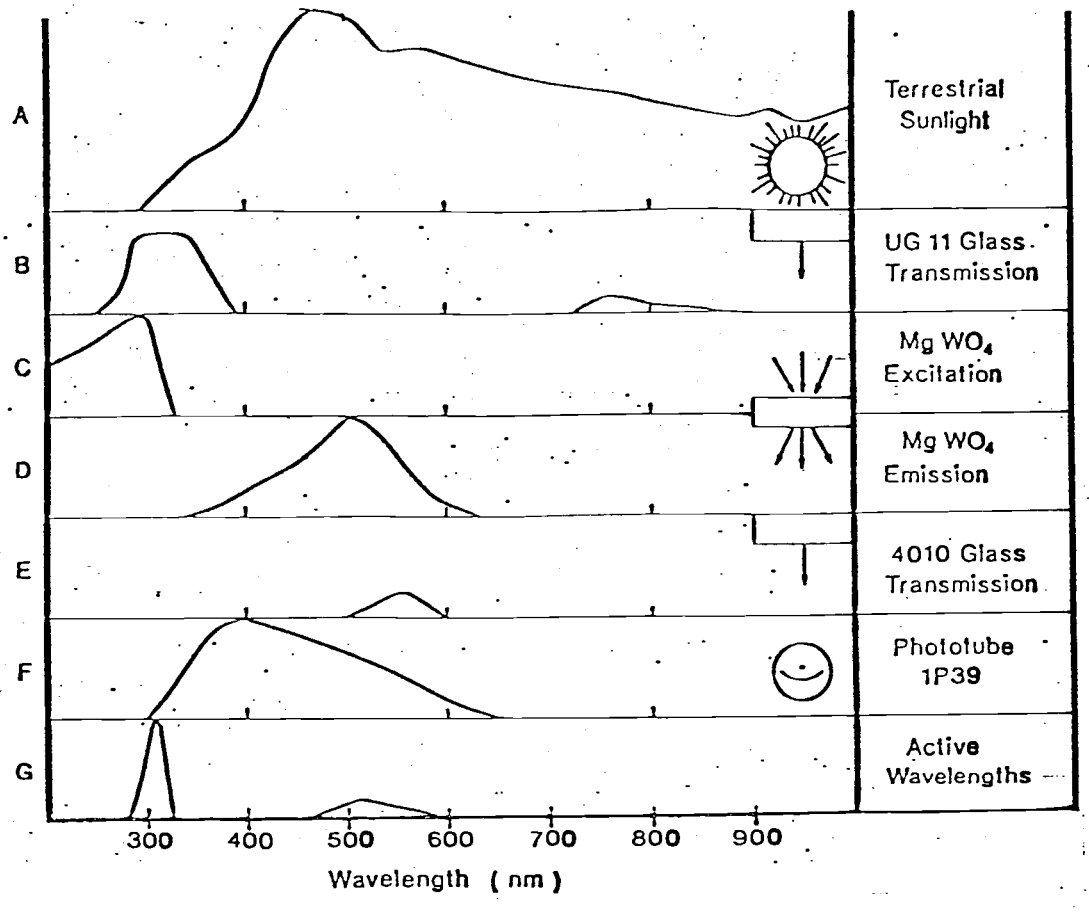

Figure 11. Principle of operation of the UV-B Meter. Panel G shows the spectral sensitivity of the sensor around 300 nm. Also shown is the shape and size of the portion of the spectrum around 510 nm which provides the final output signal. The spectral irradiance around 300 nm from the sun is instrumentally translated to a peak around 510 nm via the filter, phosphor, and photo-tube. (Berger, et al., 1975).

pre-sterilized by rinsing with 95% ethanol and then were placed under the FS-40 lamps for four hours. The containers were then sealed with autoclaved aluminum foil to minimize the entry of contaminants. Wide-mouthed polypropylene bottles of one-gallon capacity were also pre-sterilized with a rinse of 95% ethanol and were autoclaved at 15 psi pressure for 20 minutes. A supply of Instant Ocean Synthetic Sea Salts (Eastlake, Ohio) was hydrated, filtered, and autoclaved. A nutrient enrichment consisting of a 3% solution of Na_2SiO_3 in artificial seawater and 100 milliliters of Alga-Gro Concentrate (Powell Laboratories, Carolina Biological Supply, Gladstone, Oregon) was prepared and autoclaved.

Samples were collected in the sterile one-gallon polypropylene bottles from the north side of the south jetty near the mouth of the Yaquina Bay estuary at Newport, Oregon. The samples were obtained by dipping the bottles below the water surface at high tide or flood tide. Collecting samples on an incoming tide minimized the chance of collecting great amounts of terrestrial debris that may have entered the esutary at some location upstream. Also it was felt that mixing of the water column by the incoming tide would maximize homogeneity of the water column and so provide a representative sampling of the intertidal, benthic, and planktonic biological populations. The temperature of the water was determined at the time of collection by dipping a thermometer below the water surface. The filled collection bottles were kept cold in an ice chest for transportation to the laboratory in Corvallis.

In the laboratory the samples were well mixed by inverting the bottles several times. Portions from several collection bottles were dispensed into a series of sterile plastic rectangular containers so that each container received water from a number of the collection bottles. Approximately 2.5 liters of estuarine water was added to each container in this way. One hundred milliliters of the nutrient enrichment was added to each plastic rectangular container and the volume was made up to approximately 5 liters with sterile artificial seawater. This volume of water provided for a surface area to volume ratio of 0.1. The rectangular containers with the nutrient enrichment, the artificial seawater, and the estuarine water containing organisms from the estuary, having been established in this manner, were considered to be micro-ecosystems or microcosms. These microcosms were then placed into the circulating water baths which had been adjusted to the temperature of the estuarine water at the time of collection. Since the water of the Yaquina Bay estuary characteristically ranged between 9° C and 11° C the water baths were adjusted to maintain a temperature which averaged 10.5° C ± 1° C. The photoperiodicity of the fluorescent tubes was set to approximate the photoperiod existing at the time of collection of the samples. This was 14 hours of light and 10 hours of dark every 24-hour period. The height of the fluorescent tubes above the microcosms was adjusted to deliver 8000 lux (Weston Model 703 Foot Candle Meter) or 0.75 milliwats/cm^2 (YSI-Kettering Model 65 Radiometer with a YSI Model 6551 probe fitted with a YSI Model 6019 Cone Collector) to the surface of the microcosms.

The microcosms were maintained under these conditions and daily cross-inoculations were performed to minimize heterogeneity. During this "acclimation period" the concentration of the oxygen dissolved in the microcosms was determined routinely (YSI Model 54 Oxygen Meter with a YSI Model 5429 self-stirring probe). As the autotrophic populations developed the dissolved oxygen in the water in the microcosms exceeded saturation. The macroscopic appearance of the microcosms changed as the water column became more turbid and as the pigmented particles became more obvious. These macroscopic observations and the determinations of the dissolved oxygen concentration suggested that the microcosms were responding to the nutrient enrichment and the radiant energy, the result being manifest in an increased productivity. Variations in the amount of pigmented material in the water column, in the predominant color and morphology of the pigmented particulates, and oscillations in the dissolved oxygen values suggested that the microcosms were maintaining themselves and could indeed be considered micro-ecosystems.

At a time during the acclimation period, not less than seven days but not greater than nine days, exposure to UV radiation was initiated. At this point in the investigation the microcosms were divided into two groups. The group of containers remaining under the cool-white tubes only are referred to as the UV-deficient group. The others, which were exposed to ultraviolet radiation in addition to cool-white light, are referred to as the UV-enriched group. The UV enrichment was implemented at three levels, 1.7 sunburn units per hour

(SU hr^{-1}), 2.0 SU hr^{-1}, and 2.5 SU hr^{-1}. The total energy received at the surface of the UV-enriched microcosms when both the F-40 CW and the FS-40 tubes were on was adjusted to the energy received at the surface of the UV-deficient group of microcosms. The FS-40 tubes were set for a ten-hour "on" and a 14-hour "off" period which began two hours after the initiation of the cool-white photoperiod.

Cross-culturing within each of the two groups of microcosms was continued as well as routine determination of the dissolved oxygen concentration in the microcosm water.

Methods for the Assessment of the Heterotrophic Populations

The development of the micro-ecosystem was followed as the two groups of microcosms responded to the laboratory simulated environmental conditions of radiant energy, temperature, and nutrient enrichment. A modification of the light bottle-dark bottle method of determining biological oxygen demand was developed to give an overall picture of the community's metabolism. While the productivity of the system was monitored through changes in the dissolved oxygen concentrations, a more detailed evaluation of the heterotrophic populations of the community was obtained through enumeration studies of the total number of heterotrophic organisms, the total number of selected metabolic types of heterotrophic organisms, and the potential activity of the heterotrophic portion of the community.

The sampling procedure followed in this investigation may be described in the following manner (Appendix B for format). Each

microcosm to be sampled was mixed well using a sterile 30 ml glass syringe by aspirating and dispensing a syringe full of the culture several times below the surface of the microcosms so as not to forcibly introduce air into the systems. Then a 60 ml sample was withdrawn, using the syringe, from each of two microcosms in the two separate groups of microcosms. The samples from the UV-enriched systems were pooled to give a total volume of 120 ml and the samples from the UV-deficient systems were pooled to give a second 120 ml volume. Each of these two pooled samples, one for the UV-enriched microcosms and one for the UV-deficient microcosms, were mixed well by gently swirling. Portions from the two pooled samples were aseptically transferred to the first tube in a series of tubes containing sterile artificial seawater. Serial dilution of these portions allowed for the determination of total numbers of heterotrophic organisms and the total number of selected metabolic types. Portions for the assessment of heterotrophic potential were taken from the undiluted pooled samples. This sampling procedure was implemented at the following times: prior to the initiation of exposure to UV radiation; during the four to five weeks of exposure; and following the cessation of UV irradiation.

Dilution plate counts were done in duplicate using seawater agar, freshwater agar, and starch agar (Appendix A for formulations). The plates were incubated in the dark at in situ temperature and were examined for growth of colonies at one, two, three, and four weeks of incubation. Growth on the seawater agar and freshwater agar plates

provided the following information: the total number of seawater organisms per unit volume, the proportion of non-seawater-requiring organisms, the proportion of pigmented organisms, and the proportion of agar-utilizing organisms. The starch plates were flooded with Gram's iodine after ten days of incubation to determine the proportion of starch utilizers to the total number of seawater heterotrophs.

Assessment of other metabolic types followed the three tube Most Probable Number (MPN) dilution method as presented in Standard Methods for the Examination of Water and Wastewater (1960). Cellulose degradation and gelatin liquefication (Appendix A for formulation) were the two metabolic types examined using the MPN method. MPN determinations, in duplicate, were incubated in the dark at in situ temperature. The cellulose degradation tubes were examined after four weeks of incubation and the gelatin liquefication tubes were read after ten days of incubation.

The ability of heterotrophic organisms to metabolize a uniformly carbon-14 labelled organic compound provided a means for assessing the heterotrophic activity of the microcosms. This radioassay procedure was similar to that used by Harrison, et al. (1971) and may be briefly outlined here. Ten ml portions from the pooled samples were inoculated into sterile 50 ml serum bottles containing a known amount of uniformly labelled ^{14}C-glutamate. The amount of isotope used was dependent on the number of organisms present and was determined before beginning the radioassay. For this investigation 0.1 microcuries to 0.25 microcuries of the substrate was found to yield adequate results,

i.e., a rate of disintegrations which would be statistically significant when counted for a short period of time. Immediately after the introduction of the inoculum into the serum bottles a rubber sleeve-type serum bottle stopper with a needle septum was placed over the mouth of the serum bottle. The needle septum was pierced by a small plastic rod and cup assembly (Kontes Glass Company, Vineland, New Jersey, K-882320). The cup, which was suspended over the isotope-inoculum mixture, contained an accordian-folded piece of filter paper (Whatman No. 1, 2.5 x 5.0 cm).

Three such systems, i.e., three for each pooled sample, were inoculated at each sampling time. To one of the serum bottles was added 0.15 ml of 0.1 N H_2SO_4 prior to the addition of the sample in order to inactivate the micro-organisms in the inoculum. This bottle served as a blank while the other two bottles served as duplicates. Utilization of the isotope by the inoculum was allowed to proceed for two hours in the dark at in situ temperature. This length of incubation time was adequate to allow for sufficient utilization of the label without promoting an anaerobic environment within the serum bottle. At the end of the two-hour incubation period the reaction was stopped with the addition of 0.15 ml of 0.1 N H_2SO_4 injected through the needle septum. This acidification of the reaction mixture reduced the pH to about 2.2 and served to stop the reaction and release the $^{14}CO_2$ dissolved in the reaction mixture. To facilitate the absorption of $^{14}CO_2$ 0.15 ml of B-phenylethylamine (Sigma Chemical Company, St. Louis, Missouri, P-6251) was injected through the serum

bottle cap onto the folded filter paper in the cup. The reaction flasks were then swirled gently on a laboratory rotator for one-half hour to allow for the complete absorption of the $^{14}CO_2$. At the end of the shaking period the bottles were opened and the filter paper was removed and immediately placed in a scintillation vial containing 10 ml of scintillation fluor (0.001% 1,4 bis-2-4 methyl-5-phenyloxazolyl benzene and 0.04% 2,5 diphenyl-oxazole). The $^{14}CO_2$ activity was counted within one-half hour.

The reaction mixture in the serum bottles was filtered through 0.45 micron membrane filters (HAWP 02500, Millipore Corp., Bedford, Mass.) to trap organisms present in the reaction mixture. The reaction vessels were rinsed with three successive 10 ml portions of seawater and each rinse was filtered through the membrane filter. The filters were dried at 70°C for 15 minutes and then each was placed in a scintillation vial containing 10 ml of fluor.

Radioactivity of the filter paper and the membrane filters was determined using a dual channel liquid scintillation spectrometer (Packard Tricarb Model 3002). Initially quenching was corrected using the internal standardization method. While the internal standard method has its advantages it is not the method of choice when handling a large number of samples. Subsequently quenching was corrected using the channel ratio method. The procedure used to determine the optimal gain and window settings for ^{14}C isotope and to determine the relationship between channel ratio and counting efficiency followed that of Wang and Willis (1965).

A computer program for the Olivetti Programa Model 101 was written (Appendix C for format) which would calculate the channel ratio, counting efficiency, and corrected counts of a sample. The program was expanded to handle data from the radioassay procedure described above for the assessment of heterotrophic activity. By entering the counts of channel 1 and of channel 2 of the scintillation vial containing the folded filter paper and then by entering the counts in channel 1 and in channel 2 of the scintillation vial containing the membrane filter the following information was obtained: the channel ratio, counting efficiency, and corrected counts for each of the samples; the total activity of the $^{14}CO_2$ and the total ^{14}C activity of the cells on the membrane filter providing a measure of the total activity handled by the inoculum; and the proportion of the total activity respired as $^{14}CO_2$.

These methods, i.e., enumeration of the total number of heterotrophic organisms, the total number of selected metabolic types of heterotrophs, and the ability of these organisms to metabolize a labelled organic compound, provided a means for assessing the heterotrophic activities in the UV-enriched and the UV-deficient microcosms. While these methods provided an assessment of the overall physiological condition of the heterotrophic portion of the micro-ecosystems, they did not allow for the determination of specific mechanisms involved in the response of heterotrophic micro-organisms to the UV irradiation.

RESULTS

Unless otherwise specified all results reported in this section were obtained from samples withdrawn from the microcosms at two hours after the termination of the cool-white light period or four hours following the termination of the UV-irradiation period in the UV-enriched microcosms. The results reported at 2.5 SU hr^{-1} and 1.7 SU hr^{-1} pertain to the first of two experimental series. The first series of experiments were carried out during the months of July and August of 1974. A subsequent series was performed during October through November of 1974 and utilized one level of UV-irradiation, namely 2.0 SU hr^{-1}.

The initiation of UV irradiation began on day 7 for the summer series and on day 8 for the fall series. The first samples for the assessment of the heterotrophic populations were withdrawn from the microcosms immediately prior to the initiation of UV irradiation. Termination of exposure to UV irradiation was on day 28 for the summer series and on day 36 for the fall series. Exposure to cool-white radiation continued until day 36 for the summer series and until day 47 for the fall series.

Dilution Plate Counts

The total number of heterotrophic seawater organisms exhibited a 76% decrease in the first two periods of exposure to 2.5 SU hr^{-1} Figure 12.A) and a 51% decrease for a similar length of exposure at 2.0 SU hr^{-1} (Figure 12.B). The total number of seawater heterotrophs

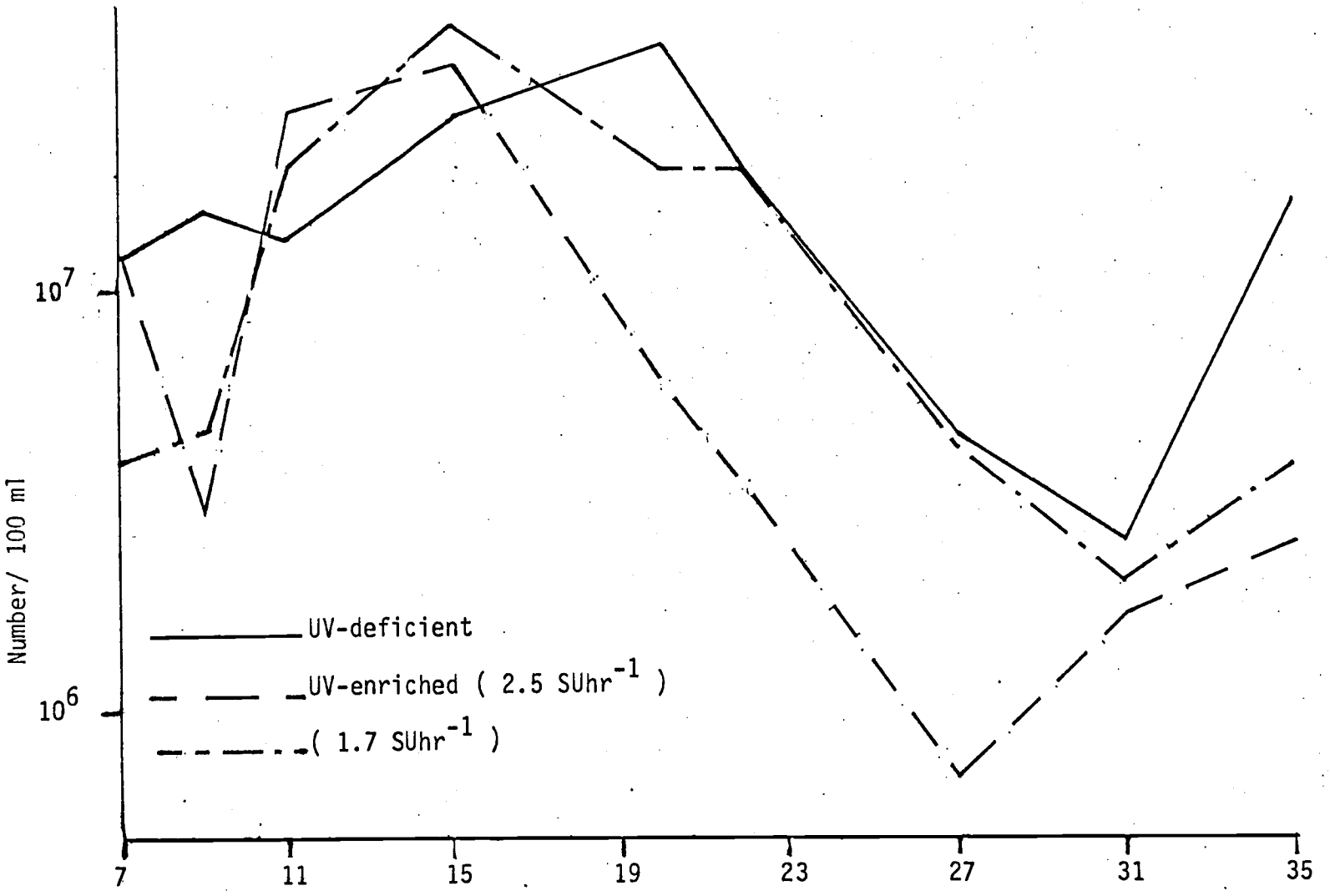

Figure 12.A. Total number of seawater heterotrophic organisms.

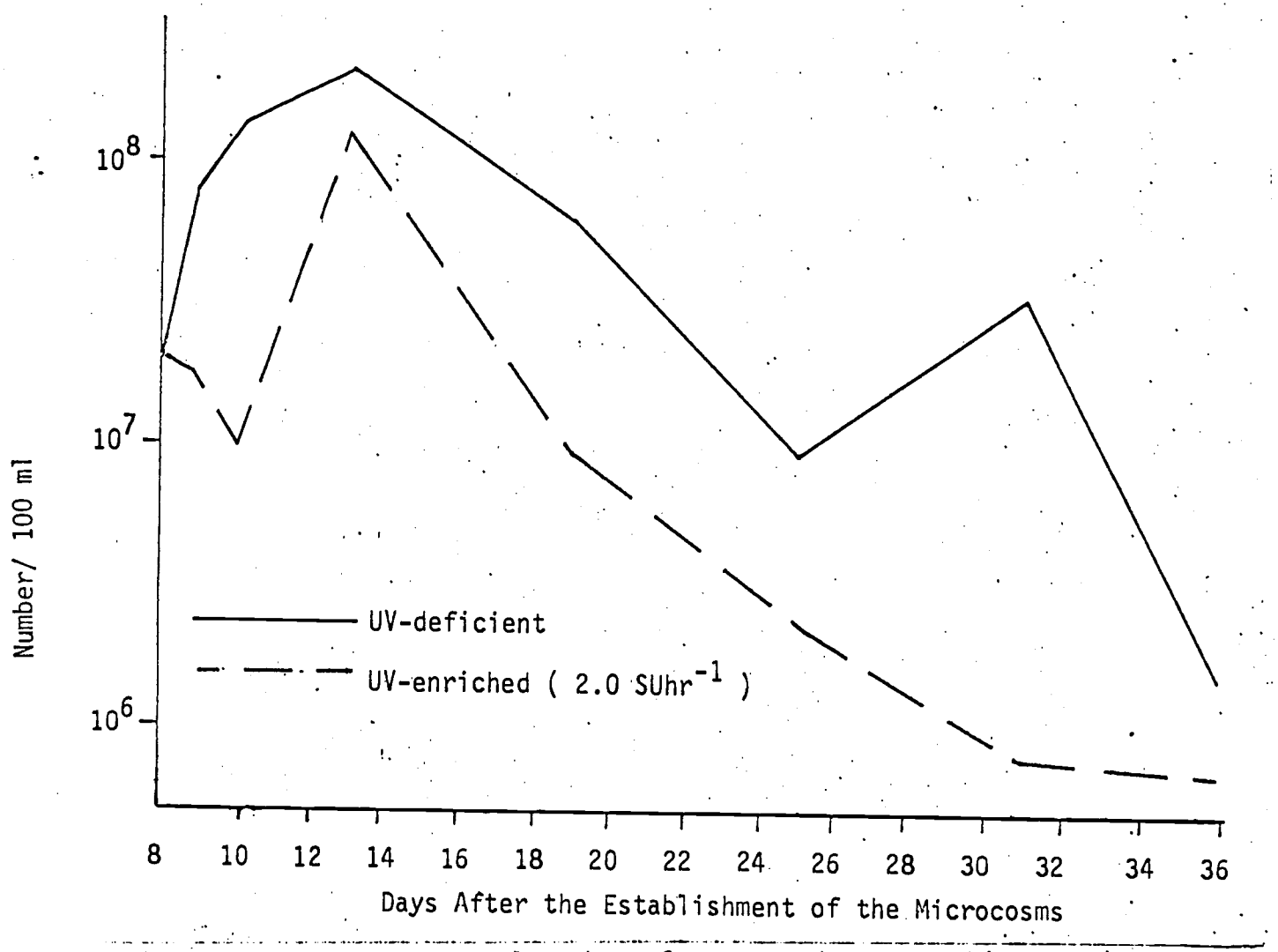

Figure 12.B. Average total number of seawater heterotrophic organisms.

increased by 18% in the microcosms exposed to 1.7 SU hr^{-1} (Figure 12.A) and increased in all UV-deficient microcosms (Figures 12.A and 12.B) in the first two periods after the initiation of UV irradiation. By 14 days following the commencement of UV irradiation the total number of seawater heterotrophs in the UV-enriched systems differed from the UV-deficient systems in the following ways: was 83% less in the microcosms exposed to 2.5 SU hr^{-1} (Figure 12.A), was 82% less in the microcosms exposed to 2.0 SU hr^{-1} (Figure 12.B), and was 26% less in the microcosms exposed to 1.7 SU hr^{-1} (Figure 12.A). After about three weeks of UV irradiation the total number of seawater heterotrophs differed as follows: 77% less in the microcosms exposed to 2.5 SU hr^{-1} than in the UV-deficient systems (Figure 12.A), 95% less in the microcosms exposed to 2.0 SU hr^{-1} than the UV-deficient microcosms (Figure 12.B), and 10% lower in the microcosms exposed to 1.7 SU hr^{-1} than in the UV-deficient systems (Figure 12.A). By 28 days of exposure to 2.5 SU hr^{-1} the total number of seawater heterotrophs was 76% less than in the UV-deficient microcosms. For the same exposure time at 2.0 SU hr^{-1} the total number of heterotrophs was 55% less than in the UV-deficient systems. Similarly, the total number of seawater heterotrophs was 69% less in the microcosms exposed to 1.7 SU hr^{-1} than in the UV-deficient microcosms.

The total number of non-seawater-requiring heterotrophic organisms exhibited oscillations with a general decrease in numbers throughout the duration of the experiment (Table X). There was no apparent UV-dose-related response in the survival of non-seawater-

TABLE IX.A. TOTAL NUMBER OF SEAWATER HETEROTROPHIC ORGANISMS/100 ml OF WATER SAMPLE.

Microcosm Group level of UV-irradiation	Days After the Establishment of the Microcosms								
	7	9	12	15	20	22	27	31	35
UV- deficient	6×10^6- 1.8×10^7	1.3×10^7- 1.8×10^7	1.2×10^7- 1.5×10^7	2.1×10^7- 3.1×10^7	3.8×10^7- 4×10^7	1.8×10^7- 2.1×10^7	3.1×10^6- 6×10^6	1.5×10^6- 3.5×10^6	6×10^6- 2.9×10^7
UV-enriched 2.5 $SUhr^{-1}$	1.1×10^7- 1.4×10^7	2.9×10^6- 3.2×10^6	2.7×10^7- 2.8×10^7	3.1×10^7- 4×10^7	4.7×10^6- 7.5×10^6	3×10^6- 3.9×10^6	6.5×10^5- 7×10^5	1.5×10^6- 2×10^6	1.5×10^6- 3.5×10^6
1.7 $SUhr^{-1}$	3.8×10^6- 4×10^6	3.2×10^6- 6×10^6	1.4×10^7- 2.6×10^7	3.6×10^7- 5×10^7	1.8×10^7- 2.1×10^7	1.7×10^7- 2.2×10^7	4×10^6- 4.5×10^6	2.5×10^6- 3.5×10^6	3×10^6- 4.7×10^6

Note 1. Hyphenated numbers indicate total count for each duplicate.

TABLE IX.B. TOTAL NUMBER OF SEAWATER HETEROTROPHIC ORGANISMS/100 ml OF WATER SAMPLES

Microcosm Group level of UV-irradiation	Days After the Establishment of the Microcosms								
	8	9	10	13	19	25	31	36	42
UV-deficient	1.5×10^7- 2.4×10^7	7×10^7- 9×10^7	1.1×10^8- 1.5×10^8	6×10^7- 3.6×10^8	5.1×10^7- 6.5×10^7	7.5×10^6- 1.4×10^7	2.9×10^7- 4×10^7	1×10^6- 2.1×10^6	1.3×10^6- 1.7×10^6
UV-enriched 2.0 $SUhr^{-1}$	1.8×10^7- 2.3×10^7	1.5×10^7- 2.1×10^7	9.4×10^6- 1.1×10^7	9.6×10^7- 1.5×10^8	7×10^6- 1.2×10^7	1.9×10^6- 2.9×10^6	8×10^5	7×10^5	2.8×10^6- 5×10^6

Note 1. Hyphenated numbers indicate total count for each duplicate.

TABLE X. TOTAL NUMBER OF NON-SEAWATER-REQUIRING HETEROTROPHIC ORGANISMS/100 ml WATER SAMPLE

Microcosm Group level of UV irradiation	Days after the establishment of the microcosms					
	7	9	12	15	20	27
UV-deficient	9.5×10^5 - 1.5×10^6	5×10^5 - 2×10^6	1×10^5 - 1.5×10^5	1×10^5 - 1.5×10^5	1.4×10^5 - 2.5×10^5	1×10^5 - 1.5×10^5
UV-enriched 2.5 SU hr^{-1}	1×10^6 - 2×10^6	2.3×10^5 - 5×10^5	9×10^5 - 1.5×10^6	3.5×10^5 - 5×10^5	1.5×10^5 - 2×10^5	8×10^4 - 1.2×10^5
1.7 SU hr^{-1}	5×10^5 - 6×10^5	8.5×10^5 - 5×10^6	*	4.5×10^5 - 5×10^5	1×10^5 - 6.5×10^5	5×10^4 - 1×10^5

*Indicates lost data point due to contaminated plate.

Note 1. Hyphenated numbers indicate total count for each duplicate.

requiring heterotrophs. Throughout the experiment the ratio of non-seawater-requiring organisms to the total number of seawater heterotrophs was generally less than 0.1.

The proportion of agar-utilizing heterotrophs to the total number of heterotrophic organisms (Tables XI.A and XI.B), and the proportion of starch-utilizing heterotrophs to the total number of seawater heterotrophs (Table XII) did not suggest a UV-related response.

The ratio of pigmented colonies to non-pigmented colonies on seawater agar exhibited the following changes in the first two exposure periods following the initiation of UV-irradiation: increased from 0.08 to 0.1 in the UV-deficient microcosms during the summer series of experiments and decreased from 0.1 to 0.02 in the UV-deficient microcosms during the fall series, increased from 0.04 to 0.63 in the microcosms exposed to 2.5 SU hr^{-1}, increased from 0.05 to 0.16 in the microcosms exposed to 2.0 SU hr^{-1}, and increased from 0.02 to 0.38 in the microcosms exposed to 1.7 SU hr^{-1} (Tables XIII.A and XIII.B). The ratio of pigmented colonies to non-pigmented colonies decreased in the UV-deficient microcosms and tended to increase and remain greater in the microcosms exposed to UV radiation throughout the duration of the experiments.

MPN Tube Dilutions

There was no apparent UV-dose-related response in the survival of gelatin-liquefying heterotrophic organisms (Table XIV).

TABLE XI.A. TOTAL NUMBER OF AGAR-UTILIZING SEAWATER HETEROTROPHS/100 ml OF WATER SAMPLE

Microcosm Group level of UV irradiation	Days after the establishment of microcosms				
	7	9	12	15	20
UV-deficient	1×10^5 - 3×10^5	2×10^5	2×10^4 - 6×10^5	1×10^5 - 2×10^5	3×10^6 - 4×10^6
UV-enriched 2.5 SU hr^{-1}	4×10^4 - 1×10^5	3×10^3 - 4×10^3	1×10^5 - 2×10^5	1×10^4 - 4×10^4	4×10^5 - 6×10^5
1.7 SU hr^{-1}	2×10^4	1×10^3 - 3×10^3	2×10^5 - 3×10^5	1×10^4	1×10^6 - 1.1×10^6

Note 1. Hyphenated numbers indicate total count for each duplicate.

TABLE XI.B. TOTAL NUMBER OF AGAR-UTILIZING HETEROTROPHS/100 ml OF WATER SAMPLE

Microcosm Group level of UV irradiation	Days after the establishment of microcosms				
	8	9	10	13	19
UV-deficient	4×10^5 - 9×10^5	9×10^5 - 3×10^6	2×10^6	5×10^5 - 5×10^6	3×10^5 - 2.5×10^6
UV-enriched 2.0 SU hr [1]	5×10^5 - 7.5×10^5	5×10^5 - 6×10^5	5×10^5	7.5×10^6 - 1.5×10^7	1×10^3

Note 1. Hyphenated numbers indicate total count for each duplicate.

TABLE XII. TOTAL NUMBER OF STARCH-UTILIZING HETEROTROPHIC ORGANISMS/100 ml OF WATER SAMPLE

Microcosm Group level of UV irradiation	Days after the establishment of the Microcosms					
	7	9	12	15	20	27
UV-deficient	3×10^6	3×10^6 - 1×10^7	1×10^7 - 1.3×10^7	2×10^6 - 3×10^6	1.6×10^7 - 2.2×10^7	1.3×10^5 - 3×10^5
UV-enriched 2.5 SU hr^{-1}	8×10^6 - 9×10^6	7×10^5 - 8×10^5	1.2×10^7 - 1.5×10^7	2×10^6 - 4×10^6	7×10^5	1.1×10^4 - 1×10^5
1.7 SU hr^{-1}	2×10^6 - 3×10^6	6×10^5 - 9×10^5	9×10^5 - 4×10^6	4×10^6 - 5×10^6	2.5×10^6 - 3×10^6	1×10^6

Note 1. Hyphenated numbers indicate total count for each duplicate.

TABLE XIII.A. TOTAL NUMBER OF PIGMENTED COLONIES ON SEAWATER AGAR/100 ml OF WATER SAMPLE

Microcosm Group level of UV irradiation	Days after the establishment of microcosms				
	7	9	15	22	27
UV-deficient	1 x 10^6	1.2 x 10^6 - 2 x 10^6	1.5 x 10^6	2.5 x 10^6 - 2 x 10^6	3 x 10^4 - 1 x 10^5
UV-enriched 2.5 SU hr^{-1}	5 x 10^5	1.7 x 10^6 - 1.9 x 10^6	2 x 10^6 - 5 x 10^6	5 x 10^5 - 9 x 10^5	1.4 x 10^5 - 2.5 x 10^5
1.7 SU hr^{-1}	1 x 10^5 - 4 x 10^6	1.5 x 10^6 - 2 x 10^6	1.6 x 10^6 - 3 x 10^6	2 x 10^6	9 x 10^5 - 1.5 x 10^6

TABLE XIII.B. TOTAL NUMBER OF PIGMENTED COLONIES ON SEAWATER AGAR/100 ml OF WATER SAMPLE

Micorcosm Group level of UV irradiation	Days after the establishment of microcosms				
	8	9	10	13	19
UV-deficient	9.5 x 10^5 - 3 x 10^6	2.5 x 10^5 - 3.5 x 10^6	5 x 10^5 - 1 x 10^7	5 x 10^5 - 2 x 10^6	3 x 10^5 - 1.5 x 10^6
UV-enriched 2.0 SU hr^{-1}	2 x 10^5 - 2 x 10^6	1.5 x 10^5 - 2 x 10^6	1.5 x 10^6 - 1.8 x 10^6	4 x 10^6 - 1 x 10^7	1 x 10^6 - 1.6 x 10^6

Note 1. Hyphenated numbers indicate total count for each duplicate.

TABLE XIV. NUMBER OF GELATIN-LIQUEFYING SEAWATER HETEROTROPHIC ORGANISMS/100 ml OF WATER SAMPLE

Days after establishment of microcosms	Microcosm Group, level of UV irradiation			
	UV-deficient		UV-enriched, 2.0 SU hr^{-1}	
	MPN index	95% C.I.	MPN index	95% C.I.
8	1.5×10^6 - 2.4×10^7	3×10^4 - 4.4×10^6 *	1.5×10^6 - 2.4×10^6	3×10^4 - 4.4×10^6 3×10^4 - 1.3×10^7
9	1.6×10^6 - 2.4×10^7	* *	1.5×10^6 - 2.4×10^6	3×10^4 - 4.4×10^6 3.6×10^4 - 1.3×10^7
13	2.4×10^7	*	2.4×10^7	*
19	1.1×10^7	1.5×10^6 - 4.8×10^7	7.5×10^4 - 4.6×10^5	1.4×10^4 - 2.3×10^5 7.1×10^4 - 2.4×10^6
24	9×10^5	1.5×10^5 - 3.6×10^6	3×10^5	*

*95% confidence interval not established.

Note 1. Hyphented numbers indicate total count for each duplicate.

Note 2. Confidence intervals from MPN tables.

The number of cellulose-utilizing heterotrophs did appear to respond to UV irradiation. The changes in the number of cellulose-degrading heterotrophs, following five days of UV-irradiation were: a 96% decrease from pre-exposure numbers in the microcosms exposed to 2.5 SU hr^{-1} (Table XV.A), a 43% decrease from pre-exposure numbers in the microcosms exposed to 1.7 SU hr^{-1} (Table XV.A). There was no change observed in the number of cellulose-utilizers in the UV-deficient microcosms during this same period. Whereas pre-exposure numbers of cellulose-utilizing heterotrophs were the same for all three groups of microcosms, i.e., 2.5 SU hr^{-1}, 1.7 SU hr^{-1}, and UV-deficient, after five periods of exposure to UV-radiation, the numbers in the 2.5 SU hr^{-1} and the 1.7 SU hr^{-1} microcosms had decreased by 97% and 81% respectively, when compared to the number of cellulose-utilizers in the UV-deficient microcosms (Table XV.A). After 20 days of irradiation the number of cellulose-utilizers was nearly 100% less in the microcosms exposed to 2.5 SU hr^{-1} than in the UV-deficient microcosms. Similarly, the numbers of cellulose-degrading heterotrophs in the microcosms exposed to 1.7 SU hr^{-1} had decreased to 97% of the number of cellulose-utilizing heterotrophs in the UV-deficient microcosms (Table XV.A).

Following the first period of exposure to 2.0 SU hr^{-1} the number of cellulose-utilizers increased. This portion of the heterotrophic population also increased in the UV-deficient microcosms during the same time interval. However, after five periods of exposure at 2.0 SU hr^{-1} the total number of cellulose-utilizing heterotrophs was 70%

TABLE XV.A. NUMBER OF CELLULOSE-UTILIZING HETEROTROPHIC ORGANISMS/100 ml OF WATER SAMPLE

Days after establishment of microcosms	Microcosm Group, level of UV irradiation					
	UV-deficient		UV-enriched 2.5 SU hr^{-1}		UV-enriched 1.7 SU hr^{-1}	
	MPN index	95% C.I.	MPN index	95% C.I.	MPN index	95% C.I.
7	1.1×10^4	1.5×10^3 - 4.8×10^4	1.1×10^4	1.5×10^3 - 4.8×10^4	1.1×10^4	1.5×10^3 - 4.8×10^4
12	1.1×10^4	1.5×10^3 - 4.8×10^4	4.3×10^2	7×10 - 2.1×10^3	1.5×10^3 - 1.1×10^4	3×10^2 - 4.4×10^3 1.5×10^3 - 4.8×10^4
20	2.4×10^4	*	4.3×10^2 - 9.3×10^2	7×10 - 2.1×10^3 1.5×10^2 - 3.8×10^3	4.6×10^3	7.1×10^2 - 2.4×10^4
27	2.4×10^3 - 1.1×10^4	3.6×10^2 - 1.3×10^4 1.5×10^3 - 4.8×10^4	$< 3 \times 10^0$	*	2.3×10^2	4×10^1 - 1.2×10^3

*95% confidence interval not established. Note 2. Confidence intervals from MPN tables.

Note 1. Hyphenated numbers indicate total count for each duplicate.

less than the number in the UV-deficient microcosms. After 11 periods of exposure at 2.0 SU hr^{-1} the number of cellulose-utilizers was 73% less than in the UV-deficient microcosms. By 18 days the number had decreased by nearly 100% of the number of cellulose-utilizing heterotrophs in the UV-deficient microcosms (Table XV.B).

Radioassay Procedure

Data from the uptake of ^{14}C-glutamate by the heterotrophic population indicate that exposure to UV radiation results in a greater proportion of ^{14}C-glutamate being respired as $^{14}CO_2$ when compared to the proportion of $^{14}CO_2$ respired by the non-VU-irradiated heterotrophs. This increased respiration was not observed for the first few periods of UV irradiation but began to appear following five periods of exposure. The difference in respiration between the UV-enriched microcosms and the UV-deficient microcosms increased for the remainder of the experiment.

The total ^{14}C activity of the CO_2 and the bacteria exhibited an immediate response to the initiation of UV irradiation. Following the first period of exposure to 2.5 SU hr^{-1} the total activity decreased by 60%. Similarly after the first period of exposure to 2.0 SU hr^{-1} the total activity decreased by 45%. The total activity of the filter paper and the membrane filters decreased by 10% following the first exposure period to 1.7 SU hr^{-1}. For the UV-deficient systems the total ^{14}C activity of the filter paper and membrane filters increased by 42% in one system and by 65% in the other during

TABLE XV.B. NUMBER OF CELLULOSE-UTILIZING HETEROTROPHIS ORGANISMS PER 100 ml OF WATER SAMPLE

Days after establishment of microcosms	Microcosm Group, level of UV irradiation			
	UV-deficient		UV-enriched 2.0 SU hr^{-1}	
	MPN index	95% C.I.	MPN index	95% C.I.
8	4.6×10^2 - 1.5×10^3	7.1×10^1 - 2.4×10^3 3×10^2 - 4.4×10^3	9.3×10^1 - 1.5×10^2	1.5×10^1 - 3.8×10^2 3×10^1 - 4.4×10^2
9	1.1×10^3 - 1.5×10^3	1.5×10^2 - 4.8×10^3 3×10^2 - 4.4×10^3	2.4×10^2 - 4.6×10^2	3.6×10^1 - 1.3×10^3 7.1×10^1 - 2.4×10^3
13	2.1×10^3 - 2.4×10^3	3.5×10^2 - 4.7×10^3 *	4.3×10^2 - 9.3×10^2	7×10^1 - 2.1×10^3 1.5×10^1 - 3.8×10^3
19	2.1×10^2 - 4.6×10^2	3.5×10^1 - 4.7×10^2 7.1×10 - 2.4×10^3	3×10^0 - 9×10^1	* *
25	1.1×10^3	1.5×10^2 - 4.8×10^3	$< 3 \times 10^0$	*
31	2.3×10^1 - 9.3×10^1	4×10^1 - 1.2×10^1 1.5×10^1 - 3.8×10^2	$< 3 \times 10^0$	*

*95% confidence interval not established.

Note 1. Hyphenated numbers indicate total count for each duplicate.

Note 2. Confidence intervals from MPN tables.

the same time period in which exposure to UV radiation began.

TABLE XVI.A. ^{14}C ACTIVITY ASSOCIATED WITH $^{14}CO_2$ AND BACTERIA (dpm x 10^4)

UV-deficient microcosms	Days after the establishment of the microcosms 7	8	9	10	12	20	22	27	31	35
$^{14}CO_2$	7.9	12.5	16.3	24.6	18.2	16.7	15.7	11.3	14.8	13.2
	8.1	13.1	18.4	22.4	18.1	16.3	15.6	10.7	13.7	12.2
^{14}C membrane filter	4.1	6.7	6.4	11.1	10.6	11.6	15.9	15.0	18.7	7.9
	4.2	7.1	6.0	11.3	10.6	11.5	16.7	14.9	17.3	10.4
Total ^{14}C activity	12.0	19.2	22.7	35.9	28.8	28.3	31.6	26.3	33.5	21.1
	12.3	20.2	24.4	33.7	28.7	27.8	32.3	25.6	31.0	22.6
% respired as $^{14}CO_2$	66	64	72	69	63	59	50	43	44	62
	66	65	76	66	63	59	48	42	44	54

TABLE XVI.B. ^{14}C ACTIVITY ASSOCIATED WITH $^{14}CO_2$ AND BACTERIA (dpm x 10^4)

UV-enriched 2.5 SU hr^{-1}	Days after the establishment of the microcosms 7	8	9	10	12	20	22	27	31	35
$^{14}CO_2$	13.8	5.4	12.7	15.7	17.5	17.4	21.7	18.6	25.8	24.2
	11.2	5.4	13.3	*	17.3	18.3	21.6	18.9	*	23.9
^{14}C membrane filter	6.6	2.6	4.6	7.0	8.2	6.8	8.2	9.7	11.5	9.6
	7.9	2.6	4.4	*	8.2	7.3	8.5	9.7	*	8.5
Total ^{14}C activity	20.4	8.0	17.3	22.7	25.7	24.2	29.9	28.3	37.3	33.8
	19.1	8.0	17.7	*	25.5	25.6	30.1	28.6	*	32.4
% respired as $^{14}CO_2$	68	67	74	69	68	72	73	66	68	72
	59	67	75	*	69	71	72	66	*	74

*Indicates lost duplicate.

TABLE XVI.C. ^{14}C ACTIVITY ASSOCIATED WITH $^{14}CO_2$ AND BACTERIA (dpm x 10^4)

UV-enriched 1.7 SU hr^{-1}	Days after the establishment of the microcosm 7	8	9	10	12	20	22	27	31	35
$^{14}CO_2$	11.1	9.6	14.0	15.1	18.1	16.9	20.4	19.3	19.5	19.9
	10.9	9.5	14.1	14.6	18.5	*	21.0	18.9	19.9	19.6
^{14}C membrane filter	4.8	4.5	5.6	7.6	7.8	7.2	9.0	12.2	12.4	10.5
	4.8	4.6	5.7	7.4	8.2	7.0	8.8	12.0	12.4	10.4
Total ^{14}C activity	15.9	14.1	19.6	22.7	25.9	24.1	29.4	31.5	31.9	30.4
	15.7	14.1	19.8	22.0	26.7	*	29.8	30.9	32.3	30.0
% respired as $^{14}CO_2$	70	68	72	67	70	70	69	61	61	65
	69	67	71	66	69	*	70	61	62	65

*Indicates lost duplicate.

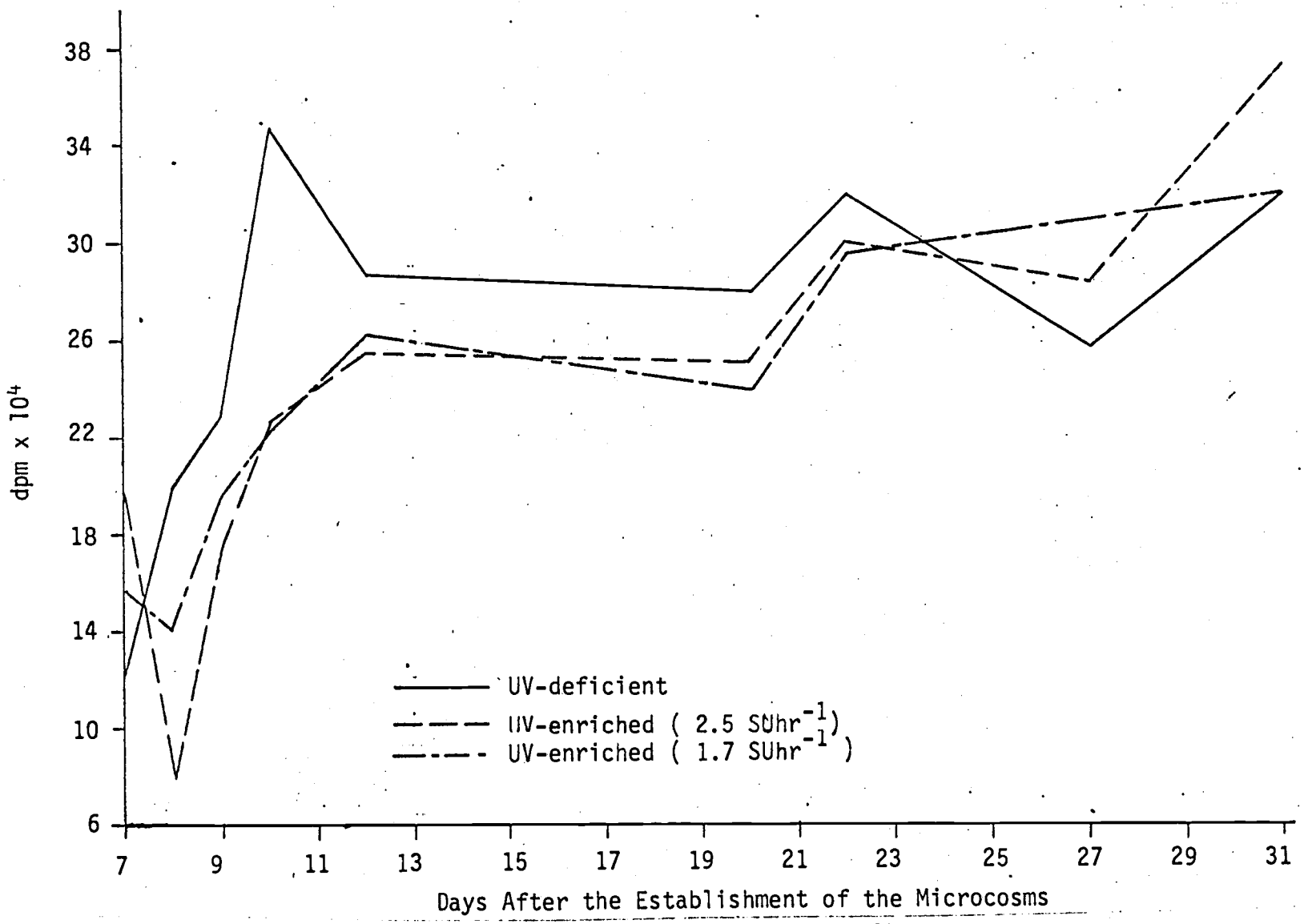

Figure 13.A. Average total ^{14}C activity associated with $^{14}CO_2$ and bacteria.

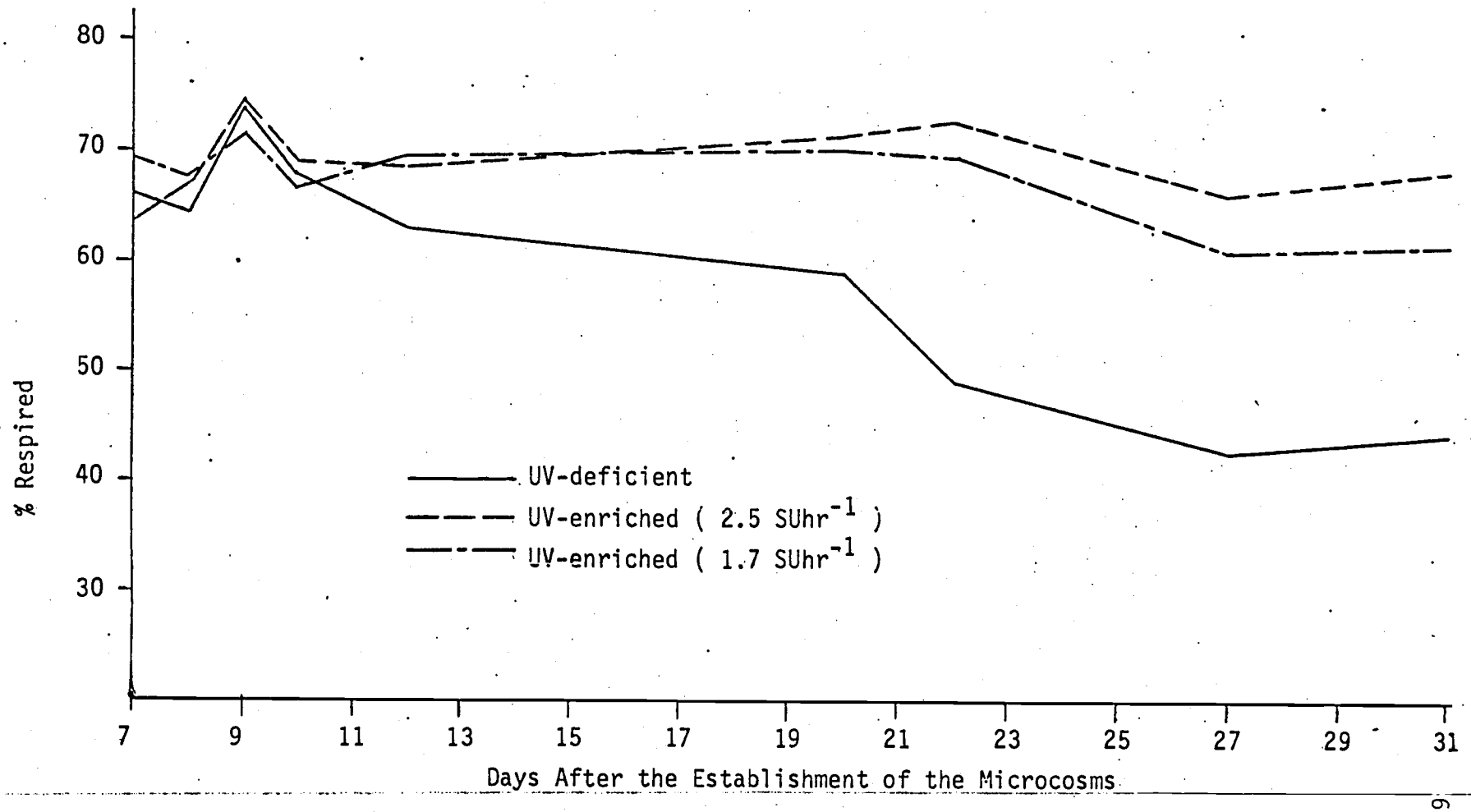

Figure 13.B. Percentage of total ^{14}C activity respired as $^{14}CO_2$.

TABLE XVII.A. ^{14}C ACTIVITY ASSOCIATED WITH $^{14}CO_2$ AND BACTERIA (dpm x 10^4)

UV-deficient microcosms	Days after the establishment of the microcosms													
	7	8	8[a]	9[b]	9[c]	9	10	13	19	24	30	35	41	46
$^{14}CO_2$	3.5	4.0	5.3	5.1	6.1	6.0	7.0	7.7	8.3	6.4	4.9	7.2	5.7	5.1
	3.5	*	4.0	4.2	5.7	5.7	7.2	6.8	7.6	5.9	5.6	7.6	5.6	4.7
^{14}C membrane filter	1.4	2.0	2.4	2.2	2.9	2.7	3.9	10.1	7.6	6.8	6.2	6.7	8.5	8.2
	1.4	*	2.5	2.3	2.6	2.6	3.9	7.1	7.7	6.9	6.5	6.7	8.3	8.1
Total ^{14}C activity	4.9	6.0	7.7	7.3	9.0	8.7	10.9	17.8	15.9	13.2	11.1	13.9	14.2	13.3
	4.9	*	6.5	6.5	8.3	8.3	11.2	13.9	15.3	12.8	12.1	14.3	13.9	12.8
% respired as $^{14}CO_2$	71	66	69	71	68	69	64	43	52	49	44	52	40	39
	71	*	62	65	69	69	65	49	49	46	46	53	40	37

[a]Microcosms sampled 2 hours after the initiation of cool-white light period.
[b]Microcosms sampled 2 hours before the termination of cool-white light period.
[c]Microcosms sampled immediately after termination of cool-white light period.
*Indicates lost duplicate.

TABLE XVII.B. ^{14}C ACTIVITY ASSOCIATED WITH $^{14}CO_2$ AND BACTERIA (dpm x 10^4)

UV-enriched 2.0 SU hr^{-1}	Days after the establishment of the microcosms													
	7	8	8[a]	9[b]	9[c]	9	10	13	19	24	30	35	41	46
$^{14}CO_2$	6.0	5.8	6.0	2.3	2.7	3.2	2.6	6.6	9.4	9.7	10.4	9.8	9.7	11.9
	6.5	*	*	2.2	2.7	3.0	3.0	7.0	8.7	9.9	10.2	11.3	9.5	11.0
^{14}C membrane filter	1.8	2.3	2.1	0.9	1.0	1.3	1.6	3.8	4.2	4.2	4.8	3.4	3.8	3.7
	1.8	*	*	0.9	1.2	1.3	1.5	4.6	4.1	4.7	4.5	3.4	3.5	3.4
Total ^{14}C activity	7.8	8.1	8.1	3.2	3.7	4.5	4.2	10.4	13.6	13.9	15.2	13.2	13.5	15.6
	8.3	*	*	3.1	3.9	4.3	4.5	11.6	12.8	14.6	14.7	14.7	13.0	14.4
% respired as $^{14}CO_2$	77	72	74	71	72	70	62	63	69	70	68	74	72	76
	78	*	*	71	69	69	66	60	68	68	69	77	73	76

[a]Microcosms sampled 2 hours after the initiation of cool-white light period.
[b]Microcosms sampled 2 hours before the termination of cool-white light period.
[c]Microcosms sampled immediately after termination of cool-white light period.
*Indicates lost duplicate.

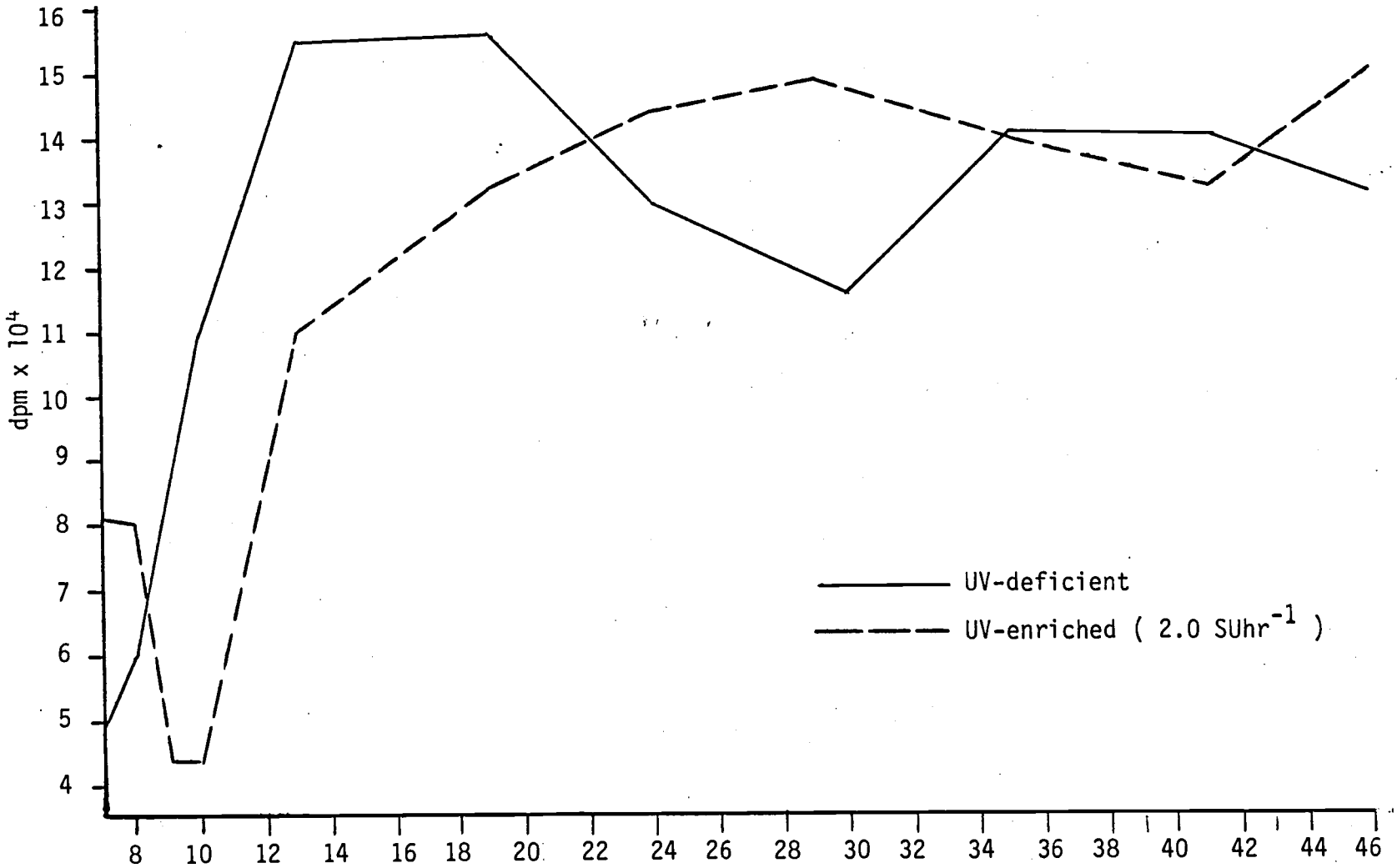

Figure 14.A. Average total ^{14}C activity associated with $^{14}CO_2$ and bacteria

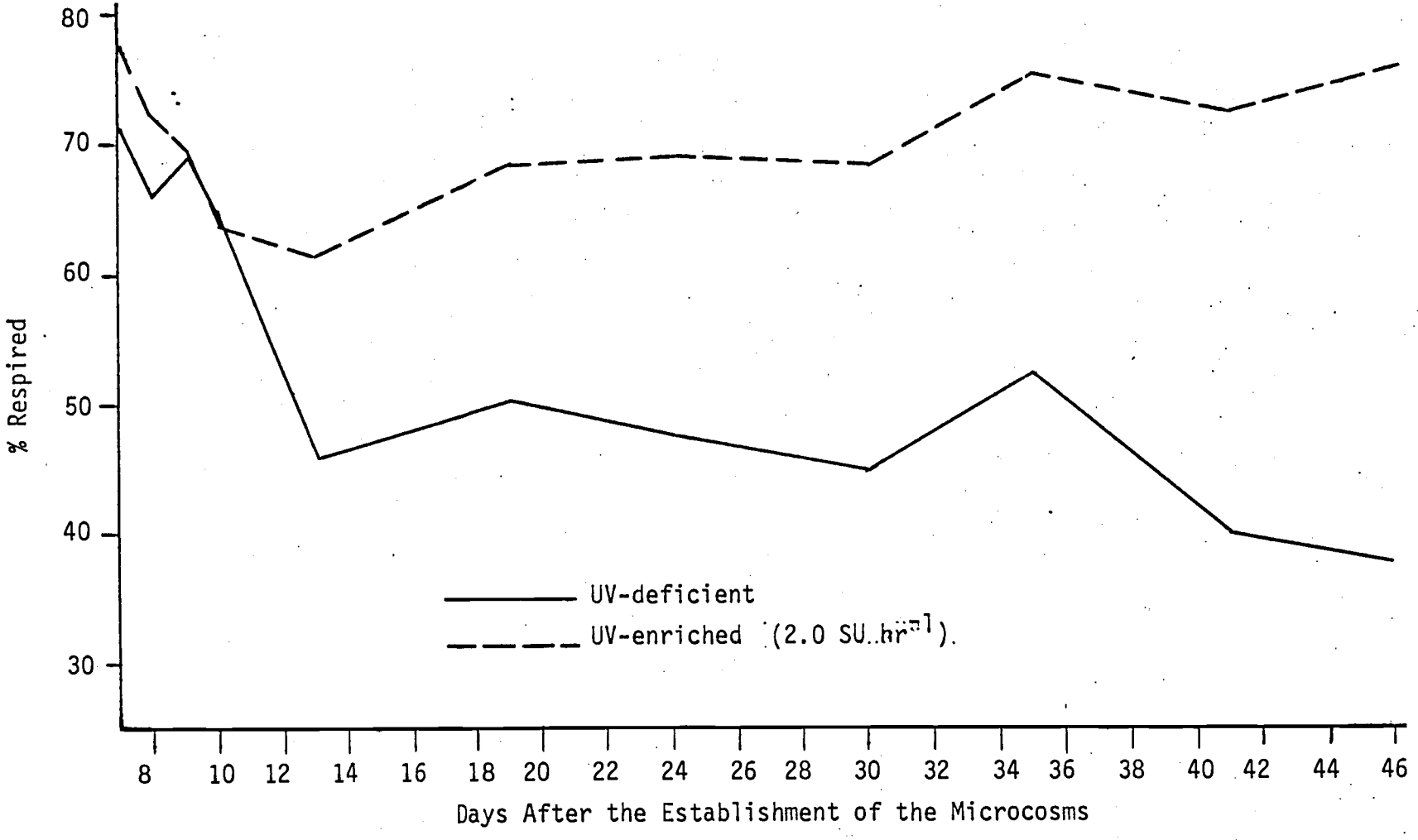

Figure 14.B. Percentage of total average ^{14}C activity respired as $^{14}CO_2$

DISCUSSION

Any area of nature that includes living organisms and non-living substances interacting so as to result in an exchange of energy between the biotic and abiotic parts may be considered an ecological system or ecosystem (Odum, 1964). Natural groupings of plants and animals are characteristic of life on earth. Each of these groupings or communities of organisms functions, in its mature state, as a self-sustaining unit in which a variety of biological interrelationships result in a condition of apparent homeostatis or steady state equilibrium (Casarett, 1968). The concept of communities of organisms emphasizes a functional unity of patterns of energy flow and a taxonomic unity of species commonly found together. Functional unity and taxonomic unity are interdependent but are not strictly tied to each other. Taxonomic unit, and therefore community structure, may change in time and space so that functionally similar communities may have different species composition (Odum, 1964). If the species composition of an established community is substantially altered, then there will result a succession of new biological interactions toward the establishment of a new stable structure which may or may not functionally resemble the original system. As long as the new community achieves some form of functional stability, even if only for a short time, it may be thought of in terms of the ecosystem concept.

The basic constituents of any ecosystem include the abiotic substances, the basic inorganic compounds of the environment, and the biotic communities. The biotic communities include producers or auto-

trophic organisms which synthesize their food from simple inorganic compounds, macro-consumers or heterotrophic organisms which ingest other organisms or particulate material, and micro-consumers or decomposers, chiefly bacteria and fungi, which break down complex organic compounds of protoplasm, absorb these decomposition products and release simple compounds (Odum, 1964). The living community and the non-living environment function together as an ecosystem.

The definition of an ecosystem is purposely broad since it seeks to emphasize functional inter-relatedness and interdependence in any area of nature. The portion of the estuarine ecosystem that has been the subject of this investigation has been referred to as a microecosystem or microcosm, although using the broad definition it may also be called an ecosystem.

While many of the conditions of the natural estuarine environment were simulated in the laboratory, the experimental conditions differed from the natural conditions in a number of ways. Instead of a sunrise and sunset period there was an immediate appearance and disappearance of radiant energy, both cool-white and ultraviolet. The radiant energy input was unchanging throughout the exposure period since there was no attempt made to simulate natural cloud cover. Tidal movement was absent and the water column was of constant and shallow depth. Alteration of the species composition was limited since no new organisms were introduced once the microcosms were established. Finally there was the problem of the selectivity of the growth media and the selectivity of the conditions under which the

microcosms were cultured. The laboratory approach allows for the study only of those organisms which will survive within the limits of the system. There may be many microbes within the natural estuarine. environment which could not tolerate the simulated conditions.

This investigation sought to detect alterations in the functional unity of a biotic community as a result of exposure to ultraviolet radiation. To facilitate this, enumeration studies and an examination of selected heterotrophic metabolic activities were performed. The results clearly indicate that some of the metabolic functions were altered. Also within the limits of the exposure regime utilized, there did not appear to be a threshold below which UV-irradiation had no effect on the functioning of the micro-ecosystems. Finally, the effects of UV-irradiation can be said to be two-fold exhibiting both short term or acute effects and long term or late effects.

There was no clear evidence for an acute effect of UV-irradiation on the number of cellulose-utilizing heterotrophs. This was due to a lack of data points immediately following the initiation of exposure to UV radiation. However after five periods of exposure the number of cellulose-degraders began to decline at all levels of UV irradiation. By three weeks following the initiation of exposure to UV radiation the number of cellulose-utilizing heterotrophs in the UV-enriched microcosms was significantly less than in the UV-deficient microcosms. This may have been an indirect effect of the UV on the heterotrophs as a result of the response of the phytoplankton to the UV irradiation. From gross estimates of the biomass and pigments of

the phytoplankton, i.e., the macroscopic appearance of the microcosms, it was apparent that there was less biomass present in the microcosms exposed to UV radiation. The apparently diminished phytoplanktonic biomass may have been the underlying cause for the decrease in the total number of cellulose-utilizing heterotrophic organisms in the UV enriched microcosms.

There was no clearly discernible effect on the ratio of non-seawater-requiring organisms to seawater heterotrophs, on the ratio of agar-utilizing heterotrophs to seawater heterotrophs, or on the proportion of starch-utilizers to the total number of seawater organisms in the microcosms exposed to UV radiation when contrasted to the UV-deficient microcosms.

The total number of seawater heterotrophic organisms exhibited a response to UV irradiation. The long term or late effect was an overall decrease at all UV exposure levels. The short term or acute response to UV irradiation was a decrease in total numbers in the first two periods of exposure to 2.5 SU hr^{-1} and 2.0 SU hr^{-1}. There was no acute response observed in the survival of the heterotrophs exposed to 1.7 SU hr^{-1}. The ability of the total number of heterotrophs in the UV irradiated microcosms to increase and attain numbers similar to the number of heterotrophs in the UV-deficient microcosms can be viewed in terms of an "acute phase recovery." This is a phenomenon which is observed in other systems subjected to radiation damage and represents the repair ability of that system. Although the system may continue to function, the recovery generally is not

complete, i.e., there is some irreparable damage. This irreparable injury may be manifest in a decreased viability of the system over the long term and may result in an overall shortening of the system's life span (Casarett, 1968). This interpretation of the effects of radiation damage comes chiefly from research on the effects of ionizing radiation, however it is of value in providing an interpretation of the response of the heterotrophic organisms in the microcosms exposed to UV radiation.

The increase in the proportion of pigmented colonies from the microcosms exposed to all levels of UV radiation may have been the result of a selection against non-pigmented colonies. This was most apparent during the first periods of exposure to UV radiation. At that time the total number of seawater heterotrophs was significantly decreased yet the total number of pigmented colonies remained relatively unchanged. As the heterotrophs in the UV-enriched microcosms recovered from the actue phase of radiation damage the proportion of pigmented colonies to non-pigmented colonies decreased. However, for the duration of the experiment, the ratio of pigmented to non-pigmented colonies remained greater in the UV-enriched microcosms when compared to the UV-deficient microcosms.

The ability of the heterotrophic organisms to metabolize uniformly labelled ^{14}C glutamate provided a means for assessing the heterotrophic activity of the microcosms. The total amount of ^{14}C glutamate handled by the heterotrophs can be estimated by summing the activity of the $^{14}CO_2$ on the filter paper and the ^{14}C activity of the

residue on the membrane filter. The amount of labelled glutamate handled by the heterotrophs from the UV-enriched microcosms exhibited an acute response at all levels of exposure. The decrease in total activity in the first two periods of UV irradiation was followed by an increase in activity. However this increase in activity of the heterotrophs in the UV-enriched microcosms did not attain the level of activity of the hetrotrophs from the UV-deficient microcosms until two weeks after the initiation of UV irradiation. The proportion of ^{14}C glutamate respired by the heterotrophs from the UV-enriched microcosms as $^{14}CO_2$ failed to show a short term effect but there was a significant late effect. The late effect was an increase in the proportion of ^{14}C glutamate being respired as $^{14}CO_2$ when compared to the respiration of the heterotrophs from the UV-deficient microcosms. This effect was observed as early as five days after the initiation of UV irradiation and continued throughout the duration of the experiment.

It may be argued that it was the heterotrophs in the UV-deficient microcosms that responded to the absence of UV radiation, rather than the heterotrophs in the UV-enriched microcosms responding to the presence of UV radiation. The latter of these two interpretations seems more reasonable. Crawford, et al. (1974) have studied the role of heterotrophic organisms in the cycling of carbon in an estuary by measuring the rate of uptake of a number of different ^{14}C labelled organic compounds. They obtained samples from the water column of Pamlico River esutary in North Carolina and ran a series of radioassays, similar to the procedure used in this investigation, within

30 minutes of sample collection. Their results indicate that over a one-year period the proportion of ^{14}C glutamate respired by the heterotrophic populations as $^{14}CO_2$ ranged between 0.42 and 0.57. Their samples may be considered to have come from a mature ecosystem. The proportion of ^{14}C glutamate respired as $^{14}CO_2$ by the heterotrophs in the UV-deficient microcosms at the later stages of the investigation agrees well with the data of Crawford, et al. (1974); however, the high proportion, 0.7 to 0.8, of ^{14}C glutamate respires as $^{14}CO_2$ by the heterotrophs in the UV-enriched microcosms may be a function of the immaturity of those systems. In effect what has been done in bringing the samples of estuarine water into the laboratory and restricting these to simulated environmental conditions was to have established a new ecosystem. This new system may or may not resemble the original system. Although one of the goals of this investigation has been to simulate as closely as possible the major environmental conditions, as has been stated above, this goal was not fully realized. The high rate of respiration of the newly established microecosystems may have been a reflection of their immature state and a reflection of the stress that the original system was subjected to by the imperfect simulation of the natural environment. However, as the new ecosystem adjusted to the new conditions its attainment of maturity may have been reflected in the decline in the respiration of the heterotrophs in the UV-deficient microcosms. Viewed in this way it can be argued that the heterotrophs in the microcosms exposed to UV radiation were subjected to additional stress before the system

had reached a mature and stable condition. The increased respiration of the heterotrophs exposed to UV radiation may have been an indication of this additional stress when compared to the respiration of the heterotrophs in the more mature and stable UV-deficient microcosms.

The increase in the proportion of ^{14}C glutamate respired as $^{14}CO_2$ by the heterotrophs exposed to UV radiation represents a possible source of increase of carbon dioxide to the marine environment. According to the contemporary models of the global carbon dioxide system a steady state equilibrium exists between atmospheric carbon dioxide, the carbon dioxide dissolved in the ocean, and the molecular species of the carbon dioxide in solution in the ocean, namely carbonate, bicarbonate and carbonic acid. The hydrogen ion concentration of the ocean is controlled by two mechanisms, according to this model, which involve the carbon dioxide system. A short term mechanism, which may respond in a matter of seconds, is called the pH buffer system; the long term mechanism, operating over centuries and exerting the ultimate control of the pH, is called the pH stat.

The pH stat system involves the interaction of hydrogen ions, either as free hydrogen or bound to carbonate or bicarbonate, with clay particles in the ocean. Perturbations in the equilibrium of the carbon dioxide system by an excess of hydrogen ions, perhaps from volcanic exhalations, would shift the available carbonate ions to bicarbonate and shift the bicarbonate ions to carbonic acid. The results of this shift in the equilibrium would be a depression of the pH. As the deep circulation of the ocean slowly brings these waters

into direct contact with clay sediments on the ocean bottom the hydrogen ions would be adsorbed at the sediment interphase. Hydrogen ions replace sodium ions in the clay structure and carbonate and sodium ions are released in the ion-exchange. This exchange, though rapid, is restricted to the water which comes in contact with the surface of the clay particles. A greater capacity for the absorption of hydrogen ions is provided by the structural rearrangement of montmorillite to kaolinite. This is a slow process, however, taking perhaps centuries, but the pH of the ocean would eventually return to equilibrium.

This conceptual model implies that the pH of the ocean has remained constant over geologic time and further implies that the carbon dioxide content of the atmosphere has also remained relatively constant. The pH stat system serves as a coarse adjustment to the pH of the ocean, however if it were the only mechanism functioning then large short term local fluctuations of the pH would be observed. That such fluctuations are not observed implies that another mechanism is involved and serves as a "fine tuner" of the pH of the ocean.

The rapid response of the carbonate pH buffer system is involved not only as a first step in the long term adjustment to the hydrogen ion concentration of the ocean but also it operates to counteract minor local changes. This mechanism is not dependent on the slow deep circulation of the ocean, as is the case with the pH stat system, and therefore operates on a shorter time basis. The pH buffer system functions to absorb or release hydrogen ions, depending upon the

conditions, from the various molecular species of carbon dioxide dissolved in the ocean through a shifting of the equilibrium existing between those species.

Using a Bjerrum diagram, which plots the concentration of the various carbonate species against pH, MacIntyre (1971) has calculated the effect of a doubling of the amount of atmospheric carbon dioxide. The pH buffer system of the ocean would function to absorb the increased carbon dioxide resulting in a 20% increase in the concentration of carbon dioxide in the atmosphere. The pH of the ocean would be lowered from an average of 8.15 to an average of 8.08. The pH stat system would function to return the pH to 8.15. This would result in a 2% increase in the concentration of carbon, in the form of carbon dioxide, in the ocean and a 2% increase in the concentration of atmospheric carbon dioxide from 320 parts per million (ppm) to 326 ppm.

MacIntyre (1971) emphasizes that the rates of the reactions of the carbon dioxide system are crucial. As an example he points out that although enough fossil fuel has been burned in the past century to have increased the atmospheric carbon dioxide content from 290 ppm to 350 ppm, the current concentration is 326 ppm. He states that the "principal removal agent is undoubtedly" the ocean. The process is slow however and the rate of production of carbon dioxide exceeds its removal by the ocean.

The contribution of additional carbon dioxide by heterotrophic organisms as a result of exposure to UV radiation may represent a

further burden to the ability of the oceans to buffer the effect of man's activity.

CONCLUSIONS

1. Exposure of the natural microbial populations in a simulated estuarine micro-ecosystem to ultraviolet radiation above 290 nm resulted in the alteration of some aspects of the functional unity of the micro-ecosystem.

2. It was not determined whether the observed alterations in the heterotrophic populations exposed to UV radiation was a transient feature which would eventually lead to the establishment of a new, although different, functional ecosystem.

3. The effects of UV irradiation on the heterotrophic populations in a simulated estuarine micro-ecosystem can be interpreted in a manner similar to interpretations rendered in the explanation of the effects of ionizing radiation on other organisms.

4. Pigmented seawater heterotrophic micro-organisms survived exposure to UV radiation more readily than non-pigmented seawater heterotrophs.

5. Exposure of the natural microbial populations in a simulated estuarine microcosm to UV radiation led to an increase in the respiration of uniformly ^{14}C labelled glutamate.

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APPENDICES

APPENDIX A

NUTRITIVE MEDIA FORMULATIONS

Partial formulation of Alga-gro Concentrate, compliments of the Powell Laboratories of Carolina Biological Supply Company, Gladstone, Oregon.

Alga-gro Concentrate when diluted 1:50 with artificial seawater (100 ml:5 liters).

Nitrate - 5.48×10^{-4} moles/liter (34 ppm)

Phosphate - 2.842×10^{-5} moles/liter (2.7 ppm)

N:P - 16:1

Cobalt - 5.67×10^{-8} moles/liter

Manganese - 2.44×10^{-6} moles/liter

Zinc - 2.55×10^{-7} moles/liter

Additional:

3% solution of $Na_2SiO_3 \cdot 9\ H_2O$ (3 ml/5 liters)

Silicate - 1.06×10^{-1} moles/liter

APPENDIX A (continued)

Seawater Agar

Bacto Peptone	1.0 gm
Bacto Yeast Extract	0.5 gm
Di-basic Potassium Phosphate	0.5 gm
Agar	15.0 gm
Filtered Artificial Seawater	1000.0 gm

pH adjusted to 7.8

Freshwater Agar

Same formulation as for Seawater Agar except replacing 1000.0 gm of filtered artificial seawater with distilled water. pH adjusted to 7.8.

Starch Agar

Soluble Starch	5.0 gm
Seawater Agar	1000.0 gm

Gram's Iodine

Iodine	1.0 gm
Potassium Iodide	2.0 gm
Distilled Water	100.0 gm

APPENDIX A (continued)

Gelatin Liquefication Media 9 ml/tube (1.0 ml inoculum/tube)

Nutrient Broth	100.0 ml
Knox Unflavored Gelatin	3.0 gm

Cellulose Degradation Media 9 ml/tube (1.0 ml inoculum/tube)

Sodium Nitrate	0.05 gm
Mono-basic Potassium Phosphate	0.05 gm
Magnesium Sulfate	0.03 gm
Ferrous Sulfate	0.001 gm
Peptone	0.005 gm
Yeast Extract	0.0001 gm
Artificial Seawater	100.0 ml

Cellulose source was cigarette rolling papers, one/tube, suspended so that one-half of the paper was above the surface of the media.

pH adjusted to 7.8

APPENDIX A (continued)

Marine Phytoplankton Culture Media (Alternative)

I. Major Nutrients - Stock Solutions

A. $NaNO_3$ - 15 gm up to 100 ml distilled water

B. $NaH_2PO_4 \cdot H_2O$ - 1 gm up to 100 ml distilled water

C. $Na_2SiO_3 \cdot 9\ H_2O$ - 3 gm up to 100 ml distilled water

D. Sequestrene-NaFe (13%) - 0.1 gm to 100 ml distilled water

E. Metal Mix - Primary Stock Solutions

1. $CuSO_4 \cdot 5\ H_2O$ 1.96% w/v in distilled water
2. $ZnSO_4 \cdot 7\ H_2O$ 4.4% w/v in distilled water

   or

   $ZnCl_2$ 2.1% w/v in distilled water
3. $CoCl_2 \cdot 6\ H_2O$ 2.0% w/v in distilled water
4. $MnCl_2 \cdot 4\ H_2O$ 36.0% w/v in distilled water
5. $Na_2MoO_4 \cdot 2\ H_2O$ 1.26% w/v in distilled water

Use 1 ml of each of the above to make up 1 liter of metal mix.

F. NH_4Cl - 26.5 gm up to 500 ml distilled water

II. Buffer Solution - 2% Sodium bicarbonate

A. 10 gm $NaHCO_3$ up to 500 ml in distilled water. Keep cold in refrigerator.

III. Thiourea

A. 100 mg up to 100 ml autoclaved distilled water. Refrigerate.

APPENDIX A (continued)

IV. Vitamins

A. Stock Mix

1 mg biotin + 1 mg B_{12} up to 20 ml distilled water

Keep frozen.

To make Vitamin mix:

1 ml biotin and B_{12} mix "A" (completely thawed) + 20 mg thiamin HCl up to 100 ml distilled water. Dispense into ampoules. Seal with torch. Autoclave for 5 min upside down in water colored with methylene blue to check for unsealed or broken ampoules. Freeze after cooling at room temperature.

B. For the Culture of Marine Phytoplankton

1. Autoclave 2 liters of seawater 15-20 min, cool quickly by running tap water on the flask for about an hour.

2. To the 2 liters of seawater add each of the following stock solutions.

$NaNO_3$	1 ml
$NaH_2PO_4 \cdot H_2O$	1 ml
$Na_2SiO_3 \cdot 9\ H_2O$	1 ml
Fe sequestrene	1 ml
Metal mix	1 ml

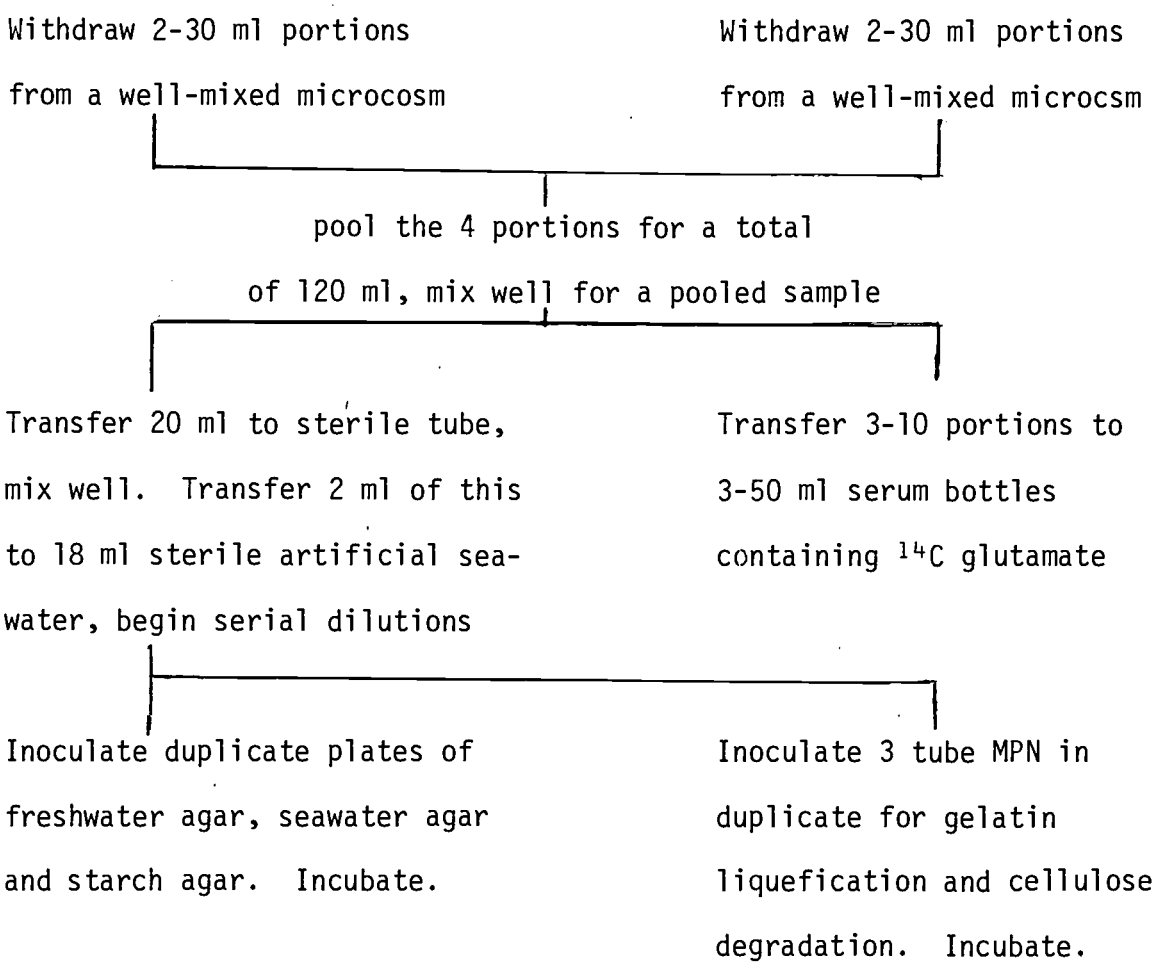
APPENDIX A (continued)

Vitamin mix	1 ml
Thiourea	1 ml
2% $NaHCO_3$	20 ml

C. Dispense medium into desired autoclaved culture flasks and keep in the cold room for at least one hour before inoculating with culture specimens.

APPENDIX B

DIAGRAMMATIC REPRESENTATION OF THE SAMPLING PROCEDURE UTILIZED FOR THE GROUPS OF MICROCOSMS



Repeat procedure for each group of microcosms to be sampled (UV-deficient and UV-enriched).

APPENDIX C

OLLIVETTI 101 PROGRAM FORMAT

Olivetti 101 program format for the calculation of channel ratio, counting efficiency, corrected disintegrations, corrected disintegrations per minute, total disintegrations per minute, and percent respired.

AV	A◊	d+
F*	S	A◊
f*	S	C‡
EV	÷	C÷
S	A◊	A◊
S	F‡	S
↓	/◊	S
S	BV	÷
S	S	A◊
B↑	S	/◊
B÷	↓	F+
/◊	S	A◊
A◊	S	f‡
EX	C↑	F↓
D*	C÷	f÷
A◊	/◊	A◊
B‡	A◊	/◊
B÷	eX	DV