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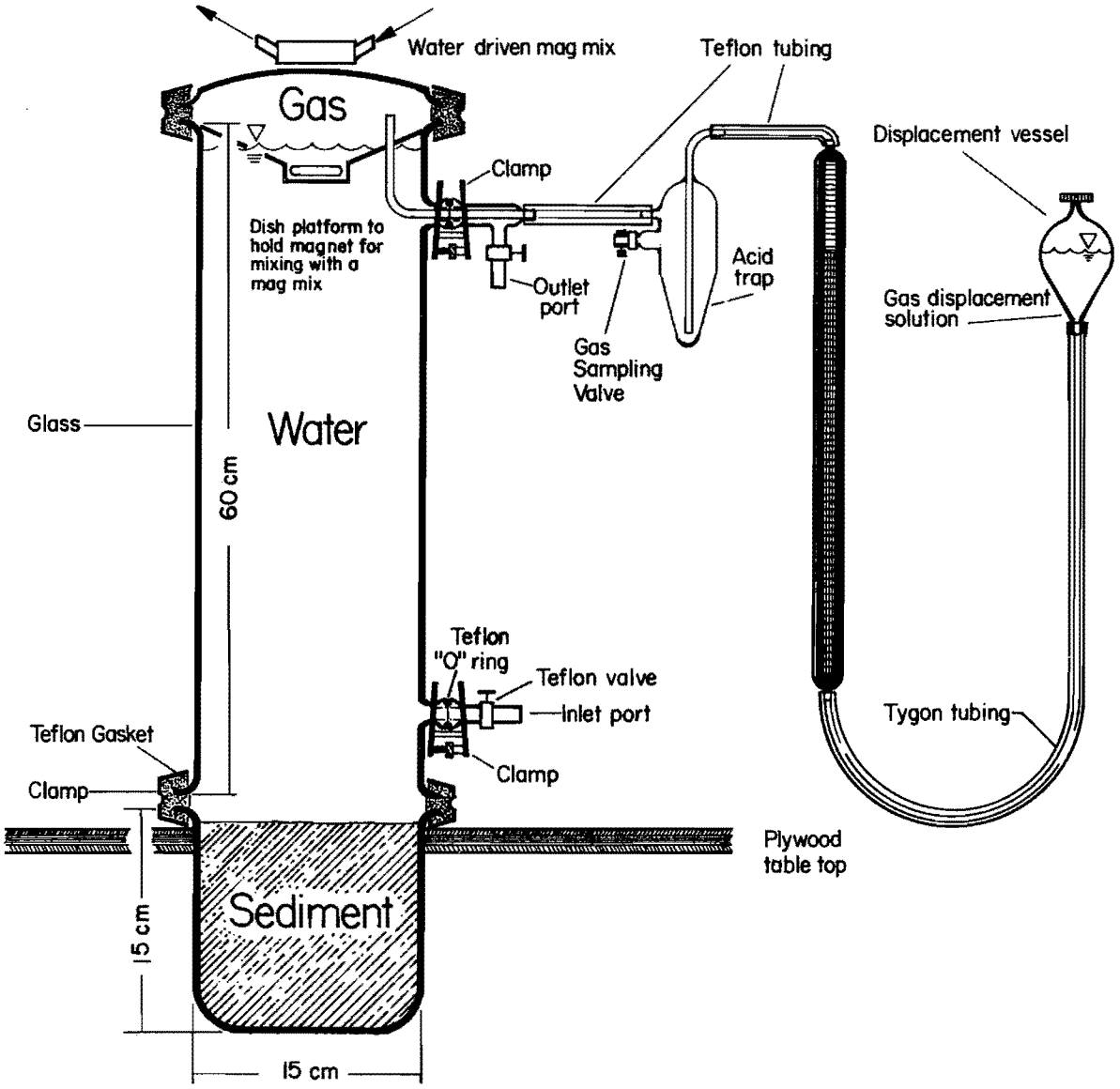

Utah Water Research Laboratory
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ABSTRACT

Small scale, three-phase microcosms were used to study the aquatic fate and effect of a polycyclic aromatic hydrocarbon (PAH), benz(a)anthracene. This compound was chosen as a model for carcinogenic PAH because it is a typical constituent of petro-chemical effluents and has middle-of-the-range physical and chemical characteristics. In a series of experiments, techniques were established for monitoring the behavior and for assessing the biological response to the pollutant. Results indicate that benz(a)anthracene has no acute effect on aquatic organisms as indicated by the parameters used to measure community structure and function (gas productivity, nutrient utilization, biomass accumulation, and species composition). Gas chromatograph/mass spectral analysis of benz(a)anthracene and metabolites in the medium sediment, and biota made it possible to trace the fate of the compound. At the end of one 60 day experiment, 76 percent remained in the sediment, 17 percent had been recovered in the medium, 1 percent had attached to the microcosm surfaces, and less than 1 percent was associated with the biota. Of the remaining 5 percent, a portion was photodegraded. There was no evidence of metabolism.

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TABLE OF CONTENTS

TABLE OF CONTENTS (CONTINUED)

TABLE OF CONTENTS (CONTINUED)

LIST OF FIGURES

LIST OF FIGURES (CONTINUED)

LIST OF FIGURES (CONTINUED)

LIST OF TABLES

INTRODUCTION

Organic chemical carcinogens reach the aquatic environment from several sources and in a variety of ways. Surface waters may receive these pollutants in effluents from manufacturing and processing plants and sewage treatment plants (Wedgwood and Cooper 1955), in runoff and erosion from forests and agricultural lands (Kraybill 1977), and through fallout and rainout of air pollution particulates (Andelman and Suess 1970). Carcinogens of natural or industrial origin adsorbed onto vegetation or soil may leach into the groundwater as well (Hueper 1960).

The fate of the polycyclic aromatic hydrocarbons (PAH) in aquatic systems has recieved a great deal of attention due to the fact that many are known to be carcinogenic to animals and probably to man (Badger 1962; Hueper and Conway 1964). The present study was initiated to further the understanding regarding the fate and effect of PAH in the freshwater environment. A three-phase (gas, liquid, sediment) laboratory microcosm was evaluated and applied. A recent survey of testing procedures that have proven useful in evaluating environmental transport, fate, and effects of trace contaminants has revealed that laboratory microcosm experiments can provide substantial information in these areas (Witherspoon et al. 1976). The use of various types of microcosms, and their advantages and disadvantages are discussed by Harris (1980) and in several papers edited by Giesy (1980).

Others have used microcosms to study the fate of complex mixtures of petroleum hydrocarbons (Lee and Takahashi 1977; Lee et al. 1978b; Gearing et al. 1980). The present study differs from the previous ones in that a single PAH was chosen for investigation and the system simulated is a freshwater, rather than a marine, environment. The idea was 1) to attempt to verify predictions for the behavior of a contaminant in simulated light and dark regions of a shallow reservoir from chemical characteristics determined in the laboratory, and 2) to evaluate this particular microcosm system as an appropriate research tool for assessing the response of the biological community to a low-dose, chronic exposure to carcinogenic PAH.

Benz(a)anthracene (BA) was the PAH chosen for study because 1) it occurs in many crude oils, refined products, and extracts of spent oil shale (Maase 1980); 2) it is a major constitutent of PAH in combustion products (NAS 1972); 3) it is on the Environmental Protection Agency's list of priority pollutants; and 4) its higher molecular weight (>4 rings) is associated with the typical physical characteristics of the heavier PAH (i.e., extremely low aqueous solubility and strong carcinogenic activity).

The objectives of this study were:

1. Establish techniques for monitoring the behavior and for assessing the biological response to BA in the aquatic environment.

2. Evaluate proposed pathways of environmental degradation and interaction of BA with light, biota, and sediment presented in the literature.

3. Determine the significance of uptake and accumulation of BA by biological organisms and the ecological magnification of the pollutant in food chains important to humans.

LITERATURE REVIEW

Use of Microcosms as a Research Tool

The basic rationale for using responses at the ecosystem-level to measure the change resulting from particular environmental perturbations is that measurements of ecosystem components, such as populations or individuals, do not adequately describe the total environmental impact. Individuals and populations do not exist in ecosystems in isolation from the surrounding biotic and abiotic components. All components are intimately interactive in the transfer of energy and materials through the system. These transfer processes determine the fate of the individuals and populations and ultimately the system as a whole.

A microcosm provides a capability to reproduce environmental diversity without the full complexity of the natural system. Specifically, with microcosms one can experiment under conditions of controlled complexity, manageable size, reproducibility, controlled environmental factors (temperature, lighting, and nutrients) and the ability to predetermine the degree to which the microcosm approaches the natural system (simulation of natural tide cycles, addition of native species and sediments, or actual placement in the field as opposed to inside the laboratory).

The reliability with which microcosms reproduce natural systems depends on the effect in disturbing community behavior of reducing scale. Scale affects many parameters. Most noticeable are the ratios of sediment or soil surface to volume and of water surface area to volume in pelagic microcosms (Giesy and Odum 1980). Also, two small or too large a consumer density may bias results (Harte et al. 1980, Bowling et al. 1980).

One application of the microcosm is in fate-and-effect studies of potentially harmful materials. The reactions and interactions of chemical carcinogens can be studied with little risk to the environment. Examples include studies by Metcalf and his colleagues on the biodegradability and ecological magnification characteristics of pesticides and carcinogens, including polynuclear aromatic hydrocarbons (PAH) (Metcalf 1975, Metcalf et al. 1971, Lu et al. 1977). Other fate-and-effect studies of PAH and petroleum mixtures have been performed in outdoor enclosures (Gearing et al. 1980, Lee et al. 1978a, Hinga et al. 1980, Lee and Takahashi 1977). Applications of the results to the fate of BA in the aquatic environment will be pursued following a review of what is known about the sources, transformations, and biotic interactions of this PAH.

Presence of BA in the Environment

Benz(a)anthracene (BA) is ubiquitous in the environment. Like other polynuclear aromatic hydrocarbons (PAH) it is found at its highest concentration near industrial centers and transportation routes. In the aquatic environment, the greatest BA burden is in rivers, estuaries, and coastal waters. Following historical precedent, most surveys of industrial pollution use benzo(a)pyrene (BaP) as the primary indicator of carcinogenic PAH. Often the only information reported in the literature are concentrations of BaP and total PAH. Recently, however, the

increased availability and use of sophisticated instrumentation and the variable proportion (1-20 percent) of BaP to total PAH (Andelman and Suess 1970), has led to measurement of individual PAH. In particular, data on BA (and frequently its isomers, chrysene and phenanthrene) can be obtained.

Literature values for the BA content of environmental samples are listed in Table 1. The largest values of BA are associated with sediments, particularly marine bay sediments. The contaminated sediments are undoubtedly a source of BA to the overlying water, but, no concurrent measurements of BA have been made in water samples to allow comparison. Likewise, the report of aqueous concentrations of BA in European river water without concurrent information for sediment samples limits use of the data to examine the persistence and ultimate fate of the compound in the freshwater environment. Reported data describing BA content of aquatic organisms are limited. Shellfish appear to accumulate more PAH from their

Table 1. Concentration of benz(a)anthracene (BA) and total polycyclic aromatic hydrocarbons (PAH) in environmental samples.[a]

Source		BA Concentration	Total Concentration of PAH Measured	Reference
River water, U.K.	Thames R.	0.14-0.39[b]	0.60-1.21	Acheson et al. 1976
River water, Germany				Borneff and Kunte 1964
	Gersprenz R.	0.0043-0.0188	0.1153-0.1164	
	Danube R.	0.011 -0.014	0.1623-0.1666	
	Main R.	0.007 -0.0162	0.1363-0.4284	
	Aach R.	0.101 -0.385	1.022 -2.252	
	Schussen R.	0.057	0.643	
Sediment, Hirakata Bay, Japan		5.-4780[c]	NA[d]	Matsushima 1979
Sediment, Saudafjord, W. Norway				Björseth et al. 1979
0-2 cm		21-2158.9[e]	NA	
2-4		0-2620.5		
4-6		7.3-2853.2		
6-8		30-1512.2		
Sediment, Charles River		3400±500[f]	NA	Tan 1979
Sediment, W. Falmouth downslope		1.1	8.12	Hites et al. 1977
W. Falmouth upslope		1.2	60.4	Ibid
Scott Lake, Ontario		6	135.7	Hilpert et al. 1978
Sediment, Buzzards Bay, Massachusetts		110-79.0	570-3,600	Giger and Blumer 1974
Mussels, Saudafjord, W. Norway,		225-25,264[f]	NA	Björseth et al. 1979
Fin Fish,				Pancirov and Brown 1977
Menhaden		<0.3	5.7	
Flounder		<1.0	30.6	
Cod fish		<2.0	15.4	
Lake Trout		<0.5	5.9	
Oysters, Norfolk, Virginia, wet wt		<10	730-1250	Cahnmann and Kuratsune 1957

[a]Concentration presented as ppb, unless otherwise stated.
[b]Includes chrysene.
[c]Concentration of BA varied as the sediment composition varied.
[d]NA=Not Available
[e]Concentration varied with distance downstream from ferro alloy smelter.
[f]89.4 percent recovery.

environment than do fish but the extent of contamination is also a function of environmental factors which will be discussed in more detail in a following section.

Several factors contribute to the worldwide presence of PAH (and BA) in the aquatic environment. These include overall magnitude of production (estimated to be 11.01 million metric tons total PAH/yr (Neff 1979)) ineffective treatment methods for removal from waste streams, efficient dispersal (due to sorption onto air or waterborne particulates), and resistance to degradation after deposition. Figure 1 depicts the mechanisms affecting the fate and transport of PAH in the aquatic environment. These processes and the roles they play in determining the fate of BA will be considered individually. In addition, data describing the effect of BA on organisms and biological communities will be presented. These discussions will be concluded by predictions of the fate and effects of BA in various aquatic environments, along with the empirical evidence used to support and refine these predictions.

Sources of Benz(a)anthracene

There are essentially three ways in which PAH are produced: high temperature (700°C) pyrolysis of organic matter, low to moderate temperature (100-150°C) diagenesis of sedimentary organic matter to form fossil fuels (Blumer 1976), and direct biosynthesis by microorganisms and plants (Neff 1979). The majority of the PAH in the environment is derived from the first process, i.e., incomplete combusion of organic matter at high temperatures (Suess 1976). In flame pyrolysis, the PAH is associated with the particulate fraction produced during combustion (soots or carbon blacks). The BA content of several such sources is shown in Table 2. These airborne particulates

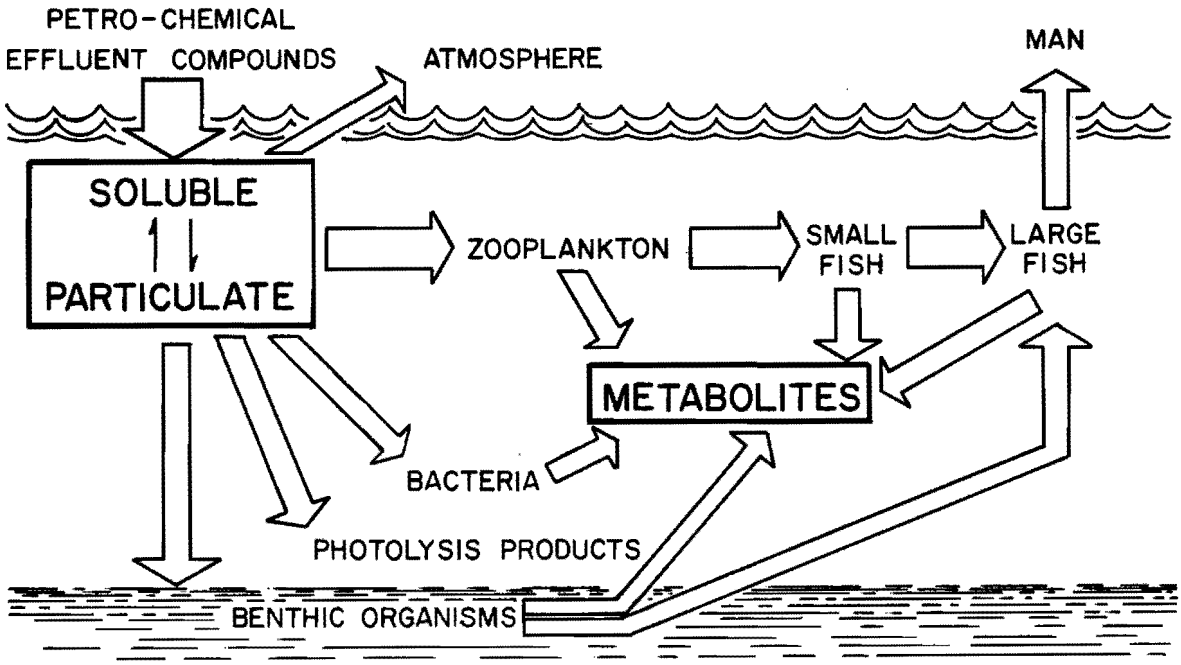


Figure 1. Transport and transformation pathways of benz(a)anthracene in the aquatic environment. (From Herbes et al. 1980.)

Table 2. Concentration of benz(a)anthracene (BA) and total polycyclic aromatic hydrocarbons (PAH) produced from various combustion emission sources.[a]

Source	BA concentration	Total Concentration of PAH Measured	Reference
Gasoline engine exhaust			Hoffmann and Wynder 1963
Exhaust tar concentration, mg/kg	61.7	4,671.7	
Concentration (μg/1-min run)	4.2	314.0	
Heat generation sources (μg/2.5 x 10^8 calories			Hangebrauck et al. 1964
Coal as fuel, pulverized	19	368	
Coal, underfeed stoker	3900	93,370	
Coal, underfeed stoker	560	95,240	
Oil, steam atomized	27	2,440	
Catalyst regeneration flue gas of a gas-oil stock from oil refining (mg/1000 m^2 gas)			Sawicki et al. 1965
Before cleaning system	270	16,047	
After cleaning system	0.10	6.93	
Air-filter samples of coke oven emissions, μg/sample			Lao et al. 1975
Sample No. 1	2740.45	51,178.42	
Sample No. 2	105.15	2,860.28	
Coal tar air emissions			Lao et al. 1975
mg/g coal	45.74[b]	537.89	
μg/m^3 air	1607.[b]	18,914.	
Municipal incinerators			Davies et al. 1976
Stack gases, mg/1000 m^3	0.72[c]	4.04	
mg/day	340.[c]	1,906.	
Cigarette smoke condensate, Kentucky reference IR1, μg/100 cigarettes	7.6	264.0	Severson et al. 1976

[a]Concentration units specified.
[b]Also includes chrysene and triphenylene.
[c]Also includes chrysene.

have sufficiently long residence times in the lower atmosphere (generally a few days) to allow dispersal of combustion products over hundreds or thousands of kilometers (Suess 1976). The particulate-sorbed PAH can enter the aquatic environment directly through fallout and precipitation or indirectly through runoff. Pyrosynthesized PAH can also enter the environment through industrial and municipal wastewater. Benz(a)anthracene has been identified in these wastewaters (Table 3) and in the effluent from the coke by-products (Wedgwood 1952) and ammonium sulfate (Wedgwood and Cooper 1954) industries.

Fossil fuels represent a much smaller source of PAH than does pyrolysis, especially to the freshwater environment. On a local basis, however, a spill of petroleum can have significant impact. The differing chemical composition of fossil fuels makes it difficult to distinguish between PAH in the original structure and those produced during processing. The BA content is compared with the total PAH in several fossil fuels in Table 4.

There is some controversy regarding PAH synthesis by biological processes.

Table 3. Concentration of benz(a)anthracene (BA) and total polycyclic aromatic hydrocarbons (PAH) in wastewater effluents.[a]

Source	BA Concentration	Total Concentration of All PAH Measured	Reference
Municipal incinerator water			Davies et al. 1976
Input	0.03	0.27	
Output	0.64	2.26	
Output µg/day	16.0	56.1	
Wastewater effluents in the GFR			Borneff and Kunte 1965
Effluents to Rotack River			
Domestic	0.191	0.798	
Industrial	0.167	5.133	
Mixed sewage, highly industrial			
Stockach R.	1.360	14.980	
Rodolfzell R.	0.343	8.558	
Domestic Sewage, Henge, GRF			Borneff and Kunte 1965
Dry weather	0.025	0.846	
During heavy rain	10.360	87.500	
Wastewater effluents			Brown and Starnes (unpub.)
Dual media filter	31.0	271.6	
Final refinery effluent	0.03	2.61	
Municipal effluents	0.0	0.18	

[a]Concentration presented as ppb, unless otherwise stated.

Table 4. Concentration of benz(a)anthracene (BA) and total polycyclic aromatic hydrocarbons (PAH) in various fossil fuels.[a]

Source	BA Concentration	Total Concentration of All PAH Measured	Reference
Petroleum materials			Pancirov and Brown 1975
S. Louisiana crude	1.7	91.1	
Kuwait crude	2.3	22.7	
No. 2 heating oil	1.2	83.5	
Bunker C	90.	656.	
Coal fluids			Pancirov and Brown 1975
Kentucky homestead feed coal	5.8	24.5	
Kentucky liquefaction product	0.2	2048.4	
Pennsylvania, manor feed coal	1.3	20.6	
Pennsylvania, liquefaction product	0.5	2295.1	
W. Virginia, Ireland mine feed coal	9.3	55.1	
W. Virginia, liquefaction product	0.4	10.0	
Processed oil shale			Maase 1980
Soxhlet extraction inorganic solvent, liquid-liquid extraction of water-developed sample, ppb	0.69-54.0[b]	14.05-1133.	
	0.5	66.8	

[a]Concentration presented as ppm unless otherwise stated.
[b]Also includes chrysene and triphenylene.

The presence of PAH has been demonstrated in a wide variety of plants from diverse sources (Guddal 1959, Borneff 1963, Grimmer 1966). PAH are frequently observed in plant meristems and have been shown to accelerate growth (in Andelman and Suess 1970). Various studies have attempted to confirm the direct synthesis of PAH by bacteria (Knoor and Schenk 1968) and algae (Borneff et al. 1968) using various substrates. Chlorella was found able to synthesize PAH (including BA, 6.9 µg/kg algae) using ^{14}C-acetate as a substrate (Borneff et al. 1968).

Considerable evidence suggests that PAH may be synthesized during the reduction of polycyclic quinone pigments by bacteria, fungi, higher plants, and some animals (Thomson 1971). This may explain the presence of PAH in locations with no history of industrial pollution. Conditions suitable for reduction synthesis of PAH from biogenic extended quinones are most favorable in anaerobic basin sediments and waterlogged soils. Perylene, benzo(ghi)perylene and coronene are apparently synthesized in this way (Rose 1977, Wakeham 1977a, Meinschein 1959).

No data were found to support the formation of BA by reduction synthesis. Generally, it is agreed that biosynthesis contributes little to the global production of PAH. However, in localized areas and under certain conditions (e.g., anoxic sediments rich in lipid precursors), biosynthesis may be significant (Neff 1979).

Transport and Transformation of Benz(a)anthracene

Various abiotic and biotic processes reduce the concentration of PAH in water. These include volatilization, hydrolysis, sorption, photolysis, oxidation, bioaccumulation, metabolism, and microbial degradation (Figure 1). The dominant pathways for transport and transformation of any given PAH are determined by its physical properties. The general physical properties of BA are given in Table 5.

Volatilization

Given its low vapor pressure, BA loss through volatilization is very slow. Using Henry's Law coefficients a river under typical environmental developed by Southworth (1979), Herbes et al. (1980) calculated the half-lives of four representative PAH compounds in a river under typical environment conditions (1 m depth, 0.5 m/sec river velocity and 1 m/sec wind velocity). The values ranged from less than a day (napthalene, 2-rings) to two months (benzo(a)pyrene, 5-rings). For BA, the expected half-life was calculated to be about 3 weeks. The half-life figure predicted by Smith and colleagues (1978) was greater than 6 weeks for four different environments (i.e., stream, eutrophic pond, eutrophic lake, and oligotrophic lake). In comparison to the other transformation processes, which shall be presented below, the contribution that volatilization makes in the removal of BA from surface waters is negligible (Southworth 1979).

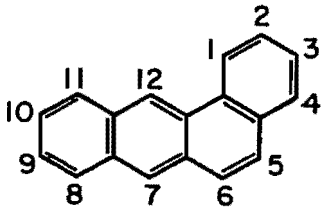
Hydrolysis

Hydrolysis does not account for any transformation of BA because this PAH contains no groups that are hydrolyzable.

Sediment partitioning of PAH

Due to its low aqueous solubility (approximately 11 µg/l at 27°C), BA quickly partitions to suspended organic and inorganic particulate matter within the aqueous environment. The distribution and persistence of the compound then become functions of the transport and fate of the particulates, and how the chemical environment of the particle surface affects the various transformation processes of the compound. The extent of partitioning of PAH is de-

Table 5. Physical properties of benz(a)anthracene.[a]



Molecular weight	228.28
Melting point, °C	155.7
Boiling point at 760 torr, °C	435 (sublimes)
Vapor pressure at 20°C, torr	5×10^{-9} (Pupp et al. 1974)
at 25°C, torr	1.1×10^{-7} (Radding et al. 1976)
Saturated vapor pressure, 20°C, torr	1333 (Radding et al. 1976)
Solubility in water at 27°C, ng/ml	11 (Davis and Parke 1942)
at 25°C, ng/ml	9.4 (May 1980)
Solubility dependence on temperature	
$0.00031\ t^3 - 0.0031\ t^2 + 0.1897\ t + 1.74$	(May 1980)
Enthalpy of solution, kcal/mole	10.71 ± 0.25 (May 1980)
Henry's law constant at 25°C	$3.28 \pm 0.46 \times 10^{-4}$ (Southworth 1979)
n-Octanol-water partition coefficient	3.98×10^5 (Southworth et al. 1978)

[a]From Smith et al. (1978).

pendent upon the nature of the particles. The factors affecting sorption are: organic matter, clay mineral content, clay mineral texture, pH, and the iron and aluminum hydrous oxide content. Organic matter is the most important factor controlling neutral organic adsorption (Aomine and Inoue 1967).

The partition coefficient, K_p, is used to describe the tendency for organic chemicals to adsorb. It is simply defined as:

$$K_p = \frac{P_s}{P_w \rho} \quad \ldots\ldots\ldots (1)$$

where P_s is the concentration of compound in supernatant at equilibrium (ppb), P_w is the concentration of compound on sediment at equilibrium (ppm), and ρ is the weight ratio of dry sediment to water (Zepp and Schlotzhauer 1979). Recorded values for the K_p for BA have ranged from 8,300 to 26,000 (Smith et al. 1978).

It is generally believed that PAH is maintained in the dissolved state through reversible desorption. Smith et al. (1978) determined K_p for both sorption and desorption of BA on sediments from Searsville Pond, California. The data showed that the K_p was the same, within experimental error, for both processes. Thus once contaminated, the sediments can be a continual source of BA to waters otherwise not having a regular input of the compound. In addition to sorbing onto sediments, BA readily sorbs onto living and dead biota (Smith et al. 1978). Herbes (1977) determined that adsorption of PAH (anthracene) may be an order of magnitude higher on suspended organic material than on mineral surfaces. Both

groups of researchers suggest sorption onto biota could be an important mechanism in biomagnification of PAH in the food chain.

Photodegradation

Photooxidation is the most important process by which BA is degraded in the aqueous environment (Herbes et al. 1976). For PAH in general, the process has been shown to be affected by several parameters including quality and quantity of radiation, oxygen content, quantity and nature of suspended material, and the presence of photosensitizers.

In early experiments, BA was found to be a photo-stable compound when exposed to the radiation output from cool fluorescent-type lights (Kuratsune and Hirohata 1962; Masuda and Kuratsune 1966). Degradation of BA was possibly observed under high levels of ultraviolet (UV) illumination (Inscoe 1964). McGinnes and Snoeyink (1974) studied the degradation of BA in aqueous solution (containing 20 percent acetone) exposed to various intensities of ultraviolet (UV) radiation (0.13 - 1.37 mW/cm^2). The decomposition rate was found to increase as a linear function of UV intensity. There did appear to be a threshold UV intensity ($\sim$ 0.25 mW/cm^2) below which no degradation occurred. These researchers also studied the degradation phenomenon of BA particles suspended in water without the addition of acetone. If acetone was not added to the solution, 1 mg/l of BA formed a suspension of variously sized particles. When a suspension of BA particles 0.625 and 1.25 m in diameter was exposed to UV irradiation, decomposition followed first order kinetics regardless of particle size. However, the decomposition rate was greater for the smaller diameter particles. The rate was also found to be a linear function of radiation intensity.

Reports on the influence of suspended solids on PAH degradation have been contradictory. Suess (1967) originally predicted that PAH would persist in turbid waters because the transmittance of UV radiation would be decreased. However, Miller and Zepp (1979) measured UV penetration in turbid water and concluded that photolysis rates within the photic zone are generally more rapid in turbid than in clear water due to the increased diffusion of light caused by scattering. McGinnes and Snoeyink (1974) studied the effects of various concentrations of suspended clay solids (0 - 1000 mg/l) on BA photo-decomposition and found that the extent of decomposition was only slightly decreased at the highest clay concentration.

Little research has been done on the use of photosensitizers to promote the degradation of PAH. However, many organic solvents (e.g. acetone and chloroform) are known to enhance PAH degradation. It is for this reason that much of the early research used to predict the persistence of PAH may underestimate the permanence of these pollutants in the natural environment. This criticism has been specifically directed toward the research of McGinnes and Snoeyink (1974) and Suess (1967) by Zepp and Schlotzhauer (1979).

The major photodegradation products of PAH compounds are endoperoxides or diones. Specifically, BA decomposes via the 7,12-endoperoxide to the 7,12-quinone (Figure 2). The degradation mechanism, however, has not been elucidated: McGuinnes and Snoeyink suspect the major degradation pathway to begin with singlet oxygen exciting BA to the triplet state. One possibility is that photooxidation-degradation process transfers energy from the triplet state of PAH, yielding singlet oxygen ($^1O_2$), which reacts with the excited PAH to yield the peroxide. Some researchers (Andelman and Suess 1970; Smith et al. 1978) have reported that photochemical transformations of certain PAH, including BA, can occur without molecular oxygen. This has led Zepp and Schlotz-

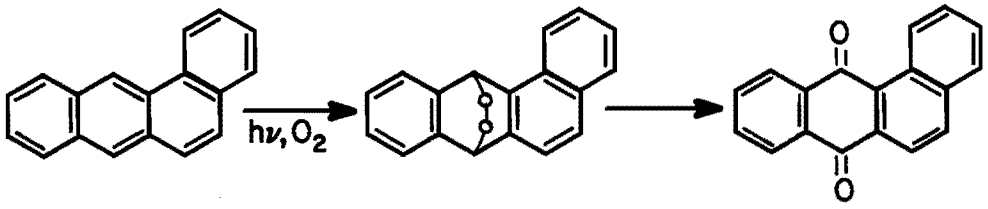


Figure 2. Photodegradation pathway of benz(a)anthracene to form benz(a)anthracene-7,12-quinone via benz(a)anthracene 7,12-endoperoxide.

hauer (1979) to suggest that the predominant primary photodegradation process of PAH in water may not involve molecular oxygen (i.e. singlet oxygen). The rate of photolysis of the PAH, pyrene, in water was studied (Zepp and Schlotzhauer 1979) comparing distilled water, water from the Gulf of Mexico, and water from the Aucilla River. The river water is known to absorb large amounts of UV and visible radiation with the production of singlet oxygen. The photolysis rates at monochromatic UV irradiation (330 nm) and under natural sunlight conditions were identical for the three waters. This finding supports the hypothesis that photosensitized oxidation of PAH is negligible. Their computations indicate that the photolysis reactions of many PAH are rapid throughout the upper mixed layers of water bodies (top 35 m), and in turbid waters the reaction is slow due to light attenuation and chemical removal through partitioning to suspended matter.

Chemical oxidation

A number of chemical oxidants degrade aqueous PAH: chlorine, ozone, peroxides, and nitrogen and sulfur oxides (NAS 1972). Since chlorine and ozone are widely used in water treatment, many investigators have studied their effects on PAH. The most thorough work was performed by Harrison and coworkers (1976a,b). Using sodium hypochlorite at a concentration of 2.2 mg/l "free chlorine" and constant conditions with regard to temperature (20°C) and pH (6.8), the degradation rates of several PAH (including BA) were observed. The rates were hyperbolic with time, and BA oxidized rapidly ($t_{1/2}$ = 25 minutes). As the free chlorine concentration increased (0.44 to 13.2 mg/l), the rate of oxidation during the initial 5 minutes of contact also increased. Temperature only slightly increased the rate of reaction between 5 and 20°C. The pH had a strong influence on the rate, most likely through its effect on the hypochlorous acid dissociation equilibrium. The maximum rate of degradation of BA occurred at the lowest pH tested, 4.5. In 5 minutes contact time, with 2.2 mg/l "free chlorine" and temperature equal to 20°C, about 45 percent of the compound was removed. Chlorination products were not identified.

Ozone reacts readily with PAH in water. The ozonolysis products for BA (and other PAH) have been studied thoroughly (Figure 3). Radding et al. (1976) used data of Il'nitskii et al. (1968) to calculate the rate constants and PAH half-lives for the reaction of 5 mg/l ozone with PAH at 25°C. For BA, the calculated half-life was 27 minutes.

Bioaccumulation, metabolism, and microbial degradation

Numerous studies have documented the ability of aquatic organisms to accumulate, metabolize, and excrete PAH. Organisms exposed to PAH (whether

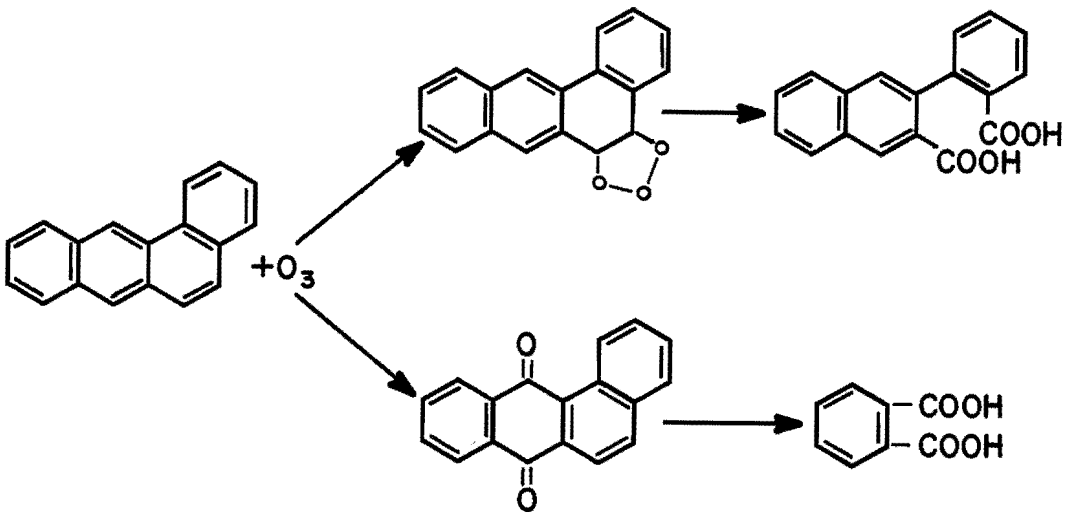


Figure 3. Proposed reactions of benz(a)anthracene with ozone. (From NAS 1972.)

in oil-water dispersions, the water-soluble fraction of crude oils, or individual radio-labeled PAH compounds) readily sequester these pollutants and store them in lipid deposits. When exposed to clean water, the parent compounds are released from tissues, but some inextractable material and metabolites remain (Malins 1977).

Lee et al. (1978a) studied the accumulation and depuration rates of several PAH by oysters. The compounds were accumulated in the order of their molecular weight. The lighter PAH (naphthalene, anthracene, and fluoranthene) were taken up to a greater extent than the heavier PAH (BA and BaP). This phenomenon is probably related to the greater water solubility of the 2- and 3-ring PAH as opposed to the 4- and 5-ring PAH. The order of release of the accumulated PAH is the same, the lower molecular weight PAH are released first (half-life 2.5 days), and BA and BaP are released more slowly (half-lives 9 and 18 days, respectively).

Species vary widely in their ability to absorb and assimilate PAH from food. In most cases, the accumulation of PAH is more efficient from water than from food or sediment. Polychaete worms (Neanthes) excrete PAH unmetabolized when it contaminates their food (Rossi 1977). Fish will assimilate PAH from food; however, the adsorption of PAH from the gut is limited and variable depending upon the species of fish, the PAH, and the type of food. Crustaceans readily accumulate PAH from contaminated food. In all instances where assimilation was demonstrated, metabolism and excretion of PAH was rapid.

Sediment-adsorbed PAH have only a very limited bioavailability to aquatic organisms. Marine polychaete worms were unable to accumulate napthalenes from the sediment although they were observed to ingest the sediment, and pass it through their digestive tracts (Rossi 1977). Similar results occurred with crude oil-contaminated sediments and sipunclid worms (Anderson et al. 1977). While clams were shown to accumulate napthalenes from the sediment, the tissue samples contained lower concentrations of the PAH than did the sedi-

ments which indicated uptake was inefficient (Fucik et al. 1977).

The enzyme system responsible for degrading PAH in mammals (known as the cytochrome P-450-dependent mixed function oxidase, the mixed-function oxidase (MFO), or the aryl hydrocarbon hydroxylase (AHH) system) renders lipophilic materials more water soluble and therefore more available for excretion. As the oxidative metabolism proceeds, the first intermediate is a highly electrophilic arene oxide. For certain PAH, these intermediates are highly toxic, mutagenic, or carcinogenic. Depending upon as yet unknown ambient conditions in the organism, the active epoxides may be transformed to less toxic products by various enzymatic or non-enzymatic reactions (Figure 4). The enzymatic reactions include the microsomal epoxide hydrases which convert the epoxides to dihydrodiols, and the glutathione-S-transferases which catalyze the formation of glutathione (GSH) conjugates. Non-enzymatic reactions are those with GSH, solvolysis to form diols, and isomerization of arene oxides to phenols (Bend et al. 1977, Corner 1975).

Other organisms that possess the cytochrome P-450 enzyme system and thus

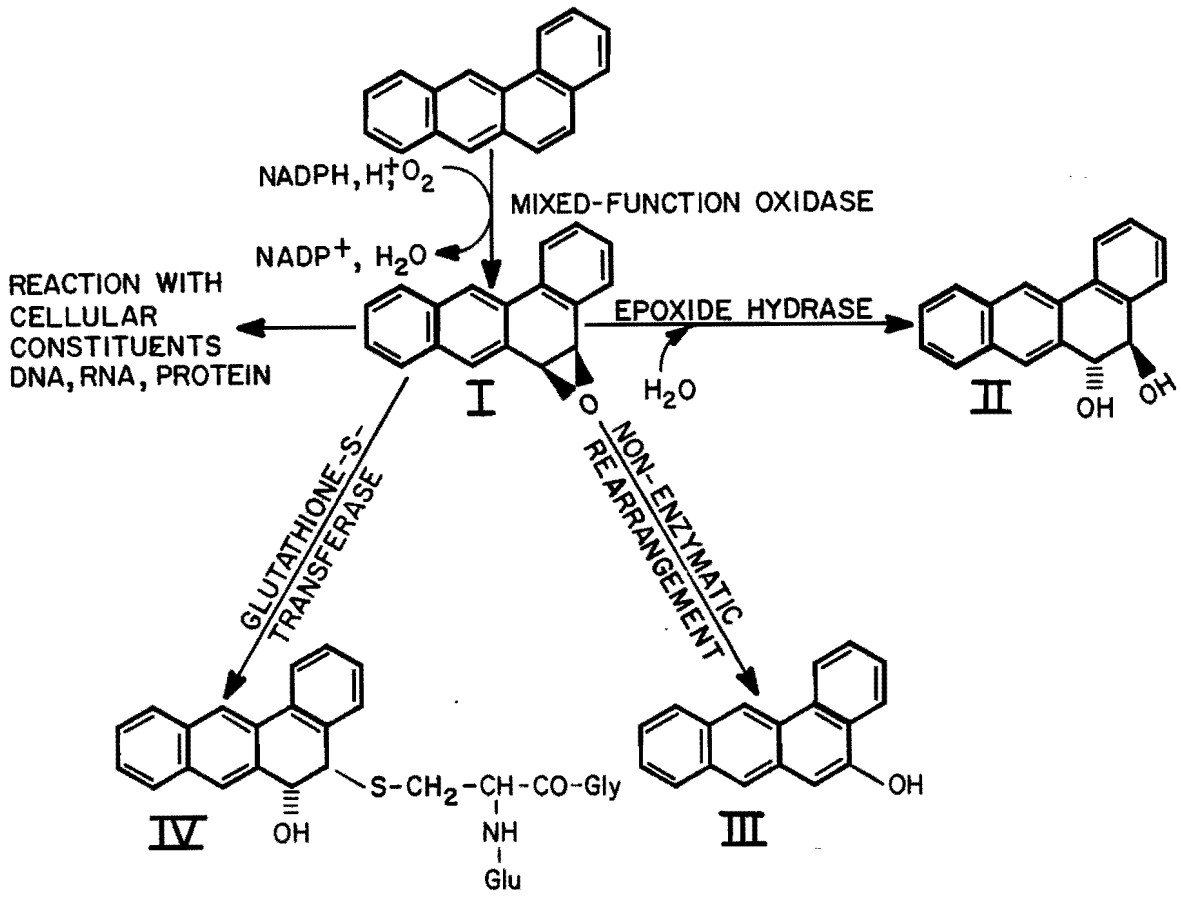


Figure 4. Proposed metabolic pathways of benz(a)anthracene degradation: I, benz(a)-anthracene-4,5-epoxide; II, trans-4,5-dihydro-4,5-dihydroxybenz(a)anthracene; III 4-hydroxybenz(a)anthracene; IV, benz(a)anthracene-4-glutathione. (From Swaisland et al. 1973.)

degrade PAH by the arene oxide pathway characteristic of mammals are fungi, most of the boney fishes (Teleosti), some amphibia, and many invertebrates. Enzyme activity has been demonstrated in invertebrates, including 12 species (all fresh water) representing five phyla: Coelenerata (Hydra), platyhelminthes (Dugesia), annelida (leech), mollusca (clam and snail), and several arthropoda (Khan et al. 1974). Included in the latter group were an isopod, an ampipod, a water flea, a copepod, a crayfish, a dragon fly larva, and a mosquito larva. There have been a few assays specifically oriented toward assessing the ability of invertebrates to utilize BA. Three members of the annelida (marine species) have been found to produce a BA metabolite that has been identified as the 5,6-dihydrodiol (Lee et al. 1978b). Another study showed that the marine polychaete (Capitella) increased the rate of BA degradation from the sediment surface (Lee et al. 1978b). The authors suggested that this organism detoxified the PAH making it more readily available to attack by other organisms. Another explanation (Neff 1979) is that worm activity provided aeration or nutrients which enhanced microbial degradation.

The enzymatic pathways for microbial PAH degradation have been elucidated. At one time it was thought that bacteria used the same MFO reaction as do mammalian systems. However, Gibson identified a different oxygenation mechanism. The process begins with incorporation of one molecule of oxygen into the aromatic nucleus with the production of a cis-dihydrodiol (Gibson 1977; Gibson et al. 1975). The hydroxyl groups may be ortho or para to one another. Further oxidation of the cis-dihydrodiol leads to the formation of catechols which are substrates for enzymatic fission of the aromatic nucleus (Gibson 1977). Figure 5 presents a comparison of bacterial and MFO-catalyzed metabolism of PAH.

Several species of bacteria are able to metabolize PAH. In most cases the higher molecular weight PAH such as BA and BaP cannot serve as a sole source of carbon (Dean-Raymond and Bartha 1975) but may be partially metabolized if an alternative growth substrate is available (Gibson 1977). A mutant produced from the native Beijerinckia isolated from a polluted freshwater stream was found to metabolize BA to cisdihydrodiols (Figure 6) (Gibson et al. 1975).

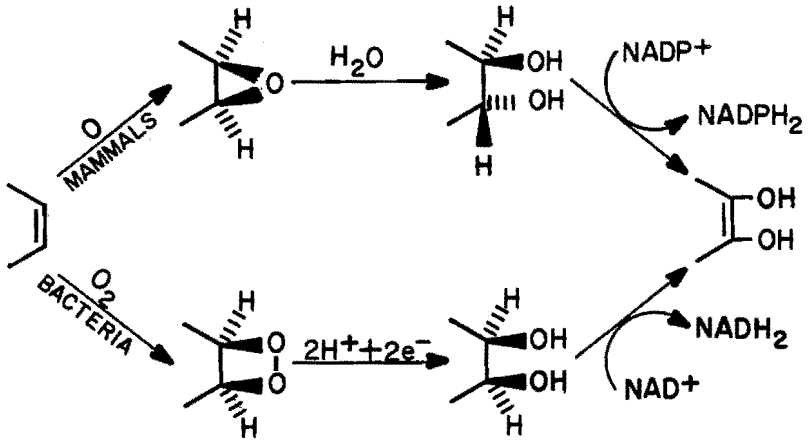


Figure 5. Comparison of the degradation pathways for aromatic structures by mammals and bacteria. (From Gibson 1976.)

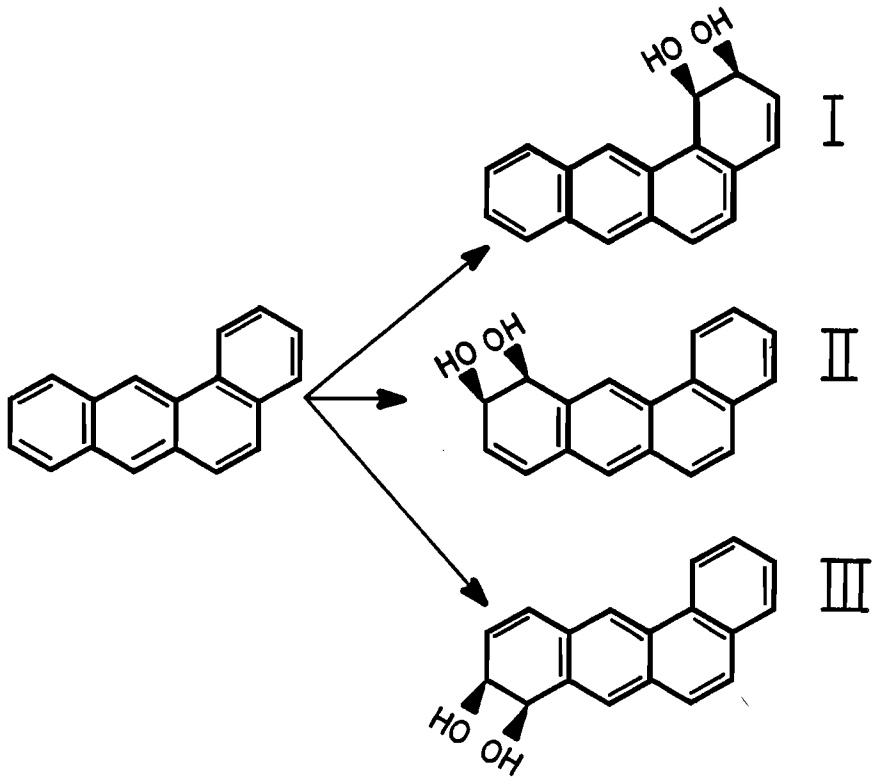


Figure 6. Cis-dihydrodiols produced from benz(a)anthracene by the mutant bacterium, Beijerinckia B-836: I, cis-1,2-dihydro-1,2-dihydroxybenz(a)anthracene; II, cis, 9,10-dihydro-9,10-dihydroxybenz(a)anthracene; III, cis, 7,8-dihydro-7,8-dihydroxybenz(a)anthracene. (From Gibson et al. 1975.)

Dean-Raymond and Bartha (1975) isolated one strain of Pseudomonas from the oil polluted estuarine waters of the Arthur Kill River (New Jersey) which was able to metabolize BA.

The ability of natural microbial populations to metabolize BA has also been examined. In some instances, prior exposure of the organism to petroleum hydrocarbons enhances the rate of degradation. This was probably the case in the research in which Walker et al. (1976a,b) demonstrated that BA could be degraded by bacteria present in Chesapeake Bay sediments (Pseudomonas and Acinetobacter). Also, their substrate was a mixture of petroleum hydrocarbons, and the BA may have been utilized in co-oxidation. Sisler and Zobell (1947) demonstrated BA metabolism (by CO_2 production) in a mixed culture of marine bacteria. Lee (1977) found that samples of polluted and unpolluted waters degraded some PAH (napthalene and BaP) but not BA. This lack of metabolism was also observed when water samples from controlled ecosystem enclosures were incubated with radiolabeled BA (Lee et al. 1978a). Degradation (as measured by $^{14}CO_2$ production) did not even occur in an enclosure which had been dosed with the water soluble fraction of No. 2 fuel oil.

No experimental work has yet confirmed an ability of algae to degrade PAH. A study of the accumulation and release of ^{14}C-napthalene by the freshwater green alga, Chlamydomonas angulosa, showed no evidence

of metabolite production (Kauss et al. 1973; Soto et al. 1975). Instead this PAH appeared to sorb onto the organism and be released when the organism was placed in a clean medium. Because napthalene (a 2-ring PAH) is much more readily degraded by many organisms (microorganisms, invertebrates, and vertebrates) than is BA, this finding supports the speculation that algae would be unable to degrade BA. In the only other species investigated, Fucus, a marine kelp, Payne (1977) found no enzyme-capability to degrade PAH (measured by aryl hydrocarbon hydroxylase activity).

Biological Effects

Organic chemical pollutants directly harm sensitive individuals or species resulting in reduced reproduction and/or growth rates, impaired functioning, abnormal behavior and development, and sometimes death. These species level effects indirectly alter ecosystem structure, function, and stability as changes in species composition change community productivity.

Most research on the biological effects of PAH describe the acute toxicity of the lower molecular weight compounds, particularly napthalene and its methylated derivatives. The biological response to the higher molecular weight PAH (BaP, BA, pyrene, and others having four or more aromatic rings) is more subtle. The explanation is that exposure is limited by the extremely low aqueous solubility of these compounds. At these levels, the danger is less in immediate toxicity and more in long term subtle (sublethal and cancerous) effects (Neff 1979). More detailed information may be found in a review article by Neff (1979).

Sublethal effects

Growth of a red alga, Antithamnion, was found to be depressed in assays using BA and four of its derivatives (Boney and Corner 1962). The response was dose-dependent, and higher concentrations (10-300 µg/l) caused more inhibition of growth. Somewhat different results were obtained with Escherichia coli as the test organism. Bacterial growth was inhibited by most of the PAH tested; however, PAH with highly angular configurations (BA and BaP) promoted growth (Hass and Applegate 1975). Growth stimulation by PAH (10-20 µg/l) has also been reported with freshwater algae. Gräf and Nowak (1966) found that the degree of stimulation of Chlorella, Scenedesmus, and Ankistrodesmus was correlated with carcinogenic activity in animals. In order of effect, these PAH were: benzo(a)pyrene > benz(a)anthracene > benzo(b)fluoranthene, indeno (1,2,3-cd)pyrene > benzo(ghi)perylene.

Abnormal development has been demonstrated in various aquatic animals exposed to the higher molecular weight. Some embryotic growth stages are more sensitive than others. For example, exposure of sea urchin eggs (Paracentrotus) to protein-bound BaP prior to fertilization results in abnormal cleavage after fertilization. Cleavage terminates at the morula stage, and embryos contain many dead cells (Ceas 1974). If embryos are exposed at a later stage (blastula or gastrula), development is normal. Similar results were found in the embryonic development of the sea urchin, Psammechinus (Bresch et al. 1972).

The behavior of certain aquatic organisms has been shown to be altered by PAH. Benzo(a)pyrene (0.2 to 1.0 µg) injected into the visceral sac of freshwater mussels (Driessena, Anodonta, and Unio) significantly reduced the rate of filtration (Haranghy 1956).

Various indicators of physiological function have been used to assess the long-term effect of PAH on aquatic organisms. While napthalene has been shown to inhibit photosynthesis in a few algal genera (Kauss et al. 1973, Vandermeulen and Ahearn 1976), this effect has

not been demonstrated for the higher molecular weight PAH.

Cancer

Organisms vary substantially in sensitivity to PAH-induced carcinogenesis. This may be due to differences in the levels of MFO-cytochrome P-450 activity and the specific functioning of these enzymes. It has been shown that aquatic animals produce the active metabolites necessary for carcinogenesis (Stegeman 1977; Payne et al. 1978) and that PAH can produce cancer-like growths and cause teratogenesis and mutagenesis in some aquatic vertebrates and invertebrates. While few studies of this nature have been conducted, a higher incidence of tumors and hyperplastic diseases are found in organisms from polluted environment than from uncontaminated areas; however, PAH has not been specifically implicated.

Overall Fate and Effect of BA in the Aquatic Environment

Predictions

Laboratory studies of the individual processes which determine the fate of BA have been used to predict its persistence and major pathway of degradation under various aquatic regimes. Similar results were obtained at Stanford Research Institute (Smith et al. 1977, 1978) and Oak Ridge National Laboratory (Herbes et al. 1980).

The investigations by Smith and colleagues (1978) suggest that BA accumulates in the sediment and biota of the aquatic environment. Desorption from contaminated sediments maintains a low concentration of BA in the overlying water. The dissolved compound is primarily transformed by photooxidation, but chemical oxidation can also occur. Although these investigators did not observe biodegradation of BA, it is possible that this may take place at very slow rates. Table 6 presents the half-lives of dissolved BA for the transformation or removal processes following a spill as calculated by an elaborate prediction model (Smith et al. 1977). Although half-lives vary among water bodies, photolysis and sorption consistently dominate the transformation and removal processes. Sorption half-lives have not been measured, but generally equilibrium is reached in less than half an hour. These data suggest that BA may be more persistent in eutrophic than in oligotrophic waters for two reasons: 1) photolysis is less effective in turbid waters and 2) sorption to suspended solids tends to remove BA from solution and become a source through desorption over a long time period.

Based on laboratory measurements of compound removal rates for four major transport processes, Herbes et al. (1980) predicted that BA is primarily removed in a shallow rapidly flowing stream through photolysis (81.2 percent), sediment sorption (17.5 percent), and volatilization (1.3 percent). Removal by microbial transformation is negligible. These researchers also predicted BA transport behavior in a clear river, a turbid channelized river, and a reservoir (Figure 7). Increases in the calculated half-lives occur from the clear river to the channelized river to the reservoir, and changes occur in the processes which dominate removal. Photolysis is important in all three systems, but it becomes less important as water becomes more turbid. Sedimentation and microbial transformation account for greater portions of BA loss as water velocity decreases. Turbidity seems to govern loss due to sediment sorption. Model validation from measurements of PAH in water and sediments downstream from a large coal coking wastewater discharge showed PAH to be at least as persistent as predicted.

Microcosm studies

The hypothesis that most of PAH, which enter the aquatic environment

Table 6. Predicted rates of transformation and transport of benz(a)anthracene in various aquatic environments.[a]

Process	Stream	Eutrophic Pond	Eutrophic Lake	Oligotrophic Lake
Photolysis, half-life (hr)	20	50	50	10
Oxidation, half-life[b] (hr)	38	38	38	38
Volatilization, half-life (hr)	>1000	>1000	>1000	>1000
Hydrolysis, half-life (hr)	NA	NA	NA	NA
Biodegradation, half-life (hr)	Slow	Slow	Slow	Slow
Half-life for all processes, except dilution (hr)	13	22	22	8
Half-life for all processes, including dilution (hr)	0.55	22	22	8
Amount BA sorbed[c] (mg m^{-3})	2.5	7.5	1.25	1.25
Percentage BA sorbed	71%	88%	55%	55%

[a] From Smith et al. (1978).

[b] Based on peroxy radical concentration of 10^{-9} M.

[c] 1 ng ml^{-1} BA is assumed in the solution phase.

partition to suspended material which accumulates in reservoir sediments, was tested in several outdoor marine enclosure microcosm studies. Of the four studies published, three were concerned with the fate of assorted compounds contained in various petroleum products, and only one of these (Hinga et al. 1980) included BA. The fourth study was designed to determine the overall biogeochemistry of BA in an enclosed marine ecosystem.

The microcosm utilized by Gearing and associates (1980) contained 13 m^3 of flowing seawater, 0.8 m^3 of silty clay sediment from mid-Narrangansett Bay and associated biota. Semi-weekly doses of no. 2 fuel oil were added in the form of an oil-seawater dispersion during a 4-month period (March to July). The average concentration of oil-derived hydrocarbons in the water column was 93 µg/l. Samples throughout the period showed the petroleum hydrocarbons to be associated with particulates in inverse proportion to their aqueous solubility. The mixing of sediments due to burrowing activity and the reduced rates of biodegradation in these zones may cause certain of these compounds, including branched alkanes, cycloalkanes, and aromatics (10-20 percent of the original sediment hydrocarbons) to persist for at least a year.

In another study (Lee and Takahashi 1977), the water soluble fraction of No. 2 fuel oil was added to different 60 m^3 enclosures at three initial concentrations (10, 20, and 40 µg/l non-volatile hydrocarbons). Water samples were taken at various intervals and depths and were analyzed for nutrients, photosynthetic productivity, species composition, and biomass. In addition,

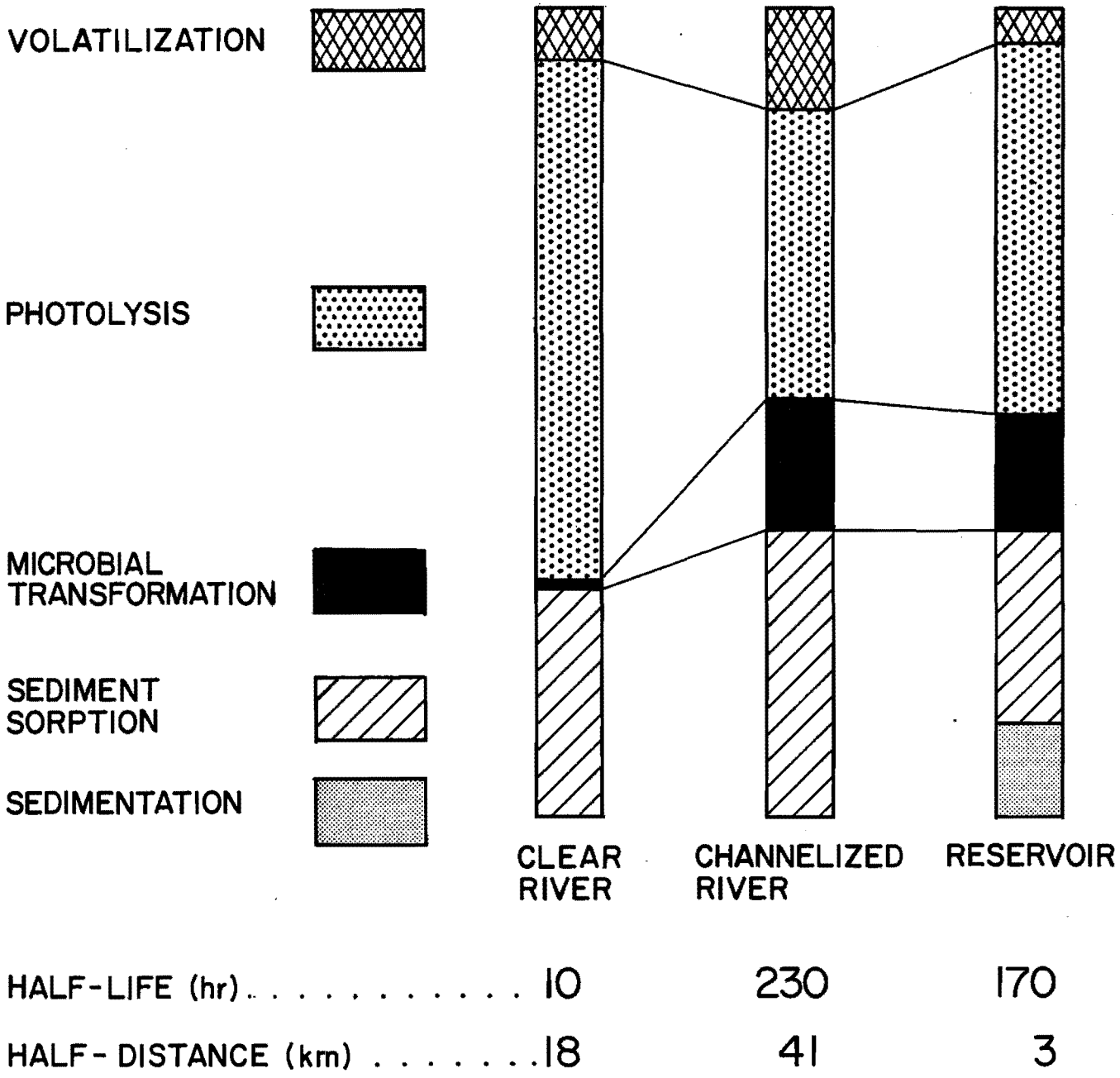


Figure 7. Predicted transport behavior of benz(a)anthracene in several representative aquatic systems. (From Herbes et al. 1980.)

bacterial degradation of hydrocarbon was measured by adding radio-labelled hydrocarbons (some PAH, but not BA) to water samples from these systems. These samples were incubated and analyzed for radioactive carbon dioxide.

Results corroborated predictions. Higher molecular PAH (represented by BaP) were lost from the water column through sorption to sinking particles including plankton. Acclimation of a microbial population that could degrade BaP was observed. The degradation rate was zero prior to the oil addition and increased slightly over 3 days. The most interesting observations were found in the change in species composition which occurred at the two higher concentrations. At 20 µg/1, there was a bloom in a microflagellate, Chrysochromulina, whose growth was shown to be stimulated by water extracts of fuel oil. The highest concentration used was 40 µg/1. Over a period of 3 days, this concentration decreased to 20 µg/1 and, again, Chrysochromulina became dominant. The control treatment plankton was dominated by diatoms. Other organisms (tintinnids and rotifers) also increased; this was not the case in the control enclosure.

The same experimental approach was also used by Lee and his colleagues (1978a). This experiment was continued for 72 days, as opposed to only 19 days in the earlier experiment. Microbial degradation of ^{14}C-labelled PAH was evaluated by measurement of $^{14}CO_2$ production. In addition, estimates were made of the rates of loss of initial PAH due to evaporation, photochemical oxidation, microbial degradation and sedimentation. The higher molecular weight PAH (BaP and BA) became associated with colloidal and detrital material, sank and accumulated in the bottom sediments. Bacterial degradation of BA and BaP was not demonstrated in either treatment or control enclosures. BA accumulated in tissues of zooplankton (primarily the copepod, Pseudocalanus) during the first 4 days; but after 9 days, no PAH could be detected in animal tissues. The authors presumed that some of the PAH were degraded. It was concluded that BA has a brief residence time in marine waters because it is removed from solution by sedimentation and transformed through photooxidation. Degradation of BA by organisms in the water column is assigned minor importance.

BA was observed in a 230 day marine microcosm study conducted by Hinga and associates (1980). Carbon-14 labeled BA (72 ng/1) was introduced into a system containing 13 m^3 seawater and a 30-cm layer of sediment. The biological system was similar to that of shallow coastal waters of the northeastern United States. Water, suspended particulates, and sediments were collected frequently and analyzed for BA and its metabolic products. The radiotracer label made it possible to follow the removal of the compound from the water column, its appearance and mixing into sediments, and the production of $^{14}CO_2$.

The results paralleled those from other marine microcosm experiments. BA was rapidly removed from the water column through sorption. The half-life of ^{14}C activity was about 50 hours. The radio-activity of the particulates (colloids, phytoplankton and zooplankton) decreased with a half-life of about 35 hours for the first 5 days and then more slowly until it became undetectable. The decrease in ^{14}C activity exceeded that expected due to the rate of washout (3.7 percent per day) in the tank.

These researchers demonstrated that the major transport mechanism for removal of BA from marine waters is sorption and sedimentation. Photooxidation was excluded as a process for BA transformation; thus this experiment represents a worst case situation. Unlike other microcosm studies, this study detected evidence of BA metabolism in the upper layer of sediment. With

much reluctance the authors extrapolated from the observed rate of CO_2 production that it would take 2-3.5 years for complete mineralization of BA. Because of uncertainties over how seasonal changes affect biodegradation, interaction of the environmental risk associated with various levels of aromatic hydrocarbons in coastal systems is difficult to predict quantitatively.

Summary

In marine ecosystems, the fate of BA and other higher molecular weight PAH is fairly well understood. Due to the hydrophobic nature of these compounds, they partition to suspended particulates and are deposited to the sediments. The solution overlying the contaminated sediments dissolves a portion of the compound, but the majority is degraded at rates largely determined by the quantity and quality of radiation which penetrates through the water column. Given sufficient time for acclimation and the required combination of environment and organisms, BA is metabolized to CO_2. At the concentrations which have been studied (1 µg/l), neither the parent compound, BA, nor its metabolic products have been shown to have a toxic effect on marine organisms.

The experiments conducted in the study reported here were designed to test the response of a freshwater community native to Lake Powell (Utah/Arizona) to a higher dose of BA. This "worst case" situation was achieved by providing sufficient BA to maintain a maximum concentration of compound in the dissolved state and by controlling environmental conditions such that the rate of photolysis would be negligible. Various parameters indicative of community structure and function were monitored.

METHODS

Microcosms

The microcosm experimental units were similar in design and operation to those used by Porcella et al. (1975) and Medine (1979) except that they were glass rather than plastic. The microcosms (Figure 8) were constructed of beaded-process pyrex pipe and consisted of a cylinder 60 cm high and 15 cm in diameter closed above by a shallow end cover and below by a deep cap. The tube and end pieces were connected and sealed airtight with gaskets: the inner gasket

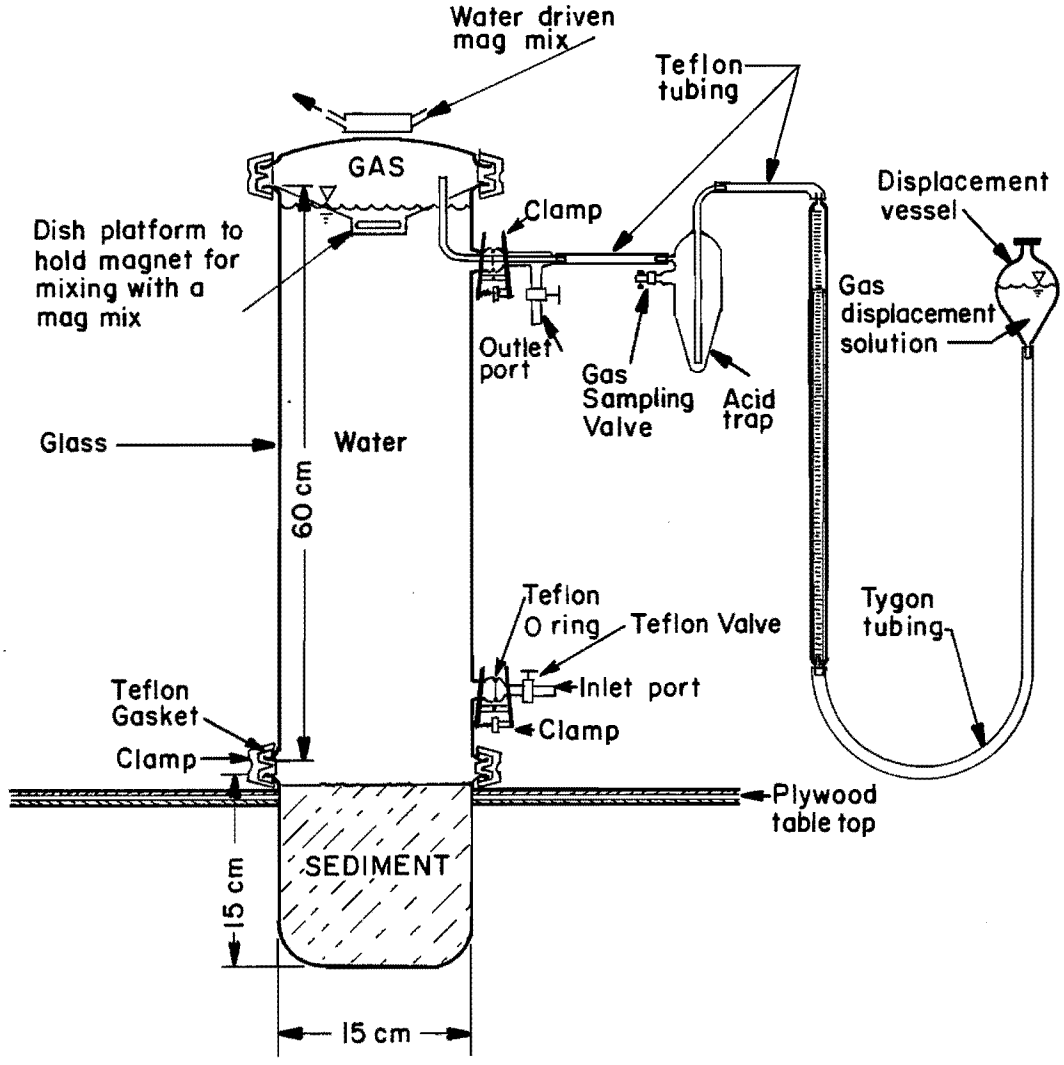


Figure 8. Schematic design of microcosm.

teflon and the outer rubber, held with a screw-tightened stainless steel band. Upper and lower ports, fitted with teflon stopcocks, were constructed in the wall of the pyrex tube for sampling the liquid phase and for supplying fresh medium. Inside the cylinder, a 6 cm diameter pyrex petri dish supported from above was positioned just beneath the water surface. It contained a teflon stir bar for mixing the microcosm liquid. The stir bar was spun by an outside water-driven magnetic mixer.

A small volume, low displacement gas-trap was installed to capture and measure gas production and consumption. A short pyrex tube (0.6 cm i.d.) passing through the upper port connected the microcosm headspace (0.9 ℓ volume) through approximately 50 cm of teflon tubing (0.6 cm i.d.) with a manometer consisting of a 50 ml buret and a leveling bulb. The manometer contained a 2.5 percent H_2SO_4 solution plus methyl red for color (Andrews et al. 1964). Between the headspace and the buret was a 50 ml volume trap which 1) delayed transfer of the acidic solution contained in the manometer into the microcosm when the pressure inside the headspace decreased rapidly (i.e., when the rate of medium coming out of the microcosm exceeded the rate going in), and 2) allowed the sampling of headspace gas composition through an attached teflon stopcock.

Experimental Design

Eight microcosms were used in four experiments to determine the most effective combination of methods for 1) handling benz(a)anthracene, BA, (introduction, extraction, and analysis), and 2) monitoring the biological response to BA (metabolism, nutrient cycling, biomass, succession, and bioaccumulation). The upstream portion of Lake Powell (Utah/Arizona) was chosen as the source of sediments, biota, and water quality parameters for nutrient medium composition. This lake area is a potential sink for energy-related effluents containing particulate-sorbed PAH carried by the White, Green, and Colorado Rivers which drain much of the coal, tar sands, and oil shale formations in the western United States.

Sediments were collected on two occasions (December 1979 and August 1980) at Hite, Utah, from a depth of approximately 10 m using an Ekman dredge. They were placed in a teflon-lined 208 liter (55 gallon) drum, sealed, and transported to the Utah Water Research Laboratory. The sediments were passed through a sieve (15.85 mm opening) to remove large rocks, plant debris, and other foreign material. The screened sediments were returned to the drum and stored at 4°C. Prior to use, the sediments were thoroughly mixed. Approximately 5 kg wet weight (3.8 kg dry weight) of sediment was added to each of the eight microcosms, filling the deep cap to within 1 cm of its upper lip (2.5 ℓ volume). The outer surface of each cap was covered with black tape to exclude light from the entire depth of the sediments. A removable tape strip was put down the length of the cap to allow observations of changes in the sediments.

Microcosms were initially filled with 11.5 liters of synthetic Lake Powell nutrient medium (Table 7), which was replaced on a semi-continuous basis at a rate of approximately one liter per day. This medium was adapted from the nutrient algal assay medium developed by EPA (USEPA 1971), but it has several modifications including a four-fold reduction in nitrogen and phosphorus, an addition of total dissolved solids (calcium, magnesium, sodium, and potassium salts), and increases in alkalinity (15 mg/l to 125 mg/l) and silica to enhance diatom growth.

Twenty liters of medium were prepared every second day from concentrated stock solutions. The medium was aerated (with compressed air) and cooled approximately 5°C below microcosm water temperature prior to medium exchange.

Table 7. Medium constituents and concentrations (from Medine 1979).

Stock Soln.		Conc. in Stock Soln. mg/l	Dil. in Feed Distilled Water	Element	Final Conc. in Microcosms µg/l
A	$NaNO_3$	2550	2.5 → 1000	N	1,050
B	K_2HPO_4	101	2.5 → 1000	P	45
C	H_3BO_3	186	1 → 1000	B	33
	$MnCl_2$	264	1 → 1000	Mn	115
	$Na_2MoO_4 \cdot 2H_2O$	7.3	1 → 1000	Mo	2.9
	$CoCl_2$	0.8	1 → 1000	Co	0.35
	$CuCl_2$	0.01	1 → 1000	Cu	0.004
D	$FeCl_3(FeCl_3 \cdot 6H_2O)$	160	1 → 1000	Fe	33
	$Na_2EDTA \cdot 2H_2O$	300	1 → 1000	$Na_2EDTA \cdot 2H_2O$	300
E	Lake Powell Synthetic Feed Solution				
	$CaCl_2 \cdot 2H_2O$	163.9	10 → 1000	Ca^{++}	80,000
	$CaSO_4$	120.5	10 → 1000	Mg^{++}	30,000
	$MgSO_4 \cdot 7H_2O$	308.0	10 → 1000	Na^{+}	101,000
	Na_2SO_4	108.7	10 → 1000	K^{+}	4,330
	K_2SO_4	9.58	10 → 1000	$CO_3^{=} + HCO_3^{-}$	177,000
	$NaHCO_3$	21,250	10 → 1000	Cl^{-}	79,000
	Silica, Si	5.0	1 → 1000	$SO_4^{=}$	284,000
				Si	5,000

The temperature differential effectively reduces column mixing and therefore contamination of microcosm water samples by fresh medium (Porcella et al. 1975, Medine 1979). The temperature stratification thus developed broke down in about 20 minutes with mixing provided by the stir bar. The addition of dissolved gases with the fresh medium was calculated according to Henry's Law (Porcella et al. 1975).

A mixed culture of Lake Powell phytoplankton was used to inoculate the microcosms after the water column had cleared of sediment fines (about 5 days). In the first two experiments, this culture was grown from the water overlying the sediments in cold storage. In the last two experiments, plankton were collected at Lake Powell and transferred to fresh medium for growth until needed.

Microcosms were subjected to two environmental light regimes. Some (either 2 or 4 depending on the experimental design) were placed in complete darkness in order to simulate hypolimnetic conditions; the dark microcosms also had slightly lower temperatures than the lighted microcosms. The remaining microcosms were subjected to a summer-like diurnal cycle of 16 hours of light and 8 hours of darkness. Lighting was provided by six 2.5 m fluorescent

bulbs (Duro-Test Corp.) positioned horizontally 40 cm above the top of the microcosms. The lamps emitted 1.2 $W \cdot m^{-2}$ of irradiation in the visible spectrum (Llambda Model LI-185 Radiometer) at a position midway down the length of the upright microcosms and less than 35 $mW \cdot m^{-2}$ in the near ultraviolet (UV-A) spectrum (Gamma Scientific Autophotometer Model 2900 with monochromator). The first two experiments were conducted at 20.0 ± 1.2°C (standard deviation), the latter two were conducted at 19.0 ± 2.0°C.

Analytical Procedures and Microcosm Response

The impact of the BA on the freshwater model ecosystems was assessed from metabolic activity, nutrient cycling, biomass, and species composition. Two estimates of metabolic activity were made. One was based on the total volume of gas produced daily, while the other was based on diurnal oxygen mass balance determinations separated into light (photosynthesis) and dark (respiration) periods. Daily leveling of the manometer attached to each microcosm, along with record of temperature and atmospheric pressure, gave the volume information used to calculate daily gas production. The measured daily gas volume was corrected to standard temperature and pressure (STP) by:

$$V_{STP},\ ml = \frac{(P - VP_{H_2O}) * V * (273.15^{\circ}C)}{T * 760\ mm\ Hg} \quad . \ . \ . \ (2)$$

where

V_{STP} = daily gas volume corrected to standard temperature and pressure, ml

P = daily atmospheric pressure, mm Hg

VP_{H_2O} = vapor pressure of water at ambient temperature, T, mm Hg

V = volume of headspace and gas trap of individual microcosm and buret, ml

T = daily microcosm temperature in °C + 273.15°C

VP_{H_2O} was determined (CRC 1976, Table D-180) for temperatures between 15.6 and 24.0°C as

$$VP_{H_2O},\ mm\ Hg = -1.3649 + 0.93653 * T_{water},\ ^{\circ}C \quad . \ . \ . \ (3)$$

Weekly or semi-weekly gas compositional data were used to calculate atmospheric oxygen (O_2), carbon dioxide (CO_2), and methane (CH_4) production.

Gas samples were collected with a syringe and analyzed on a Hewlett-Packard 5750 research gas chromatograph. Instrument operating conditions were:

Detector - Thermal Conductivity

Columns - 1.8 m x 3 mm o.d. stainless-steel, containing 60-80 molecular sieve 5A (O_2, N_2, CH_4)
- 1.8 m x 3 mm o.d. stainless-steel, containing 100-120 Porapak S (CO_2)

Carrier Gas - Helium

Flow Rates
Carrier Gas - 35 ml/min
Tank Pressure - 55 psig

Temperature
Column - 55-60°C
Detector - 115°C
Injector Port - 50-55°C

Known standards of all gases detected and air samples were run on a routine basis in conjunction with samples analyzed from the microcosms. The area under each peak was calculated by an electronic integrator (Hewlett-Packard model 3380A) and used to estimate the mole fraction of each gas.

The equation used to calculate the total mass of O_2 in the headspace was

$$\text{Mass } O_2, \text{ mg} = V_{STP}, \text{ ml} * \frac{1 \text{ mole } O_2}{22{,}415 \text{ ml}} * \frac{O_2\%}{100\%} * \frac{32{,}000 \text{ mg } O_2}{\text{mole } O_2} \quad . \; . \; . \; (4)$$

The calculation of total net O_2 production was corrected for the dissolved oxygen content of the microcosm (Eq. 5, Porcella et al. 1975; Medine 1979) by

$$\left[\begin{array}{l}\text{Net in } O_2 \\ \text{content in} \\ \text{microcosm} \\ \text{during 1} \\ \text{day period}\end{array}\right] = \left[\begin{array}{l}\text{Total } O_2 \text{ content} \\ \text{(all phases) in} \\ \text{microcosm before} \\ \text{medium exchange} \\ \text{on current day}\end{array}\right] - \left[\begin{array}{l}\text{Total } O_2 \text{ content} \\ \text{(all phases) in} \\ \text{microcosm after} \\ \text{medium exchange} \\ \text{on previous day}\end{array}\right] \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; (5)$$

Linear interpolation provided values between days for which gas composition was computed.

Throughout the duration of the experiment, microcosm productivity was measured by diurnal oxygen mass balance determination. In a 24-hour period, analysis of dissolved and atmospheric oxygen content was determined three times: immediately prior to lamps coming on, following lamps going off, and again after the dark period before the lamps came on. Daytime oxygen mass production and nighttime oxygen consumption were calculated by adding the change in mass of oxygen in the dissolved and gaseous phases during the respective periods, and correcting for the dissolved oxygen added and removed during medium exchange (Equations 2-5).

Water quality analyses were conducted on microcosm medium samples at regularly scheduled intervals. The analyses performed and the methods used are listed in Table 8.

Biomass was measured by weekly samples analyzed for total organic carbon. Planktonic species composition was determined by microscopic examination of weekly samples prepared by the technique of McNabb (1960). The frequency of 12 common species (or species groups in the case of diatoms) was used as a measure of comparison.

Introduction of BA into Microcosms

Four methods for introducing BA into the microcosms were attempted with the primary objective to provide a constant low level concentration of dissolved BA:

Method 1. Prior to medium exchange approximately 10 µg of BA per liter of medium were added in an emulsion with benzene. This emulsion was accomplished by injecting 1 ml of the BA in benzene solution (10 µg/ml) into 1 ℓ of medium under the cone of a sonifier at a slow rate (1 ml/min). The contents were continually stirred during medium exchange.

Method 2. Lake Powell sediment was dried overnight, then ground and sieved (No. 100 sieve). Ten grams were measured into 125 ml Erlenmeyer flasks fitted tightly with ground glass stoppers. BA dissolved in benzene (10 µg in 20 ml) was added to each flask. The flasks were covered with aluminum foil and placed on a shaker table and mixed for 16 hours. After a period of settling, the solvent was decanted. The contaminated sediment was allowed to dry on a watchglass in the dark. Based on

Table 8. Procedures for analyses performed at the Utah Water Research Laboratory.

Parameter	Method	Reference
pH	Glass Electrode	APHA 1975
Alkalinity	Potentiometric	APHA 1975
Organic Carbon	Ampoule Method	Oceanography International Model 0524B Carbon Analyzer User Manual
Dissolved Oxygen	Winkler Method, Azide Modification	APHA 1975
Nitrate	Cadmium Reduction	APHA 1975 (Automated)
Nitrite	Diazotization	APHA 1975 (Automated)
Ammonia	Indophenol	Solorzano 1969
Orthophosphorus	Ascorbic Acid	Strickland and Parsons 1968
Total Phosphorus	Acid Digestion	USEPA 1976
Total Bacterial Count	Pour Plate	APHA 1975

the initial BA input and the quantity remaining in solution (determined fluorometrically) the quantity of contaminated sediment required to provide 2 mg BA to each of the treatment microcosms was determined. The amount of BA initially added was calculated using literature values for BA solubility, sediment-water partition coefficients, the estimated length of experiment, and washout rate. The contaminated sediment (3.6 g) was thoroughly mixed with four separate samples of wet sediment (approx. 500 g dry weight) in 400 ml pyrex beakers and layered over the 4.5 kg (wet weight) of sediment previously placed in the bottom of each microcosm cap. This contaminated upper sediment layer was approximately 2.5 cm thick. Four treatment microcosms were contaminated in this way. The four control microcosms were treated in the same manner with benzene-treated sediment only.

Method 3. A small quantity (83.6 g) corresponding to 50 ml of wet sediment was weighed into each of four 250 ml pyrex beakers. Two milligrams of BA in methanol (4 ml of 0.5 mg/ml) totalling 2 mg were added to the sediment, hand-mixed with a glass rod, and allowed to sit in the dark at room temperature overnight. The next day, another 83.6 g of uncontaminated sediment was added to each beaker, the total contents of each beaker was mixed and then layered over the 5 kg (3.8 kg dry weight) of sediment previously placed in each microcosm. The approximate 100 ml volume of sediment added represents contamination of the top 0.5 cm. Four treatment microcosms were contaminated this way, while four control systems were treated likewise, but with methanol-contaminated sediment only.

Method 4. A small quantity (20 g) of wet sediment was weighed onto each of four pyrex watchglasses. An additional 2 g of dried and sieved (No. 100 sieve) sediment were placed on top. The dried sediment was contaminated with BA in benzene solution (200 µl of 10 mg/ml)

for a total of 2 mg BA per microcosm. The benzene was allowed to evaporate. The contaminated sediment was mixed with 90 g of wet sediment, wetted slightly to allow pouring, and spread on top of 5 kg (3.8 kg dry weight) of wet sediment previously measured into the bottom of the microcosm. Four treatment systems were prepared in this way. Four control systems were treated identically with benzene addition only.

Extraction of BA from Medium and Sediments

Reagents

Practical or technical grade solvents were used to minimize costs. Solvents were purified (see procedures for n-hexane and benzene, Vogel 1939) and/or redistilled in an all-glass still before use. Solvents were stored in glass containers and were protected from contact with plastics or rubber at all times.

Benz(a)anthracene (99+ percent pure) and benz(a)anthracene-7, 12-dione were obtained from Eastman Kodak Co. (Rochester, N.Y.). Anthracene (98+ percent pure) was obtained from Aldrich Chemical Co. (Milwaukee, Wis.). These chemicals were used without further purification.

Sample preparation and storage

Water samples were collected from the microcosms in amber-colored (2.2 ℓ) glass bottles and immediately subjected to liquid-liquid extraction. Sediment cores were collected by inserting glass tubing (2.5 cm i.d.) through the sediment, sealing the upper end with a cork, removing the tubing from the sediments, and corking the bottom end. These cores were stored in the freezer. The frozen cores were cut into segments (0-1 cm and 1-2 cm) placed into cellulose thimbles (which had been subjected to soxhlet extraction for 4 hours with dichloromethane). The cores and thimbles were wrapped in aluminum foil, and stored in the freezer. Algal samples to be extracted were filtered onto pre-washed, pre-combusted, and pre-weighed glass fiber filters (Whatman GF/C). The filters were placed in pre-extracted cellulose thimbles, wrapped in aluminum foil and stored in the freezer. Other aquatic organisms (chironomid flies and daphnia) were collected on prepared glass fiber filters (Whatman GF/C), placed on aluminum filter containers and frozen.

Extraction

To avoid possible photodecomposition, all extraction and purification procedures were carried out under fluorescent lamps that were covered with a clear plastic film (Llumar manufactured by Chemplast, Inc., Wayne, N.J.) which eliminates radiation of wavelengths less than 400 nm.

Liquid-liquid extraction of microcosm medium was performed in 1 ℓ, separatory funnels with n-hexane. Initially three volumes of 10 ml each were used (Experiments 1-3), but the amount was increased to 30 ml for more efficient extraction of the internal standard (anthracene at 85 percent). Hexane extracts from several (3 to 7) days were combined in 250-ml volume separatory funnels for a weekly average. Excess water was drained out of the funnels, and the hexane was concentrated by roto-evaporation (temperature less than 30°C) to about 10 ml. Any remaining water was either physically removed by pipet or frozen. The solvent was then further concentrated under a gentle stream of N_2 gas to 0.5 ml, and stored in the dark in small glass vials (4 ml capacity, with teflon caps) until analysis.

Sediments were subject to two soxhlet extraction procedures:

Method 1. Sediments were soxhlet extracted for 24 hours in methanol (600 ml). Benzene was added (150 ml), and the solution was allowed to extract for

24 more hours. The extract was concentrated using the roto-evaporation apparatus to near dryness, and the flask was then rinsed five times with approximately 7 ml n-hexane each time. These rinsings were combined in a clean round-bottomed 500 ml flask, and concentrated by roto-evaporation to about 5 ml and then to 0.5 ml under a gentle stream of N_2 gas.

Method 2. Sediments were soxhlet extracted in a 1:1 solution of methanol and benzene (150 ml each). An internal standard (20 µg anthracene) was added prior to beginning extraction. After 24 hours, another volume of benzene was added (150 ml) and the solution allowed to extract for another 24 hours. The extract was placed in a separatory funnel. The organic solvent layer (benzene) was washed with tap water to remove salts while the aqueous layer (methanol-water) was extracted several times (3-5) with small aliquots (30 ml each) of benzene. The benzene extracts were collected, and concentrated by roto-evaporation and N_2 gas to 0.5 ml.

Algae collected on filters and stored in soxhlet thimbles were extracted in the same manner as the sediments in method #2.

Flies and daphnia collected on glass fiber filters were placed in small glass vials filled with n-hexane. The vials were wrapped in aluminum foil and allowed to shake for several days at room temperature. The hexane was then concentrated to 0.5 ml under N_2 gas and the samples stored.

Analysis of BA

Two methods were tried and evaluated for identification and quantification of BA. Fluorescence of the compound (excitation wavelength, 286 nm, and emission wavelength, 386 nm) using a Varian Model SF-330 spectrofluorometer was found to be a sensitive and accurate (to 10 ng/ml) for quantification of BA in pure solutions.

Computerized gas chromatography/mass spectrometry (GC/MS) was the alternative. A Hewlett Packard Model 5985A GC/MS system was used for GC separation in conjunction with mass spectrograph identification and quantification of peaks. Specific conditions were:

Column	-	10 m glass capillary coated with SP2100 (Supelco, Inc., Bellefont, Pa.)
Carrier Gas	-	Helium
Flow Rate	-	1 ml/min
Temperature	-	Oven - 100-250°C at 10°/min Injector - 150°C Detector - 200°C
Sample size	-	1 µl
Ionization Mode	-	Electron Impact
Electron Energy	-	70 eV
Emission	-	300 Amps
Ion Source Pressure	-	8×10^{-6} Torr

RESULTS

The first three experiments proved to be learning experiences that made possible the accomplishment of the project objectives in the fourth experiment. For the first three, the results presented are the lessons learned. For the fourth, the experimental findings are presented in greater detail.

Results of the First Experiment

In the first experiment, BA was added directly to the medium at a rate of 10 µg/l in 1 ml of benzene per day. A problem soon became apparent in that the dissolved oxygen (DO) and the atmospheric oxygen (O_2) concentrations steadily declined (for an example see Figure 9). Although there appeared to be some O_2 production in the lighted microcosms, after day 40 there was essentially no difference between the oxygen contents of the light and dark systems and no difference between treatment (BA) and control.

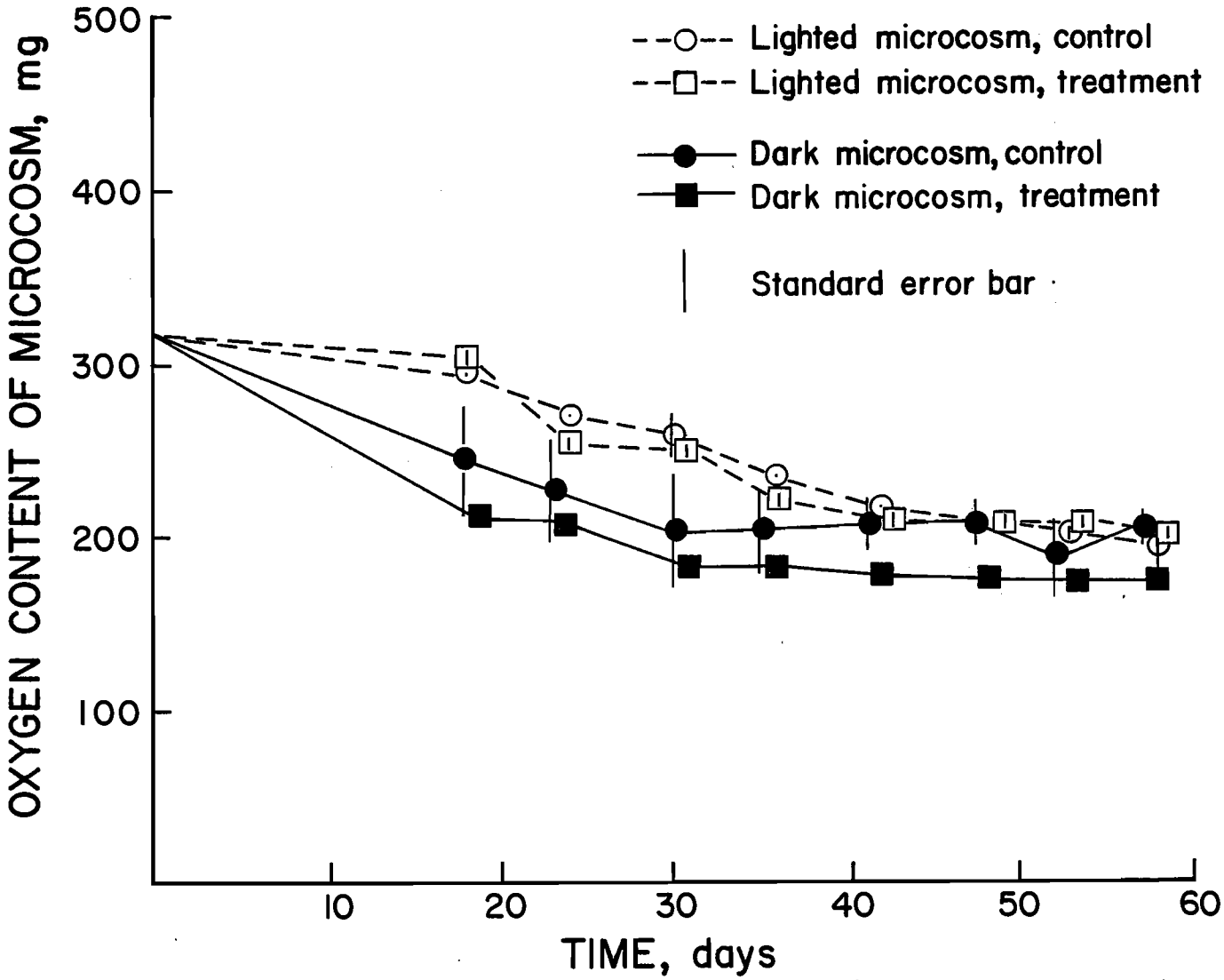


Figure 9. Total oxygen content (gaseous and dissolved) of microcosms during experiment #1.

Benzene is known to inhibit bacterial cell division at 92 mg/l and algal growth at 525 mg/l (Verschueren 1977). Before the concentration of benzene reached toxic levels, the lighted microcosms showed growth of algae. As the concentration of benzene increased, presumably the algae consumed oxygen, died, and exerted an oxygen demand. System dynamics essentially ceased when DO levels dropped below 1 mg/l.

In addition, this procedure was time consuming and relatively hazardous due to benzene volatilization. This methodology also failed to provide a constant concentration of dissolved BA in the system. During the first and second weeks, no BA was detected in the extracted medium samples. In the third and fourth weeks, estimated average BA levels (both light and dark systems) increased from less than 100 to about 550 ng/l; however, the accuracy of these values is questionable because of high background fluorescence. Overall the anoxic conditions overwhelmed any treatment effects.

Results of the Second Experiment

The second approach to introducing BA into the microcosm was to contaminate the sediments and rely upon the slow release of the compound to the aqueous phase dictated by the chemical equilibrium. BA was added to sediments in a solution of methanol (4 ml of 0.5 mg/ml solution). Again, the experiment was terminated because the oxygen in the closed system was rapidly declining (Figure 10).

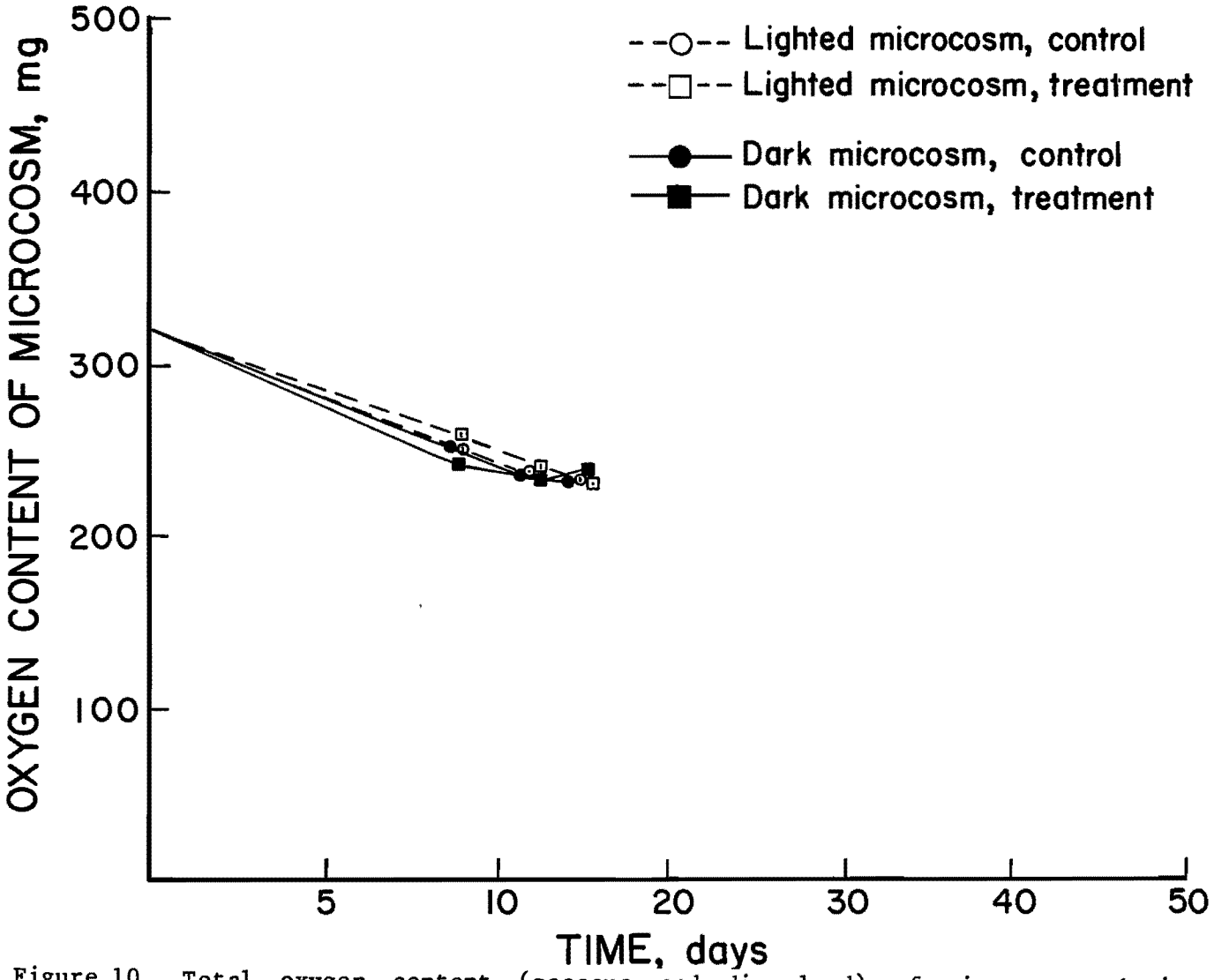


Figure 10. Total oxygen content (gaseous and dissolved) of microcosms during experiment #2.

The methanol concentration in the microcosms (0.28 g/l) was probably not sufficient to be toxic to algae and bacteria. The lethal dose for the bacterium, Pseudomonas, is 0.6 g/l and for the alga, Scenedesmus, it is 10 g/l (Verschueren 1977). However, methanol exerts a 5-day biochemical oxygen demand of 0.76-1.12 mg O_2/mg. At saturation, the maximum dissolved oxygen content of each microcosm was 90 mg, which is only 3 percent of that required for complete metabolism of the quantity of methanol present.

The experiment was terminated after 15 days. On day 11, the results indicate the average BA contents were 3.8 and 3.3 µg/l for the light and dark treatments, respectively. The slight difference is probably not significant. However, these values attest to the effectiveness of the contaminated sediments in introducing a significant quantity of BA into the microcosm medium.

Results of the Third Experiment

In the third experiment, the sediment was contaminated by adsorping BA from a solution of benzene. To avoid toxicity, the benzene was allowed to evaporate from the initially dry sediments prior to their mixing with the remaining sediment. The experiment ran for 47 days, and no signs of toxicity were observed. Also, further improvements were made in the analytical procedures, and sampling schedule was modified for more effective monitoring of system response and compound mass balance. These improvements provided data for evaluations of the techniques for measurement of metabolic activity and of the overall effect of treatment.

From Figure 11, the rate of oxygen production under light in the microcosms is shown to be greater during the active growing phase in the control microcosms than in the single microcosm treated with BA. (One of the two treatment microcosms was lost on day 10 due to an acid spill). This early difference in oxygen production rate does not appear to lead to a significant difference in steady state (days 25-47) oxygen content.

For determining diurnal patterns, productivity was measured frequently in order to estimate community photosynthesis and respiration. The data (Figure 12) show large variations (as indicated by vertical lines (standard error) superimposed on the data points) and do not correspond to the total estimated oxygen accumulation (Figure 13). It was determined that duplicate samples should be taken for DO measurement and be taken more frequently for diurnal pattern analyses.

BA could be detected in the liquid-liquid extracts of the medium. Extraction of 6 ℓ of medium proved sufficient for quantification of BA by fluorescence. In the lighted microcosms, the average BA concentration in the medium was 16.8 ng/l; and in the dark microcosms, it was 10.4 ng/l. Since these values were based on spectrofluorometric measurements, they may be low. Based on these estimates and GC/MS estimates of the BA content of the upper two centimeters of the sediments, a mass balance was calculated (Figure 14). As much as 33 percent of the compound was not accounted for. Some of the compound may have been undetected in the medium, a small amount may have been sorbed to the glass walls of the microcosms, and the remainder could possibly be deeper within the sediment.

The problems encountered in this experiment led to several methodological changes. Fluorescence, as a method to identify and quantify BA in liquid extracts, was abandoned due to interference from background fluorescence. Instead, the GC/MS was used for analysis. Samples were refrigerated for storage and analyzed as soon as possible. Another factor which may have

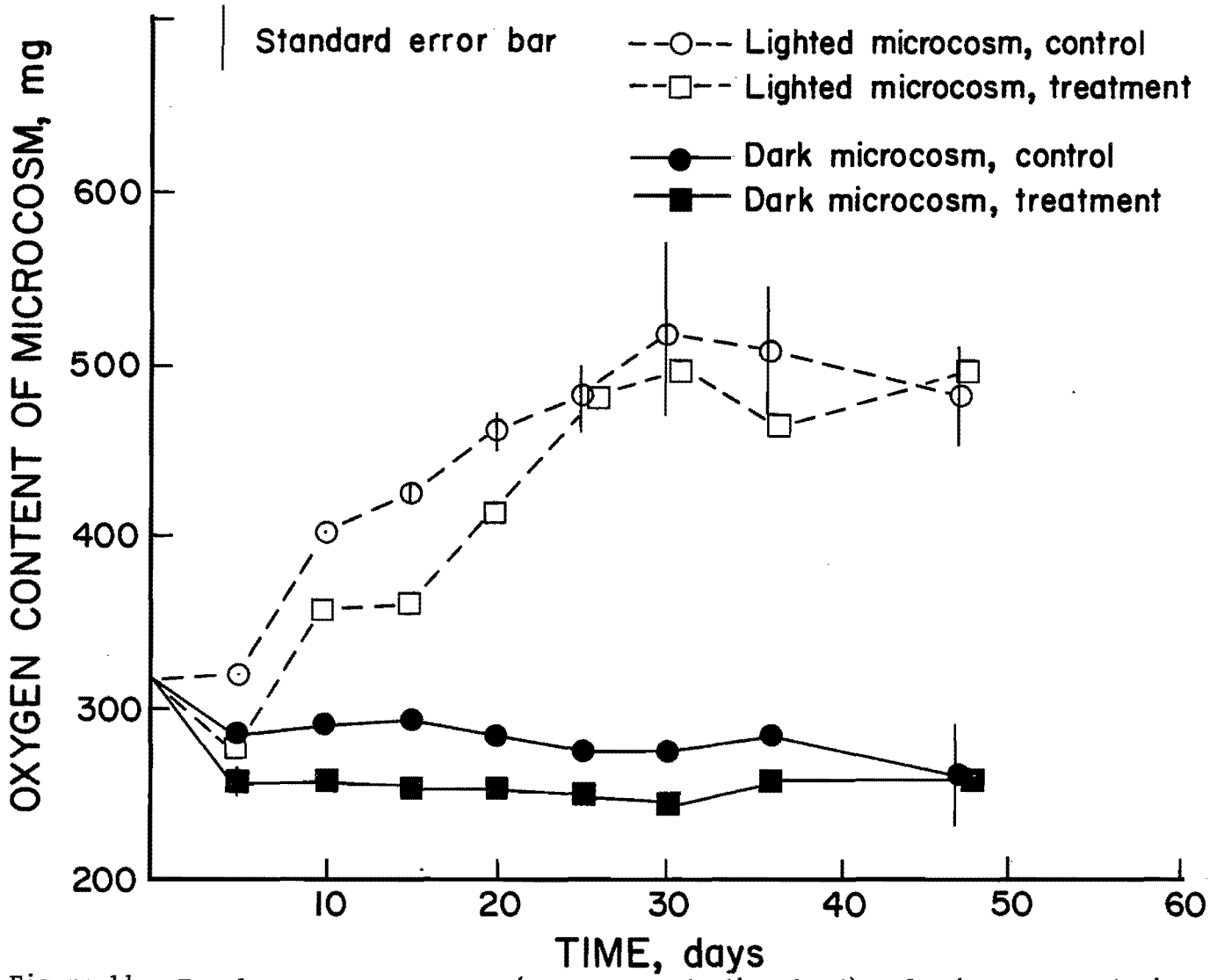


Figure 11. Total oxygen content (gaseous and dissolved) of microcosms during experiment #3. (No standard error bar indicates less error than the width of the data point.)

contributed to the low levels of BA in solution was its depth in the soil due to the relatively thick (2.5 cm) layer of contaminated sediment. Consequently, a thinner layer (1 cm) was contaminated in the next experiment. Also, the sediment extraction should have gone to depths at least twice that which was contaminated as benthic organisms are active in mobilizing PAH to deeper sediment depths (Hinga et al. 1980). Finally, there was a potential problem of accumulation of oxygen in the lighted microcosms to supersaturation levels (> 18 mg/l DO and >35 percent O_2). A procedure for purging the aqueous phase with breathing air was used when a predetermined maximum DO and O_2 content was reached. Also, since the procedure required the microcosms to be opened, the current method was inappropriate to compare system productivity based on total system oxygen content. Instead, the data were presented as total oxygen production for a given period. Each time the microcosm was opened, the mass of oxygen produced was added to that already accumulated.

Procedures for the Fourth Experiment

In the final experiment, the sediments were again contaminated with BA in a solution of benzene. The

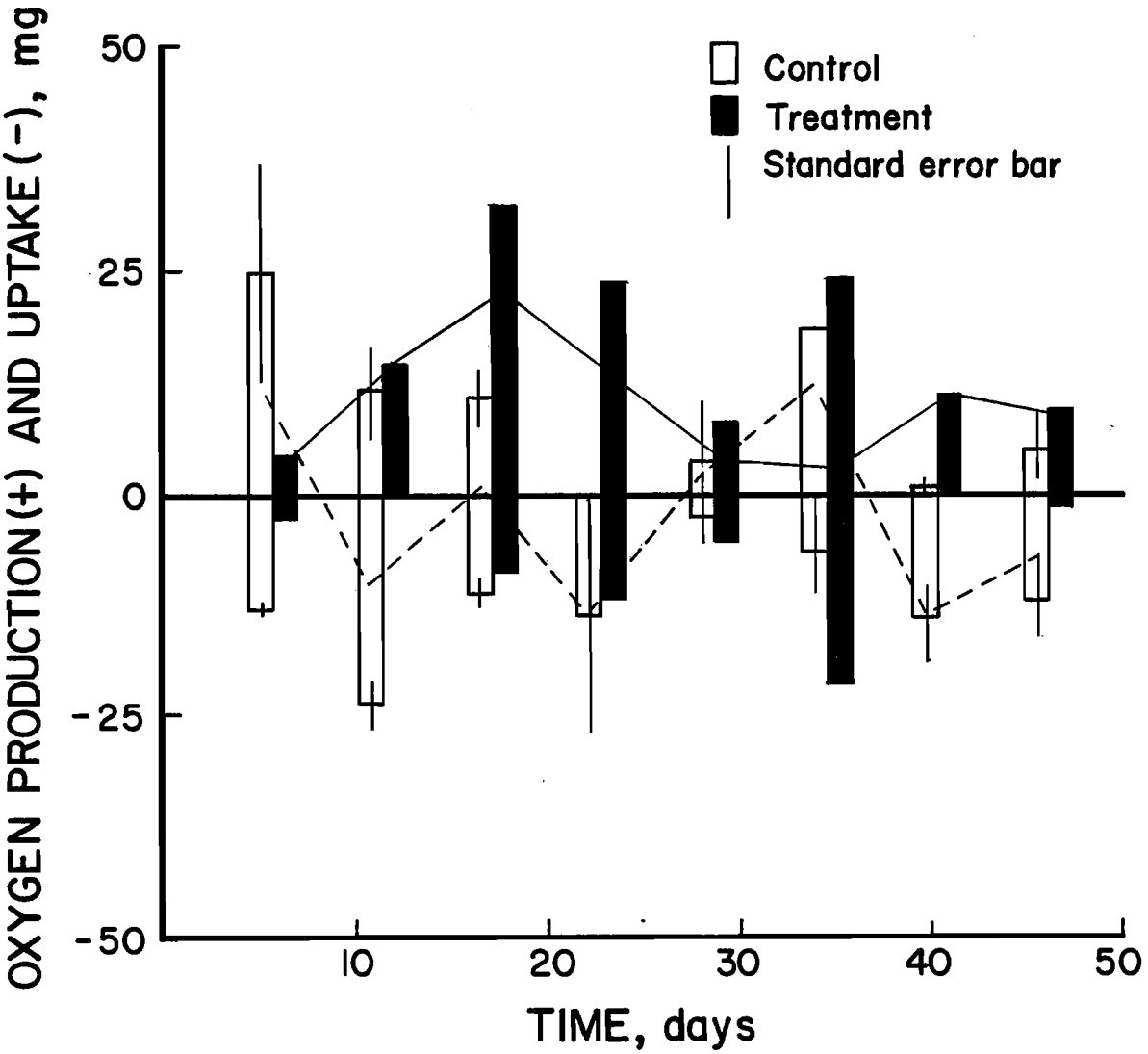


Figure 12. Diurnal oxygen production (indicated by bar length above the x-axis) and utilization (indicated by bar length below the x-axis) for control (#1 and #3) and BA treatment (#2) lighted microcosms. The lines (---) or (——) connecting bars indicate net oxygen production.

solution (200 µl of 10 µg BA/µl for a total of 2 mg), was added directly to the dried sediments, then the benzene was allowed to evaporate prior to mixing with the remaining wet sediments. The systems were purged, as previously described, on three days (14, 26, and 41) when the DO exceeded 15 mg/l and O_2 concentration reached 30 percent.

Diurnal productivity measurements were taken semi-weekly for the first 4 weeks and weekly for the final 4 weeks. The analysis was based on two replicate DO samples and three replicate O_2 samples. Nutrients, alkalinity, total organic carbon, and pH were analyzed on a weekly basis. Liquid-liquid organic solvent extractions were performed on 3 ℓ of medium/microcosm/week, and the extracts combined and analyzed by GC/MS on a weekly basis. In order to determine extraction efficiency, an internal standard (approximately 20 µg anthracene) was spiked into the first liter of medium extracted for that week. In addition to extracting the medium and any suspended material therein, 1 ℓ per

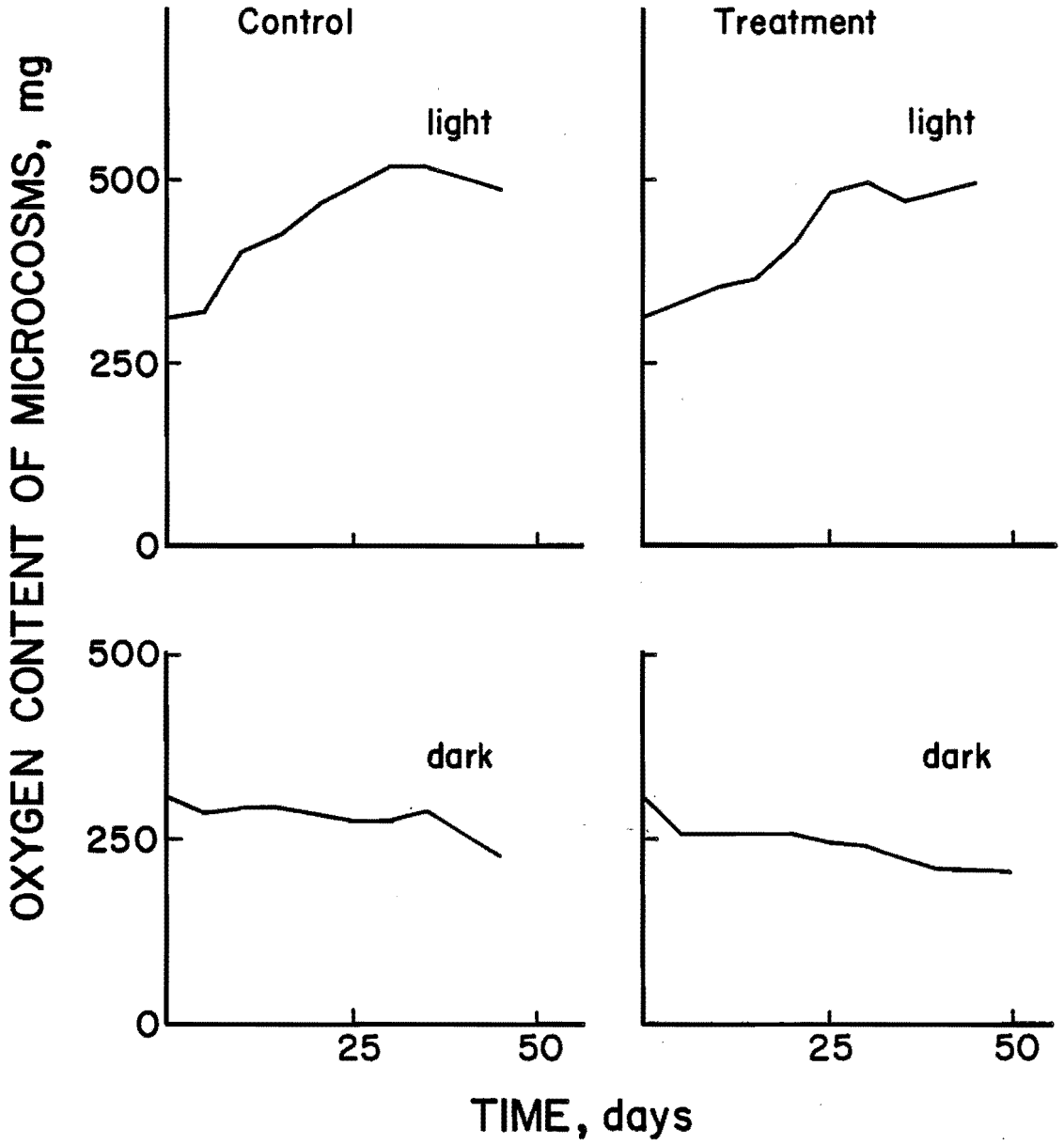


Figure 13. Total oxygen content (gaseous and dissolved) of control (#1 and #3, lighted; #5 and #7, dark) and BA treatment (#2, lighted; #6 and #8 dark) microcosms during experiment #3.

week was filtered through glass fiber filters, and the mass accumulated on the filter was extracted by shaking with hexane in a 4 ml vial for several days.

At the end of the experiment, 8 ℓ of medium were extracted, and the remainder was filtered. The material thus collected (algae) and the wall growth were combined and extracted using the soxhlet procedure developed for the sediments (second procedure outlined in the methods section). Sediments were cored, frozen, sectioned (0-1 cm and 1-2 cm depths) and soxhlet extracted. Finally the glass walls of the microcosm were washed down with benzene and the rinses were concentrated and analyzed.

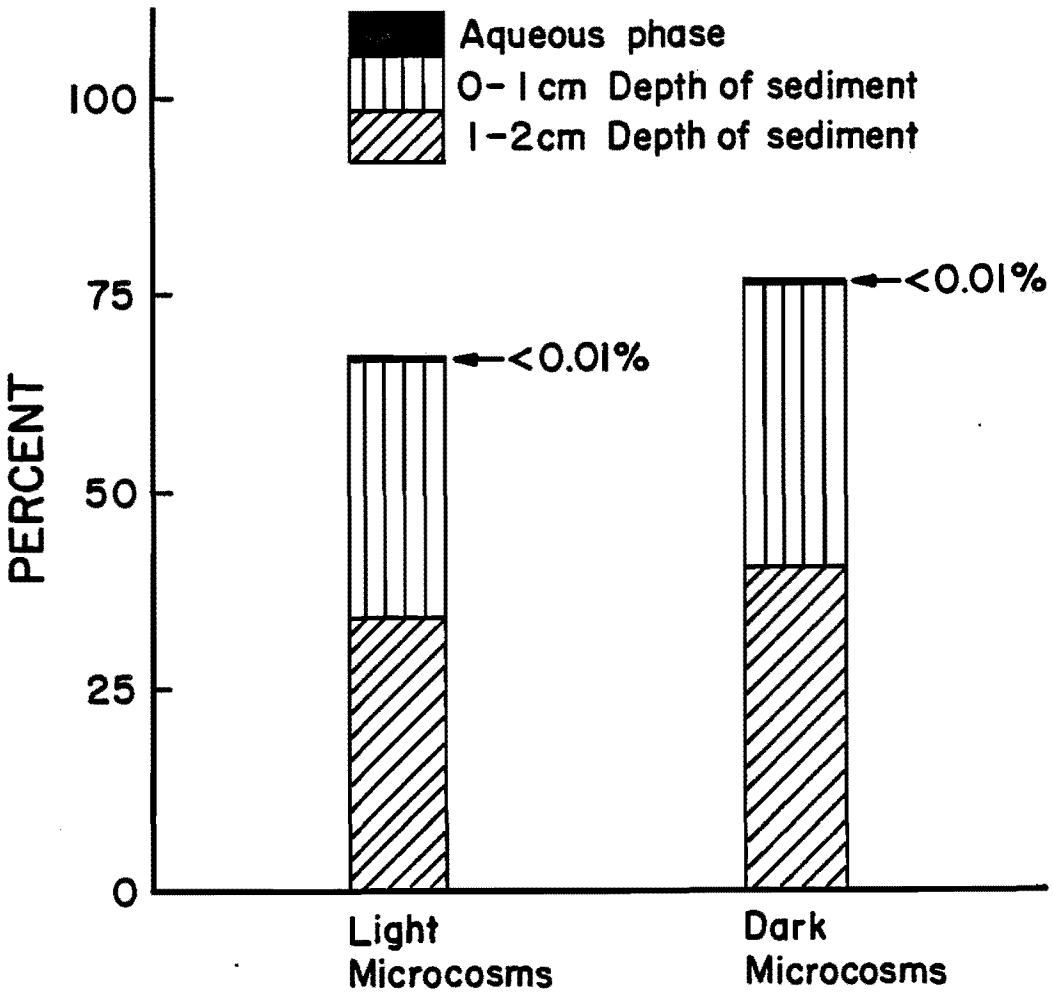


Figure 14. Mass balance of BA in lighted (#2) and dark (#6 and #8) treatment microcosms during experiment #3.

Results of the Fourth Experiment

The lighted microcosms displayed similar patterns of cumulative gas production (Figure 15). During an initial lag-phase (days 0 to 5), net gas production was minimal. This was followed by a period of greater gas production (days 5-15). In four of the six lighted systems, gas production gradually leveled off (after day 15) until daytime gas production during photosynthesis just compensated for nighttime gas uptake in respiration. In the remaining two systems the rate of gas production displayed during the early growth phase was maintained to day 60 when the experiment was terminated.

There was considerable within-treatment variation in the maximum gas production rate achieved, the length of time this production was maintained, and thus, the final total gas production achieved varied between treatments (Appendix B). In fact, the responses displayed by replicate microcosms varied so much that the difference due to treatment could not be considered significant.

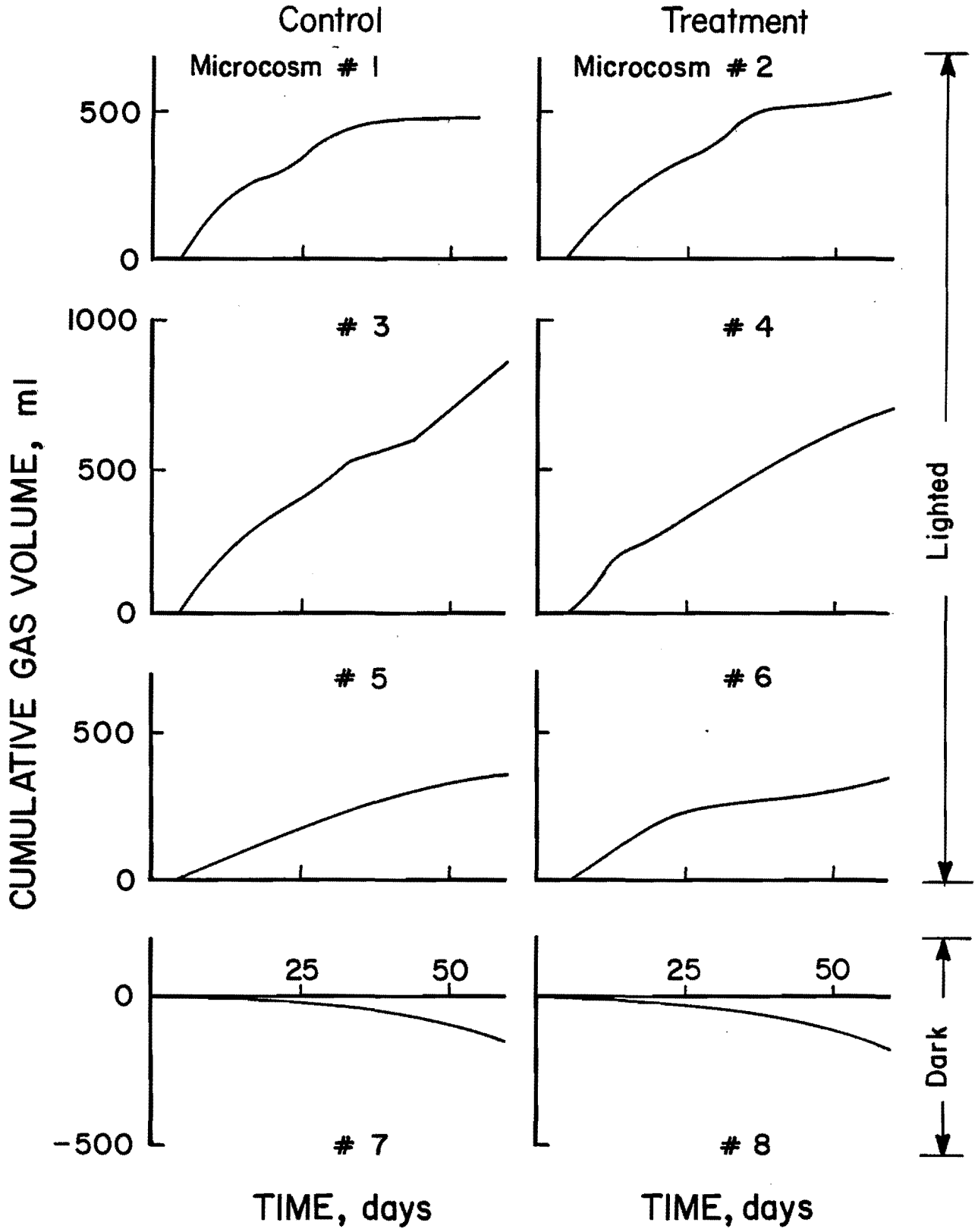


Figure 15. Cumulative gas volume of control (#1,#3,#5, and #7) and BA treatment (#2,#4,#6, and #8) microcosms during experiment #4.

A tendency for the response of the microcosms to "pair up" (#1 with #2, #3 with #4, and #5 with #6) led to investigation of the environmental factors (light, temperature) which could be responsible. No explanatory patterns could be found. Temperatures varied by 0.5°C among the systems (microcosms #1 and #6 were slightly cooler than the other lighted systems). Radiation intensities measured at a point midway between the table and the top of the microcosm ranged from 1.23 to 1.38 watts·m^{-2}. Neither of these parameters correlated with the observed pairing. Overall, the energy levels (radiation) were very low (<1 percent sunlight intensity) and the effects of stray light or possible interactions among temperature, radiation, and other environmental variables are unknown.

The dark microcosms showed a net uptake of gas. While the total gas ultimately removed was nearly the same (138 ml and 155 ml for #7 and #8, respectively), the rates differed. Microcosm #7 had a nearly steady uptake rate after day 15 (3 ml/day). Microcosm #8 had a small gas utilization rate for the first 35 days (1 ml/day) but this significantly increased after day 35 (4 ml/day).

Specific Gas Composition

Oxygen

Oxygen (O_2) production (Figure 16) followed the same patterns as did total gas production. As with total gas production, there was no trend particular to treatment. Microcosm #3 produced the greatest quantity of O_2 (325 mg), microcosm #4 was the second highest producer (255 mg), #1 and #2 were intermediate (175 mg and 210 mg, respectively) and #5 and #6 produced the lowest totals (120 mg each). The dark microcosms consumed O_2 as was expected due to their respiratory state. It appeared that the control microcosm (#7) began biological activity prior to the treatment microcosm as evidenced by the later starting date in #8 (approximately day 22 versus day 13) for O_2 consumption to become measurable.

Carbon dioxide and methane

Net production of carbon dioxide (CO_2) increased in the lighted microcosm during the experiment (Figure 17). Of the control systems, microcosm #3 demonstrated the highest level of production (final value 0.46 mg). Microcosms #1 and #5 had lower amounts of CO_2 accumulation (0.169 and 0.219 mg, respectively). The treatment microcosms, on the whole, had greater accumulation of CO_2 than did the control systems. Microcosms #2 and #4 displayed very similar patterns in terms of period of maximum CO_2 production (between days 12 and 19) and maximum amounts accumulated (0.33 mg for #2 and 0.34 mg for #4). Microcosm #6 showed a faster rate of CO_2 production initially (0.26 mg/day between days 12 and 19 versus 0.019 mg/day and 0.020 mg/day for #2 and #4, respectively), but the net production leveled off and resulted in a smaller final quantity (0.257 mg).

Methane was not detected in any of the microcosms until day 43 (Figure 18). Production was greatest in the two microcosms (#3 and #4) which had previously displayed maximum rates of O_2 and CO_2 production. These two microcosms, one of which is a control system and the other a treatment system, obviously had the most active biological communities.

The dark microcosms (microcosms #7 and #8) had somewhat lower CO_2 production rates (Figure 19). It is probable that sufficient time had not elapsed for these systems to have developed a large heterotrophic community. Relatively small quantities of O_2 were consumed. The level of CO_2 rose only slightly above the initial ambient concentration. Although methane was produced in the dark microcosms, its concentration was lower than that

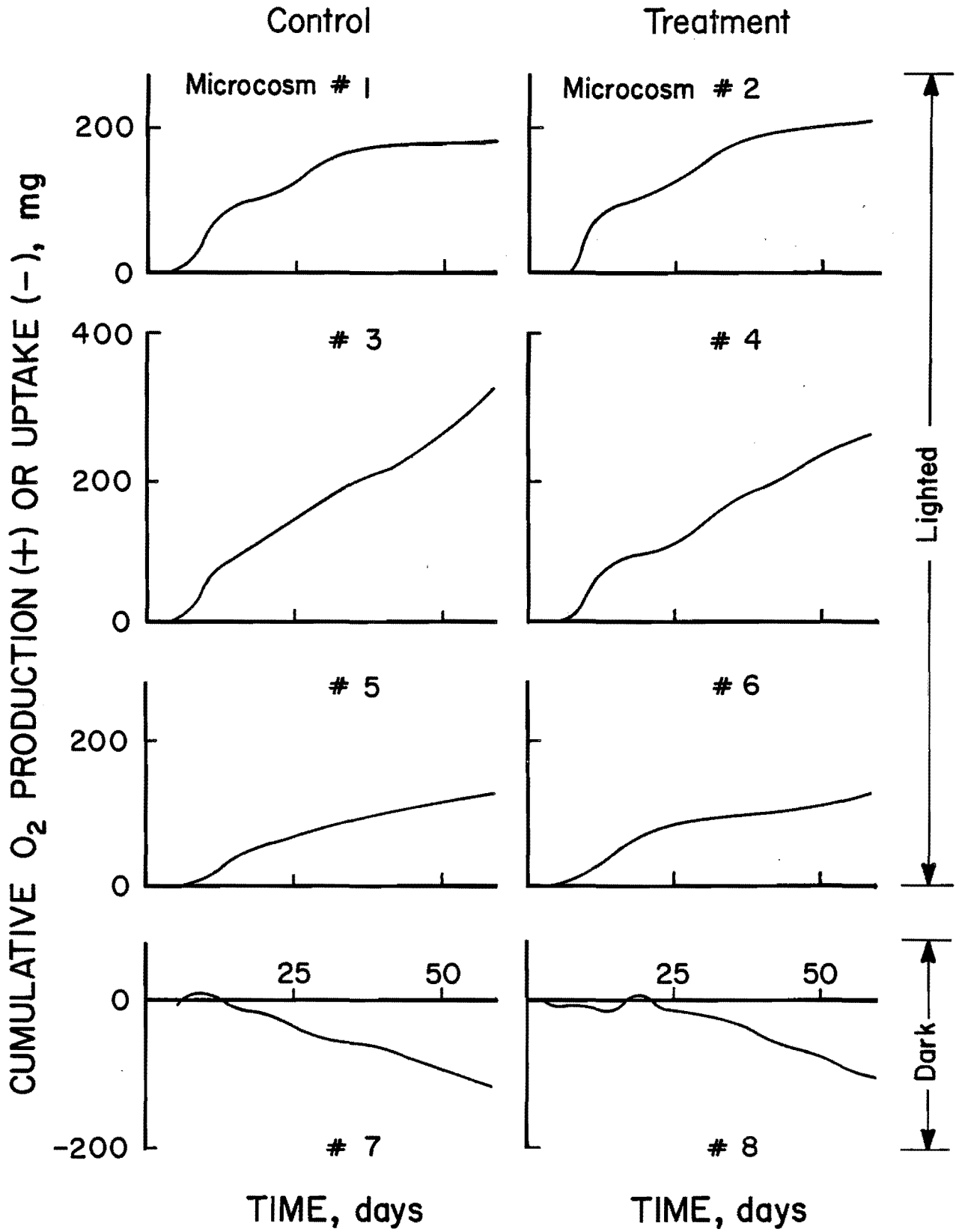


Figure 16. Cumulative oxygen production or uptake by control (#1,#3,#5, and #7) and BA treatment (#2,#4,#6, and #8) microcosms during experiment #4.

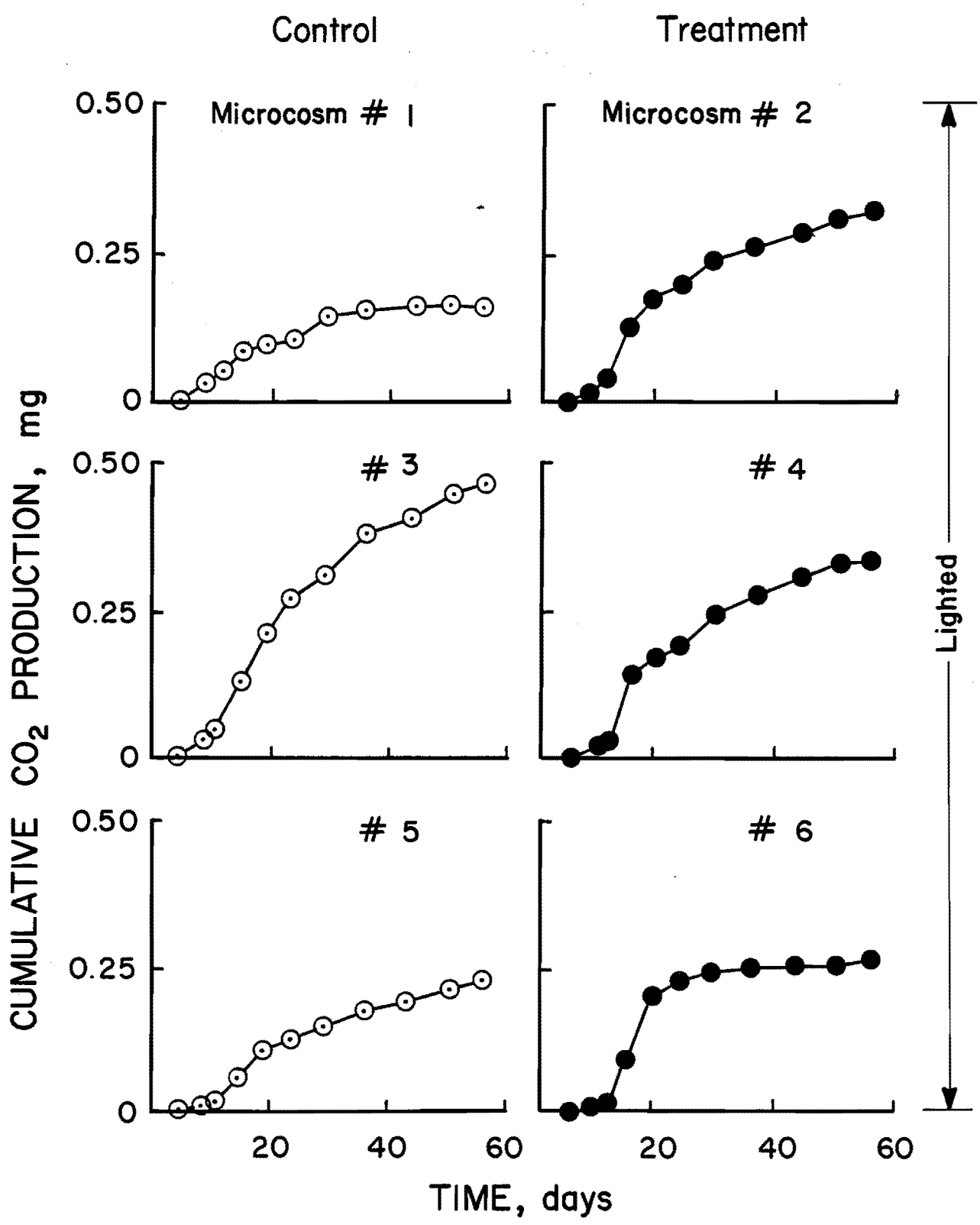


Figure 17. Cumulative carbon dioxide production by control (#1,#3, and #5) and BA treatment (#2,#4, and #6) lighted microcosms during experiment #4.

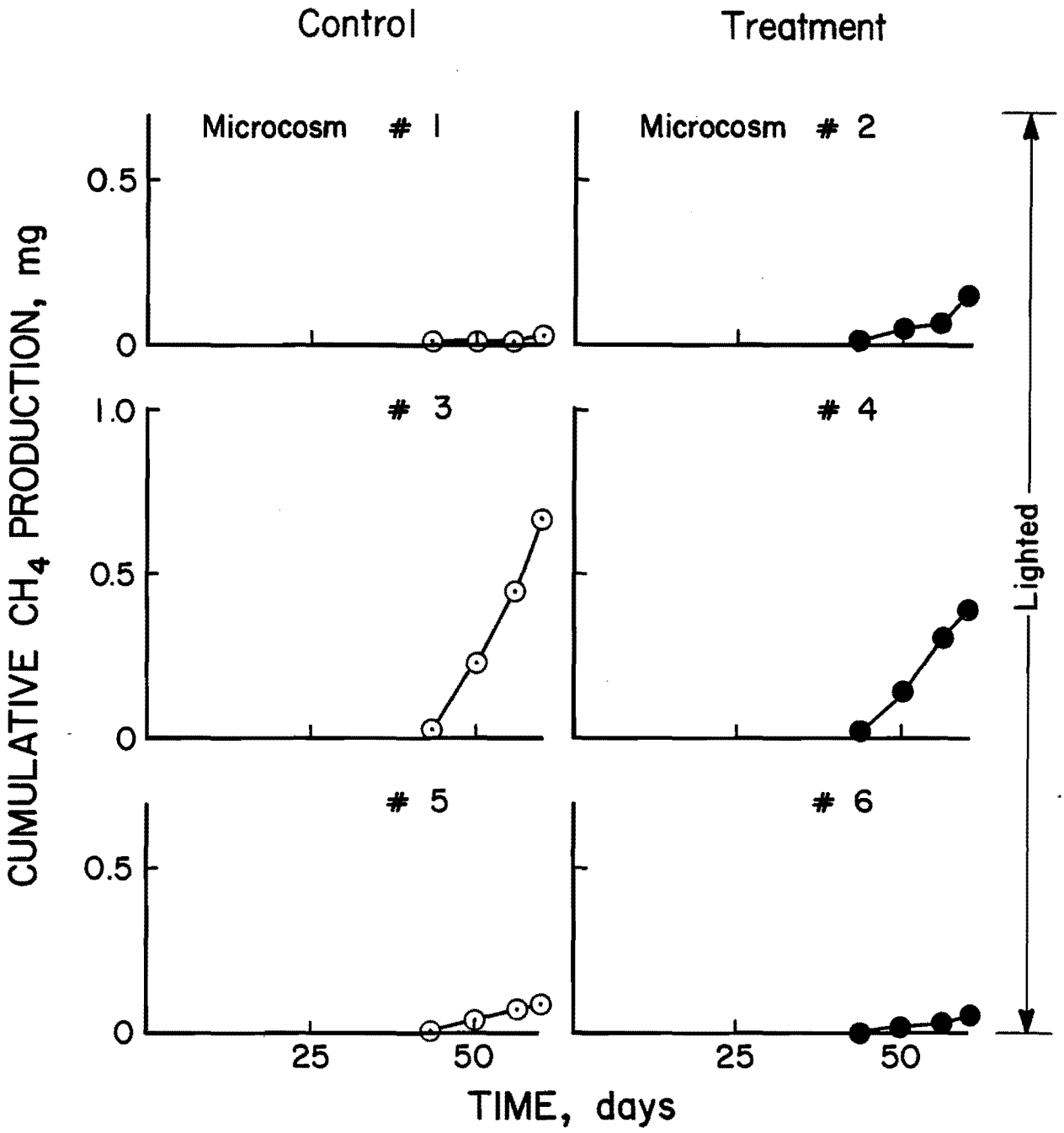


Figure 18. Cumulative methane production by control (#1,#3, and #5) and BA treatment (#2,#4, and #6) lighted microcosms during experiment #4.

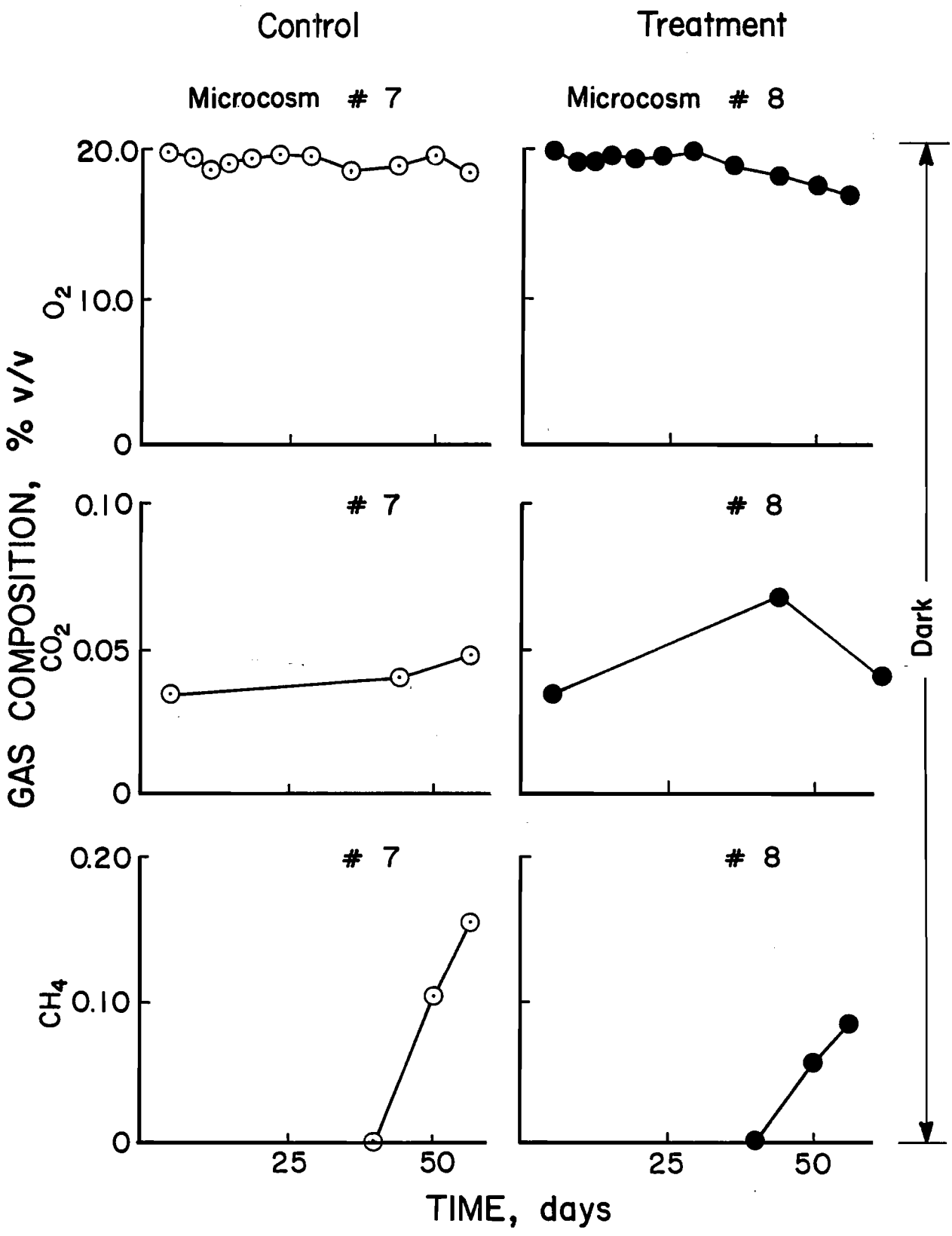


Figure 19. Gas composition of control (#7) and BA treatment (#8) dark microcosms during experiment #4.

observed in the lighted microcosms. For example, on day 56 methane concentrations were, in order beginning with microcosm #1, 0.14, 0.33, 0.31, 0.22, 0.40, and 0.16 percent methane by volume, versus 0.15 and 0.06 percent for microcosms #7 and #8, respectively.

Diurnal Productivity Measurements

The change in total oxygen mass (dissolved and gaseous) during the 16 hr daylight period and the 8 hr night period determined for ten 1 day periods during the 60 day experimental interval was used as the measure of microcosm productivity. The pattern of rapid initial oxygen evolution indicated in Figure 16 was reproduced in the diurnal rate measurements (Figure 20). Typically, the high gross production rates (17-33 mg O_2 produced/daylight period) between days 8 and 15 were accompanied by high respiration rates (5-19 mg O_2 utilized/night period), although the figures for O_2 uptake during the dark were quite variable. On some days (day 19, microcosms #4 and #6; day 23, microcosms #1, #2, and #3; day 35, microcosms #1, #2, and #4; day 42, microcosm #4; and day 49, microcosm #4), a slight amount of oxygen was calculated as produced during the night period. Perhaps significant errors are introduced by extrapolating to total dissolved oxygen mass from samples taken from the upper portion of the water column, or perhaps mixing is incomplete in the water column.

Average net O_2 production for the replicate control and treatment microcosms are shown in Figure 21. In this form, the data indicate a difference in means (control greater than treatment net production) on day 12. This difference is significant using the t-test statistic ($\alpha \leq 0.05$, two-tailed test). In addition, the day at which the maximum net production occurs for the control microcosms (day 12) is earlier than that for the treatment microcosm (day 15). These two observations suggest that one effect of the treatment may be to delay total community productivity by reducing the magnitude of the initial production rate and by delaying establishment of primary producers. However, a summation of the areas underneath the curves reveals that the total production during the 60 day experimental period as measured by net oxygen evolution is nearly the same (812 mg and 832 mg, respectively) for control and treatment microcosms.

Nutrient Status

Phosphorus

The dynamics of ortho-phosphate (ortho-P) in both treatment and control lighted microcosms were quite similar (Figure 22). On the first sampling date (day 4) up to 80 percent (range 53-80 percent) of the ortho-P concentration added with the medium was recovered in the medium exchange. After day 11, however, the concentration of ortho-P in all six lighted microcosms was near zero.

As nutrients were utilized, the bulk of the total phosphorus (total P) occurred as particulate P (i.e., phytoplankton). Early during the experiment, the algae were suspended (see notes in Appendix A, day 9) in the water column. This was corroborated by the total P data (Figure 23) which shows a wash-out of particulate P at this time. Gradually, the algae community became dominated by periphyton thus explaining the more or less gradual reduction in total P with time even though ortho-P was being utilized. By day 46 the total P in the water column of microcosms #3, #4, and #5 began to increase again. This occurred simultaneously with the observation that the water columns in these three microcosms were green with suspended algae (see notes in Appendix A).

The average composition of the various forms of phosphorus in the microcosms was determined directly

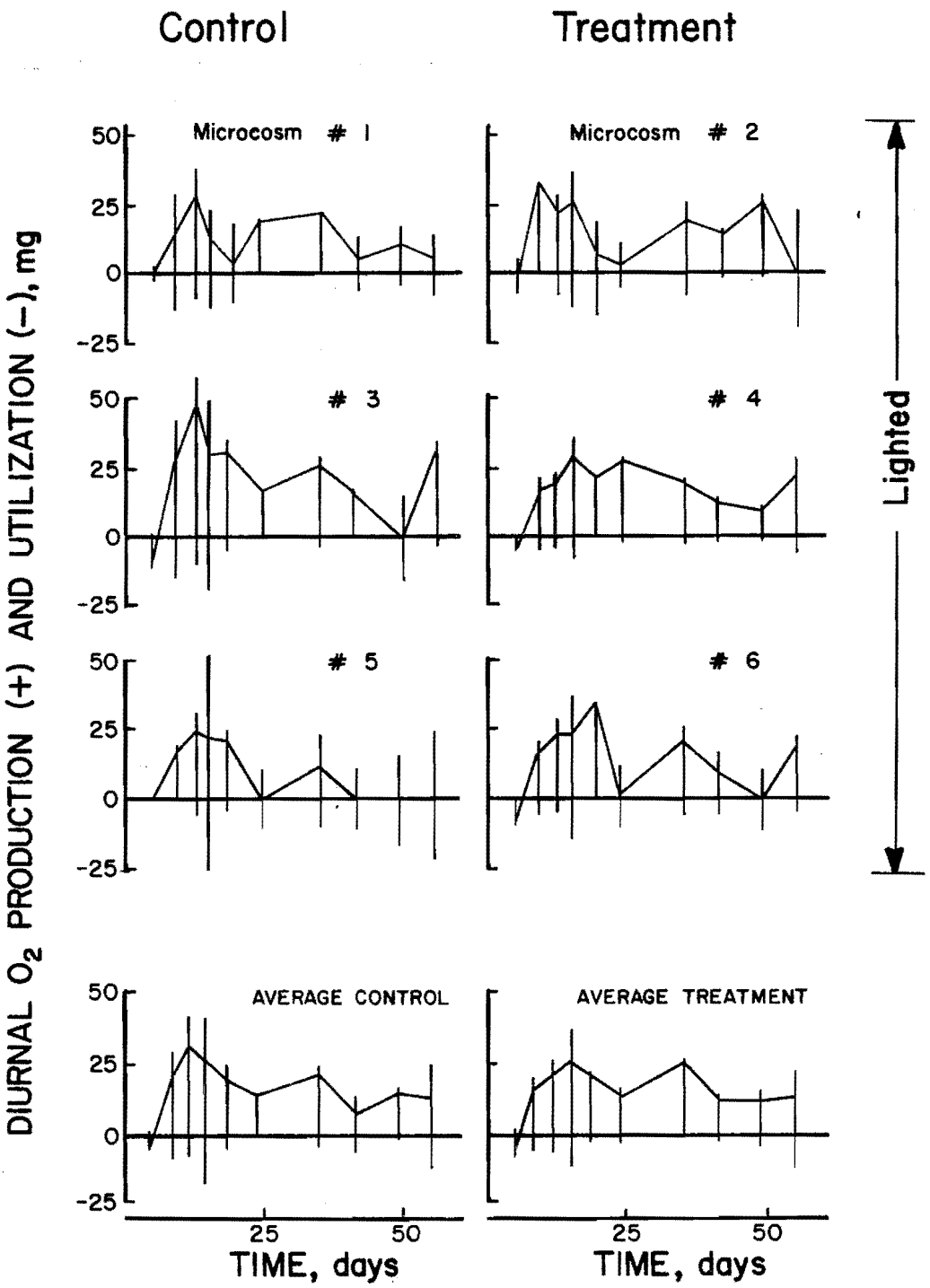


Figure 20. Diurnal oxygen production (indicated by bar length above the x-axis) and utilization (indicated by bar length below the x-axis) for control (#1,#3, and #5) and BA treatment (#2,#4, and #6) microcosms during experiment #4. The line (——) connecting bars indicates net oxygen production.

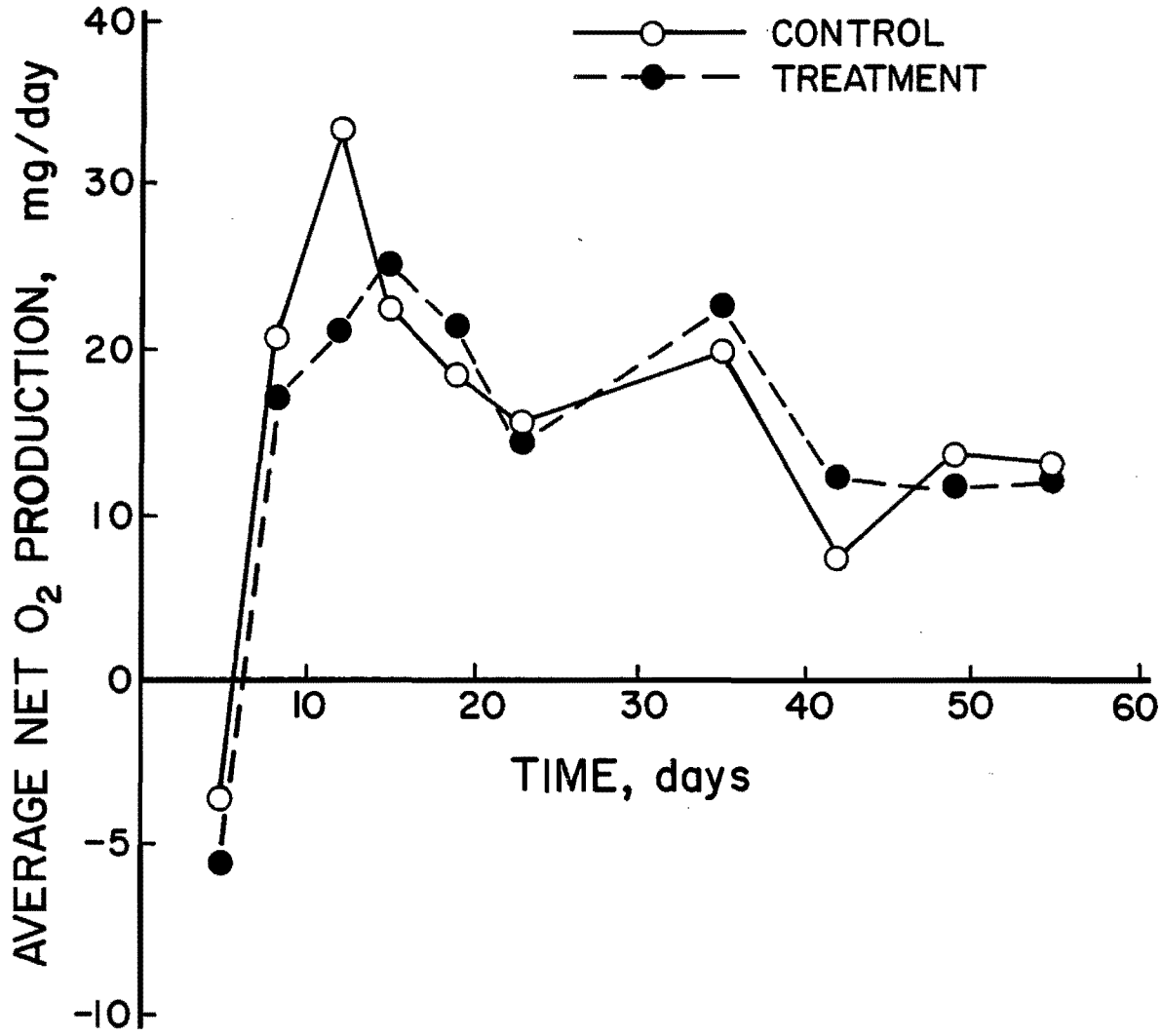


Figure 21. Average net oxygen production rate for control and BA treatment microcosms during experiment #4.

(ortho-P and suspended particulate P) or indirectly (attached particulate P) from mass balance analyses and plotted in Figure 24. Attached particulate P was not measured. It was estimated as the difference between the quantity of ortho-P added to, and the ortho-P and particulate P removed from, the microcosms during medium exchange.

Initially the microcosms were filled with 0.51 mg of phosphorus (as ortho-P). In the mass balance calculations, only 80 percent, on the average, of this P could be accounted for as ortho-P, particulate P, and attached P. Two possible causes were adsorption of orthophosphate or limited precision of the analysis.

The P dynamics displayed in Figure 24 clearly show the concentration of ortho-P in the liquid phase to decline rapidly with a concurrent increase in particulate P. The attached particulate P (a calculated quantity) appears to account for about 25 percent of the P. The portion declined as more of the

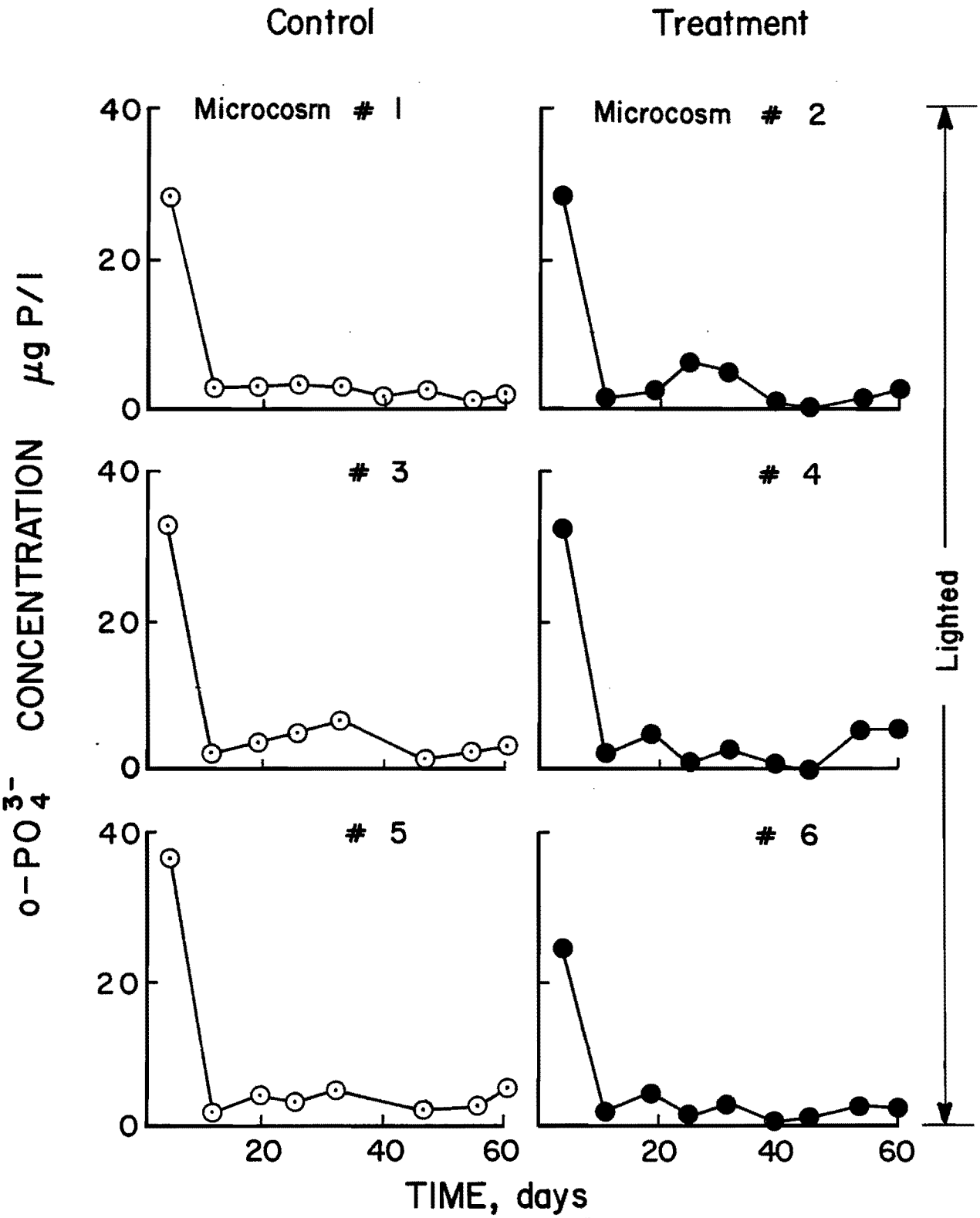


Figure 22. Orthophosphate concentration in control (#1,#3, and #5) and BA treatment (#2,#4, and #6) lighted microcosms during experiment #4.

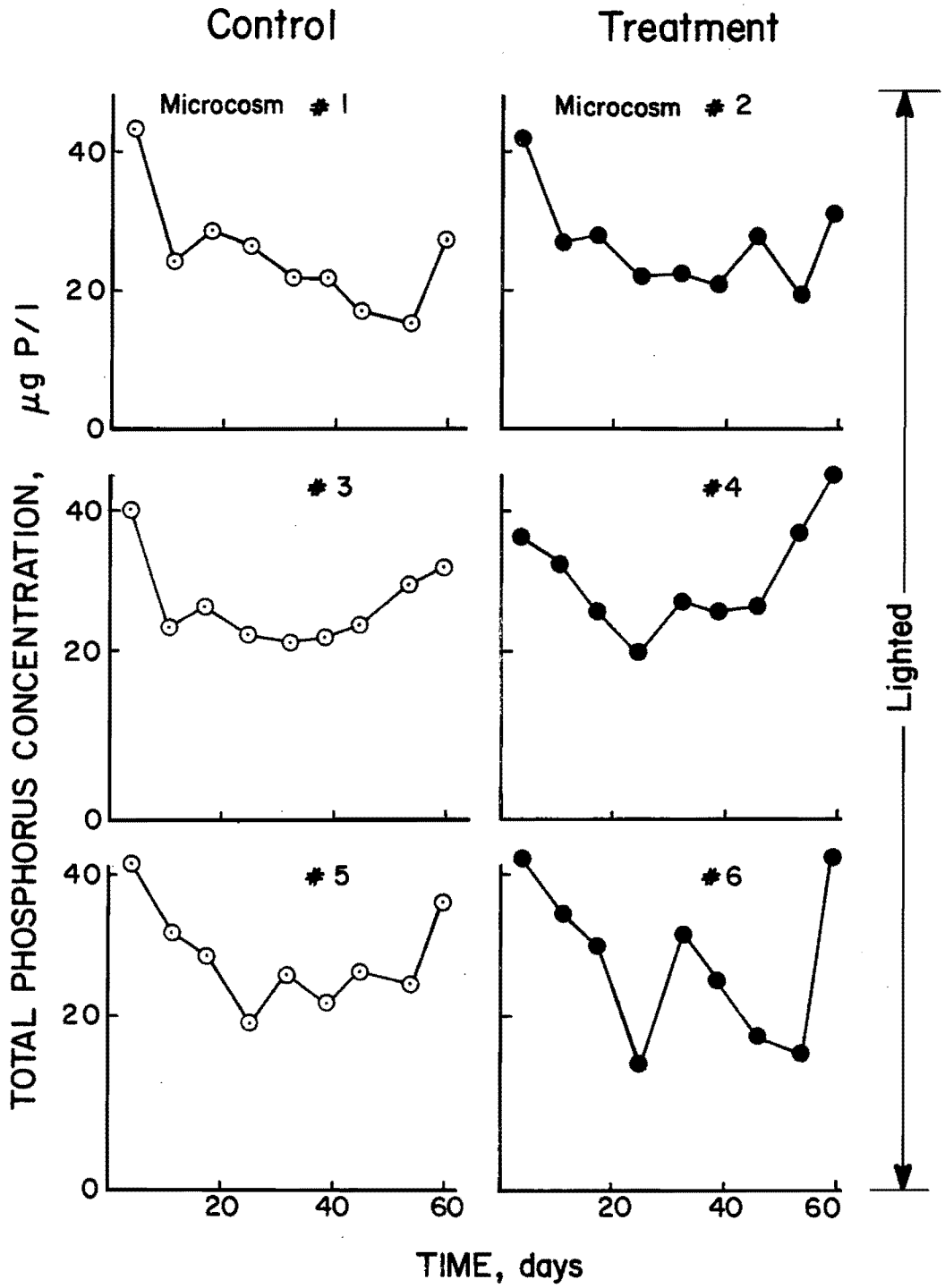


Figure 23. Total phosphorus concentration in control (#1,#3, and #5) and BA treatment (#2,#4, and #6) lighted microcosms during experiment #4.

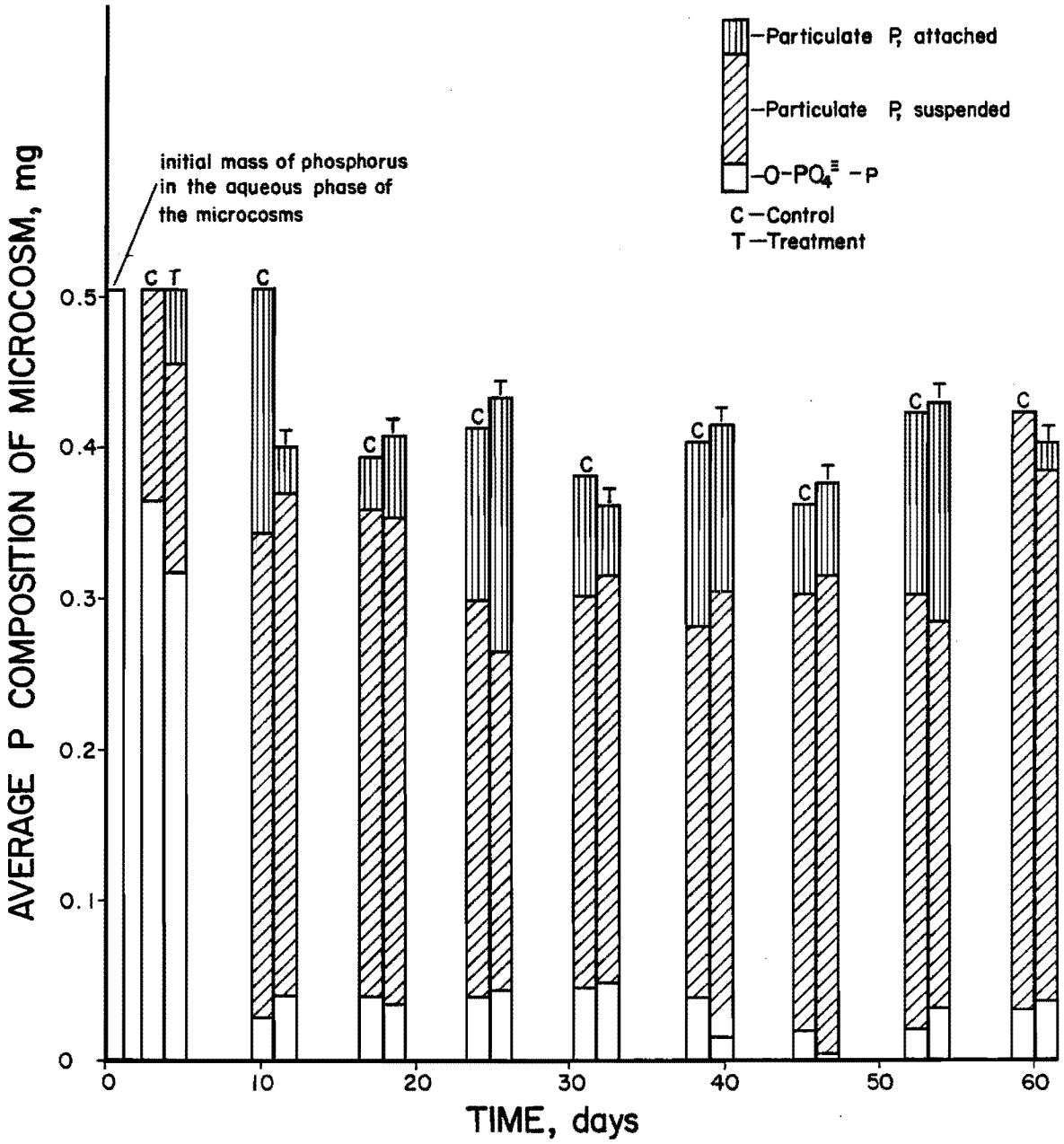


Figure 24. Average phosphorus composition based on mass balance analyses for the control and BA treatment lighted microcosms during experiment #4.

input P could be accounted for in the particulate P removed.

The dark microcosms utilized little ortho-P in the feed medium (Figure 25) beyond day 18. A comparison of the ortho and total P dynamics indicates that P occurred as ortho-P. Prior to day 18, little inorganic P was detected. It may have been assimilated into periphyton or adsorbed directly to the glass walls of the microcosm.

Nitrogen

Nitrogen (N) was added to the microcosms as nitrate to an average concentration in the medium of 1.05 mg-N/1. After the first sampling period (day 4) the combined concentration of

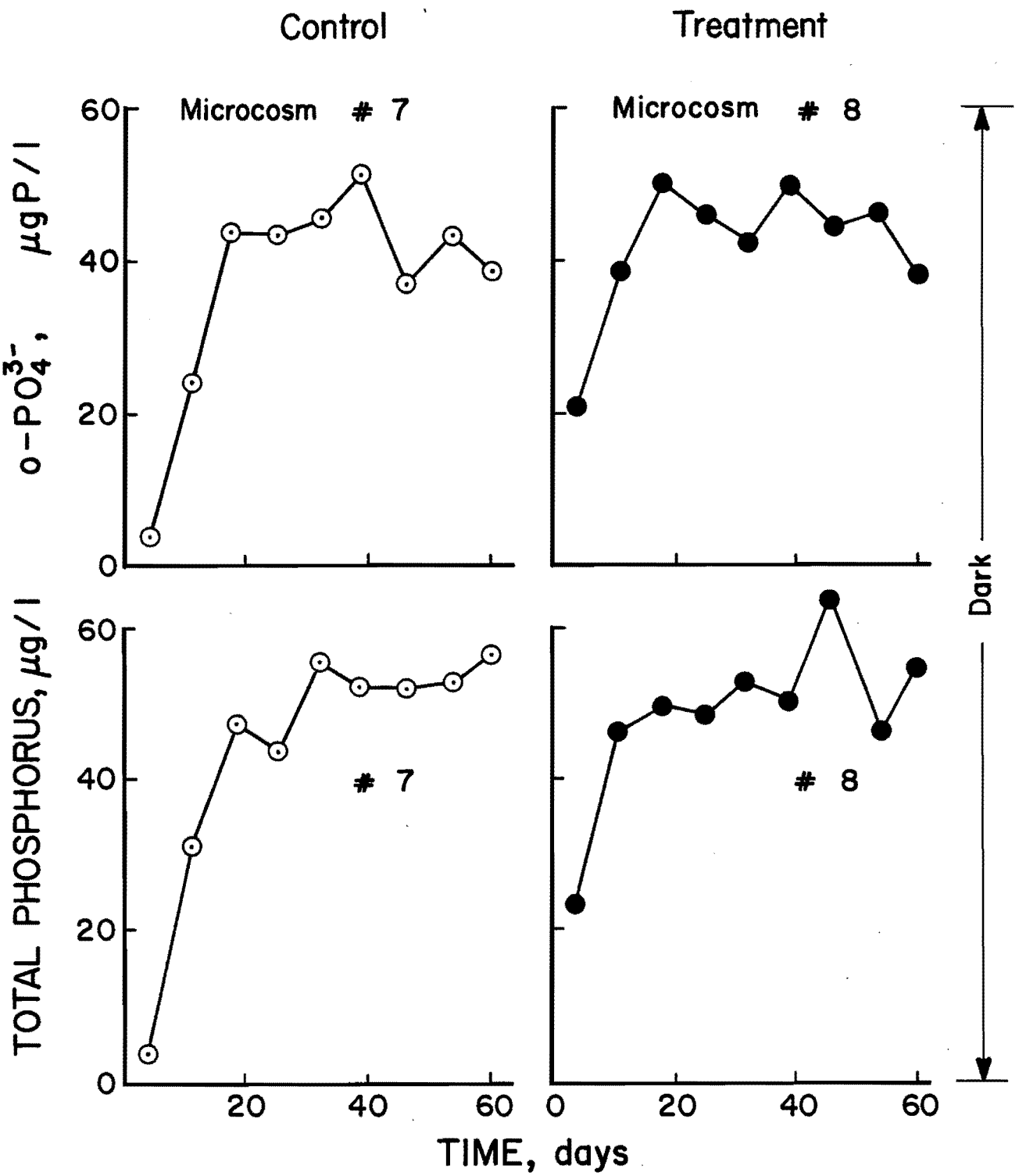


Figure 25. Ortho- and total phosphorus concentrations of control (#7) and BA treatment (#8) dark microcosms during experiment #4.

nitrate and nitrite (Figures 26 and 27) was near the detection limit of the analysis (0.04 mg NO_3-N/l), indicating that the nutrient was rapidly assimilated.

Generally, the net production of ammonia (NH_3-N) in the microcosms (both lighted and dark) was low. (Figure 28). Four lighted microcosms (one control, #5, and all three treatment microcosms) produced low quantities of NH_3 early in the experiment (prior to day 25), probably from the decomposition of sediment organic matter. Three microcosms (#1, #2, and #3) displayed mid-period ammonia production, but the amounts are so narrowly above the detection limit that they may be due to experimental error.

Average daily compositions of nitrogen species, comparing control with treatment microcosms, is shown in Figure 29. The figure clearly shows how the nitrate initially present in the medium was rapidly assimilated. The particulate N was calculated from measured particulate phosphorus using an experimentally-derived mass ratio for phosphorus-to-nitrogen of the mixed phytoplankton collected from the microcosms (1:7.1). Again, the attached particulate organic N was that portion of incoming mass that could not be accounted for as output and must therefore have accumulated in periphyton biomass.

Ammonia constituted a very small portion of the total nitrogen. It is possible that NH_3 production increased later in the experimental period and that its concentration remained low due to concomitant uptake by nitrifiers, resulting in the observed net production of nitrate. As in the case of phosphorus cycling, there were no obvious distinctions in nitrogen dynamics with treatment.

Biological Community

Biomass

The treatment did not have a significant effect on final biomass accumulation in the microcosms. Averages for control and treatment systems were 0.672 and 0.669 g, respectively.

Plankton composition

The major plankton species were monitored in the microcosms for the duration of the experiment to assess the influence of treatment on phytoplankton composition and succession (Appendix B). Most species were unicellular green algae (Ankistrodesmus, Cosmarium, Golenkinia, Pediastrum, and Scenedesmus). There was one multicellular green alga (Coelastrum) which frequently occurred as a unicell, and one filamentous green alga (Mougeotia). There was one member of the chryptophyta (Cryptomonas), several genera of diatoms (pinnate-Fragillaria, Medlosira (rare), Navicula, and Nitzschia, and centrate-Cyclotella and Stephanodiscus), and one blue green filament (Lyngbya). In addition, one rotifer (Monostyla) was detected.

The results of the plankton survey are displayed as average values for the three replicate microcosms by date within each treatment (Figure 30). The occurrence of a species or group is indicated by a particular pattern in the bar graph. Its frequency of appearance is indicated by the length of a section of bar. Generally, the control and treatment microcosms had similar species' occurrence and frequency. They had about the same variety of plankton species (averages for control and treatment were 8.2 and 7.2 species, respectively). Both displayed an early peak in species abundance (maxima

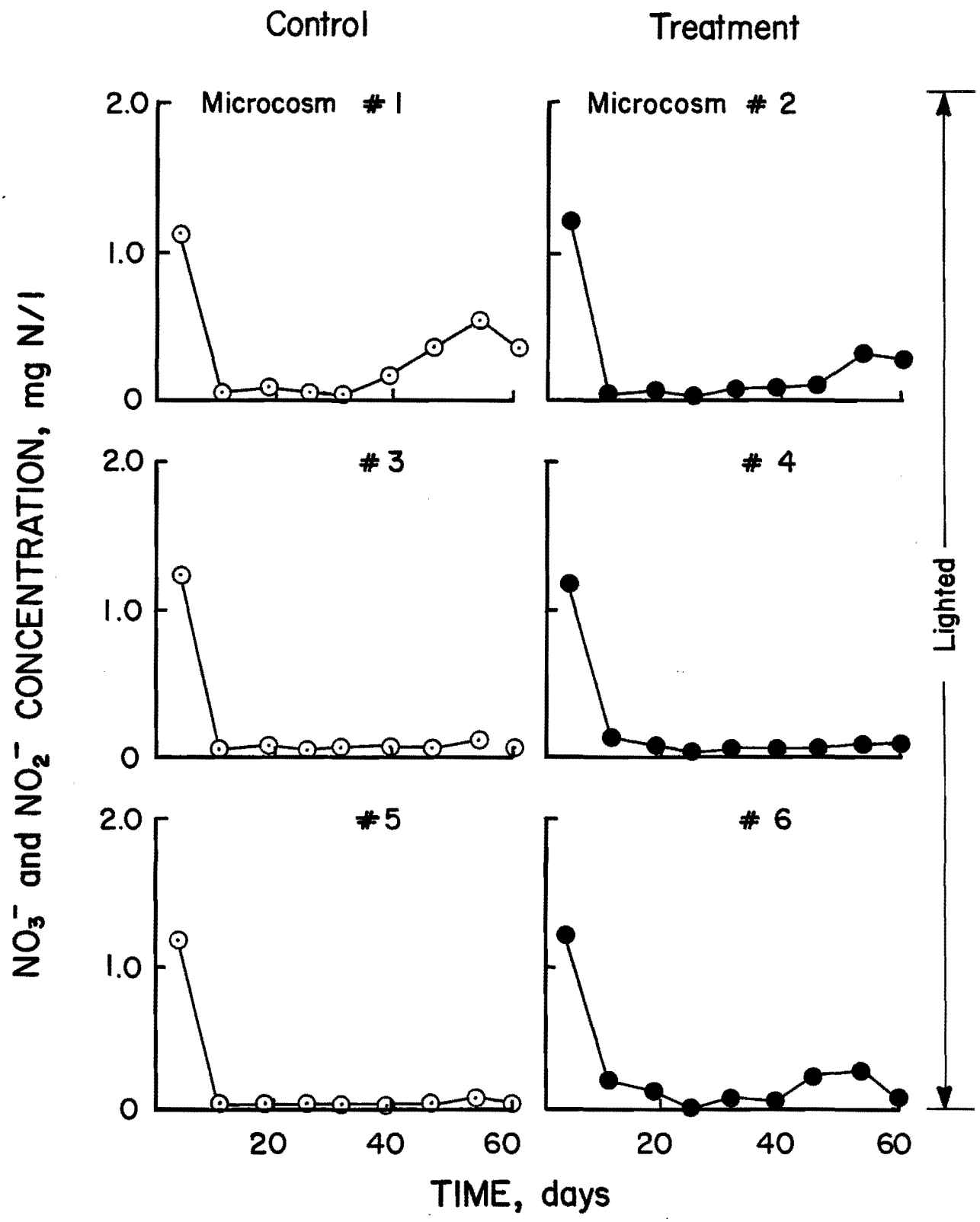


Figure 26. Nitrate (NO_3-N) and nitrite (NO_2-N) nitrogen concentration of control (#1,#3, and #5) and BA treatment (#2,#4, and #6) lighted microcosms during experiment #4.

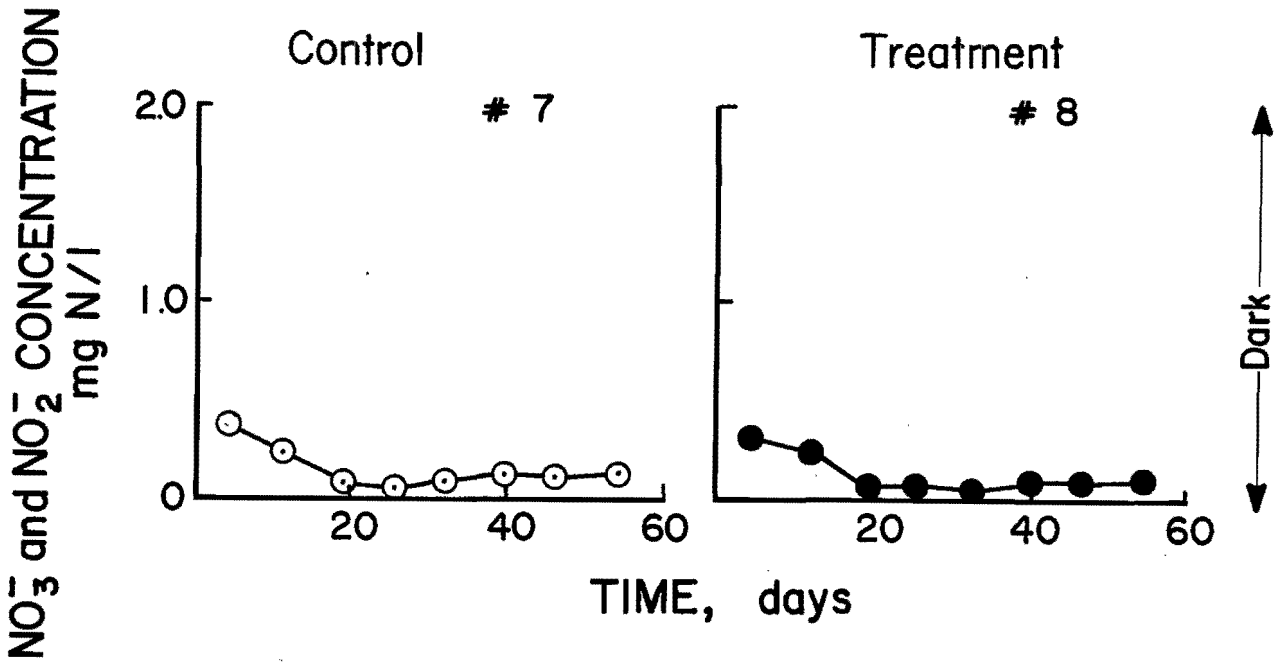


Figure 27. Nitrate (NO_3-N) and nitrite (NO_2-N) nitrogen concentration of control (#7) and BA treatment (#8) dark microcosms during experiment #4.

occurred on day 18 for control and day 11 for treatment systems). Finally, the microcosms were similar in the timing of introduction and persistence of species. Common to both control and treatment systems were species which were found on every sampling date (Scenedesmus, pinnate diatoms, Coelastrum, and Golenkinia) those found on most sampling dates (Ankistrodesmus and centrate diatoms), those species which were present early in the experiment and decreased in abundance through time (Scenedesmus, Ankistrodesmus, Mougeotia, Coelastrum, and Cryptomonas), and those species which were more abundant later in the experiment (Cosmarium, Monostyla and Lyngbya).

There a very few differences between control and treatment microcosms. Pediastrum was more abundant and sometimes occurred only in treatment microcosms (days 18, 26, and 32), Cosmarium occurs earlier (day 46) in treatment microcosms than in control (day 60), and Cryptomonas persisted in control microcosms throughout the experiment, but was not found in treatment microcosms after day 39.

Invertebrates

In addition to a complex phytoplankton composition, copepods, chironomid flies, daphnia, ostracods, and tubificid worms were observed in the microcosms. The final experiment was fairly typical of the pattern seen in earlier experiments for emergence of these organisms. Worm activity was first observed on day 5 (see Appendix A), and by day 11 all systems (light and dark) showed evidence of burrowing. The organisms were not observed once an algal mat formed in the lighted systems. Copepods and ostracods were seen in the microcosms but their occurrence was rare. Daphnia were more common but only occurred in two of the lighted systems BA-treated microcosms (#2 and #6). Chironomid flies emerged from the

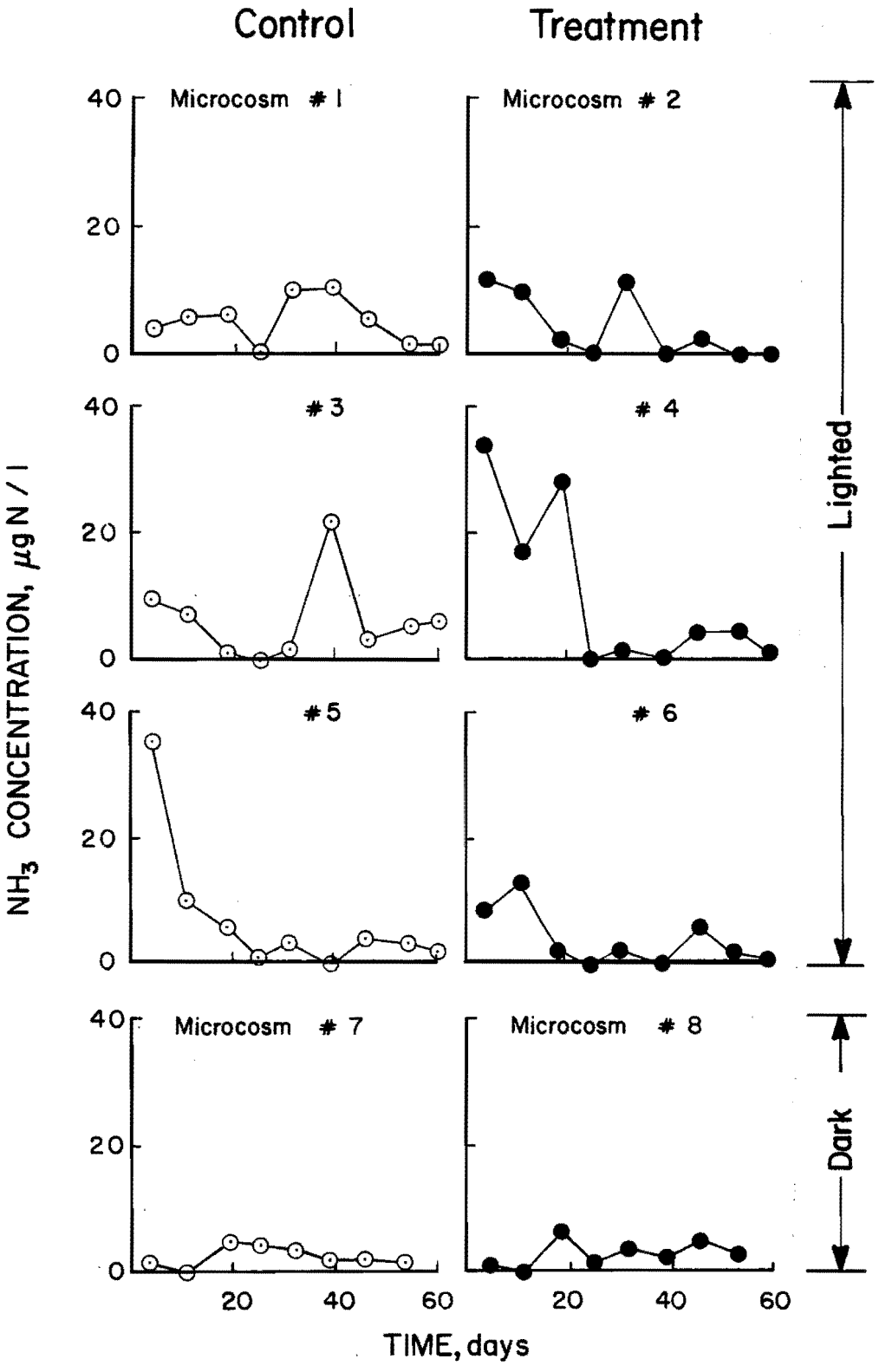


Figure 28. Ammonia (NH_3-N) nitrogen concentration of control (#1,#3, #5, and #7) and BA treatment (#2,#4, #6 and #8) lighted and dark microcosms during experiment #4.

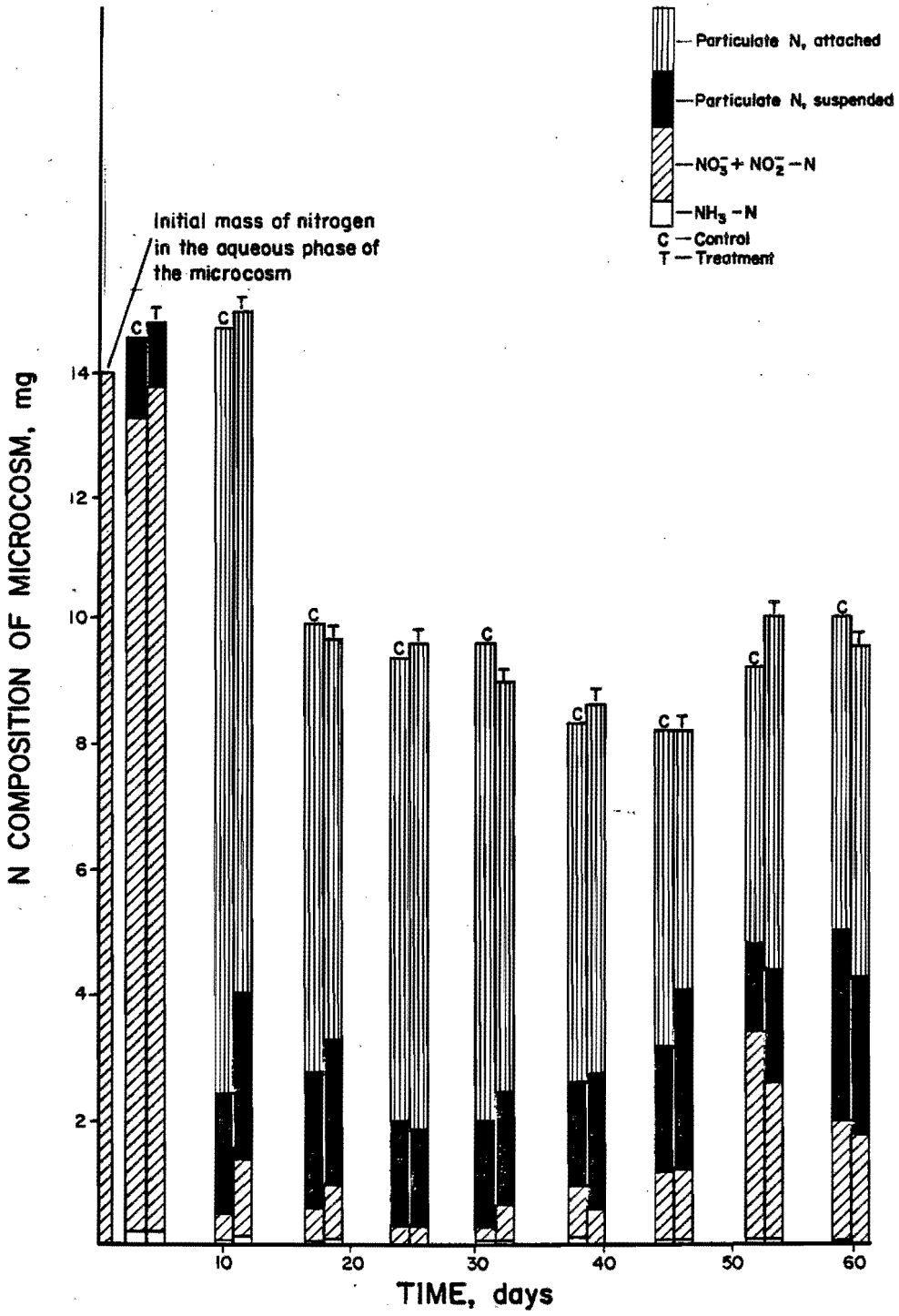


Figure 29. Average nitrogen composition based on mass balance analysis for control and BA treatment lighted microcosms during experiment #4.

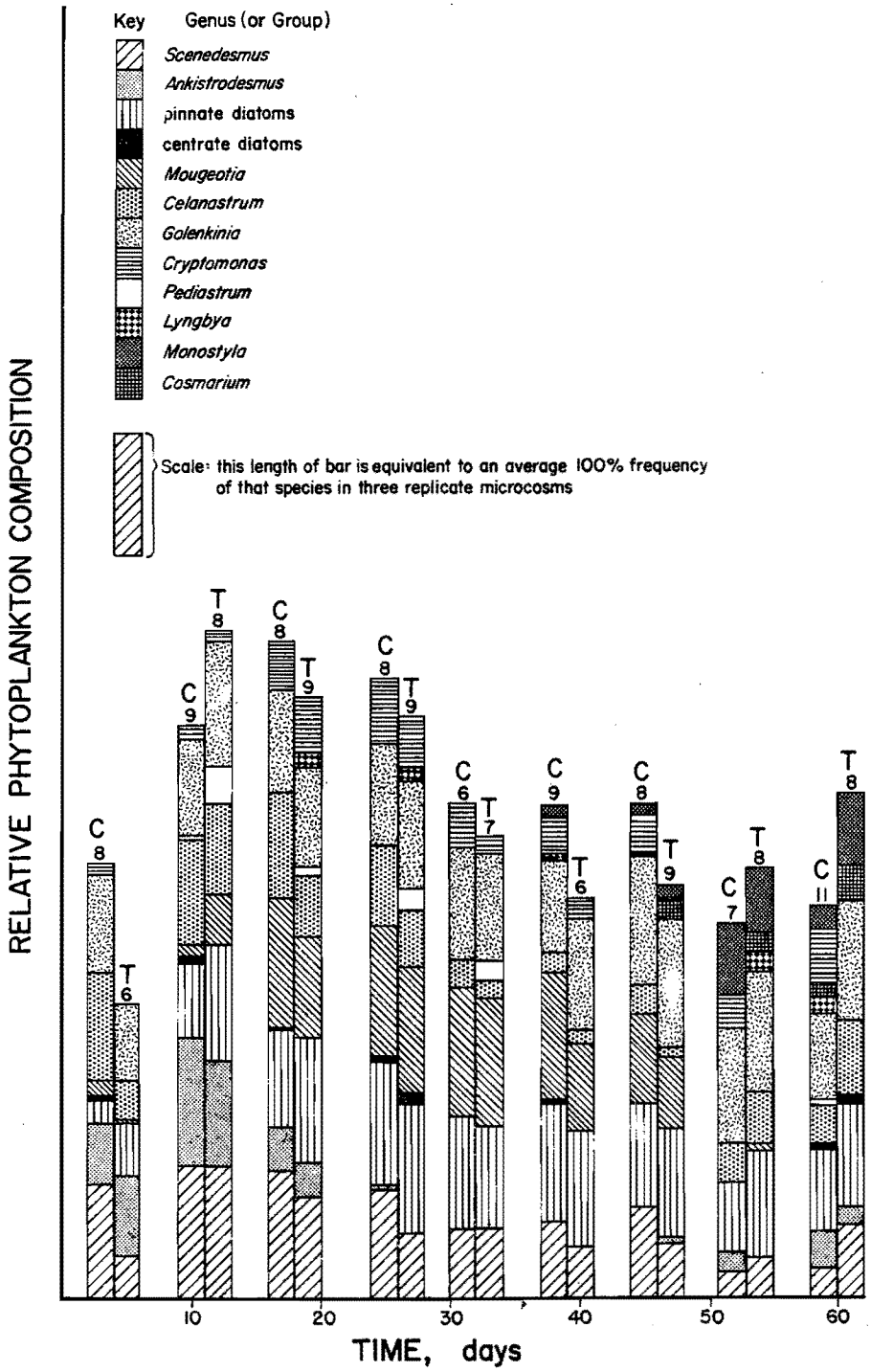


Figure 30. Average phytoplankton composition and relative frequency in control and BA treatment lighted microcosms during experiment #4. (After Prescott 1964, see Appendix Table B-5.)

sediments during the 7th week in two treatment systems, #2 and 6, and one control system, #1. Within another 10 days, flies were observed in two of the remaining three lighted microcosms but never in treatment system #4.

Bacteria

Total bacterial counts were measured weekly (Figure 31). Initial (day 4) average counts were 1.24 and 1.22 x 10^5 bacteria/ml in control and treatment systems, respectively. By day 11, the number of suspended bacteria decreased to 0.25 and 0.15 x 10^5 bacteria/ml for control and treatment, respectively, and remained about this density for the duration of the experiment. The difference is not statistically significant.

Biomass measurement

Total organic carbon was used as an indicator of weekly changes in biomass within the microcosms. The pattern displayed by this parameter (Figure 32) was similar to that of the bacterial counts. This confirms the observation that the periphyton community dominated throughout the later phase of the experiment.

BA Mass Balance

As others found in earlier experiments, desorption from contaminated sediments gave a chronic low level concentration of BA in the aqueous phase of the microcosm. However, instead of maintaining BA at or near its solubility limit (6.6 µg/l at 20°C) for the duration of the experiment, the concentration was initially high (14.5 to 18.5 µg/l) and decreased exponentially to a more or less constant concentration (3.0 µg/l) after the fifth week (Figure 33). One explanation is that the suspended material in the medium samples may have concentrated PAH from solution.

The lighted microcosms displayed nearly identical concentrations of BA through time. The initial concentrations, measured on day 6, averaged 14.5 µg/l and ranged from 14.1 to 14.7 µg/l; the rates of decline in concentration was similar (coefficients were -0.031, -0.032, and -0.030 day^{-1} for microcosms #2, #4, and #6, respectively); and the equilibrium concentrations, established after day 40, were all about 3 µg BA/l.

The single treatment microcosm in the dark (#8) had a greater concentra-

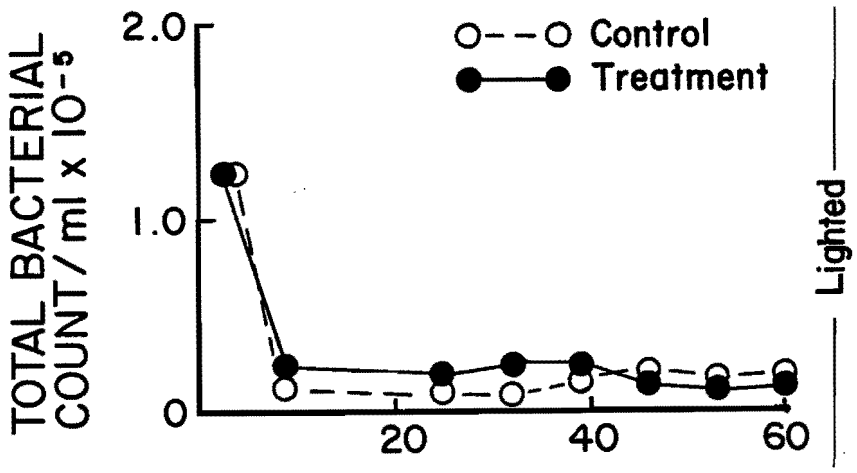


Figure 31. Average total bacteria count in control and BA treatment microcosms during experiment #4.

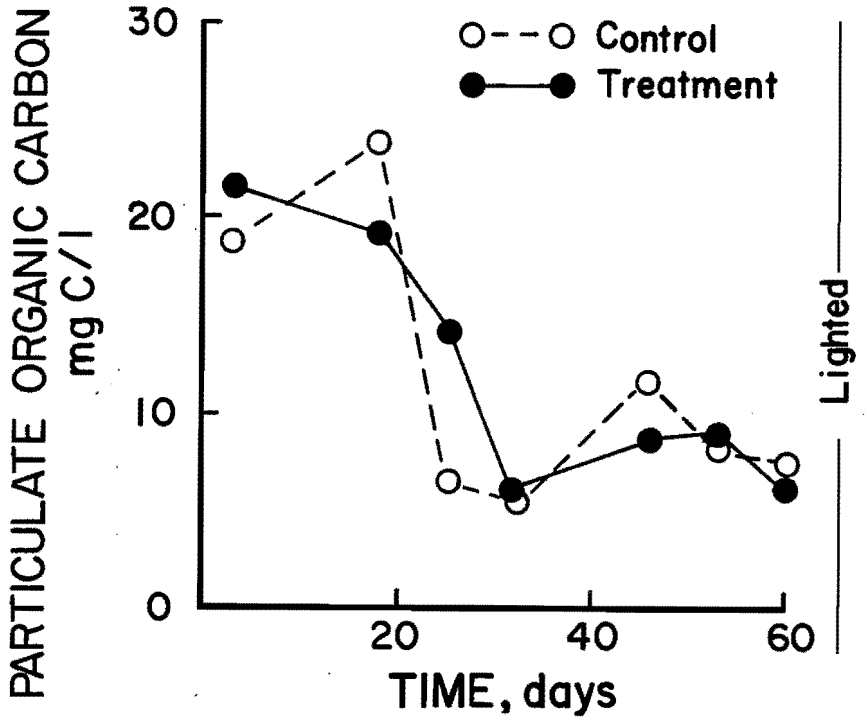


Figure 32. Average organic carbon in control and BA treatment microcosms during experiment #4.

tion of dissolved BA (initially 18.4 µg/l) than found in the lighted microcosms. This difference continued throughout the first 6 weeks of the experiment, but after day 46, the BA concentrations reached approximately the same equilibrium level (3 µg/l) as the lighted microcosms.

The lower concentration of dissolved BA in the lighted microcosms may have been due to removal by sorption onto suspended and attached biota or to transformation by photooxidation. Evidence that both processes were occurring was the recovery of trace amounts of BA (<1 percent of the original BA spike) in the extracts of algae from all the lighted microcosms and in chironomid flies from microcosms #2 and #6 and of small quantities of the major photooxidation product, BA-7,12-dione, in the microcosms on the final sampling date (when 8 ℓ were extracted).

The fate of BA in the microcosms was evaluated from proportions of the parent compound recovered from the liquid and solid phases (Figure 34). The quantity of dissolved BA removed daily during medium exchange was calculated from the exponentially decreasing concentration regressions for the four treatment microcosms (Figure 33). The accumulated mass of BA in the dissolved phase and that determined to be present at the end of the experiment sorbed to sediment biota and glassware accounted for approximately 87 percent and 98 percent of the initial 2 mg spike in the light and dark microcosms, respectively. A portion of compound which was not accounted for in the lighted microcosms was probably photodegraded. As mentioned previously, a compound likely to be BA-7,12-dione (a known photolysis product of BA) was detected in the medium extracts from the light microcosms (this compound had the

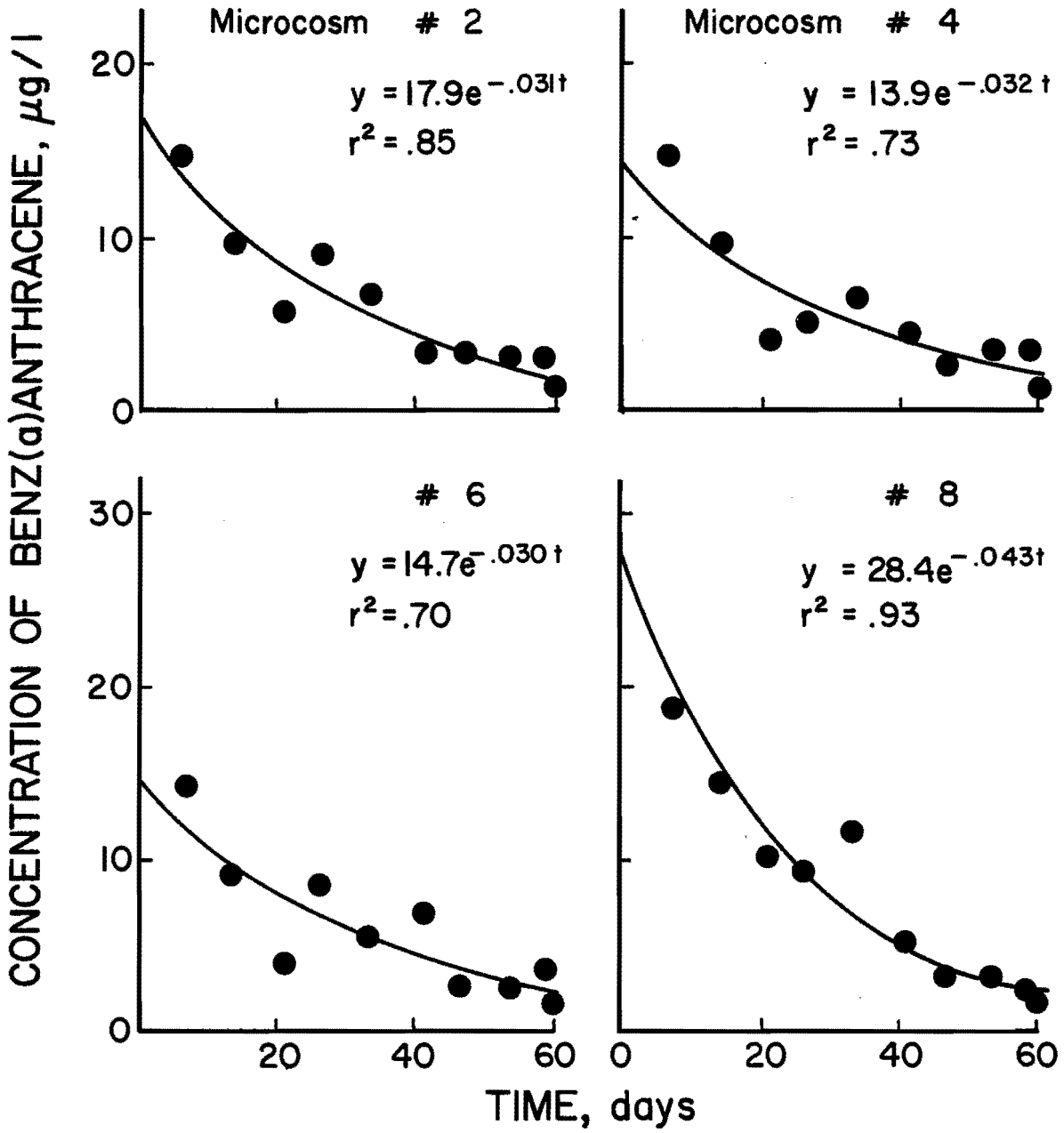


Figure 33. Benz(a)anthracene concentration in lighted (#2,#4, and #6) and dark (#8) BA treatment microcosms during experiment #4. Actual values are indicated by the symbol (o). The line and its equation represent the least squares best approximation to the data.

same mass spectrum and GC retention time as BA but could not be positively identified due to low concentrations). Other losses could have been the result of movement of the compound to greater depths in the sediment (>2 cm) which was not analyzed. Volatilization was assumed to be negligible (Henry's Law coefficient, K_h=3.28 x 10^{-4} at 25°C (Southworth 1979)).

The overall fate of BA in the replicate lighted microcosms was very similar. Approximately 67 percent (1314 - 1360 µg) of the compound remained in the upper centimeter of the sediment. A

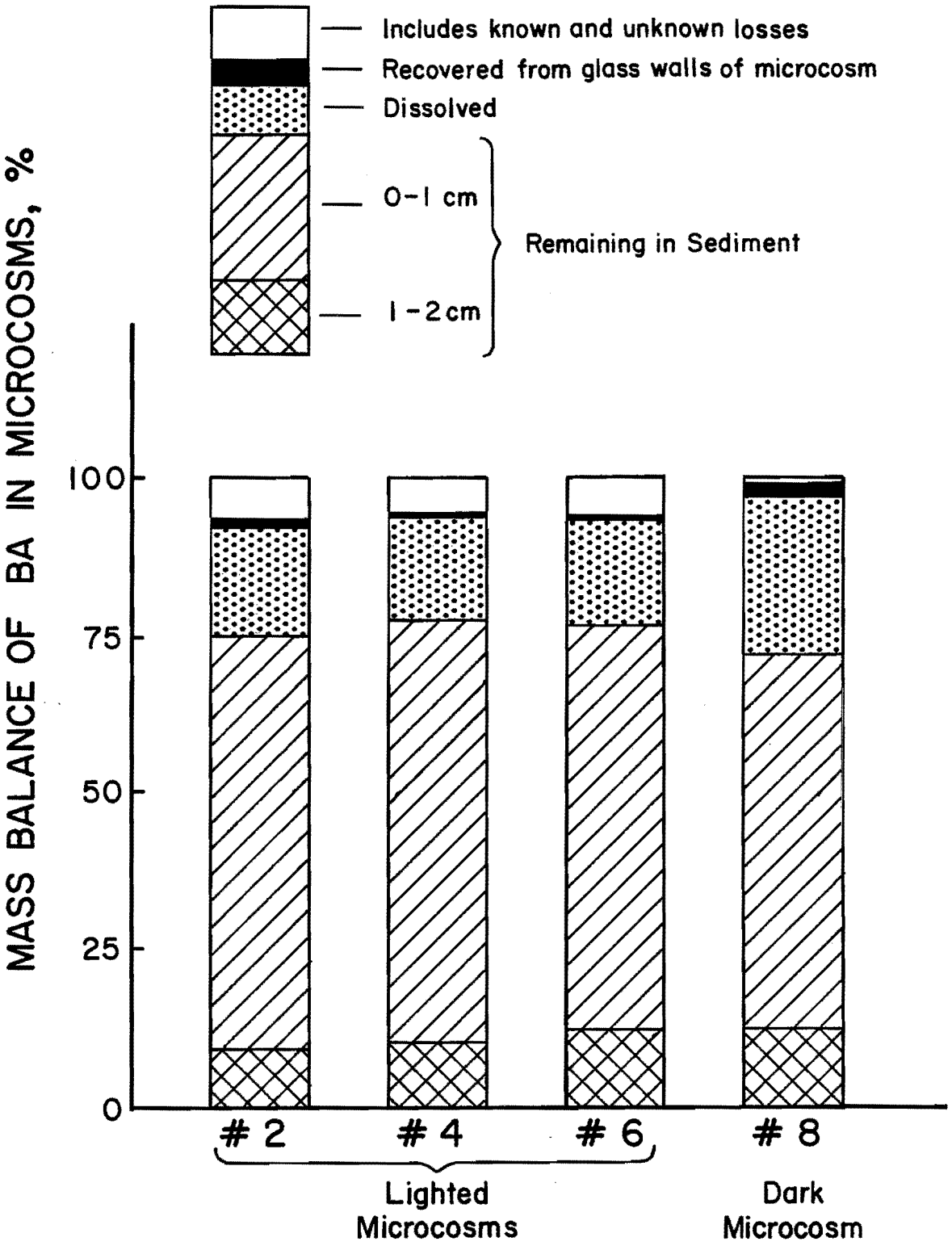


Figure 34. Mass balance of benz(a)anthracene in lighted (#2,#4, and #6) and dark (#8) treatment microcosms during experiment #4.

portion (9 percent, 182 - 185 μg) of the original BA in the upper sediment layer was mobilized within the sediment and could be found immediately below the surface layer (1 - 2 cm depth). Throughout the experimental period, the total mass of BA removed in the liquid phase ranged from 318 - 365 μg (approximately 17 percent). The fate of BA in the dark microcosm differed from that in the lighted microcosms, especially in the quantity of BA dissolved in the aqueous phase and removed during medium exchange (25 versus average of 18 percent, for dark and light microcosms, respectively).

DISCUSSION

Results of Microcosm Experiments

Biological effects

One objective was to study the effect of a long-term, low-dose exposure of a polycyclic aromatic hydrocarbon on the development of the summer biotic community of Lake Powell. The system approach was taken in order to assess the response in terms of community metabolism, nutrient cycling, biomass, and species composition. In general, the introduction of benz(a)anthracene caused very few differences in the way the microcosms functioned. No significant differences could be found in oxygen production, nutrient utilization, species composition, nor biomass accumulation. These findings support earlier experiments in which BA was found not to be acutely toxic to aquatic organisms, probably because it is relatively insoluble (Neff 1979).

Fate of BA in the aquatic environment

The microcosm experiment indicated that under conditions which exclude photolysis, the majority of the BA stays sorbed to sediment particles and other surfaces. Desorption processes maintain dissolved levels of BA at around 3 µg/l when the washout rate is approximately 10 percent. At these rates of desorption and washout, the half-life of BA is more than 270 days in a lighted and 240 days in a dark microcosm environment. The small difference in persistence seems to be due to the higher initial concentration of BA in the aqueous phase. Other minor pathways included sorption to the biota (flies and algae), and photodecomposition.

These removal processes are consistent with those presented in the literature. However, in an environment where radiation levels are higher, photooxidation becomes the primary transformation pathway for dissolved BA, and its persistence in the lighted or euphotic environment is greatly reduced. For this study, radiation capable of causing the photolysis of BA was eliminated in order to test the "worst case" one where this process was not removing BA from the system. Other researchers have detected BA metabolism in microcosm enclosures, but only after a long period of acclimatization (Hinga 1980).

Utilization of the Three-Phase Microcosm for Environmental Fate-and-Effect Research

Evaluation of procedures

This study provided opportunity for testing techniques and assembling a list of procedures that work. The final methods for introduction and monitoring of PAH were very effective. Contaminated surface sediments simulated the desired chronic low-dose contamination which would take place either with a continual low level waste discharge or following an accidental petroleum spill. The schedule used for sampling and extraction was highly successful for analysis of the parent compound. Unfortunately these procedures were not sufficient to detect degradation products as well. It was not until the microcosms were dismantled and a larger sample (8 ℓ) was extracted, that a well-known BA degradation product was detected. A different approach may be required to trace compounds that are readily photodegraded and/or biodegraded. Radio-labeled compounds would

probably make mass balance analyses more effective in overall recovery and accuracy.

A sophisticated instrument such as a GC/MS is definitely required to achieve the degree of analytical accuracy needed for this type of experimentation. Fluorescence has certain advantages in conserving time in analysis and sample preparation, but the results are not reliable when the medium contains an assortment of organic molecules and degradation products which contribute to a high level of background fluorescence. This is a problem observed in other PAH studies, especially those involving photodecomposition of PAH (McGinnes and Snoeyink 1974).

In these experiments, the traditional microcosm operational procedures were supplemented with diurnal oxygen mass balance experiments in an attempt to obtain more information on the metabolic state of the microcosms. The variability observed in mass balance computations was disconcerting and points to several potential problems. For example, errors could have risen through extrapolation of total DO content in the microcosm from that measured in a sample of the top fluid layer. Considering the effort required and the quality of information received, the diurnal analyses are not recommended.

Evaluation of three-phase microcosm

Several features of the microcosm were used to advantage: 1) the closed system made it possible to perform mass balance analyses for important elements, 2) the semi-continuous input of medium provided samples for continual measurement of pollutant level, and 3) the flexibility of the microcosm structure facilitated changing the mode of pollutant introduction.

Predictions based on the literature on marine microcosm experiments on the effect and fate of BA were confirmed in these small-scale laboratory microcosm experiments. Even at relatively high BA concentrations (3 µg/l), there was no evidence of any effect, either positive or negative, on the biological community. There may, however, have been sublethal responses that were undetected. In the absence of sufficient radiation for photooxidation, there was essentially no difference in persistence of BA between the lighted and dark environments. This is the case because photooxidation is the primary transformation pathway for dissolved BA.

While the microcosms used in these experiments were not particularly suited to detect sublethal responses of aquatic organisms, they worked well in determining the fate of relatively insoluble organic contaminants. In a recent symposium (Giesy 1980), the types of microcosms, their appropriate use, and experimental limitations are discussed. In general, microcosms having structurally simple ecosystems are best for determination of chemical fate (mechanisms, rates) or chemical toxicity. Complex ecosystems are more suitable to study the effects of contaminants on biological structure and functions and on interactions among organisms that affect bioaccumulation.

The results of the present study fit the above generalization. The system had relatively simple trophic structure. The compound was not toxic, and no significant differences in the biological response could be detected. The best data described the fate of the compound given a contamination procedure, physical environment, and compound recovery scheme.

Given this information on microcosm use post facto, it is unfortunate that energy sources such as UV radiation were excluded from this study. Photodegradation is the primary transformation mechanism determining the fate of BA in aquatic systems (Herbes et al. 1976), and a realistic physical/chemical environment was not in effect for valid

measurement of its effect on compound degradation.

Other precautions should be taken in microcosm design (Giesy 1980) in scaling down the environment for experimental advantage. Two important problems are related to the disproportioned surface-to-volume and sediment-to-liquid ratios (Harris 1980). Of special concern was how the attachment of phytoplankton to microcosm walls can bias experimental results. This phenomenon occurred early (by the second week) in the experiment, hampered by the assessment of biomass and bioaccumulation, and potentially altered the response of the community.

Evaluation of the Hazard to Humans from BA in the Aquatic Environment

Trace quantities of BA were detected in extracts of algae and flies. The concentration in the algae was just slightly above that in solution (partition coefficient, K_p=1.1). This suggests that the compound is merely being sorbed to the algae as opposed to being actively assimilated. On the other hand, the flies in microcosm #6 contained a much higher concentration of BA (partition coefficient, K_p=10) than that found in the medium. This observation was not confirmed by data from the other two lighted microcosms, probably because there were fewer flies (0.5, 1.3, and 4.4 mg in microcosms #2, #4, and #6, respectively), and thus a BA concentration below the detection limit of the GC/MS (approximately 5 ng/μl). The data suggest that fly larvae concentrate BA from the algae-food source. Further experimentation is required to distinguish bioaccumulation from passive adsorption by organic matter.

In experimental studies of the accumulation of PAH through the ingestion of food, it is generally found that the majority of the PAH is excreted unmetabolized. This is not always the case. Ability to absorb and assimilate PAH from food depends upon the organism, the PAH, and the type of food. Neff (1979) concluded that food-chain biomagnification requires that the material must be readily absorbed from the food and relatively resistant to metabolism or excretion.

In the present experiment, the conditions existed for assimilation to occur. Given the small bioaccumulation factor (K_p=10) and the relatively high concentration of BA both in the sediments and the water, the potential for humans to become exposed to BA in food is low.

These overall results support speculation by Neff (1979) that BA (and probably other higher molecular weight PAH) are relatively unavailable for biomagnification in aquatic food chains. Potential pathways to humans that bare watching include consumptions of shellfish recently exposed to PAH-containing effluents and of water taken from the hypolimnion of a reservoir receiving PAH from contaminated sediments.

Significance of Research

The results presented in this report have furthered our understanding of both the behavior of trace organic chemical contaminants and the appropriate way to study this behavior. Previously, small-scale microcosms, similar to the one used in this study, had been used to study both the fate and effect of materials which had an acute effect (stimulative or detrimental) on community productivity (Medine 1980, Porcella et al. 1975, Stube et al. 1976). The present study indicated that the three-phase microcosm could also be used to study the effect of environmental elements on the behavior of non-toxic potentially hazardous pollutants in long-term experiments.

The results of long-term experiments of this nature could be used to reduce the hazardous impact of organic

chemicals. For example, 1) knowledge of the pollutants which are most harmful to humans and natural resources and under what conditions they have their effect, would permit a concentrated effort put toward their containment; 2) understanding the behavior of potentially dangerous compounds would allow a priority sampling scheme for monitoring pollutants so that surveys could be more effective in warning of contamination; and 3) information about the way the hazardous chemical degrades under different physcial and chemical environments, would lead to increasingly effective treatment methods.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The response of the Lake Powell aquatic environment to an input of benz(a)anthracene (BA) was simulated in sediment, water, gas, biota microcosms. Conclusions were:

1. No toxic effects due to BA addition were demonstrated by reduced biological activity or survival of organisms.

2. BA addition did not affect the final biomass or productivity of the aquatic community.

3. Mass balance analyses on the lighted microcosms indicated that approximately 76.1 percent of the BA introduced was found in the sediments, 17.3 percent in the water, 0.1 percent in the algae, 0.1 percent in the flies, and 0.8 percent was sorbed to the glass walls of the microcosm. The remaining 5.6 percent was not accounted for; the majority of this was thought to have been transformed through photolysis.

4. Mass balance analyses of the single dark microcosm indicated that 71.4 percent of BA was found in the sediments, 25.9 percent in the water, and 2.1 percent was sorbed to the glass walls of the microcosm. The remaining 0.7 percent was not accounted for.

5. Literature predictions of the persistence of BA in reservoirs and of sorption as the major pathway of removal from the aquatic system were confirmed in these microcosm experiments.

6. Chironomid flies accumulated BA to a concentration of 660 ppm (partition coefficient, K_p=10). It is not known whether this level of compound in the organisms was assimilated from the food source (algae) which contained a BA concentration of 4-5 ppm.

7. Metabolic products of BA were not detected.

8. Recommendations on experimental techniques include:

a. Sediment contamination by applying a solution of volatile organic solvent to dried sediment as opposed to direct injection into the medium or sediment contamination using a solution of a relatively non-volatile solvent.

b. GC/MS for detection and quantification as opposed to fluorescence for which there is interference due to high levels of background fluorescing compounds.

c. Daily gas volume and weekly gas compositional measurement to determine community productivity as opposed to bi-weekly diurnal oxygen mass balance experiments.

d. No technique was found adequate to monitor algal biomass through time.

9. Advantages of using the three-phase model ecosystem to test predictions of the overall fate and effect of trace organic pollutants, such as BA, include:

a. Daily monitoring of community productivity.

b. Weekly measurement of nutrient status and other chemical parameters (pH, dissolved oxygen).

c. Weekly measurement of dissolved pollutant concentration.

d. Daily observation of algal growth and invertebrate activity.

10. Disadvantages include:

a. Most of the algal biomass was periphyton, and this parameter can only be measured after the experiment is terminated; thus the effect of treatment on the rate of biomass growth cannot be assessed while the experiment is in progress.

b. The fate of the compound can only be deduced from initial and final analyses of microcosm phases and weekly measurement of dissolved concentration. No continuous sampling of sediment nor biota can be used to clarify the behavior of the compound.

c. Since the microcosm is designed to measure the effect of treatment in terms of productivity and aquatic chemistry, only compounds and elements which affect photosynthesis will be labeled as contaminants.

Recommendations for Further Research

1. Microcosms structured and monitored as recommended above should be used to study the effects on chemical fate of critical environmental features (particularly radiation intensity and its portion in ultraviolet wave lengths).

2. Radio-labeled compounds should be used with these experimental microcosms to identify and trace metabolic products.

3. Direct or indirect measurement of algal biomass is needed in microcosm studies to determine the effect of treatment on growth and species composition as well as to investigate sublethal effects on cell structure.

4. Additional effort should be made to increase the sensitivity of the microcosm as a tool to detect small differences in treatment. More replication or more care during initial experimental startup is needed to reduce variation within treatment.

5. In situ studies in Lake Powell using large volume microcosms are needed to try to determine the biogeochemistry of BA in the natural reservoir environment, including its long term effect on organisms.

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APPENDIX A

GENERAL OBSERVATIONS ON THE RESPONSE AND OPERATION OF THE MICROCOSMS

Table A-1. Experiment #1.

Elapsed Days	Date	Observation	Microcosm 1→4 light Microcosm 5→8 dark Odd numbers = control Even numbers= BA Treatment
	7/7	Sediment placed in caps	
	7/14	Systems filled with media	
1	7/15	Water is cloudy (sediment fines) in all light microcosms. #1, 2, and 3 have burrowing worm activity	
2	7/16	#5 drew in 100 ml acid, emptied and refilled with enough NaOH to neutralize (pH 7.1); #6 bottom port broke, liquid emptied sucking 20 mls acid, replaced port, neutralized with NaOH and refilled	
3	7/17	#1, 3, and 4 petri dish has algal growth. #6 lost 1 liter, refilled	
4	7/18	Had to reset all manometers because the microcosms leaked	
5	7/19	Sediment surface green with algal growth for all light microcosms (#1, 2, 3, and 4); #2 bubbles at sediment surface	
6	7/20	#1 bubbles on sediment surface, #2 less green than others (1, 3, and 4). Dark microcosms (#5, 6, 7, and 8) nearly identical in appearance --water is clear and sediments are unchanged	
7	7/21	#1 and 3 bubbles from sediment	
8	7/22	Analysis day	
9	7/23	#1, 2, and 4 Sediment very green as well as lower 1/3 of liquid. #6, 7, and 8 all becoming cloudy	
10	7/24	Cross inoculate 30 percent of media exchange (within treatment with respect to BA and light)	
11	7/25	#1, 2, and 4 sediment covered with algal growth, #4 scum lifting off sediment, all dark microcosms are cloudy	

Table A-1. Continued.

Elapsed Days	Date	Observation	Microcosm 1→4 light Microcosm 5→8 dark Odd numbers = control Even numbers= BA Treatment
15	7/29	Dark microcosms still show no change	
18	8/1	Analysis day and Diurnal	
20	8/3	#1 and 3 liquid is clear, but patchy growth on sediment surface #2 and 4 cloudy, sediment surface fully covered with algal growth	
22	8/5	#2 and 4 still cloudy, with small white particles (diatoms?) suspended, #3 dark objects on petri dish (diatoms)	
23	8/6	Diurnal	
25	8/8	#2 still cloudy	
26	8/9	#1, 3, and 4 all clear, #2 getting cleared #1 and 3 have brownish growth on petri dish	
28	8/11	Analysis day and Diurnal, #2 nearly clear	
30	8/13	#1 and 2 have patchy covering of algal mat on sediments and a brown growth on petri dish. #2 and 4 sediment surface is completely covered with algal mat	
31	8/14	#5 sediment has black colored zone 2 cm below interface	
33	8/16	Diurnal #1 sediment is full of channels throughout upper half; #2, 3, and 4 sediment has channels throughout entire depth; dark microcosms have channeling throughout, some black zones in #5 and #6	
34	8/17	Lighted microcosms beginning to have wall growth, especially in #1, #5 broke gas sampling septum in trap	
37	8/20	#7 has strange goldish-green coloration to liquid that other dark microcosms don't have	

Table A-1. Continued.

Elapsed Days	Date	Observation	Microcosm 1→4 light Microcosm 5→8 dark Odd numbers = control Even numbers= BA Treatment
38	8/21	Analysis day; #1 and 3 brown growth on petri dish, #2 and 4 white growth on petri dish, #7 still gold-green color	
39	8/22	Diurnal, growth on walls of lighted microcosms becoming more obvious, #3 has white particles suspended in it	
40	8/23	#5 replaced acid trap	
44	8/30	Began applying vacuum to media to try to remove benzene (1/2 hr)	
45	8/31	Analysis day	
47	9/2	#3 white growth on petri dish, sediment surface rough with small patches of algae raised above the surface, inside wall appears coated with algae, #5 water clear, slightly yellow in color, little sediment activity, #6 water is nearly clear, little channeling in sediment	
50	9/5	Experiment terminated and microcosms dismantled	

Table A-2. Experiment #2.

Elapsed Days	Date	Observation	Microcosm 1→4 light Microcosm 5→8 dark Odd numbers = control Even numbers= BA Treatment
	9/19	All microcosms filled with sediment. Layered contaminated sediment 167.6 g total/microcosm and placed tubes on. Filled all with media (22°C). Checked for leaks. Overlying water looks clear.	
1	9/21	All looks well	
5	9/25	Cross inoculation (within treatments, 300 ml)	
7	9/27	Cross inoculated (300 ml)	
9	9/29	Diurnal	
11	10/1	#1, 2, 3 + 4 all have algal growth on sediment	
12	10/2	Diurnal #1, 2, 3 + 4 sediment surface is green in patches	
15	10/5	Diurnal	
17	10/7	Experiment terminated	

Table A-3. Experiment #3.

Elapsed Days	Date	Observation	Microcosm 1→4 light Microcosm 5→8 dark Odd numbers = control Even numbers= BA Treatment
	10/9	Sediments added to microcosm cap	
1	10/10	Contaminated sediments added to the treatment microcosms. Systems partially filled with media and checked for leaks	
5	10/14	Microcosms filled rest of way with media, algae added and systems sealed	
6	10/15	All sediments have oxidized layer due to sitting out for 3 days prior to filling with contaminated sediments	
7	10/16	#1 had dark spots (anaerobic zones) in sediment below oxidized zone	
8	10/17	#1 and #5 have one copepod each, has bubbles on surface, put more algae in with media	
9	10/18	#1 has one burrowing worm, still see copepod, liquid turning green. #2 has worm, turning brown, #3 has 2 worms, is yellow-green, #4--no activity, brown like #2. Acid spill in #4, replaced media. Dark microcosm--generally nothing happening	
10	10/19	Diurnal and Analysis day	
11	10/20	#1-sediment covered with algal mat, lots of bubbles beneath; liquid green, fine filament on walls, #3 same as #1 but not as well developed, #4 lost	
12	10/21	#1 algal mat has broken away from sediment surface with bubbles, liquid really green, 3 copepods; #2 beginning to green up; #3 lots of suspended algal growth; #5 has 2 copepods	
15	10/24	Diurnal	
18	10/27	#1 lower 1/4 of glass wall is covered with growth, mostly filamentous	

Table A-3. Continued.

Elapsed Days	Date	Observation	Microcosm 1→4 light Microcosm 5→8 dark Odd numbers = control Even numbers= BA Treatment
20	10/29	Diurnal and Analysis Day; #1 and #3 several copepods	
27	11/4	#7 sediment surface partly black lost of burrowing activity with depth; #8 sediment surface reddish same as initially, very little activity with depth	
30	11/7	Diurnal and Analysis Day	
34	11/11	#7 burrowing worm	
35	11/12	#7 three worms seen. Diurnal	
	11/13-11/18	All microcosms open	
41	11/18	Microcosms closed; Diurnal and Analysis Day	
	11/19-11/29	All microcosms opened	
48	11/26	#2 and #3 sediment algal mat lifting up	
50	11/28	Analysis Day	
51	11/29	Microcosm closed; Diurnal	
52	11/30	Experiment terminated	

Table A-4. Experiment #4.

Elapsed Days	Date	Observation	Microcosm 1→4 light Microcosm 5→8 dark Odd numbers = control Even numbers= BA Treatment
1	12/11	Systems open; all were inoculated with algal culture; waiting for petri dish for #4; had to refill #8 due to leak	
2	12/12	Cross inoculated control with control and treatment with treatment lighted systems; put petri dish in #4	
3	12/13	Petri dish broken in #6, had to remove some media, remove dish parts; sediments got stirred up; cross inoculated lighted systems as on 12/12 (500 mls)	
4	12/14	Analysis Day	
5	12/15	Diurnal, cross-inoculated (500 ml); #6 low alkalinity added 57.5 mg $NaHCO_3$. Sediment surface slightly green (in order of intensity) #3,1,2,5,4,6; #2 and #3 have worm activity; #3 bubbles coming from sediment; evening--checked alkalinity of #6, ok	
8	12/18	Diurnal; #6 scum beginning to lift off sediment surface; not as green as #1 and #3	
9	12/19	All lighted microcosms are green with suspended algae	
10	12/20	#3 opened to clean acid out of trap	
11	12/21	Analysis Day; #2 and #4 scum floated from sediment to water surface has filamentous algae (Lyngbya); #7 and #8 worm activity in sediment	
12	12/22	Diurnal	
13	12/23	#1,2,3,4,5 sediment scum has floated to top of water column	
14	12/24	Purged all lighted microcosms (#1-6) 4 minutes with breathing air	
15	12/25	Diurnal	

Table A-4. Continued.

Elapsed Days	Date	Observation	Microcosm 1→4 light Microcosm 5→8 dark Odd numbers = control Even numbers= BA Treatment
18	12/28	Analysis Day	
19	12/29	Diurnal	
20	12/30	All lighted systems--lots of algal colonies of walls especially lower 1/3 and lots of bubbles from sediment #2 suspended shite particles (diatoms ?) in upper 1/3 of column; 1 daphnia, 1 copepod #4 white particles same as #2, scum mat an irridescent green color; #5 column greener than other systems; #6 white particle same as #2 1 daphnia; #8 opened to clean out acid trap	
23	1/2	Diurnal	
25	1/4	Analysis Day	
26	1/5	#1,2 and 3 flies at liquid surface; purged all lighted microcosms (#1-6) for 4 minutes with breathing air	
29	1/8	Diurnal; power off for 4.5 hours during light period	
32	1/11	Analysis Day	
33	1/12	#2 has 6-7 daphnias	
36	1/15	Diurnal	
39	1/18	Analysis Day; #1 no algal mat, dispersed colonies of algae on walls, stirbar is covered; #2 little bit of algal mat on sediment, scum on walls; #3--no algal mat, scum on walls; #4 little mat on sediment, algal colonies on walls of microcosm; #5 mat on surface, column green with suspended algae; #6 surface scum attached but floating; new scum on surface and walls	
41	1/20	#1,2 and 3--flies; #2 still has daphnia; all lighted systems were purged with breathing air for 5 minutes	

Table A-4. Continued.

Elapsed Days	Date	Observation	Microcosm 1→4 light Microcosm 5→8 dark Odd numbers = control Even numbers= BA Treatment
42	1/21	#6--saw one ostracod	
43	1/22	Diurnal	
46	1/25	Analysis Day; #1 growth is sparse; #2 lots of growth on walls; #3 some filamentous algal growth on walls; #3,4,5 all still very green with suspended algae; #6 has growth on walls like #2	
47	1/26	#6 has green filamentous algae in column in upper 1/3	
50	1/29	Diurnal #1,2 and 6 larvae (chironomid flies) in sediment and on glass walls; #3 dark green filamentous alga; #6 light green filamentous alga; all through liquid phase	
53	2/1	Analysis Day; #4 and 5 not much wall growth as in #1,2,3 and 6, but lots of suspended algae; #6 filamentous growth well developed	
54	2/2	#1 bright green algal growth same as noticed in #6 on 1/26	
55	2/3	#1 green filament becoming more prominent, larvae in sediment not on walls; #2 some patches of green algae attached to wall; #3 and 4 little growth; #5 more algal growth than 3 and 4 but not much; #6 very green, lots of filamentous green, walls with scum, also lots of worm activity	
56	2/4	Diurnal; #3 filament up from sediment in long streamers attached to sediment and extending 1/2 way up the wall	
60	2/8	Analysis Day; #1 lots of green filamentous growth throughout, pupal cases (empty) at top; #2 daphnia of all sizes, pupal cases at top, some filamentous algae as in #1; #4, 5 and 6 pupal cases; #5 can see flies	

Table A-4. Continued.

Elapsed Days	Date	Observation	Microcosm 1→4 light Microcosm 5→8 dark Odd numbers = control Even numbers= BA Treatment
62	2/10	Microcosms dismantled #1 saved algae and flies; #2 had 150 flies, daphnia also collected; #3 had 6 flies (not collected)	
63	2/11	Microcosms dismantled #5 has filamentous growth on sediment surface; #6 has some daphnia	

APPENDIX B

EXPERIMENTAL DATA

Table B-1. Mass balances of gas detected in microcosms accumulated over period of study.

Day	Microcosm #1 Net Volume (ml at STP)	Microcosm #1 O_2 (mg)	Microcosm #1 CO_2 (mg)	Microcosm #2 Net Volume (ml at STP)	Microcosm #2 O_2 (mg)	Microcosm #2 CO_2 (mg)
5	2.11	0.59	0.01	0.13	0.04	0.0
8	68.49	21.66	0.034	34.46	11.06	0.016
12	183.21	65.78	0.052	178.81	66.25	0.046
15	238.90	83.28	0.086	234.64	83.20	0.135
19	272.96	95.20	0.100	267.05	94.24	0.176
23	312.28	110.84	0.107	306.18	110.01	0.200
29	388.76	139.15	0.145	388.01	138.17	0.244
36	452.21	163.50	0.164	487.55	176.30	0.269
43	469.72	170.02	0.170	519.93	188.20	0.283
50	469.91	170.09	0.170	546.79	197.24	0.312
56	469.87	170.08	0.169	556.24	200.55	0.328
59	484.00	174.72	N.A.[a]	587.56	212.00	N.A.
	Microcosm #3			Microcosm #4		
5	4.88	1.35	0.003	4.28	1.16	0.003
8	64.86	20.70	0.028	34.89	10.82	0.017
12	197.67	70.74	0.049	160.36	57.37	0.034
15	253.95	88.28	0.134	217.21	76.07	0.141
19	309.24	107.92	0.207	241.79	84.43	0.177
23	353.26	130.81	0.269	274.54	91.64	0.193
29	436.77	156.36	0.302	367.31	129.09	0.246
36	528.51	192.77	0.329	465.95	169.92	0.276
43	581.20	211.28	0.356	537.33	197.65	0.303
50	693.89	254.96	0.398	609.29	224.09	0.332
56	776.10	290.92	0.412	654.44	241.71	0.339
59	843.90	322.95	N.A.	691.92	256.83	N.A.
	Microcosm #5			Microcosm #6		
5	2.86	0.79	0.002	-1.74	-0.48	0.0
8	9.93	3.12	0.004	12.50	4.02	0.005
12	49.30	17.71	0.008	83.17	29.02	0.015
15	75.68	26.35	0.059	119.24	39.54	0.093
19	107.67	36.80	0.102	189.08	63.35	0.197
23	138.04	46.71	0.122	220.75	75.70	0.218
29	183.77	62.05	0.146	256.34	86.73	0.239
36	239.24	84.78	0.163	273.41	92.60	0.248
43	266.42	98.32	0.179	286.10	97.19	0.249
50	307.24	109.87	0.202	292.43	99.79	0.250
56	330.01	118.55	0.219	331.82	115.29	0.257
59	337.46	121.55	N.A.	344.60	120.83	N.A.
	Microcosm #7			Microcosm #8		
5	-4.30	-3.85	-0.003	-5.63	-5.03	-0.004
8	2.72	+2.31	0.001	-1.34	-1.23	0.0
12	3.10	+2.61	0.001	-6.53	-5.67	-0.005
15	-4.98	-4.27	-0.004	-11.19	-11.73	-0.011
19	-14.44	-12.58	-0.011	-12.83	+1.47	-0.013
23	-32.96	-28.97	-0.024	-24.24	-8.6	-0.022
29	-52.41	-46.24	-0.039	-41.82	-24.31	-0.038
36	-63.68	-55.48	-0.048	-53.43	-34.09	-0.049
43	-81.66	-70.6	-0.063	-85.00	-60.04	-0.079
50	-106.16	-92.04	-0.071	-111.71	-77.36	-0.105
56	-135.11	-116.27	-0.096	-147.08	-104.6	-0.141
59	-139.10	-119.43	N.A.	-151.78	-108.13	N.A.

[a] N.A. = data are not available.

Table B-2. System parameter and nutrient measurements over period of study.

	Day	pH	Alk. mg/l as $CaCO_3$	Dissolved Oxygen mg/l	NH_3-N mg/l	$NO_2 + NO_3$-N mg/l	PO_4-P mg/l	Total P mg/l	Total Bacteria #/ml x 10^{-5}	TOC mg/l
Microcosm #1	4	7.6	125	8.4	<0.010	1.12	0.027	0.043	2.10	30.64
	11	8.1	121	12.9* [a]	<0.010	0.02	<0.010	0.024	0.48	34.53
	18	8.0	124	11.1*	<0.010	0.07	<0.010	0.028	N.A. [b]	32.21
	25	8.0	138	10.3*	<0.010	0.03	<0.010	0.032	0.35	22.64
	32	8.2	126	10.2	<0.010	0.01	<0.010	0.022	0.52	14.41
	39	8.9	122	10.2*	<0.010	0.13	<0.010	0.022	0.31	15.10
	46	7.6	134	7.9*	<0.010	0.34	<0.010	0.018	0.14	9.54
	53	7.5	134	8.4	<0.010	1.11	<0.010	0.015	0.05	12.08
	60	7.6	122	8.8	<0.010	0.37	<0.010	0.027	N.A.	10.89
Microcosm #2	4	8.1	91	7.6	0.012	1.19	0.027	0.043	2.80	21.78
	11	8.5	113.5	13.8*	0.010	0.02	<0.010	0.027	0.16	40.77
	18	8.2	121	14.4*	<0.010	0.04	<0.010	0.028	N.A.	38.86
	25	8.1	139	12.6*	<0.010	0.03	<0.010	0.022	0.10	34.87
	32	8.3	122	11.5	0.012	0.04	<0.010	0.022	0.52	22.35
	39	8.0	118	10.5*	<0.010	0.08	<0.010	0.021	0.21	20.08
	45	7.6	134	8.6*	<0.010	0.19	<0.010	0.028	0.34	19.31
	52	7.6	133	9.3	<0.010	1.13	<0.010	0.019	0.09	11.97
	60	7.6	132	10.0	<0.010	0.28	<0.010	0.026	0.33	10.30
Microcosm #3	4	8.0	82.5	7.7	<0.010	1.20	0.028	0.042	0.73	20.23
	11	8.4	116.0	12.7*	<0.010	0.02	<0.010	0.028	0.12	46.01
	18	8.2	127	11.2*	<0.010	0.03	<0.010	0.031	N.A.	30.73
	25	8.2	131	11.0*	<0.010	0.02	<0.010	0.027	0.23	29.69
	32	8.4	126	11.2	<0.010	0.03	<0.010	0.025	0.63	18.98
	39	8.1	119	11.2*	<0.010	0.04	<0.010	0.026	0.24	16.30
	46	7.8	133	10.4*	<0.010	0.03	<0.010	0.028	0.18	17.23
	53	8.0	132	13.3	<0.010	1.08	<0.010	0.034	0.07	22.35
	60	8.3	132	15.7	<0.010	0.07	<0.010	0.031	0.07	15.28
Microcosm #4	4	8.3	117	7.7	0.034	1.19	0.032	0.032	0.78	26.15
	11	8.5	127.5	13.0*	0.017	0.11	<0.010	0.037	0.19	48.16
	18	8.3	121	11.1*	0.028	0.07	<0.010	0.029	N.A.	28.31
	25	8.1	132	11.1*	<0.010	0.03	<0.010	0.024	0.05	28.60
	32	8.5	138	11.8	<0.010	0.02	<0.010	0.031	0.05	20.36
	39	8.4	113	12.6*	<0.010	0.03	<0.010	0.030	0.16	20.70
	46	8.0	127	10.2*	<0.010	0.04	<0.010	0.031	0.18	19.40
	53	7.8	132	11.1	<0.010	0.08	<0.010	0.041	0.30	27.85
	60	7.9	131	11.8	<0.010	0.08	<0.010	0.050	0.06	23.01
Microcosm #5	4	8.3	107	8.3	0.035	1.16	0.036	0.036	0.89	21.75
	11	8.4	129	13.2*	0.011	0.03	<0.010	0.037	0.14	38.32
	18	8.5	128	11.6*	<0.010	0.03	<0.010	0.034	N.A.	46.24
	25	8.3	131	8.7*	<0.010	0.03	<0.010	0.024	0.08	26.70
	32	8.4	135	10.9	<0.010	0.02	<0.010	0.031	0.24	26.36
	39	8.4	124	13.2*	<0.010	0.05	<0.010	0.016	0.18	28.02
	46	8.0	131	10.3*	<0.010	0.04	<0.010	0.031	0.06	24.52
	53	8.0	135	11.3	<0.010	0.10	<0.010	0.029	0.22	14.00
	60	7.9	133	12.2	<0.010	0.06	<0.010	0.042	0.21	12.89
Microcosm #6	4	7.4	N.A.	7.2	<0.010	1.20	0.023	0.036	1.3	16.56
	11	8.2	107	11.9*	0.013	0.21	<0.010	0.036	0.09	43.58
	18	8.1	125	10.6*	<0.010	0.15	<0.010	0.031	N.A.	38.85
	25	8.2	137	8.3*	<0.010	0.03	<0.010	0.018	0.18	24.08
	32	8.1	134	9.6	<0.010	0.08	<0.010	0.037	0.19	18.57
	39	8.1	124	11.0*	<0.010	0.08	<0.010	0.030	1.3	15.17
	46	7.8	132	9.2*	<0.010	0.25	<0.010	0.022	0.17	14.62
	53	8.0	134	11.6	<0.010	0.22	<0.010	0.020	0.12	13.30
	60	8.2	132	13.6	<0.010	0.10	<0.010	0.044	0.17	13.92

[a] * refers to times when the aqueous phase of the littoral microcosms were purged with breathing air for 4 minutes.

[b] N.A. = data are not available.

Table B-2. Continued.

	Day	pH	Alk. mg/l as $CaCO_3$	Dissolved Oxygen mg/l	NH_3-N mg/l	$NO_2 + NO_3$-N mg/l	PO_4-P mg/l	Total P mg/l	Total Bacteria #/mlx10^{-5}	TOC mg/l
Microcosm #7	4	8.4	121	6.7	<0.010	1.25	0.013	0.013	0.48	23.73
	11	8.0	131	7.2	0.033	1.14	0.024	0.031	0.09	30.27
	18	7.6	123	6.8	0.054	1.19	0.044	0.047	N.A.	43.36
	25	7.3	133	7.8	<0.010	1.17	0.044	0.044	8.6	19.55
	32	7.1	146	6.7	<0.010	1.05	0.044	0.055	2.7	21.35
	39	7.4	122	6.4	<0.010	1.00	0.044	0.047	1.3	1.47
	46	7.2	132	5.8	<0.010	1.07	0.038	0.053	1.2	10.28
	53	7.1	129	5.6	<0.010	1.08	0.044	0.053	0.58	10.93
	60	7.5	126	5.8	<0.010	1.01	0.039	0.057	0.15	16.96
Microcosm #8	4	8.4	121	7.2	0.010	1.26	0.021	0.024	0.75	25.91
	11	7.9	133	7.3	0.034	1.14	0.039	0.046	0.13	27.42
	18	7.6	125	7.3	0.104	1.2	0.044	0.047	N.A.	45.85
	25	7.5	133	7.3	0.010	1.17	0.045	0.047	1.9	22.99
	32	7.1	144	6.8	0.010	1.08	0.043	0.053	2.0	16.09
	39	7.4	121	6.5	0.010	1.04	0.045	0.045	2.7	32.49
	46	7.0	131	6.0	0.010	1.04	0.045	0.060	1.2	16.01
	53	7.0	133	5.6	0.010	1.13	0.045	0.045	0.82	27.13
	60	7.4	128	5.6	0.010	1.03	0.039	0.054	0.84	14.22

Table B-3. Mass balance of benz(a)anthracene in aqueous phase of microcosms.

Microcosm #2				Microcosm #4			
Day	Extraction Efficiency (%)	BA Concentration (µg/l)	Accumulated Mass to Date (µg)	Day	Extraction Efficiency (%)	BA Concentration (µg/l)	Accumulated Mass to Date (µg)
7	N.A.[a]	14.7	41.6	7	N.A.	14.5	37.1
14	47	9.7	115.5	14	98	9.0	102.4
21	80	5.7	178.3	21	89	4.0	157.3
26	42	9.0	213.8	26	100	5.0	188.6
33	100	6.6	250.1	33	100	6.3	218.9
41	100	3.4	282.8	41	100	4.1	246.9
47	100	3.3	305.5	47	100	2.8	266.3
53	100	3.1	324.5	53	100	3.5	281.4
58	84	3.0	335.0	58	91	3.4	292.7
60	84	1.1	365.5	60	97	1.1	317.8
Microcosm #6				Microcosm #8			
7	N.A.	14.1	39.6	7	N.A.	18.8	65.8
14	100	9.0	109.7	14	73	14.5	190.0
21	100	4.3	169.6	21	81	10.1	282.0
26	87	8.7	203.6	26	81	9.3	332.6
33	100	5.4	237.3	33	100	11.7	387.5
41	50	7.5	268.8	41	100	5.1	433.0
47	100	2.7	290.8	47	100	3.2	458.3
53	100	2.8	308.0	53	100	3.1	477.7
58	65	3.8	321.0	58	94	2.6	490.4
60	68	1.5	351.0	60	86	1.6	516.8

[a] N.A. = data are not available.

Table B-4. Final mass of benz(a)anthracene in non-aqueous phases of microcosm.

Source	Microcosm			
	#2	#4	#6	#8
SEDIMENT[a]				
0-1 cm	1314	1360	1343	1188
1-2 cm	182	185	182	240
GLASSWALLS	20.2	10.7	13.6	42.1
ALGAE	2.4	2.5	3.0	0
FLIES	0	0	2.9	None

[a]Extraction efficiencies:

Sediment, 0-1 cm average 81%, range 70-87%
1-2 cm average 82%, range 74-94%

Table B-5. Plankton species composition.[a]

	Day	Species[b]											
		Scenedesmus	Ankistrodesmus	Pinnate Diatoms	Centrate Diatoms	Mougeotia	Coelastrum	Pediastrum	Golenkinia	Lyngbya	Cosmarium	Cryptomonas	Monostyla
Microcosm #1	4	30	17	1	0	2	29	0	30	0	0	0	0
	11	30	30	8	2	30	30	0	30	0	0	0	0
	18	27	23	24	2	30	28	0	29	1	0	0	0
	25	18	2	28	1	30	17	0	30	0	0	0	0
	32	1	0	28	0	30	9	0	30	0	0	0	0
	39	5	0	30	2	30	0	0	30	4	0	0	0
	45	12	0	23	1	29	7	0	30	3	0	0	4
	52	10	15	8	28	0	9	1	21	0	1	0	2
	59	17	29	26	0	0	15	3	6	12	7	0	1
Microcosm #2	4	26	14	9	0	1	21	0	10	1	0	0	0
	11	30	30	28	0	11	30	26	30	0	0	0	0
	18	23	9	30	0	30	15	5	22	0	0	0	0
	25	24	0	29	4	30	9	12	25	10	0	0	0
	32	15	0	29	0	30	3	12	25	0	0	0	1
	39	2	0	30	0	27	3	1	20	0	0	7	0
	45	7	1	29	0	11	0	0	30	0	1	24	8
	52	20	0	28	1	2	19	0	24	15	4	0	29
	59	21	0	24	1	0	22	0	21	0	8	0	19
Microcosm #3	4	25	13	7	1	5	21	0	11	0	0	0	0
	11	30	30	30	0	30	30	7	27	0	0	0	0
	18	30	9	20	0	30	23	0	12	0	0	0	0
	25	27	0	30	2	30	22	0	16	1	0	15	0
	32	17	0	27	0	30	7	0	19	0	0	0	27
	39	18	0	25	0	30	7	1	19	0	0	0	27
	45	18	0	28	0	30	3	0	30	0	0	0	30
	52	6	0	23	0	0	11	0	29	0	0	20	30
	59	2	0	20	0	0	6	0	27	0	2	2	30
Microcosm #4	4	no sample											
	11	30	29	19	0	24	30	0	30	0	0	0	1
	18	30	13	24	0	30	5	0	29	0	0	6	0
	25	25	0	30	1	30	18	0	27	9	0	30	0
	32	12	0	30	0	30	21	2	21	1	0	4	0
	39	19	0	24	0	30	4	0	30	0	0	2	0
	45	13	0	17	0	30	3	0	29	0	0	9	0
	52	0	0	17	0	0	1	0	29	0	0	3	20
	59	3	0	18	5	0	2	0	30	1	0	0	21
Microcosm #5	4	24	13	7	2	3	25	0	29	0	0	8	0
	11	30	30	15	0	20	30	0	30	0	0	30	0
	18	30	0	22	0	30	26	0	30	0	0	30	0
	25	29	1	30	0	30	17	0	27	0	0	30	0
	32	30	0	23	0	30	4	1	27	0	0	6	0
	39	30	0	26	0	30	6	0	30	0	0	0	1
	45	30	0	26	0	30	10	0	30	0	0	0	0
	52	2	0	19	3	0	3	0	29	0	0	0	22
	59	1	0	10	0	0	5	0	28	0	0	0	14
Microcosm #6	4	4	11	9	1	1	30	0	14	0	0	0	0
	11	30	30	30	0	0	30	0	30	0	0	0	0
	18	22	14	28	0	9	11	0	30	0	8	0	0
	25	11	0	30	1	30	9	0	30	0	30	0	0
	32	15	0	30	0	30	2	3	29	0	1	1	0
	39	14	0	29	0	2	3	0	30	1	3	1	0
	45	19	2	29	0	9	3	0	30	12	0	1	0
	52	10	0	30	0	0	15	0	30	10	1	0	5
	59	28	12	29	0	0	29	0	30	19	0	8	11

[a] As indicated by the number of views in which the species was sited out of 30 views/sample.

[b] Reference: Prescott, G. W. 1964. How to Know the Freshwater Algae. Wm. C. Brown Co. Publishers, Dubuque, Iowa. 348 p.

Table B-6. Results of 24-hour diurnal productivity experiments.

Day	Period[a]	Microcosm					
		#1	#2	#3	#4	#5	#6
5	L	3.29[b]	4.85	0.11	0.39	0.88	2.37
	D	- 2.88	- 9.39	-10.00	- 4.33	- 1.47	-10.01
8	L	28.34	26.21	41.57	21.41	19.74	20.26
	D	-12.00	6.16	-14.60	- 4.31	- 2.29	- 6.26
12	L	38.04	28.06	57.23	22.42	30.67	28.17
	D	- 8.68	- 7.52	-10.69	- 4.30	- 6.39	- 4.98
15	L	22.27	37.13	49.71	36.49	53.66	37.75
	D	-10.58	-12.27	-20.33	- 8.27	-25.55	-14.56
19	L	16.21	19.46	34.93	19.97	25.67	29.42
	D	-10.62	-14.25	- 4.54	2.85	- 5.91	5.52
23	L	7.47	10.33	3.76	28.16	10.05	11.15
	D	12.95	6.33	15.27	- 2.23	-10.79	-10.79
29	L	No data due to power failure					
	D						
35	L	19.62	24.58	28.88	22.08	23.18	26.89
	D	2.91	9.33	- 3.80	1.49	-10.94	- 5.41
42	L	12.58	14.01	17.16	13.06	11.12	17.37
	D	- 6.89	- 3.45	- 0.07	2.53	-11.27	- 6.58
49	L	16.96	27.23	14.17	10.72	16.64	10.69
	D	-14.86	- 4.17	-14.16	2.75	-17.22	-11.24
55	L	13.66	20.47	36.89	27.80	24.12	22.17
	D	- 8.84	-21.16	- 4.39	- 6.34	-22.49	- 4.81

[a] L = Light period, 12 hr.
D = Dark period, 8 hr.

[b] Net oxygen mass gain or loss (-) in all phases of the microcosm.