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Carbon Sources and Algal Community Structure and Metabolism in a Reservoir Undergoing Eutrophication by Domestic and Industrial Effluents

Submitted to

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by

Troy C. Dorris
Professor of Zoology and Director, Oklahoma
Reservoir Research Center
Oklahoma State University

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Although the name of only the principal investigator is listed on the cover page, others made major contributions to the project. They include:

Ernest M. Hodnet, Ph.D., Professor of Chemistry
Louis P. Varga, Ph.D., Associate Professor of Chemistry
Jerry L. Wilhm, Ph.D., Associate Professor of Zoology
Sterling L. Burks, Ph.D., Research Associate Zoology
Frederick L. Spangler, Ph.D., Graduate Research Assistant
Rex L. Eley, M.S., Graduate Research, Zoology
Kenneth A. Kochsiek, M.S., Graduate Research, Zoology
John Ransom, Graduate Research, Zoology

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KEYSTONE RESERVOIR, THE STUDY AREA

Keystone Reservoir was formed in 1964 by construction of a dam across the Arkansas River just below the confluence of the Cimarron and Arkansas Rivers west of Tulsa, Oklahoma, (Figure 1). It was constructed by the U. S. Army Corps of Engineers for multiple uses including flood control, generation of hydroelectric power and recreation. Filling of the reservoir was completed in April, 1965.

Total watershed is 1.94×10^7 hectares. Surface area of the reservoir at power pool level (elevation 220 m) is 1.06×10^6 hectares. Gross storage capacity at that level is 8.18×10^8 m^3. Maximum storage capacity at flood stage (elevation 230 m) is 2.32×10^9 m^3.

The Arkansas River receives effluents from urban areas and oil refineries in Oklahoma and Kansas. The Cimarron River receives effluents from a smaller number of urban areas and oil refineries in Oklahoma. High dissolved solids in the Cimarron River are derived from salt deposits in northwestern Oklahoma.

The Reservoir was continuously stratified throughout 1965 and part of 1966 because of a chemical density gradient (Eley 1967). Cimarron River water contained higher dissolved solids and was denser than the Arkansas River water. The dense Cimarron River water underflowed the lighter Arkansas River water, forming a stable "monimolimnion."

The Cimarron River headwaters are in a semi-arid region in which New Mexico, Colorado, and Kansas are adjacent to the Oklahoma panhandle. Elevation in this region ranges from 915 to 1,370 meters and average annual precipitation is less than 46 cm (Gray and Galloway, 1959). In northwestern Oklahoma the Cimarron River flows through mixed-grass prairie with average annual precipitation of 76 cm and elevation of 305 to 427 meters. Some soil areas of northcentral Oklahoma, particularly the Grant-Pond Creek-Nash association (Gray and Galloway, 1959), have accumulations of soluble salts which result in high conductivity, one of the peculiar features of the Cimarron River. Average annual precipitation in north central Oklahoma is about 97 cm. A wide range of variation occurs from year to year, however.

Keystone Reservoir is a harsh environment for aquatic flora. High turbidity greatly restricts the depth to which photosynthesis may occur. Attached macrophytes are not found at any point although heavy growths of Cladophora develop at surface level on rocky bluffs. Water level fluctuates greatly thus making even more difficult the establishment of bottom flora. Ice covered Station I to a depth of about two cm on one occasion and to a lesser depth on several others. Ice did not cover the other sampling sites at any time.

ORGANIC COMPOUNDS FROM OIL REFINERY AND DOMESTIC SEWAGE EFFLUENTS

A part of the present project was to investigate the organic chemical composition in the water in Keystone Reservoir in Oklahoma and to separate these compounds quantitatively as to source - either industrial effluents or domestic sewage. A major objective of the study which developed later was to adapt present advanced analytical techniques to analyses of trace aqueous organic compounds.

The accomplishments of the study of organic chemical compounds in Keystone Reservoir were: (1) determination of the comparative semi-quantitative concentration of dissolved organic compounds at different locations within the reservoir and (2) identification of a trace aqueous organic compound with gas chromatography-mass spectrometry.

The identification of organic chemical compounds in the aquatic environment has been the objective of many investigations (Vallentyne, 1957). Knowledge of the organic compounds in different types of aquatic environments is necessary to fully understand interactions between organisms and the surrounding aqueous medium. The number of natural and synthetic organic compounds positively identified in natural waters is less than 50 whereas the number of unknown compounds probably is very much greater. Analytical research is needed to identify the unknown aqueous organic compounds.

Methods for Dissolved Organic Compounds

Concentration Techniques

The techniques that have been developed to concentrate organic chemical compounds from aqueous solutions can be categorized as solvent extraction, distillation, adsorption, and freeze concentration. All of the techniques will effectively concentrate organic compounds from an aqueous solution but all have certain limitations.

Solvent extraction is an efficient technique for extracting and concentrating organic compounds from small volumes of water. Large volumes of water, however, require large volumes of solvent for extraction of organic solutes. The large volume of solvent is difficult to evaporate and may leave a residue of organic impurities in the final sample (Hoak, 1962).

Vacuum, steam, and fractional distillation techniques are useful for separating and concentrating relatively volatile organic compounds from an aqueous solution. Distillation is usually performed on small volumes of water, and is therefore limited as a technique for concentrating trace organic compounds from large volumes of water.

Freeze concentration of organic solutes has been investigated as a technique for concentrating organic compounds from water (Shapiro, 1961, Baker, 1965). The technique apparently did not alter the chemical structure of the compounds, inhibited bacterial degradation, and reduced the loss of volatile compounds. Quantitative recovery might be achieved if the operating conditions were carefully controlled. This technique should be valuable to future research on trace organic compounds in water.

Adsorption of dissolved organic compounds by activated carbon has been used to concentrate organic compounds which cause tastes and odors in water supplies (Braus, Middleton, and Graham, 1951). The carbon adsorption method (CAM) has been accepted as a tentative standard method by the American Water Works Association Subcommittee on Standard Methods of Organic Analysis (1962). The CAM has been used to collect several trace aqueous organic compounds which were identified (Rosen, Skeel, and Ettinger, 1963) and has given impetus to qualitative investigations of aqueous organic compounds (Ettinger, 1965).

The main component of the CAM is a glass column filled with granular activated carbon which adsorbs dissolved organic compounds from water passed through the column. The adsorbed organic compounds are extracted from the activated carbon by reflux distillation with chloroform and ethanol. Chloroform, a relatively nonpolar solvent, dissolved nonpolar organic compounds and ethanol, a relatively polar solvent, dissolves polar compounds. The ratio of the two extracts can be used as a somewhat subjective index to the type of organic matter involved, i.e., the polar compounds result from biological processes whereas the nonpolar compounds are derived from petrochemical processes.

The CAM is reproducible at ± 10 per cent (Anon. 1962, Booth, 1965). The rate of organic adsorption by activated carbon varies inversely with the flow rate of water through the carbon column (Booth, 1965). Hoak (1962) reported quantitative adsorption of phenol from a prepared solution, but solvent desorption recovered only 70 to 80 per cent of the carbon-adsorbed phenol. Ultra violet spectra of the desorbed phenol indicated changes in the chemical structure. Golden, et al. (1956) reported 72.7 per cent recovery of adsorbed ^{14}C-labeled phenol from activated carbon.

Activated carbon may differentially adsorb certain organic compounds. Baker (1964) used a gas chromatograph to quantify the carbon column influent and effluent of a prepared organic solution. 14 Grams of activated carbon (Nuchar C-190) removed 99.8 per cent of n-butanol from 590 ml of prepared solution (3,240 mg/liter). Adsorption efficiency dropped rapidly when larger volumes of solution were passed through the carbon column. Nuchar C-190 adsorbed 99.9 per cent of both solutes from a binary solution of n-butanol and n-amyl acetate before breakthrough of the n-butanol. The carbon continued to adsorb all of the n-amyl acetate but n-butanol was partially desorbed.

Daniels, et al. (1963) utilized the CAM to quantify dissolved organic compounds in Lake Michigan and Lake Huron. The ratio of the alcohol extract to the chloroform extract fluctuated seasonally with changes in water level and temperature of the lake.

The mean annual concentration of aqueous organic compounds collected from Lake Mandota by carbon adsorption was 614 µg/liter (Lee, Kumke, and Becker 1965). This is only five per cent of the quantity collected from Lake Mendota by Birge and Juday (1926) using centrifugation, evaporation, and combustion. Lake Mendota does not receive many organic effluents and was considered to be relatively low in organic contant, yet the CCE concentration in Lake Mendota exceeded the CCE concentration in the Ohio River (Lee, et al., 1965).

Rock, et al., (1966) concluded that turbidity at natural pH did not affect the qualitative recovery of organic chemical compounds from water, but removal of turbidity by sand prefilters improved the reproducibility of the quantitative results.

Weber and Morris (1963) reported that the rate-limiting factor in the carbon adsorption process was intraparticle diffusion of the solute molecules within the micropore structure of the granular activated carbon. The rate of adsorption of various organic compounds indicated than an inverse relationship existed between the rate of equilibrium attainment and size of the molecule. Hassler (1951) concluded that larger molecules were adsorbed more completely than smaller molecules of a homologous series of compounds.

The adsorption process appears to be related not only to molecular size which affects the intraparticle diffusion rate but also to solubility of the compound in water which affects the initial surface adsorption of the compound by the activated carbon. A slightly soluble organic compound with a structurally large molecule will be rapidly adsorbed from aqueous solution but will diffuse slowly within the micropore structure of the carbon. Thus the compound will rapidly saturate the carbon surface and the adsorption rate will decline rapidly.

Coughlin and Ezra (1968) reported that the surface acidity of activated carbon exerted a major influence upon the capacity of carbon to adsorb organic compounds. The authors reduced the adsorption capacity of activated carbon by a factor of eight by oxidizing the carbon. Subsequent reduction of the oxidized carbon restored only seven per cent of the original adsorptive capacity. The results were interpreted as confirming that organic solutes are adsorbed as a multimolecular layer on the surface of activated carbon.

In summary, the main limitation of the CAM is the variable recovery efficiency, which is affected by temperature, pH, turbidity, composition and concentration of organic solutes, contact time (flow rate), volume of water filtered, and surface acidity of the activated carbon. The CAM data may be interpreted as providing only semiquantitative indices to the concentration of organic compounds.

Despite its limitations the CAM was selected as a concentration method in the present study, since it is readily adapted to continuous field monitoring of dissolved organics and can concentrate dissolved organic compounds from a large volume of water.

Sampling sites were located at the upper and lower ends of the Arkansas and Cimarron arms of the reservoir (Fig. 1). A fifth sampling site was located on the Arkansas River approximately 27 kilometers below Tulsa, Oklahoma, 47 kilometers downstream from the reservoir.

Portable samplers based on the design used by the Robert A. Taft Sanitary Engineering Center were installed at each sampling station (Fig. 2). Malfunction of the constant head tank (D) and metering pump (E) necessitated installation of a direct line from the column effluent end (C) to the volumetric measuring tank (G). A flow valve was installed on the carbon column effluent line to control flow rate. This modification permitted continuous operation without daily attention. Water normally was pumped continuously through the carbon column for a week before the column was recharged with fresh carbon. Most samples were collected without prefiltration since there appeared to be no appreciable effect on qualitative recoveries and only a minor effect on quantitative reproducibility (Kumke, 1965, Booth, 1965).

Pyrex glass tubes 7.6 cm by 45.7 cm served as containers for the activated carbon. A 40-mesh stainless steel screen in a neoprene gasket prevented the granular carbon from being washed from the column. The end plates were constructed from 0.64 cm brass or plexiglass. Teflon tape was used to seal all joints and polyethylene tubing was used for water supply lines.

The pyrex tubes were filled with Nuchar C-190* (30 mesh) activated carbon without tamping. Water flow rate was normally adjusted to 1 liter/minute or less. The liquid level control was calibrated to dump the volumetric measuring tank at 1000 ml (± 5 ml) and actuate a digital counter.

The adsorbed organic compounds were sequentially extracted in a large-capacity Soxhlet extractor (Corning # 3885) for 48 hours with distilled chloroform and 95 per cent ethanol. The carbon chloroform (CCE) and carbon alcohol extract (CAE) were concentrated to approximately 250 ml by distillation and filtered through a 0.45-micron membrant filter* to remove particulate carbon from the sample. All of the chloroform and ethanol was removed by evaporation at 48 and 65° C, respectively. The quantity of organic compounds contributed by activated carbon blanks was determined by chloroform and ethanol extraction, and subtracted from the gross CCE and CAE. Net weight of CCE and CAE was converted to concentration by the equation:

$$\mu\text{g/liter of CCE or CAE} = \frac{\text{net grams of CCE or CAE} \times 10^6}{\text{liters of water filtered}}$$

The CCE and CAE concentrates were stored at 2 to 4° C until subsequent analytical tests could be performed.

Analytical Techniques

The complexity of the mixture of trace aqueous organic compounds has been a major obstacle to identification of individual compounds.

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*West Virginia Pulp & Paper Co., New York, N. Y.

Classical solubility separations (Cheronis and Entrikin, 1963) are time consuming, and usually are not adequate for separating complex mixtures such as exist in natural waters. Solubility separations are useful for separating the complex mixture into smaller, less complex fractions which can then be separated into individual compounds. The final separations can be performed by column, paper, thin-layer, or gas chromatography. Of these methods, gas chromatography and thin-layer chromatography have the greatest resolution capacity. Quantitative and qualitative analyses of micro-quantities of organic compounds have been aided in recent years by gas-liquid chromatography, and many successful applications to water pollution investigation have been reported (Baker, 1962, Cochran and Bess, 1966, Collins, 1966, Hindin, May, and Dunstan, 1965).

Compounds resolved by gas-liquid chromatography can be positively identified with infrared, nuclear magnetic resonance, mass spectrometry, or other analytical techniques. Infrared spectrometry has been used to identify compounds collected by the carbon adsorption method (Rosen, Skeel, and Ettinger, 1963). The list of compounds identified illustrates the complexity of aqueous organic chemical compounds; naphthalene, tetralin, styrene, acetophenone, ethyl-benzene, bis(2-chloroisopropyl) ether, 2-ethylhexanol, bis(2-chloroethyl) ether, di-isobutylcarbinol, phenylmethylcarbinol, and 2-methyl-5-pyridine. A combination of GLC, infrared, nuclear magnetic resonance, and mass spectrometry was used by Medsker, Jenkins, and Thomas (1968) to identify geosmin from blue-green algae and actinomycetes.

Gas-liquid chromatography retention time has been used to "fingerprint" volatile organic compounds which caused malflavors in drinking water (Caruso, Bramer, and Hoak, 1966). The suspected compounds had the same GLC retention times as phenol and napthalene. Swinnerton and Linnenbom (1967) used GLC to compare retention times of C_1 to C_4 standard hydrocarbons with retention times of hydrocarbons collected from Chesapeake Bay and identified ethane, ethylene, propane, propylene, isobutane, butene, n-butane, isopentane, and n-pentane. All of these compounds except butene were also identified in water collected from the Bahamas.

Sugar and Conway (1968) used GLC retention times on columns of different polarity to identify organic compounds in petrochemical wastes, both before and after biological treatment. The most positive information was obtained by mass spectral analysis of the GLC resolved peaks. 1-Hexanol was identified in the petrochemical waste by this procedure.

The utility of GLC in analyses of aqueous organic compounds has been demonstrated. However, complete elucidation of the complex mixture of organic compounds will require much additional research. New techniques and modifications of existing techniques must be developed before the organic composition of aquatic environments can be determined. The present study of aqueous organic compounds in Keystone Reservoir, in Oklahoma, utilized the carbon adsorption method for collection and gas chromatograph-mass spectrometry for qualitative analysis.

Initially, a separation based on solubility differences was used to separate the complex mixture of chloroform extractable compounds (USPHS, 1965). The neutral fraction was then chromatographed on a silica gel (Davidson code-950) column and eluted successively with

80 ml each of iso-octane, benzene, and chloroform:methanol (1:1).

Neither the solubility nor the column chromatography fractions could be completely resolved by GLC since the fractions contained many compounds with similar GLC characteristics and most of the compounds were not volatile enough to elute from the GLC at a maximum temperature of 300° C for several hours.

Steam distillation with continuous ether extraction of the steam distillate was investigated as an alternative procedure to separate a simple group of volatile organic compounds from the complex mixture. This procedure proved to be successful and was faster than solubility separation.

Two gas chromatographs, F & M Model 810 equipped with hydrogen flame ionization detector and F & M Model 700 equipped with a thermal conductivity detector, were used to resolve the volatile compounds. Various columns were used to resolve the mixture (Table I). The maximum operating temperature of a GLC column is determined by the stability of the liquid phase. The maximum temperature used in this study was 300° C. All GLC columns were pre-conditioned at 25 to 50° C above the anticipated maximum operating temperature. The percentage of liquid phase was reduced by "bleed-off", thus the actual percentage of liquid phase was less than reported.

Compounds resolved by GLC were collected by insertion of a capillary glass tube into the exit port of the gas chromatographic column (Kabot 1967). Condensation of the gaseous vapors generally occurred upon contact with the air-cooled capillary tube, but in some cases the capillary tube had to be cooled with an acetone-dry ice bath. The collected compound was transferred to a 1.5 mm KBr disc for

TABLE I

GAS LIQUID CHROMATOGRAPHY COLUMNS UTILIZED IN THIS INVESTIGATION

Material	Dimensions	Per Cent Liquid Phase	Stationary Solid Support
**Cu	1/8" x 6'	10% Se 30	80-100 mesh Diatoport S
*Glass	1/4" x 6'	5% Se 30	60-80 mesh Chrom W-AW-DMCS
*Glass	1/4" x 6'	5% OV-1	60-80 mesh Chrom W-AW-DMCS
*Glass	1/4" x 8'	5% OV-1	60-80 mesh Chrom W-AW-DMCS
**Cu	1/4" x 6'	20% Apiezon L	60-80 mesh Chrom W-AW
*Glass	1/4" x 6'	5% Apiezon L	60-80 mesh Chrom W-AW-DMCS
*Glass	1/4" x 8'	5% Polymethphenyl ether (6 ring)	" "
*Cu	1/8" x 12'	5% Carbowax 20M	" "
**Stainless Steel	1/4" x 20'	1% Carbowax 20M	60-80 mesh Diatoport S

* Prepared on a weight liquid phase/weight of support percentage, but column conditioning probably reduced the percentage of the liquid phase.

** Purchased prepacked from F & M Scientific Division of Hewlett Packard.

subsequent infrared analysis.

Infrared spectra were obtained with a Perkin-Elmer Model 137 infrared spectrophotometer. The instrument was equipped with a beam condensing unit which permitted spectra to be obtained from microquantities of organic compounds pressed into a 1.5 mm KBr disc. Liquid NaCl cells with a 0.1 mm path length were used when sufficient quantity of sample was available.

A combination gas chromatograph-mass spectrometer (GC-MS) instrument (Waller 1967) was utilized to supplement the infrared spectra. The combination GC-MS used the resolving capacity of the GLC to separate mixtures of organic chemical compounds and determines the mass spectra of the separated compounds as they elute from the GLC. The mass spectrum of a compound can be used to determine the molecular weight (M+). The fragmentation pattern produced upon electron impact can be utilized to aid in elucidating the structure of the compound. The GC-MS instrument allows positive identification of a trace compound without the necessity of purifying several milligrams of the unknown compound. If a sufficient quantity of the unknown is available, the GC-MS identification can be supplemented by infrared and nuclear magnetic resonance spectrometry.

RESULTS AND DISCUSSION

The concentration of CCE and CAE in the Arkansas River below Tulsa, Oklahoma, and also in Keystone Reservoir varied sporadically during this study (Fig. 3,4,5,6, and 7). Apparently, organic wastes of varying concentration and volume were occasionally discharged into the receiving streams above and below the reservoir. The temporal variation in concentration in the river was reflected by changes in the reservoir water despite quenching factors such as dilution, sedimentation, etc. The Cimarron River arm had less variation in organic concentration than the Arkansas River arm of Keystone Reservoir. Greatest variation occurred in the Arkansas River below Tulsa, Oklahoma. Variation in concentration of organic compounds apparently was indicative of the quantity and quality of organic effluents to receiving streams.

A multiple linear regression analysis was made of the CCE and CAE values, treating water flow rate and quantity filtered as covariables. A month x station interaction was detected for the CCE variable but not for CAE. CCE compounds must have entered the reservoir from point sources, since there was low correlation in rate of change in concentration among stations from month to month. The month x station interaction prevented a meaningful test for differences among annual station CCE means by multiple linear regression. A linear regression analysis within months and among stations was performed to detect the nature of the interaction (Table II).

TABLE II

MEAN CCE ADJUSTED FOR COVARIANCE OF FLOW RATE
AND VOLUME OF WATER FILTERED

Months	Station Monthly Mean (grams) A	C	D	E	F	$F^{*}_{cal.}$	Probability of larger $F_{cal.}$
12	64.6461	0.5100	0.7107	0.0547	1.1804	2622.69	(P<.0005)
1	1.3137	0.1402	2.6598	1.7761	0.7048	2.02	(.50>P<.25)
2	0.6857	0.4393	1.1953	0.7567	0.7483	0.14	high
3	1.8994	0.7623	0.4613	0.4583		3.62	(.25>P<.1)
4	2.0092	1.2989	1.8483	0.1942	1.3849	0.40	high
5	2.1283	0.0207	1.5572	0.8161	3.0305	5.10	(.10>P<.05)
6	2.5087	1.1110	1.6049	0.5498	0.4148	1.58	(.50>P<.25)
7	2.2613	-0.9752	0.0970	0.8422	0.8183	7.34	(.10>P<.05)
8	1.5190	0.6862	1.9675	0.8055	0.8733	1.05	(.50>P<.25)
9	1.4577	1.5125	0.5298	0.2986		1.25	(.50>P<.25)

* $F_{cal.}$ = Adjusted treatment mean square divided by error mean square.

Null Hypothesis: No difference among treatments after adjusting for covariance.

The mean weights of CCE were directly comparable, since adjustments for flow rate and volume of water filtered had been made in the analysis.

The concentration of CAE varied dependently among stations. CAE compounds must have entered the reservoir from many sources, causing a corresponding relative change in concentration at all stations from month to month. The multiple linear regression analysis of annual CAE means showed that there was a difference among stations ($.05>P<.025$). The deviation of station adjusted means from the total adjusted mean (Table III) was used to detect specific differences between stations (Table IV).

TABLE III

DEVIATION OF STATION CAE MEANS FROM ADJUSTED TOTAL MEAN

Station	Deviation from Total Mean (grams)
A	0.98258
C	-0.69317
D	0.56277
E	0.02479
F	-0.87697

TABLE IV

SPECIFIC DIFFERENCES BETWEEN ADJUSTED CAE MEANS

Contrast	Students' T	Probability of Larger T
A vs C,D,E, and F	3.863	($P<.001$)
C and D vs E and F	1.1814	($.2>P<.3$)
E vs F	2.2019	($P<.001$)
C vs D	-3.6384	($.02>P<.05$)

Null Hypothesis: No difference among treatments after adjusting for covariance.

An aggregate annual mean concentration of CCE and CAE was determined by dividing the total quantity of organic compounds collected by the total volume of water filtered (Table V). The aggregate was useful for visualizing the differences among the stations and permits the investigator to interpret differences shown by multiple linear regression analysis. Also, the aggregate mean can be compared with published values.

TABLE V

AGGREGATE MEAN CCE AND CAE

	CCE (µg/liter)		CAE (µg/liter)	
Station	Mean	Range	Mean	Range
Ark. below Tulsa	2,325	202 - 19,639	1,133	299 - 2,416
Reservoir Stations				
Upper Cimarron	262	6 - 752	734	153 - 3,087
Lower Cimarron	142	39 - 686	456	139 - 1,176
Upper Arkansas	302	44 - 6,241	538	221 - 1,732
Lower Arkansas	167	13 - 661	834	205 - 5,468

The CCE compounds are considered to be more detrimental to water quality than the CAE compounds (Middleton, Grant, and Rosen 1956). Station A located on the Arkansas River below Tulsa, Oklahoma, contained higher concentrations of CCE than the other stations (Table V). The CCE concentration of 19,639 μg/liter was the highest concentration collected and was higher than any published values.

The highest mean CCE concentration among reservoir stations occurred at the upper end of the Arkansas arm. The mean CCE concentration from the Arkansas River arm exceeded that from the Cimarron River arm of the reservoir. The lowest mean concentration of CCE was from the lower part of the Cimarron arm. There was a reduction in mean CCE concentration from the upper to the lower end of both arms in Keystone Reservoir. The reduction was 54 and 55 per cent in the Cimarron and Arkansas arms, respectively.

The highest aggregate mean concentration of CAE occurred in the Arkansas River below Tulsa, Oklahoma. However, the maximum CAE sample value was collected from the lower Arkansas River arm of the reservoir.

Within the reservoir, the lowest mean CAE concentration occurred at the lower end of the Cimarron River arm. The mean concentration of CAE decreased from the upper to the lower end of the Cimarron River arm. In contrast, the mean concentration of CAE increased from the upper to the lower end of the Arkansas River arm of the reservoir. The aggregate mean CAE concentration from the Arkansas River exceeded that from the Cimarron River arm of the reservoir.

There was no apparent correlation between visible evidence such as oil slicks and high concentration of either CCE or CAE. On two occasions, the surface of the Arkansas River below Tulsa was partially covered by an oil slick. There was a two-fold increase in CCE concentration on one occasion but not on the second occasion

Anoxic conditions developed in the stable hypolimnion in the Cimarron arm of the reservoir during the summer, 1966 (Eley 1967). Hydrogen sulfide and ammonia were produced during this period and were suspected to be the cause of a fish kill in the Arkansas River below Tulsa, Oklahoma. Although water from 10 meters depth from the lower end of the Cimarron River arm of the reservoir had a characteristic hydrogen sulfide odor, and some yellow crystals were obtained in the CCE, there were no radical fluctuations in either CCE or CAE concentration in this arm of the reservoir during the anoxic period. No apparent correlation existed between the fish kill and organic concentration.

The reduction in CCE concentration during passage through the reservoir indicates that some degradation of industrial wastes occurs in the reservoir. The amount of degradation is unknown, since the effects of dilution and sedimentation upon the concentration of CCE was not determined.

The increase in CAE concentration from the upper end to the lower end of the Arkansas River arm of Keystone Reservoir was probably due to the addition of municipal effluent at Cleveland, Oklahoma, and organic compounds from natural sources.

The aggregate mean concentration of CCE from Keystone Reservoir was relatively high compared to published values from other locations (Table VI). The drainage basin of Keystone Reservoir is dominated primarily by an agricultural economy. In contrast, the Ohio, Kanawha, and Missouri Rivers are in highly industrialized regions.

Five CAM samples were collected from an oil refinery effluent located on the Arkansas River above Keystone Reservoir (Table VII). The aggregate mean CCE concentration from the oil refinery effluent

TABLE VI

CONCENTRATION OF CARBON-ADSORBED ORGANIC COMPOUNDS FROM KEYSTONE RESERVOIR, ARKANSAS RIVER, AND OTHER LOCATIONS

Water Body	Reference		CCE µg/liter	CAE µg/liter
Arkansas River (below Tulsa)	Present Study	$\bar{X}$	2,325	1,133
Keystone Reservoir Upper Cimarron	" "	$\bar{X}$	262	734
Keystone Reservoir Lower Cimarron	" "	$\bar{X}$	142	456
Keystone Reservoir Upper Arkansas	" "	$\bar{X}$	302	538
Keystone Reservoir Lower Arkansas	" "	$\bar{X}$	167	834
Arkansas River Ponca City, Okla.	Nat. Water Qual Net. 1958-1959	X_i	108	135
" "	" 1962 "	X_i	58	76
Ohio River	" 1961 "	X_i	144	198
Columbia River	" "	X_i	28	68
Lake Mendota	Kumke 1963	$\bar{X}$	197	424
Kanawha River	Middleton & Lichtenberg 1960	X_i	1,800	311
Missouri River	Myrick & Ryckman 1963	$\bar{X}$	58	100
Sewage Effluent	Myrick & Ryckman 1962	X_i	7,000	22,000
Sacramento River	Greenberg 1965	$\bar{X}$	81	150

X_i = Single sample value

$\bar{X}$ = Mean value

TABLE VII

CARBON-ADSORBED ORGANIC COMPOUNDS FROM AN OIL REFINERY EFFLUENT

Date 1967	Water filtered Liters	CCE Concentration μg/liter	CAE Concentration μg/liter
6/23-6/29	3,653	6,735	1,728
6/29-7/6	3,850	7,396	1,509
7/6-7/13	814	6,369	3,656
7/13-7/27	4,942	6,557	1,326
7/27-8/3	3,297	7,566	1,733

was 6,983 μg/liter and the mean CAE concentration was 1,653 μg/liter. Petrochemical wastes have been considered to be mostly non-polar hydrocarbon type compounds with large CCE/CAE ratios. However, the CCE concentration from the refinery effluent was only about four times the CAE concentration. Bio-oxidation treatment of petrochemical wastes will result in formation of some metabolically oxidized compounds, which would be soluble in the alcohol extract.

The effluent had been treated in an API oil separator, bio-oxidation system, and held in oxidation lagoons before being discharged to the receiving stream. The aggregate mean CCE concentration was less than that reported for a municipal waste treatment plant (7,000 μg/liter) (Myrick and Ryckman 1962). The effluent had apparently been thoroughly treated but bio-oxidation cannot remove all of the organic compounds since many are refractory (Ludzack and Ettinger 1960). Dilution and some degradation of the oil refinery waste occurred before it reached the upper end of the reservoir.

Adsorption Efficiency

Two carbon columns were installed in series to determine the amount of organic compounds not adsorbed by the first column (Table VIII). The range of CCE and CAE compounds adsorbed by the second column was 1.3 to 60.8 and 3.8 to 83.2 per cent respectively of the compounds adsorbed by the first column in the series. These results indicate that a significant amount of organic compounds may not be adsorbed by a single column and that the semi-quantitative data might be low by a factor of as much as two in some cases.

TABLE VIII

CARBON ADSORPTION DATA FOR COLUMNS IN SERIES

Date 1966	Flow rate l/min.	Liters filtered	CCE 1st. column	CCE 2nd. column	CAE 1st. column	CAE 2nd. column
8/26-8/31	0.31	1,046	401	244	2,279	1,228
8/31-9/6	0.66	2,151	599	55	1,601	178
9/6-9/13	0.63	1,433	502	7	981	38
9/13-9/29	0.80	2,745	354	19	927	772
9/29-10/10	0.75	3,776	576	25	903	270

In order to determine the effect of flow rate on adsorption of organic compounds, carbon columns were operated in parallel at different flow rates (Table IX). The effect of sampling continuously for two weeks as compared to collecting weekly samples was also

TABLE IX

CARBON ADSORPTION DATA FOR COLUMNS IN PARALLEL

Date 1966	Flow rate l/min	Liters filtered	CCE	CAE	Flow rate l/min	Liters filtered	CCE	CAE
	Column A				Column B			
4/20-4/27	0.80	4,395	219	700	0.46	3,676	202	776
4/27-5/4	0.51	2,776	229	931	*			
5/4-5/13	0.20	5,362	254	962	1.07	7,866	247	788
5/13-5/20	1.00	1,264	458	744	*			
5/20-5/25	0.95	6,975	275	451	0.97	6,859	322	418
5/25-6/1	1.07	5,610	484	543	0.42	138	361	806
6/1-6/8	1.01	5,463	318	706	0.68	4,153	279	754
6/8-6/15	1.07	3,789	293	400	*			
6/15-6/22	0.83	3,909	457	890	0.63	3,334	302	564
6/22-7/5	0.95	5,837	274	879	0.50	4,492	270	lost
7/5-7/15	0.97	1,615	481	1,838	0.50	501	242	3,087
9/13-9/28	0.53	3,769	68	760	0.83	6,671	66	584

* A single column B was used to continuously sample for two weeks, while column A was replaced with a fresh column weekly.

determined. A reduction in flow rate increased the CAE concentration but appeared to have a slight, negative effect on the CCE concentration. The maximum amount of leakage of chloroform soluble compounds occurred at a very slow flow rate of 0.80 liters/min. Booth (1965) and Kumke (1963) showed that a slower flow rate increased the adsorption efficiency of a single column for both CCE and CAE compounds. The maximum leakage of CCE compounds at low flow rates found in the present study may be an anomoly.

Analytical Results

Five chloroform extracts were subjected to solubility separation to resolve the complex mixture of organic compounds (Table X). The neutral fraction was the largest in each separation. Gas chromatography analysis on a column containing 20 per cent Apiezon L on

TABLE X

SOLUBILITY SEPARATION OF CARBON CHLOROFORM EXTRACTS FROM KEYSTONE RESERVOIR, ARKANSAS RIVER, AND AN OIL REFINERY

	Weight of Solubility Fractions in Grams					
Sample	Water Solubles	Ether Insol.	Strong Acids	Weak Acids	Bases	Neutrals
*A-11	0.0636	0.0380	0.0318	0.1578	0.0072	0.8273
*A-27	Lost	0.0293	0.1610	0.2655	0.0199	0.7571
**F-13	0.1095	0.0879	0.0432	0.1229	0.0019	0.2534
**F-22	0.0648	0.0333	0.0301	0.0785	0.0124	0.3225
***G-5	0.5412	0.0695	0.5415	1.7686	0.2819	11.4254

* Samples A from the Arkansas River below Tulsa, Okla. A-11 collected March 11-22, 1966, and A-27 collected September 29 - October 10, 1966.

** Samples F from the Arkansas River above Keystone Reservoir. F-13 collected June 8-22, 1966, and F-22 collected October 31, 1966 - January 3, 1967.

*** Sample G from an oil refinery on the Arkansas River above Keystone Reservoir, collected July 27 - August 3, 1967.

Chrom W-AW failed to resolve the mixtures into individual components. To further subdivide the organic mixture, the neutral solubility fractions of A-11 and A-27 were separated into aliphatics, aromatics, and oxygenated fractions by column chromatography on a Silica Gel G column. Sample A-11 contained 0.3724 g of aliphatics, 0.0997 g of aromatics, and 0.1301 g of oxygenated compounds. 72.79 per cent of the neutral fraction was recovered from the column. Sample A-27 was collected as 120 two milliliter fractions and was not weighted. The mixtures were still too complex to resolve by GLC.

The mixture of organic compounds contained many compounds that were difficult to elute from the gas chromatographic column. A maximum operating temperature of 300° C, determined by the stability of the stationary liquid phase, would not elute all of the compounds in several hours. An alternative separation procedure was thus selected to separate volatile compounds from the non-volatile compounds.

The activated carbon was treated by steam distillation-ether extraction to remove the volatile compounds from the carbon. The quantity of organic compounds removed by this technique was approximately 10 to 100 mg. Twenty-seven samples were treated and 21 samples yielded volatile compounds that could be resolved by GLC.

The GLC retention times of the steam-volatile compounds resolved at nearly identical operating conditions were used to compare composition of the samples (Table XI). Column A contained a highly polar Carbowax 20M liquid phase. Column B contained a non-polar liquid phase, silicon gum rubber (GE Se-30).

GLC peak # 2 (Column A) occurred in the samples collected from the Arkansas arm but not detected in samples from the Cimarron arm of Keystone Reservoir. The source of the compound was probably on the Arkansas River above Keystone Reservoir. The persistence of the compounds in samples from the lower portion of the reservoir and below the reservoir indicate that these compounds were refractory or were contributed to the reservoir from many different sources.

GLC peak # 4 (Columns A & B) was present at all stations at various times of the year. It was not detected in carbon blanks which had been steam distilled-ether extracted.

The chromatogram of a sample collected from an oil refinery effluent located on the Arkansas River above Keystone Reservoir contained 26 major peaks. The retention times of several peaks (Table XI, Column B, Sample G) were nearly identical with peaks in samples collected from the Arkansas River arm of the Keystone Reservoir. Oil refinery wastes have been shown to be refractory (Myrick and Ryckman 1963), and since several refineries discharge wastes into the Arkansas River above Keystone, some of the compounds could have persisted in waters passing through the Reservoir.

The quantity of organic compounds removed by the steam distillation technique was too small to permit separation and isolation by conventional methods. An attempt to collect the resolved compounds as they were eluted from the gas chromatographic column by using a glass capillary tube for condensor was only partially successful. The major problem encountered was contamination of the isolated compound with liquid phase "bleed-off" from the gas chromatograph column.

TABLE XI

COMPARISON OF POLARITY (RETENTION TIME) OF COMPOUNDS COLLECTED FROM KEYSTONE RESERVOIR, ARKANSAS RIVER, AND AN OIL REFINERY

Column A

1/8 in. x 12 ft. Cu; 5% Carbowax 20M on 60-80 mesh Chrom W-AW-DMCS
Temp. Prog. 50° → 250° @ 10°/min.

Station and date	Peak Number										
	1	2	3	4	5	6	7	8	9	10	11
A-Jan., 1966		13.2	14.0			18.7		23.1		24.9	
A-Mar., 1966		13.1		14.5		18.9			24.2		26.3
C-Oct., 1966			13.5	14.7					24.3		26.2
D-Oct., 1966				15.0	16.0	18.9	20.8	23.4		25.5	
E-June, 1967		12.7		14.6	16.1						
F-May, 1966			13.9	14.7		19.0			24.1		26.1
F-June, 1966		12.8		14.8	15.9				24.3		26.3
F-July, 1966			14.0		16.3				24.4		26.5
F-Jan., 1967	10.6	12.8		14.4	16.4	18.9	20.9	23.2			26.9
Reagent BHT		12.2									

Column B

1/8 in x 6 ft. Cu; 10% Se-30 on 80-100 mesh Diatoport S.
Temp. Prog. 100° → 250° C @ 10°/min.

Station and Date	1	2	3	4	5	6	7	8	9	10	11
A-Jan., 1966			11.1		14.1		17.3	18.3		23.2	
A-Mar., 1966				11.6	14.6		17.7	18.6			24.0
A-Oct., 1966				11.7				18.0			24.7
C-Dec., 1965					14.1		17.4	18.4		23.3	
C-Jan., 1966					14.0		17.1	18.1		23.1	
D-Oct., 1966		10.3	11.0	11.6	14.1		17.6		21.0	23.0	
E-June, 1967	8.6			11.6			17.5	18.5			23.8
E-June, 1967				11.5		16.7	17.6		21.5		
F-Apr., 1966							17.3	18.2		23.4	
F-May , 1966				11.4			17.5				
F-June, 1966				11.6	14.3		17.5		20.5	23.5	
F-June, 1966	8.7	10.2				16.3			21.0		
F-July, 1966				11.6	14.1		17.8	18.7			24.1
F-July, 1966		10.5				16.5	17.1		20.9		
F-Sep., 1966		10.6				16.4			21.2		
F-Oct., 1966						16.5	17.2		20.9		
F-Jan., 1967	8.8					16.3			21.9		
Reagent BHT	8.6										
C-4	8.5	10.1	10.9	11.4							

Identification of a Trace Aqueous Organic Compound

Two steam-volatile samples from Keystone Reservoir were analyzed on the combination gas chromatograph-mass spectrometer (GC-MS). The steam volatile samples from the upper and lower ends of the Arkansas arm of the reservoir contained six peaks separable by GLC (Figs. 8 and 9). Only peaks 1, 2, and 3 were analyzed by GC-MS, since column conditions on this instrument were restricted to isothermal temperatures and it was observed that peaks 4, 5, and 6 were not cleanly resolved (Figs. 10 and 11).

Mass spectra of the first GLC peak from both samples indicated that each peak was a mixture of two compounds, one with molecular weight (M^+) 218 and the other with molecular weight (M^+) 220 (Figs. 12 and 13). Either two compounds were present or two hydrogen atoms were lost due to catalytic decomposition in the sample prior to reaching the ion source (Waller and Kinneberg 1968).

The second GLC peak from both samples had a molecular weight (M^+) of 220 (Figs. 14 and 15). The base peak was 205 and the fragmentation pattern was similar to that of 2,6-ditertiary-butyl-4-methylphenol (BHT) (API uncertified Mass Spectrum # 595). The GLC retention time of standard BHT (Fig. 16) and the unknown (Fig. 17) and also the mass spectra (Figs. 18 and 19) were identical. It was concluded that the unknown compound collected from both the upper and lower end of the Arkansas arm of the reservoir was BHT. Confirmatory evidence was obtained by co-chromatography of the unknown and BHT on a Carbowax GLC column. The retention time of BHT (Fig. 20) was similar to peak # 3 of the unknown sample (Fig. 21), whereas the retention time of the mixture showed one peak which corresponded to peak # 3 (Fig. 22).

However, a shortening of the retention time by approximately one minute was observed in the mixture.

The third GLC peak in the steam volatile samples from the reservoir (Figs. 8 and 9) had a molecular weight of 123 (Figs. 23 and 24). The compound was not identified, however, the odd molecular weight indicates that the compound contained an odd number of nitrogen atoms. The fragmentation pattern indicates that it was probably aromatic.

BHT was not detected in samples from the Cimarron arm of the reservoir, which may indicate that the source of the compound was on the Arkansas River above Keystone Reservoir. BHT is used as an antioxidant in the manufacture of rubber and gasoline. It is also added to some foods, such as dry cereals, to retard spoilage (Merck Index 1960).

Pharmacological investigations indicate that BHT is not toxic at concentrations of 100 to 200 mg/liter in food consumed by rats (Gaunt, Gilbert, and Martin 1965) and by chickens (Frawley, Kay, and Calandra 1965). A concentration of 500 mg/liter of BHT in the diet of laying hens led to deposition of 20 mg/liter of BHT in the fat of the eggs (van Stratum and Vos 1965). At a dosage of 500 mg/Kg of rat body weight BHT reduced the level of glucose-6-phosphatase, increased the level of glucose-6-phosphodehydrogenase, and increased the size of the liver (Feuer, Gaunt, Goldberg, and Fairweather 1965). Recovery from the effects was rapid when it was removed from the diet.

PHYTOPLANKTON (ALGAE) POPULATIONS

From the standpoint of community metabolism, photosynthesis is the most important activity of plants. Study of photosynthetic pigments provides insight into the physiological aspects of aquatic systems and to community structure. Early work relating photosynthesis and chlorophyll was undertaken by Wilstatter and Stoll (1918). According to Odum, et al. (1958) the first use of chlorophyll as a measure of photosynthesis was by Harvey (1934). Since then photosynthesis has been studied extensively (Rabinowitch, 1945, 1951, 1956; Calvin, 1958, and others).

Chlorophyll may be used as an indicator of standing crop but light conditions influence the relationship (Strickland, 1960). Difficulty arises in quantitatively relating chlorophyll to productivity because of the large range in the ratio of photosynthetic rate to chlorophyll (assimilation number). However, assimilation numbers tend to vary around a constant value which may be used to compute gross productivity from chlorophyll and light data (Ryther and Yentsch, 1957).

Odum (1957, Lorenzen (1963), Gibor and Meehan (1961), and others report significant diurnal variation in chlorophyll concentration. Yentsch and Ryther (1957), and Shimada (1958) report chlorophyll maxima at dawn in marine waters. Ichimura (1958) found a diurnal variation in assimilation number. Lorenzen (1963) found diurnal variation in photosynthesis to be due to changes in pigment concentrations in cells. He also observed diurnal variation in assimilation number. In field studies diurnal variation in chlorophyll cannot satisfactorily be accounted for. Sampling at the same time of day on each collecting trip is assumed to circumvent the problem for the most part.

Chlorophyll typically has a maximum concentration at some depth which may or may not be within the euphotic zone (Lorenzen, 1965). When the maximum concentration occurs at low light intensity deep below the surface the assimilation number is usually low (Steemann Nielsen and Hansen, 1959). Maxima below the euphotic zone may be accounted for by differential sinking rates of cells (Steele and Yentsch, 1960). Shade adaptation may greatly influence chlorophyll concentration in some instances (Yentsch and Lee, 1966). Whether these relationships apply in relatively shallow, well mixed bodies of fresh water is not well established.

A consequence of the aging and sinking of phytoplankters is that pigments begin to be degraded. Available methods for distinguishing between active and detrital chlorophyll are less than adequate. Yentsch and Menzel (1963) proposed a fluorescence method of determining phytoplankton chlorophyll and magnesium-void pigments. Chlorophyll and chlorophyllide (chlorophyll molecule with magnesium but without the phytol group) have the same absorption spectra in the visible light region (Yentsch, 1965a), and a convenient method of separating them is not available.

Phaeophytin is one of the major products of degradation of chlorophyll and is nonphotosynthetic. A phaeophytin is a magnesium-void compound with phytol. Removal of the phytol yields phaeophorbide. Yentsch (1965b) demonstrated that exposure of dark-adapted cells to light results in a decrease of phaeophytin and an increase in chlorophyll content indicating a direct conversion. Subjecting cells low in phaeophytin to darkness leads to an increase in phaeophytin and a corresponding loss of capacity for light uptake of ^{14}C and lowered efficiency of pigment.

Chlorophyll amount per unit area tends to be adjusted for maximum absorption of available light (Odum, et al., 1958). Light-adapted cells at the surface tend to have relatively low amounts of chlorophyll and high assimilation numbers (Gessner, 1949). Conversely, shade-adapted cells have relatively larger amounts of chlorophyll and low assimilation numbers. For relatively great surface light intensity the chlorophyll per unit area is expected to be high as are efficiency and assimilation number. This suggests that in the southwestern United States, with long periods of bright light, productivity in aquatic systems may be greater than anticipated in view of the fact that turbidity is typically very high.

Since many aquatic plants have optimum light intensities for photosynthesis, a mid-day reduction in photosynthetic rate may occur (Rabinowitch, 1951). When an extensive chlorophyll containing zone is present the reduction may be obscured by production by shade adapted cells in deep water. Steemann Nielsen (1954) and Verduin (1956) report that community photosynthesis does not drop as low as might be expected under conditions of diminished light. Odum, et al. (1958) recognize the following types of communities based on chlorophyll adaptation to light: stratified, shaded, and mixing communities and thin cultures with bright light. In the mixing community, cells are adapted to intermediate light. Communities of most lakes of southwestern United

States, and Keystone Reservoir in particular, are probably of this type.

The objectives of this study were to observe spatial and temporal distribution of chlorophylls a, b, and c, non-astacin carotenoids (phytoplankton pigments), and astacin-type carotenoids (animal carotenoids), and to examine the results for ecological implications. Quantitative and qualitative pigment determinations reveal numerous relationships which constitute the ecology of a body of water.

PHYTOPLANKTON METHODS

The methods of Richards with Thompson (1952) and Creitz and Richards (1955) were combined for concentrating plankton. Water was centrifuged at the rate of one liter per seven minutes with a Foerst plankton centrifuge. Remaining water was removed from the centrifugate with Type AA Millipore filters with pore size 0.45 μ. Extraction was carried out in darkness in 90 per cent acetone under refrigeration for 18 to 24 hours. Samples were centrifuged for 15 minutes at 2,000 rpm prior to determination of absorbancies. In some cases a longer centrifugation time was required for removal of turbidity. A Beckman DB-G recording spectrophotometer was used to determine absorbancies in the wavelength range 400 to 700 mμ (Figure 25). Readings were also taken at 750 mμ for correction of errors due to turbidity (Strickland and Parsons, 1965). Dilutions frequently were necessary since solutions must have absorbancies less than 0.8. Cells of four cm path length were used except for a short time at the beginning of the study when one cm cells were the only ones available. In cases in which the chlorophyll a peak did not occur at exactly 665 mμ the readings were taken at the peaks. Other readings were taken relative to the chlorophyll a peak even if its location was displaced laterally (Banse and Anderson, 1967). Pigment quantities were computed by methods of Richards with Thompson (1952) and Parsons and Strickland (1963). Since specific absorption coefficients are unknown for chlorophyll c and the carotenoids, results of quantitative determinations are in millispecific pigment units, represented by the letters MSPU. An MSPU is approximately equal to one mg (Richards with Thompson, 1952).

The Foerst centrifuge is less than 100 per cent efficient in concentration of plankton (Hartman, 1958; Lasker and Holmes, 1957; Reinhard, 1931). Parallel series of samples comparing the centrifuge to the Millipore filter showed that amounts of chlorophyll a obtained by the filters were higher by 21 per cent. Therefore, results of quantitative pigment determinations were multiplied by 1.21. Pennak (1949) applied a blanket correction factor of 25 per cent. Hartman (1958) showed that after three centrifugings up to 11 per cent of the organisms may remain in the water. Thus repeated centrifuging was not used in this study.

Ash-free weight (loss on ignition) determinations were made on samples preserved in 5 per cent formalin. One- to five-hundred ml of water were centrifuged and diluted with distilled water and commercial formalin to 10 ml final volume.

Conductivity and temperature were measured in the field with a temperature-compensated Industrial Instruments Solu Bridge, Model RB 3-3341. Some conductivity measurements were made in the laboratory with an Industrial Instruments Model RC1B conductivity meter. Turbidities were determined using a Bausch and Lomb Spectronic-20 colorimeter.

Solar radiation measurements (langleys per day) at Stillwater, Oklahoma, were supplied by the Oklahoma State University Geography Department. Data for some days were not available from that source and values obtained at Oklahoma City, Oklahoma, were substituted.

Solar radiation data were used to estimate daily production of organic material by the method of Ryther and Yentsch (1957).

Correlation coefficients were computed for some parameters (Steele and Torrie, 1960). The Wilcoxon matched-pairs signed-ranks test (Siegel, 1956) was used to test for significance of differences between annual means of pigment concentrations.

This study was conducted on the Cimarron branch of the reservoir. Four stations were marked with permanent buoys (Figure 26). The upstream station, Station I, was shallow (0.5 to 4.5 m) with high turbidity, high flow rate, high conductivity, and rapid temperature change relative to other stations. Station II, next downstream, varied in depth from eight to ten meters, was turbid much of the year and had reduced flow rate. One large creek, House Creek, enters between Stations I and II. Chemical stratification developed at Station II was absent at I. Station III was about 15 meters deep and the water was generally less turbid than at upstream stations. Chemical stratification was better developed than at I and II. Station III was located at a constricted region where the channel makes a sharp bend. Two large backwater areas are located between Stations II and III and undoubtedly exert an influence on conditions at III. Large backwaters are also situated between III and IV and adjacent to IV.

QUANTITATIVE PIGMENT ESTIMATES

Chlorophyll a

Annual means of chlorophyll a concentrations decreased downstream and with depth (Table XII). All but two of the differences tested were significant at the 95 per cent level and all but four were significant at the 99 per cent level. More differences between annual means of concentrations were significant for chlorophyll a than for other pigments. The difference between annual means of chlorophyll a concentrations in surface samples at Stations I and II was great but its level of significance was lower than expected on the basis of its magnitude. The same result was found for differences between annual means of concentrations of the other pigments at those stations and can be accounted for by the extreme variability of pigment concentrations, particularly at Station I. The pattern of decrease of annual means of concentrations was the same for all pigments and reflects the long-term spatial distribution. On any given date, however, distribution may have been considerably different. This result was expected on the basis of Kosminski's classic chlorophyll distribution study (1938).

The small water mass at Station I was subject to rapid and large changes in conditions and weekly variation in pigment amount was high (Figure 27). Chlorophyll a concentration exceeded 100 mg m^{-3} on ten occasions at Station I, three at Station II, one at Station III, but never at Station IV. Since Station I was very shallow, changes in concentration of pigment at the surface closely approximated changes in pigment amount on an area basis.

TABLE XII

ANNUAL MEANS OF CONCENTRATIONS (MG M^{-3}) AND AMOUNTS (MG M^{-2}) OF CHLOROPHYLL A

Depth (Meters)	I		II		III		IV
0	84.81	*	46.23	**	29.81		23.06
			**		**		**
2			38.72	**	28.36	**	20.17
			**		**		**
4			33.95	**	23.25	**	18.38
					*		
6					20.98	**	17.60
					**		**
10					17.38	**	15.01
							**
16							10.81
mg m^{-2}	92.30	**	272.75		281.27		264.54

One asterisk (*) between two means indicates that the means are significantly different at the 95 per cent level. Two asterisks (**) indicate differences significant at the 99 per cent level.

Maxima in chlorophyll a amounts were observed at Station I in October, December, March and April (Figure 27). None of the peaks was sustained, however. Each peak consisted of a single observation. Amounts of chlorophyll a were generally less than 100 mg m^{-2} at Station I and greater than 100 mg m^{-2} at other stations. Changes in amounts were consistently small throughout January, February, and most of March.

Unidirectional periods of change in amount of chlorophyll a at Station II lasted longer than at Station I, thus seasonal trends were more distinct. A fall peak of 606.4 mg m^{-2} on 21 September was particularly well defined (Figure 27). A general decrease until 8 February followed but minor peaks were distinct in December and January. Variability was high in February and March. A low level was reached in early April but was followed by several weeks of continuous increase. A spring maximum of 503.9 mg m^{-2} occurred on 7 June. In one week, however, the amount fell to 77.3 mg m^{-2}, the minimum for the year. Five weeks of continuous increase followed.

At Station III unidirectional periods of change in amount of chlorophyll a were usually only one or two weeks long. The result was numerous peaks spaced a few weeks apart. A fall high of 533.7 mg m^{-2} occurred in November. A peak in January was well defined at Station III, less evident at II and lacking at I and IV. From the yearly minimum of 141.6 mg m^{-2} in February there was a general increase until mid-May. In May, June, and July variability was great but amounts were generally high.

Station IV showed the best defined long-range trends in changes in amount of chlorophyll a (Figure 27). A fall maximum occurred in September and was followed by a general decrease until mid-December. Weekly variation in December and January was low and amounts of chlorophyll a were low. From late February until May amounts increased regularly with few exceptions. After the May peak, amounts were reduced drastically for several weeks. A second spring peak, 632.2 mg m^{-2}, observed on 28 June was high for the year and was followed by the yearly low, 75.3 mg m^{-2} three weeks later.

Chlorophyll b

Chlorophyll b occurred in much smaller amounts than the other chlorophylls (Table XIII). The trend was to lower means downstream and with depth but several notable exceptions occurred. Greatest significance in differences was between means at Stations II and III. Nine pairs of means were different at the 95 per cent level and seven at the 99 per cent level.

Temporal distribution of chlorophyll b at Station I differed from that at other stations (Figure 28). Amounts were very low throughout the year and exceeded 10 mg m^{-2} only three times. During fall maxima at other stations chlorophyll b was absent at Station I. A notable peak of 38.6 mg m^{-2} occurred on 30 March. The only prolonged period of increase took place from 7 June to 19 July and terminated in a high of 15.6 mg m^{-2}.

Changes in amount of chlorophyll b followed a common pattern at the three downstream stations (Figure 28). At the beginning of the study amounts were low. Rapid increase occurred earliest at Station II. A change from 4.6 mg m^{-2} to 53.9 mg m^{-2} occurred within two weeks in September. Over the next four weeks the amount fell to 21.3 mg m^{-2}. A two-week period of increase then resulted in the maximum for the year, 60.6 mg m^{-2}, on 9 November. These changes produced two fall peaks. Nine measurements during this time were higher than the highest measurement during the spring. From 2 December to 6 January chlorophyll b fell from 53.8 mg m^{-2} to 0 mg m^{-2}. An amount greater than 10 mg m^{-2} was not observed until 15 March. The pigment nearly disappeared late in December and did not appear in appreciable amounts until March at each of the downstream stations. A very decided period of decrease preceded this minimum at each station. Coincidence of periods of change at the three stations was great.

TABLE XIII

ANNUAL MEANS OF CONCENTRATIONS (MG M^{-3}) AND AMOUNTS (MG M^{-2}) OF CHLOROPHYLL B

Depth (Meters)	I		II		III	IV
0	1.77		2.92	**	2.05	1.30
2			1.93	**	2.26	1.28
			**		**	*
4			1.34	**	1.14	0.93
					**	
6					0.68	0.82
						**
10					0.49	0.46
						*
16						0.29
mg m^{-2}	2.77	**	16.33	**	12.54	12.81

One asterisk (*) between two means indicates that the means are significantly different at the 95 per cent level. Two asterisks (**) indicate differences significant at the 99 per cent level.

An early spring peak of 34.2 mg m^{-2} occurred on 22 March at Station II. One week later the amount fell to 10.3 mg and it remained at that level until late June except for two occasions. A summer peak of 24.4 mg m^{-2} was observed on 5 July. Corresponding peaks occurred at the other stations but were of greater magnitude at Stations III and IV.

Autumn peaks of 41.3 and 57.8 mg m^{-2} were observed at Station III on 2 November and 25 November. A high September peak corresponding to the one at Station II did not appear. A single, one-week period of decline occurred between the two fall peaks. From 25 November to 19

December the amount of chlorophyll b fell to 1.43 mg m^{-2}. Appreciable amounts did not appear again until 15 March. From then until 15 June amounts were within the range 5 to 15 mg m^{-2} except on three occasions when they fell to about 3 mg m^{-2}. On 15 June 29.5 mg m^{-2} was observed and a decrease immediately followed. The drastic nature of changes in amounts of phytoplankton pigments is exemplified by the occurrence of 201.9 mg m^{-2} on 10 July. That measurement is more than three times greater than any other for the entire study. One week later the amount was near zero.

From 30 September to 7 October chlorophyll b increased more than six-fold at Station IV (Figure 28). In two weeks the amount dropped from 47.8 to 21.8 mg m^{-2} and then increased in one week to 56.0 mg m^{2} on 29 October, the maximum for the year. For about a month, until 25 November, a plateau was maintained at about 30 mg m^{-2}. Then a four-week period of decline ended on 19 December when the winter level near zero was reached. In one week chlorophyll b increased from 2.1 to 14.6 mg m^{-2} on 15 March. Three weeks of gradual decline followed. On 12 April the amount was 36.4 mg m^{-2}, an early spring peak. Until 28 June, when 46.7 mg m^{-2} was measured, 8 mg m^{-2} was not exceeded. A steady decline to zero occurred from 28 June to 25 July.

Chlorophyll c

Annual means of chlorophyll c concentration decreased with depth and distance downstream (Table XIV). Differences between means were significant at the 95 per cent level in only half of the 20 cases tested.

TABLE XIV

ANNUAL MEANS OF CONCENTRATIONS (MSPU M^{-3}) AND AMOUNTS (MSPU M^{-2}) OF CHLOROPHYLL C

Depth (Meters)	I		II		III	IV
0	18.62		11.13	**	7.55	6.47
					**	*
2			9.90	**	6.32	5.42
			*		**	
4			9.02	**	5.05	4.42
6					4.90	4.32
					*	*
10					3.53	3.11
						*
16						2.44
MSPU m^{-2}	20.68	**	68.16	**	58.14	63.91

One asterisk (*) between two means indicates that the means are significantly different at the 95 per cent level. Two asterisks (**) indicate differences significant at the 99 per cent level.

Variation in amount of chlorophyll c was low at Station I (Figure 29). Throughout August, September, October, and much of November amounts ranged between 5 and 25 MSPU m^{-2}. Twenty-five MSPU m^{-2} was exceeded once in November and once in December. In late December, January, and February amounts were below 10 MSPU m^{-2}. There was a general increase in March and April with the yearly maximum of 87.4 MSPU m^{-2} occurring on 12 April. Two weeks of decline followed the maximum. A gradual, stepwise increase then led to a summer peak in mid-July.

Except for a few one-week periods of decline, the amount of chlorophyll c increased steadily from August until mid-January at Station II (Figure 29). Steady decline until mid-March followed. Two weeks of sharp increase followed by two of decrease resulted in a distinct peak of 153.9 MSPU m^{-2} on 22 March. Amounts increased during April and early May but were variable in May and June.

At Station III amounts of chlorophyll c were generally less than 50 MSPU m^{-2} from August to mid-March (Figure 29). Exceptions occurred in October, November, and January when some values were near 100 MSPU m^{-2}, suggesting peaks in plankton populations. After 15 March chlorophyll c was present in amounts greater than 50 MSPU m^{-2} except for some days in April and June. A peak of 142.9 MSPU m^{-2} occurred on 20 May and was followed by a general decline for four weeks. The maximum for the year was 359.2 MSPU m^{-2} on 10 July after four weeks of steady increase.

Amounts of chlorophyll c at Station IV were less than 50 MSPU m^{-2} from August to March except for a three-week period in October and November when a peak of 174.1 MSPU m^{-2} was attained (Figure 29). December and January had particularly low amounts whereas amounts were relatively high during those months at Stations II and III. At Station IV there was a general increase in amount of chlorophyll c from December to 15 March when a high of 118.2 MSPU m^{-2} was observed. In late March, as at Stations II and III, a sharp decline occurred until early April. The highest value of the year, 228.2 MSPU m^{-2}, was observed on 12 April. Amounts decreased to near 25 MSPU m^{-2} for three weeks in June but rose again to a peak of 186.0 MSPU m^{-2} in early July.

Non-Astacin Carotenoids

Non-astacin carotenoids were present in greater amounts than any pigment except chlorophyll a (Table XV). Concentrations decreased downstream and with depth with no exceptions. Differences between annual means were significant at the 99 per cent level in most cases. The null hypothesis that surface means at Stations I and II (41.6 and 28.1 MSPU m^{-2} respectively) were equal had a probability of 0.29 despite their relatively great difference in magnitude. Differences between four and six meter means at Staions III and IV were not significant at the 95 per cent level nor were they great in relative magnitude.

TABLE XV

ANNUAL MEANS OF CONCENTRATIONS (MSPU M^{-3}) AND AMOUNTS (MSPU M^{-2}) OF NON-ASTACIN CAROTENOIDS

Depth (Meters)	I		II		III		IV
0	41.57		28.09	**	16.61	**	12.65
			**		**		**
2			21.42	**	15.46	**	10.60
			**		**		**
4			19.34	**	12.81	**	9.66
6					11.43	**	9.33
					**		**
10					6.85	*	7.88
							**
16							5.21
MSPU m^{-2}	48.74	**	153.67		150.78		134.89

One asterisk (*) between two means indicates that the means are significantly different at the 95 per cent level. Two asterisks (**) indicate differences significant at the 99 per cent level.

Seasonal trends in amount per unit area were apparent but variation within any time period was great, particularly at Station I (Figure 30). Large amounts (up to 163.2 MSPU m^{-2}) constituting a fall peak at Station I were observed in October, November, and December but were interspersed with low totals. A general range of 5 to 50 MSPU m^{-2} was maintained study except for one date in July. From 22 March until 12 April amounts varied between 62.3 and 221.3 MSPU m^{-2}.

At Station II a well defined peak of 365 MSPU m^{-2} occurred in September (Figure 30). Two hundred MSPU m^{-2} was exceeded only six other times during the year. Three of them constitute a minor but well defined peak in January. From 11 January until 5 April there was a general decrease with the longest interruption being two weeks. Amounts then increased to a high of 236 MSPU m^{-2} in a single week. Gradual, uninterrupted increase to the end of July followed.

Numerous two- to four-week periods of unidirectional change in amount of non-astacin carotenoids characterized Station III. Amounts greater than 200 MSPU m^{-2} were observed in August, October, November, January, May and July. Each such peak was preceded by a general increase and followed by a general decrease with few abrupt changes. The maximum for the year, 312.5 MSPU m^{-2}, occurred on 23 November. Maxima in August, November, January and June may be said to be summer, fall, winter, and spring peaks although division into seasons is not clear.

Three well defined peaks, two extending over more than four weeks each, characterized Station IV. Variation within peaks was great however. Maxima of more than 300 MSPU m^{-2} occurred in September, October, May, and June. The September and October maxima appeared to be a part of a general high with one low observation separating them. A range of 50 to 100 MSPU m^{-2} was not exceeded from 2 December to 22 February. In contrast, Stations I, II, and III had relative maxima during that time.

Astacin-Type Carotenoids

This group of pigments is often referred to as the animal carotenoids (Crustacea in particular). They are of special interest in plankton studies because their abundance relative to that of plant pigments, particularly chlorophyll a, may indicate grazing by zooplankters. Grazing may significantly affect the standing crop or influence succession (Fogg, 1965). Wetzel (1964) states that grazing is indicated by an inverse relationship between astacin and plant pigments. Evidence supporting this is given by Anderson, et al. (1955), Langford and Jermolajev (1966) and others.

In most cases concentrations of astacin-type carotenoids decreased downstream and with depth (Table XVI). However only a few of the differences tested were significant at the 95 per cent level. This result was not unexpected since the means do not differ greatly in magnitude and zooplankton distribution does not have the same controlling factors that phytoplankton distribution has. Variation in astacin-type carotenoid concentration with depth is not expected to vary in the same manner as that of the plant pigments.

Animal carotenoids were absent much of the time early in the study at Station I (Figure 31). High turbidity was suspected as being a cause. Astacin peaks, however, occasionally occurred at times of extremely high turbidity. Amounts of astacin were less than 5 MSPU m^{-2} until 15 March with one exception. After 15 March the amount ranged from 4.3 to 68.5 MSPU m^{-2} except on four dates, three of which occurred consecutively in late May and early June. The high for the year, 68.5 MSPU m^{-2}, followed by one week the spring chlorophyll a maximum. The late May--early June low followed a sharp chlorophyll a decline. Grazing activity is indicated by these relationships.

Seasonal trends were more apparent at Station II with astacin-type carotenoids present at all times. Amounts were lowest from late December to the end of March with two distinct minor peaks, both inversely related to chlorophyll a changes observed during that period. Highest amounts (greater than 20 MSPU m^{-2}) occurred in the intervals from September to December and May to June. Maxima were not maintained although levels were generally higher than in the winter.

TABLE XVI

ANNUAL MEANS OF CONCENTRATIONS (MSPU M^{-3}) AND AMOUNTS (MSPU M^{-2}) OF ASTACIN-TYPE CAROTENOIDS

Depth (Meters)	I		II		III		IV
0	3.35		2.44	**	1.34	**	1.74
			*				
2			1.98	**	1.25		1.50
							*
4			1.95	**	1.30		1.26
6					1.25		1.23
10					1.17		0.98
16							1.15
MSPU m^{-2}	5.25	**	15.77		16.33		20.17

One asterisk (*) between two means indicates that the means are significantly different at the 95 per cent level. Two asterisks (**) indicate differences significant at the 99 per cent level.

Changes in astacin amounts at Station III corresponded to those at Station II. High values for the year were observed in August, November, May and July. Several distinct lesser peaks appeared. Lowest values occurred in the period from December to March. Astacin--chlorophyll a relationships indicating grazing are illustrated in Figure 32.

At Station IV amounts of astacin were high in September, October, and July. Well defined peaks also occurred in March and May. Unidirectional periods of change were usually not longer than two weeks, the longest being three and four weeks. As with plant pigments, greatest variability was in the fall and spring when amounts were generally highest. Evidence of grazing by zooplankton is given by the inverse relationship to chlorophyll a (Figure 32).

ECOLOGICAL RELATIONSHIPS

Chlorophyll To Plant Carotenoid Ratio

Non-astacin carotenoids constituted about 40 per cent of the total phytoplankton pigment amount (Table XVII). Maximum ratios for Stations I to IV were 6.96, 5.10, 4.95, and 7.52. Minimum ratios were 1.35, 1.58, 1.43, and 1.74. Large ratios indicate relatively low amounts of carotenoid pigment. Although seasonal differences were not great, there was generally relatively less carotenoid pigment in the spring and summer and relatively more in fall and winter months. This result is consistent with the anticipated ecological succession in which high ratios prevail at times favorable to phytoplankton growth (Yentsch, 1959). Before 30 September only one ratio lower than 1.9 was observed. From October through March ratios less than 1.9 were very common. Only one ratio less than 2.0 occurred at any station after 5 April. Wetzel (1964) found that concentrations of chlorophylls a and c and non-astacin carotenoids maintained constant proportions throughout the year.

Chlorophyll Fractions

The fraction of a particular chlorophyll at a given time is defined as the amount (or concentration) of the chlorophyll divided by the sum of the amounts (or concentrations) of all of the chlorophylls at that time (Table XVII). Units for the numerator and denominator must be the same but may be either on an area or volume basis. Some long-term similarities are apparent in changes in chlorophyll fractions among stations (Figures 33 and 34).

TABLE XVII

ANNUAL MEANS OF PIGMENT FRACTIONS

NATC = Non-Astacin Type Carotenoids; ATC = Astacin Type Carotenoids

Station	Chlorophylls			Carotenoids		Total Chlorophyll
	A / Total	B / Total	C / Total	ATC / Total	NATC / Total	NATC
I	0.793	0.022	0.185	0.122	0.878	2.675
II	0.759	0.043	0.198	0.103	0.897	2.520
III	0.825	0.027	0.148	0.098	0.902	2.350
IV	0.784	0.034	0.182	0.154	0.846	2.685

In late September and early October the chlorophyll a fraction was higher than its annual mean (Figure 33). At Station I the "a fraction" fell below its annual mean for several weeks beginning in early November. At other stations reduction of the "a fraction" began late in October. Then a period during which it was higher than the annual mean for several consecutive weeks during December and January was observed at each station except Station II. Pronounced reductions in the "a fraction" occurred simultaneously at all stations in early March. Increases followed at all stations until values reached the annual means around which they varied until mid-June. At that time general dropoffs occurred, particularly at Stations I, III, and IV.

At Station I the "b fraction" was not appreciable until February. Chlorophyll b appeared then and was present for several weeks during which it was absent at other stations. The "b fraction" was maximum for the year at Station I on 30 March but decreased to zero over the next three weeks. It remained low until June and then increased until the end of July.

Changes in the "b fraction" paralleled each other closely at the three downstream stations (Figure 34). High values near 0.10 were maintained during November at Stations II, III, and IV and simultaneous lowering followed in December. Most striking was the absence of chlorophyll b for several weeks in December, January, and February at Stations II to IV. In late March and early April minor plateaus near 0.05 were observed for several weeks. Values near zero separated the plateaus from June and early July highs. The "b fraction" was near zero at all three downstream stations in July.

The chlorophyll c fraction, when graphed, appears to be nearly a mirror image of the "a fraction" because of the low contribution of b to the total.

Light Penetration

Turbidity

Turbidity--standing crop relationships are not well understood although there is general agreement that turbidity is limiting in some cases (Verduin, 1954). Harris and Silvey (1940) found maximum production and minimum turbidity in some cases and minimum production and maximum turbidity in others in Texas reservoir lakes. In a study of Oklahoma waters, Claffey (1955) found that numbers of algal cells decreased with increasing turbidity. In Japanese lakes Ichimura (1956) found a curvilinear relationship between transparency and chlorophyll content except in cases in which organic matter content was extremely high or wind resulted in large amounts of suspended inorganic material.

In Keystone Reservoir turbidity changed rapidly, particularly at Stations I and II. Mean turbidity decreased downstream as evidenced by increase in euphotic zone depth. On some dates when standing crop was high turbidity was great. The opposite was also observed. It is possible that the first result was observed because a large crop was produced in relatively clear water which suddenly became turbid. Verduin (1954) suggests that a large crop may develop in a shallow euphotic zone if circulation is great enough to maintain organisms in the euphotic zone a sufficient part of the daylight hours.

Euphotic Zone

Euphotic zone depth showed long-term changes at all stations (Figure 35). Changes at Stations I and II paralled each other closely as did those at Stations III and IV. Annual mean euphotic zone depth increased downstream (Figure 36).

At Station II there was a general increase in euphotic zone depth until 1 March. A general decrease followed until 10 May. At other stations changes in one direction were less continuous. Stations III and IV had the most pronounced seasonal differences with well defined lows in September, October, and April and prolonged maxima in the winter.

Pigment amounts, particularly of chlorophyll a, were generally inversely related to euphotic zone depth. A possible explanation is that large plankton populations contribute greatly to turbidity. In some cases, particularly at Stations I and II, there were deep euphotic zones and low chlorophyll indicating that clay was the major source of

TABLE XVIII

CHLOROPHYLL A--EUPHOTIC ZONE RELATIONSHIPS

Station	Maximum Euphotic Zone Depth meters	Minimum Euphotic Zone Depth meters	Mean Depth Of Euphotic Zone meters	Euphotic Zone As Fraction Of Total Depth	Chlorophyll a In Euphotic Zone As Fraction Of Total In Water Column
I	1.67	0.03	0.55	0.55	0.45
II	3.29	0.41	1.63	0.23	0.26
III	3.75	0.57	2.32	0.15	0.21
IV	4.52	0.63	2.64	0.15	0.20

turbidity. Pigment maxima when euphotic zone is shallow probably are allowed by changes in other conditions. Table XVIII summarizes some of the relationships between euphotic zone depth and chlorophyll a.

At Station I the euphotic zone extended to the bottom on several occasions. Differences between fraction of chlorophyll a in the euphotic zone (relative to total chlorophyll a in the water column) and the euphotic zone fraction of the water column may be regarded as evidence of unequal vertical distribution of pigment. At Station I the euphotic zone comprised more than half of the water column but possessed less than half of the chlorophyll a. At Station IV the euphotic zone was 15 per cent of the water column but has 20 per cent of the chlorophyll a. Chlorophyll a probably was equally distributed between euphotic zone and the lower water mass at some point near Station II (Figure 37).

Estimated Gross Primary Productivity

Since chlorophyll a is the major photosynthetic pigment there is good reason to seek a relationship between it and gross photosynthesis and light intensity. Several authors have investigated the relationship (Strickland, 1960). In this study the method of Ryther and Yentsch (1957) was applied. The method is based on an average ratio of 3.7 mg carbon fixed per hour to 1.0 mg chlorophyll. Strickland (1960) reported a range of about 1 to 10 mg carbon fixed per hour for each mg chlorophyll with an average of about four. Results must be considered with this range of variability in mind.

Productivity increased downstream to Station III and then decreased at Station IV (Table XIX). These findings might have been expected on the basis of the changes in euphotic zone depth and chlorophyll a in the euphotic zone from upstream to downstream. From

Station II to III euphotic zone depth increased 52.3 per cent, amount of chlorophyll a decreased 12.7 per cent, and productivity increased 4.5 per cent. The increase in productivity occurred despite lower chlorophyll a amount because of the proportionately large increase in the zone in which light was sufficient for photosynthesis.

From Station III to IV euphotic zone depth increase was only about one-fourth as great as between II and III. Chlorophyll a in the euphotic zone decreased 14.6 per cent, and productivity decreased 12.7 per cent because the relative increase in extent of photosynthetic zone was not great enough to allow it to increase.

TABLE XIX

CHLOROPHYLL-BASED ESTIMATES OF GROSS PRODUCTIVITY

	grams of carbon fixed m^{-2} day^{-1}		
Station	Means	Extremes	
		Maximum	Minimum
I	0.592	3.071	0.010
II	0.852	2.985	0.044
III	0.890	2.184	0.069
IV	0.768	2.184	0.069

Seasonal trends in gross productivity (Figure 38) were indicated at all stations but were not well defined. Since they were computed using chlorophyll amounts as a factor, rates varied much the same as pigment varied. Daily incident solar radiation values were low in December and January (18 to 309 ly day^{-1}) with values as high as 710 ly day^{-1} in other times of the year. Day to day variations in solar radiation were great, especially in spring and summer and did not correlate well with variations in pigment amounts. However, pigment amounts and production rates were low during the months when radiation was low. Correlation coefficients were computed with surface samples of chlorophyll a and the means of incident solar radiation for sample dates and the four days preceding each. By station, going downstream, correlation coefficients were -0.01, -0.28, 0.32, and 0.32.

Eley (personal communication) studied productivity in Keystone Reservoir using the light--dark bottle method from August, 1965, to April, 1966, and found monthly means of productivity ranging from zero to 1.50 grams carbon fixed m^{-2} day^{-1}.

Ash-Free Weight

Ash-free weight was determined once or twice a month at each depth at each station. Annual means displayed the same pattern of spatial and temporal distribution displayed by the pigments. There was a general decrease downstream and with depth (Table XX). Some irregularities in the pattern occurred however. The surface mean at Station I was nearly four times greater than the next highest mean. Undoubtedly much of the organic material at Station I was allochthonous.

Ash-free weight at Station I showed different temporal variation than at the other stations. The minimum amount was observed at the same time as at other stations, however, and an April 26 peak corresponded to peaks at Stations III and IV. Even though ash-free weight per unit volume was relatively much greater at Station I, the amount per unit area was considerably lower than at any other station except on two occasions. On 26 April ash-free weight at Station I exceeded that at all other stations and on 21 June it exceeded that at stations

II and III.

Clay turbidity was frequently high at Station I and may have interfered with ash-free weight determinations. Clay particles may retain moisture in oven dried samples. Retained moisture is lost on ignition and the weight loss is credited to loss of organic material.

TABLE XX

ANNUAL MEANS OF ASH-FREE WEIGHT, GRAMS M^{-3}

Depth (Meters)	Station I	II	III	IV
0	41.12	11.61	7.31	6.78
2		9.49	6.29	7.36
4		9.15	5.18	5.74
6			5.63	6.56
10			6.86	6.51
16				5.23

In order to separate the contributions of detritus and zooplankton from the total ash-free weight it was assumed that 35 per cent of the ash-free weight was contributed by phytoplankton (Pennak, 1955; Wright, 1959). This must be considered to be a rough approximation of a factor which undoubtedly varies with time and location.

The general relationship 1.0 µg chlorophyll a = 0.14 mg ash-free weight was arrived at. Ash-free weights of all surface samples for the year were summed and 65 per cent was subtracted. The result was divided by the corresponding sum of chlorophyll a determinations. This relationship agrees well with ratios of 0.12 and 0.11 reported by

Wright (1959). Worthy of note, however, is that the ratio computed for the three downstream stations alone is only 1.0 µg chlorophyll a = 0.09 mg ash-free weight.

The ratio of chlorophyll a and ash-free weight increases distinctly with depth (Table XXI) and can be accounted for by the presence of large amounts of detritus at lower depths and by degradation of chlorophyll in lower water masses. Since no correction could be made for detrital chlorophyll it is assumed that the relationship 1.0 µg chlorophyll a = 0.14 mg ash-free weight applies at all depths. That being the case, computation of degree of reduction of phytoplankton contribution, or of increase of zooplankton-detritus contribution to ash-free weight was possible. Results are shown in Table X as decreasing phytoplankton contribution to ash-free weight with increased depth.

Strickland (1960) approached the chlorophyll--ash-free weight relationship through the equations

$$\text{mg C} = F \times \text{mg chlorophyll}$$

and

$$\text{mg C} = (0.5 \pm 0.05) \times \text{mg ash-free weight}$$

where mg C is organically combined carbon and F is a constant to be computed for each situation. Values of F are a means of comparing data reported in the literature. By computing mg C from ash-free weights and using pigment data, an F was found for each station (Table XXI). Strickland reports F values of 20 to 130 for mixed populations and suggests that, as a rule of thumb, F = 30 for natural populations without nutrient deficiencies. For Wright's data (1959) yielding the relationship 1.0 µg chlorophyll a = 0.12 mg ash-free weight Strickland found that F = 60. The value F = 82 at Station I in this

TABLE XXI

RELATIONSHIP BETWEEN CHLOROPHYLL AND ASH-FREE WEIGHT

	Station											
	I			II			III			IV		
Depth (meters)	mg Ash-Free Wgt. / µg Chlorophyll a	F	% Ash-Free Wgt. Contributed by Phytoplankton	mg Ash-Free Wgt. / µg Chlorophyll a	F	% Ash-Free Wgt. Contributed by Phytoplankton	mg Ash-Free Wgt. / µg Chlorophyll a	F	% Ash-Free Wgt. Contributed by Phytoplankton	mg Ash-Free Wgt. / µg Chlorophyll a	F	% Ash-Free Wgt. Contributed by Phytoplankton
0	0.47	83	35	0.23	42	35	0.26	41	35	0.31	45	35
2				0.26		30	0.25		36	0.46		24
4				0.35		23	0.24		37	0.39		28
6							0.30		31	0.47		23
10							0.45		22	0.64		17
16										0.68		16

study is about twice as high as that for the other stations. It may be a representative value but the data suggest that the arbitrary assignment of 35 per cent to the phytoplankton contribution to ash-free weight is questionable.

Chlorophyll a - Dry Weight Relationship

Using Strickland's (1960) equation

$$\text{mg ash-free organic matter} = F \times \text{mg dry weight}$$

where $F = 0.6 \pm 0.2$ for mixed populations, the chlorophyll a relationship to dry weight was examined. Using annual means of pigment estimates in surface samples chlorophyll a was found to be in the range 0.23 to 0.94 per cent of dry weight. For the stations in order downstream the ranges determined were 0.23 to 0.48, 0.46 to 0.91, 0.46 to 0.94, and 0.40 to 0.77 per cent. Of particular interest is that even though the ranges are rather wide, that for Station I barely overlaps those of the other stations which coincide quite completely. If the relationship 1.0 μg chlorophyll a = 0.14 mg ash-free weight is used for the calculation, the range is found to be 0.28 to 0.57 per cent. The same calculation on Wright's data (1959) yields a range of 0.33 to 0.67 per cent.

Rabinowitch (1945) reports a range of 0.09 to 2.0 per cent of dry weight according to a tabulation of results of several workers. McConnell and Sigler (1959) found chlorophyll a to be 0.15 to 2.4 per cent of dry weight in river algae. Wetzel (1964) gives a range of 0.25 to 2.0 per cent for Borax Lake, California. The per cent in green algae is reported to range as high a 6.0 (Atkins and Jenkins, 1953).

Margalef gives a range of 0.31 to 0.64 per cent for some artificial lakes in Spain.

Pigment Diversity

A pigment diversity index proposed by Margalef (1957) was applied in this study. The index is the ratio of optical density readings on 90 per cent acetone extracts at 430 and 665 mu. Yellow pigments absorb heavily at 430 mu and green pigments (chlorophyll a) absorb heavily in the 665 mu region. Thus, the ratio is a "yellow/green" ratio and gives an indication of the number of molecules of one type relative to those of the other. Physiological states of populations and successional changes are reflected in changes in the pigment diversity index. Aging and nutrient conditions are the major factors influencing both total and relative amounts of pigments (Ketchum, et al., 1958). Pigment proportion is more dependent on aging than on light (Margalef, 1958, 1963). The ratio D_{430}/D_{665} tends to be highest in old, stable ecosystems and lowest for young, growing populations (Odum, 1963). Margalef (1964) reported ratios of 4.32 to 6.98 during summer for artificial lakes in Spain. Knudson (unpublished) found ratios as high as nine for clear ponds in north central Oklahoma.

Pigment diversity was computed for all samples collected during the study period. The greatest differences between stations occurred during the cold months (Figure 39). Pigment diversity values were highest in January and February with the highest ratio (4.15) at Station II early in January. Stations III, IV, and I had successively lower peaks of 3.55 late in January, 3.25 early in February, and 2.80 late in December, respectively. The ratio decreased rapidly at all stations early in March during which time biomass began to increase after a steady, winter-long decline (Figure 39). Throughout the study pigment diversity was consistently lower at Station I than at other stations. Except in winter months there were only small differences in pigment diversity among stations. Table XXII gives annual means of pigment diversity.

TABLE XXII

PIGMENT DIVERSITY ANNUAL MEANS

Depth (Meters)	Station I	 II	 III	 IV
0	2.53	2.82	2.73	2.81
2		2.76	2.72	2.78
4		2.77	2.73	2.77
6			2.79	2.79
10			2.72	2.79
16				2.98

Low diversity at Station I may be an effect of high turbidity. Knudson (unpublished) found that diversity did not reach high values at any time throughout the year in turbid Oklahoma farm ponds. Succession may be prevented from proceeding to maturity. River currents at Station I could have the same effect, however.

Succession

Seasonal variation in pigment amounts is partly a result of changes in species composition. The Richards with Thompson method, however, does not give adequate differentiation of pigments for taxonomic sorting (Strickland, 1960). Margalef (1958) showed that chlorophyll a relative to biomass decreases with natural development of a plankton community. He divided succession into three stages and found the ratio to be indicative of the stage. In this study only Station IV yielded ratios changing in a manner suggesting succession (Figure 40). Ash-free weight data were probably not obtained frequently enough for thorough analysis. Well defined maxima occurred in December and June. The yearly minimum was in March at about the middle of a long general increase in amount of chlorophyll a. The December peak occurred early in the prolonged winter chlorophyll a minimum. Changes in the chlorophyll a to ash-free weight ratio suggest succession but numerically do not fall into Margalef's categories.

The expected succession is: diatoms, green algae, bluegreen algae (Blum, 1956; Fogg, 1965). Chlorophyll c peaks occurred at the upper three stations in December and January indicating relatively great abundance of Chlorophyta or Euglenophyta at that time.

Trophic Classification

The reservoir may be classified with regard to trophic state on the basis of chlorophyll data. In general, deep, oligotrophic lakes have less chlorophyll and shallow, eutrophic lakes have more (Sakamoto, 1966). Aruga and Monsi (1963) regarded lakes having 30 to 120 mg m^{-2} in the euphotic zone as being eutrophic. Ichimura (1956) classified Japanese lakes having 10.6 to 57.5 mg chlorophyll m^{-3} above the compensation point as eutrophic. Keystone Reservoir falls into this category with annual mean chlorophyll a in the euphotic zone ranging from 33 to 59 mg m^{-2}.

Aruga (1966) considered the ratio between annual maximum and minimum to be a clue to trophic type with higher ratios characteristic of eutrophic lakes. Ratios for Stations I to IV were 18.8, 7.9, 4.0, and 8.5 respectively.

Oligotrophic waters are characterized by ultraplanktonic forms and larger organisms are more predominant in eutrophic waters (Wetzel, 1964). In Keystone Reservoir large organisms appeared frequently at Station I and seldom at Station IV.

Evidence indicates that the degree of eutrophication decreases downstream. However, physical conditions differ greatly among the stations, particularly between Station I and the others, and criteria must be applied judiciously.

SUMMARY AND CONCLUSIONS

The carbon adsorption method was selected for continuous collection of semi-quantitative samples of trace aqueous organic compounds from Keystone Reservoir and the Arkansas River below Tulsa, Oklahoma. Qualitative analyses of the steam volatile compounds were performed on a combination gas chromatograph-mass spectrometer.

The concentration of organic compounds from the Arkansas River below Tulsa, Oklahoma exceeded that in Keystone Reservoir and also most published values from other locations. The Arkansas River arm contained somewhat higher concentrations than the Cimarron River arm of the reservoir. There was a decrease in concentration of CCE in both arms of the reservoir. CAE decreased from the upper to the lower end of the Cimarron River arm. The observed reductions in concentration were possibly due to dilution, sedimentation, or bio-oxidation. The concentration of CAE in the Arkansas River arm increased from the upper to the lower end of the reservoir. The increase may have been caused by sewage outfalls between the stations or by organic compounds from natural sources.

2,6-Ditertiary butyl-4-methylphenol (BHT) was identified in extracts from the upper and lower ends of the Arkansas River arm of Keystone Reservoir. This had not been detected as a persistent organic contaminant in a main stream reservoir. Sewage effluents may have contained the compound, since it is a widely used anti-oxidant, and it probably was introduced into the Arkansas River upstream from the reservoir. Since BHT was detected at the lower end of the reservoir, it may be concluded that it was not amenable to metabolic oxidation. Identification of BHT in this study with the combination gas chromatograph-mass spectrometer is among the first successful applications of this instrument to analysis of an organic contaminant in surface receiving waters.

Weekly plankton pigment concentrations were measured at depth intervals at four stations on Keystone Reservoir, Tulsa, Oklahoma. Ash-free weight (loss on ignition) was determined once or twice each month. Light, temperature, and turbidity were measured each week. Daily river discharge and solar radiation records were obtained from the U. S. Geological Survey and the Geography Department of Oklahoma State University. Mean temperature of the water mass varied seasonally but did not change much in any short time period. Thus, division of the year into seasons was not warranted on the basis of temperature. Turbidity decreased and depth of the euphotic zone increased progressively downstream.

The contributions of chlorophylls a, b, and c to total chlorophyll were 75 to 82, 2 to 4, and 14 to 20 per cent respectively. Astacin-type carotenoids made up 9.8 to 15.4 per cent of total carotenoids. Annual means of chlorophyll a in the euphotic zone ranged from 33 to 59 mg m^{-3}. On the basis of these values and literature reports of eutrophic lakes, Keystone Reservoir must be considered to be eutrophic. Chlorophyll a was estimated to have constituted 0.23 to 0.94 per cent of dry weight of phytoplankton.

The relationship 1.0 μg chlorophyll a = 0.14 mg ash-free weight (surface samples) was arrived at. It was applied at all depths in computing relative contributions of plankton and detritus to ash-free weight. Phytoplankton contributed less to ash-free weight as depth increased.

Pigment diversity was usually lowest at Station L. Most pronounced differences between stations occurred in January and February when Station II had the highest values.

Gross productivity (grams C fixed m^{-2} day^{-1}), estimated from chlorophyll a data, increased downstream to Station III but was lower at Station IV than at III. The range of annual means was 0.01 to 2.98 g C m^{-2} day^{-1}.

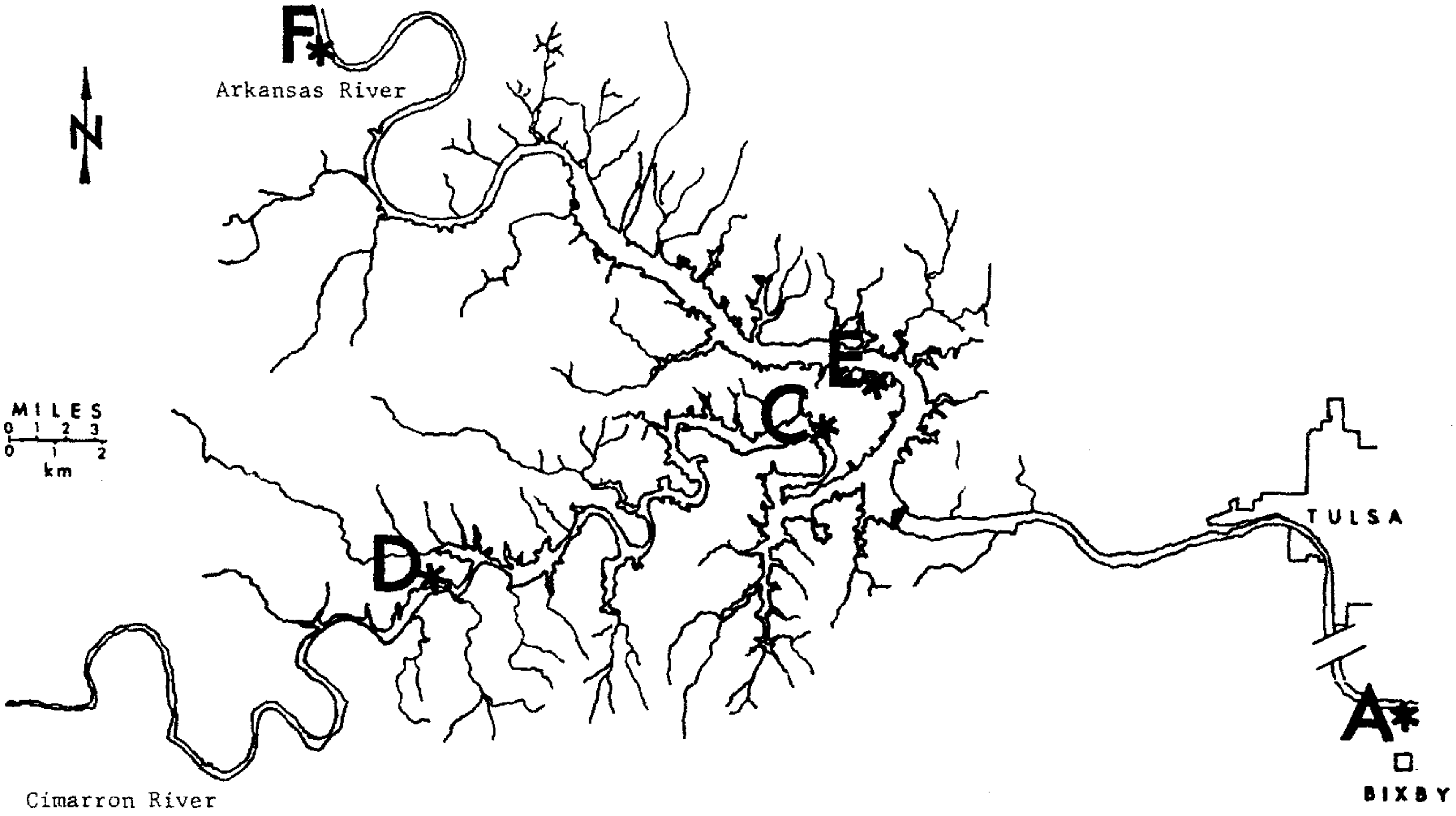
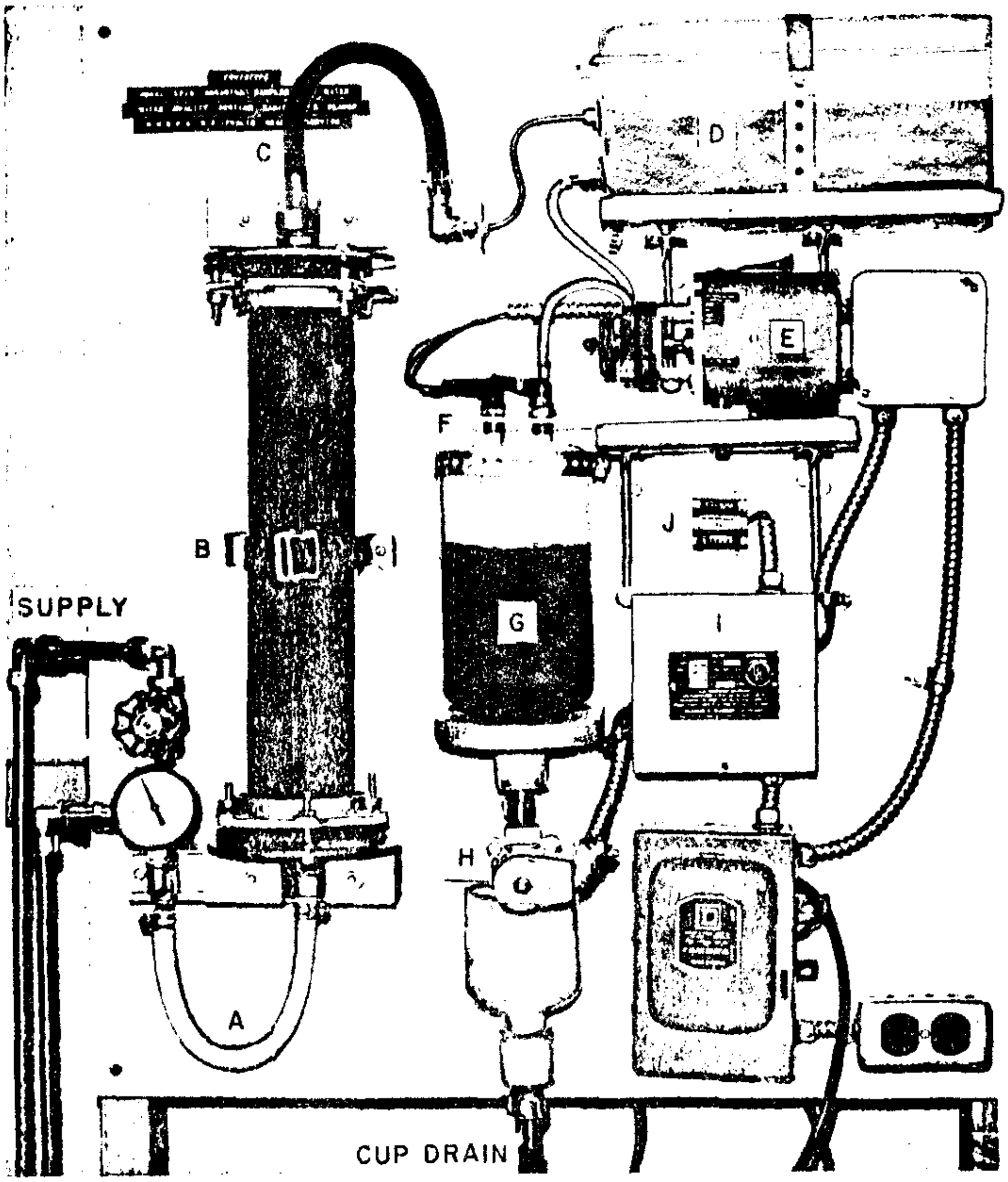

F
Arkansas River
N
MILES
0 1 2 3
0 1 2
km
E
C
D
TULSA
A
BIXBY
Cimarron River

Figure 1. Keystone Reservoir, Oklahoma. A through F Sampling Stations.



MODEL LF-2 ORGANICS SAMPLER FOR WATER

A- TEFLON HOSE
B- WEB STRAP
C- RUBBER HOSE
D- CONSTANT HEAD TANK
E- METERING PUMP
F- PROBE HOLDER BRACKET
G- VOLUMETRIC MEASURING TANK
H- SOLENOID VALVE
I- LIQUID LEVEL CONTROL
J DIGITAL COUNTER

Figure 2. Low Flow Rate Organic Sampler. Source: Specifications Manual, USPHS, Water Quality Section, 1964

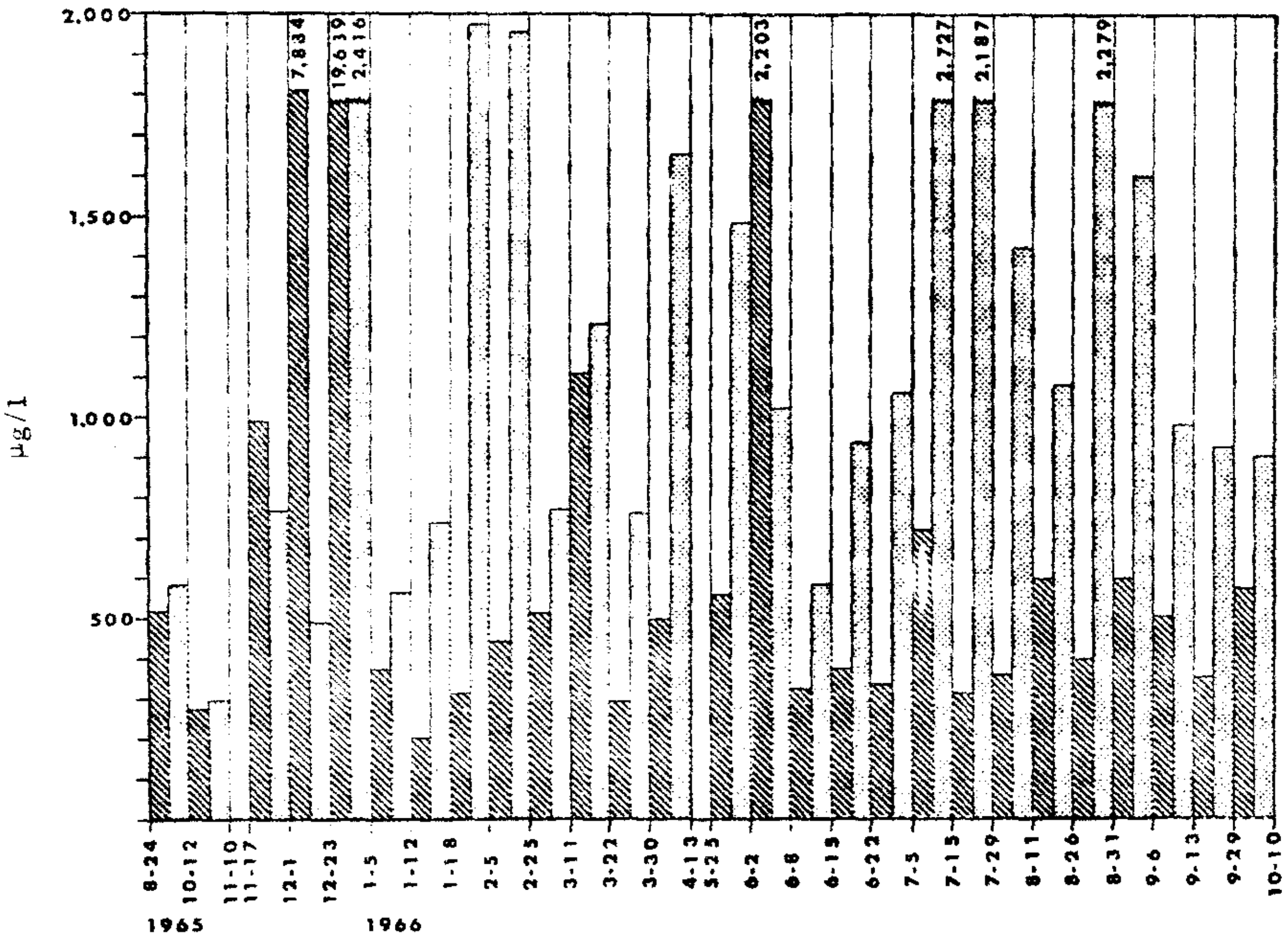


Figure 3. Concentration of Carbon Chloroform Extract (Diagonal) and Carbon Alcohol Extract (Stippled) Collected from (A) Arkansas River below Tulsa, Oklahoma.

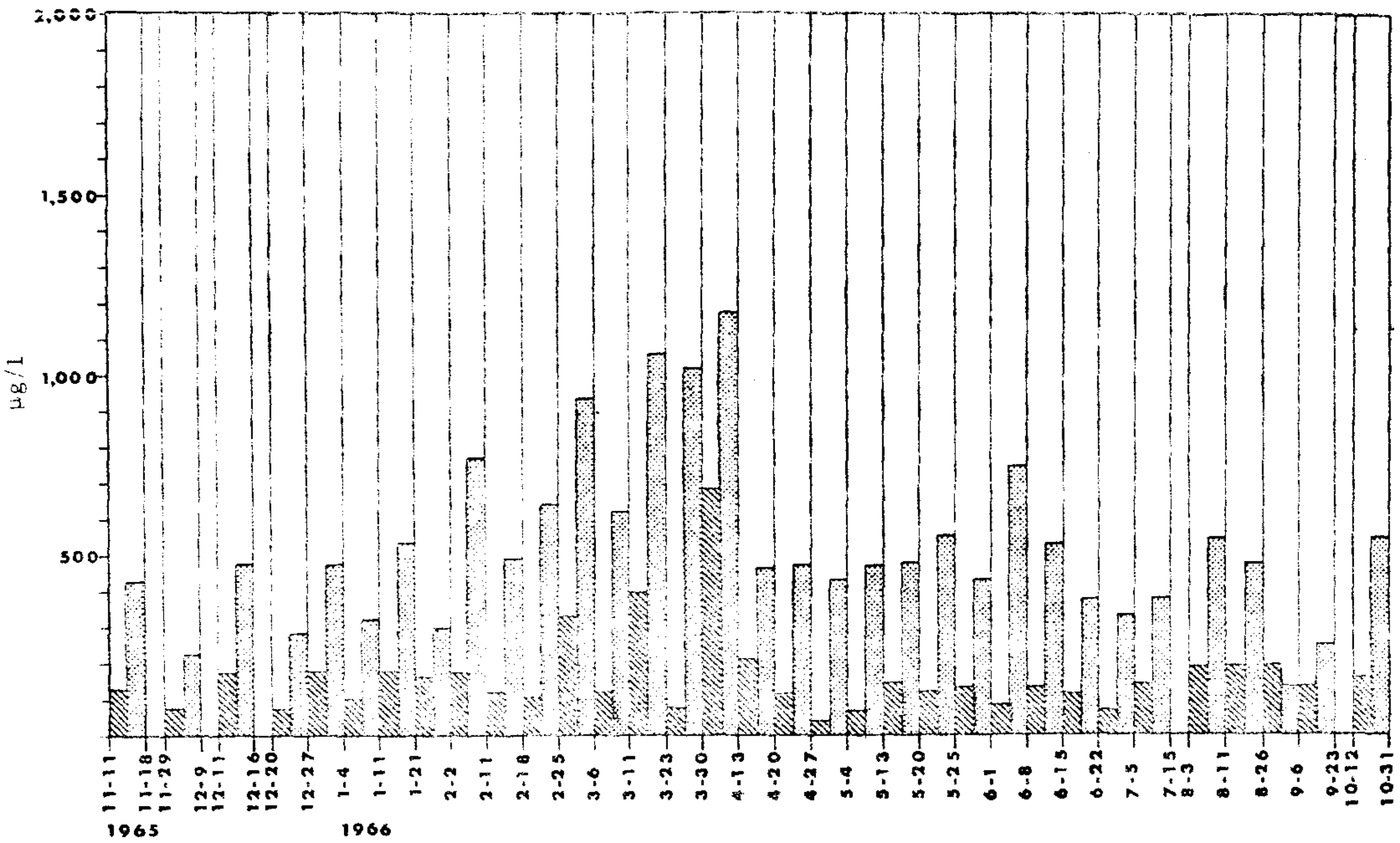


Figure 4. Concentration of Carbon Chloroform Extract (Diagonal) and Carbon Alcohol Extract (Stippled) Collected from (C) Lower End of Cimarron River Arm of Keystone Reservoir.

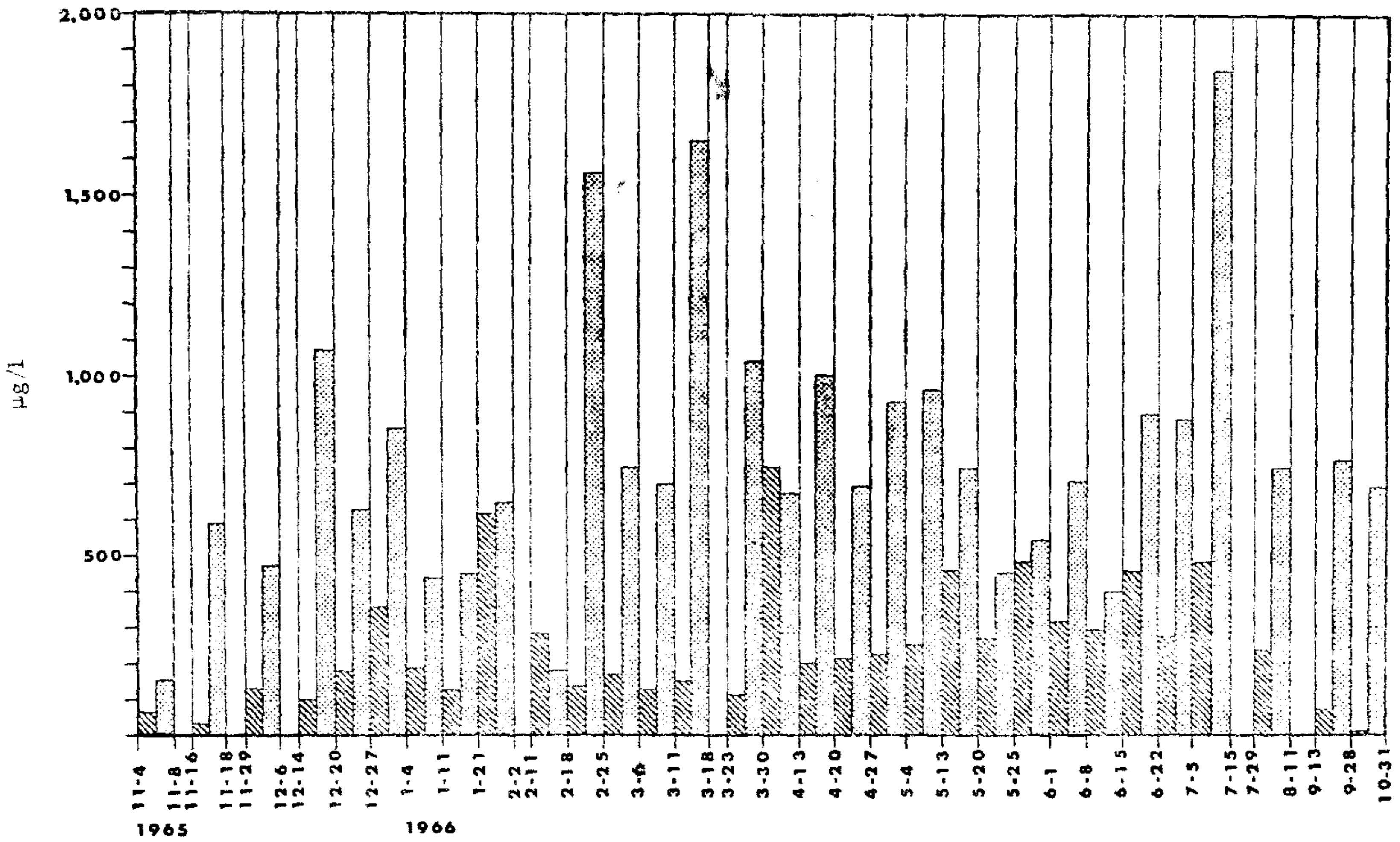


Figure 5. Concentration of Carbon Chloroform Extract (Diagonal) and Carbon Alcohol Extract (Stippled) Collected from (D) Upper End of Cimarron River Arm of Keystone Reservoir.

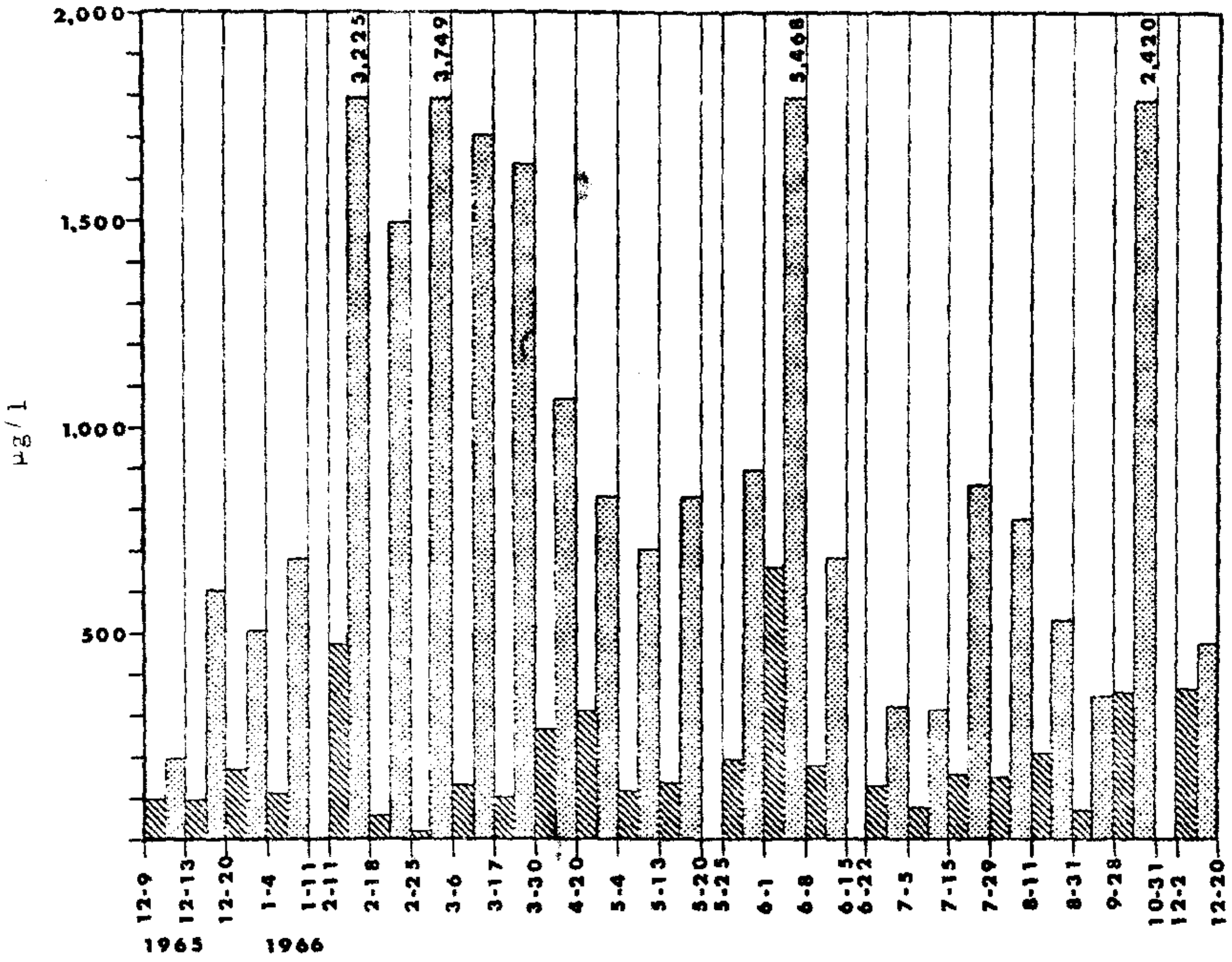


Figure 6. Concentration of Carbon Chloroform Extract (Diagonal) and Carbon Alcohol Extract (Stippled) Collected from (E) Lower End of Arkansas River Arm of Keystone Reservoir.

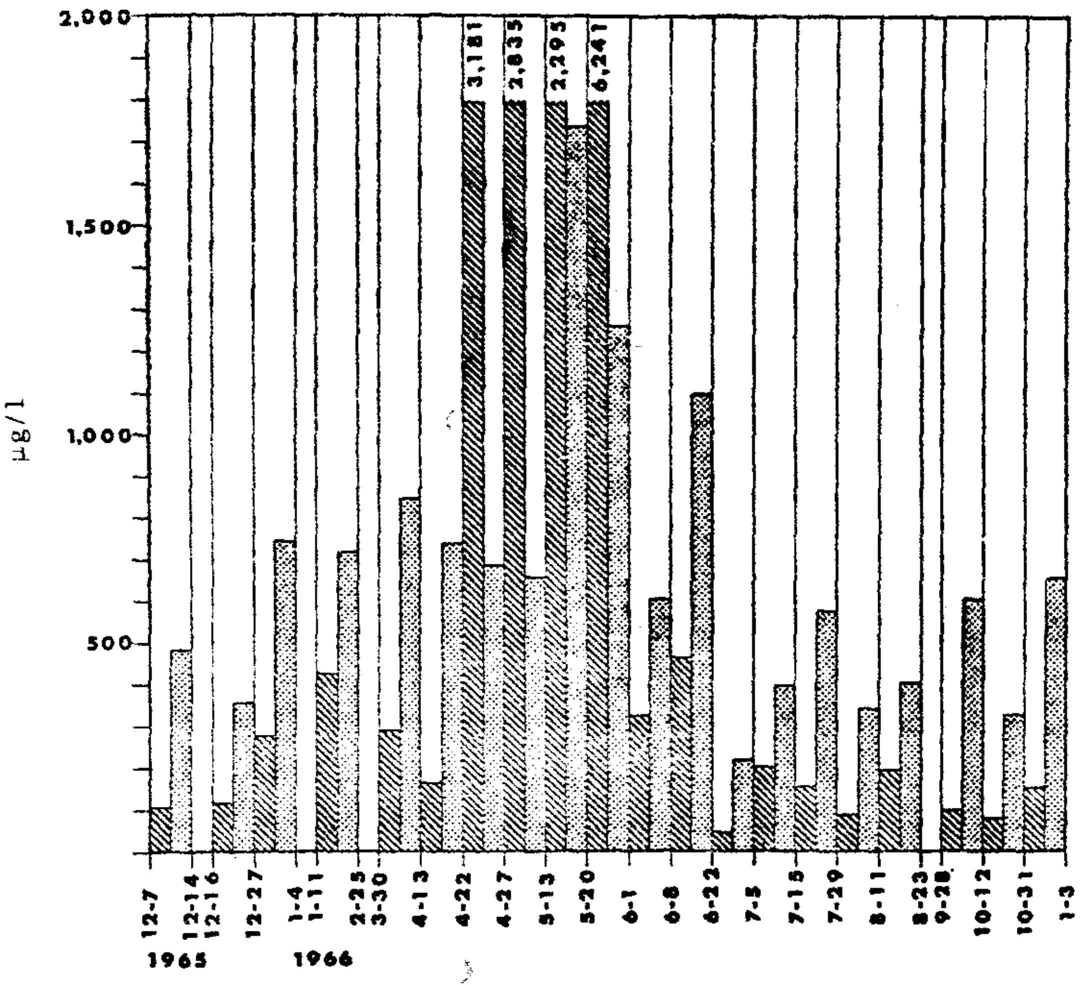
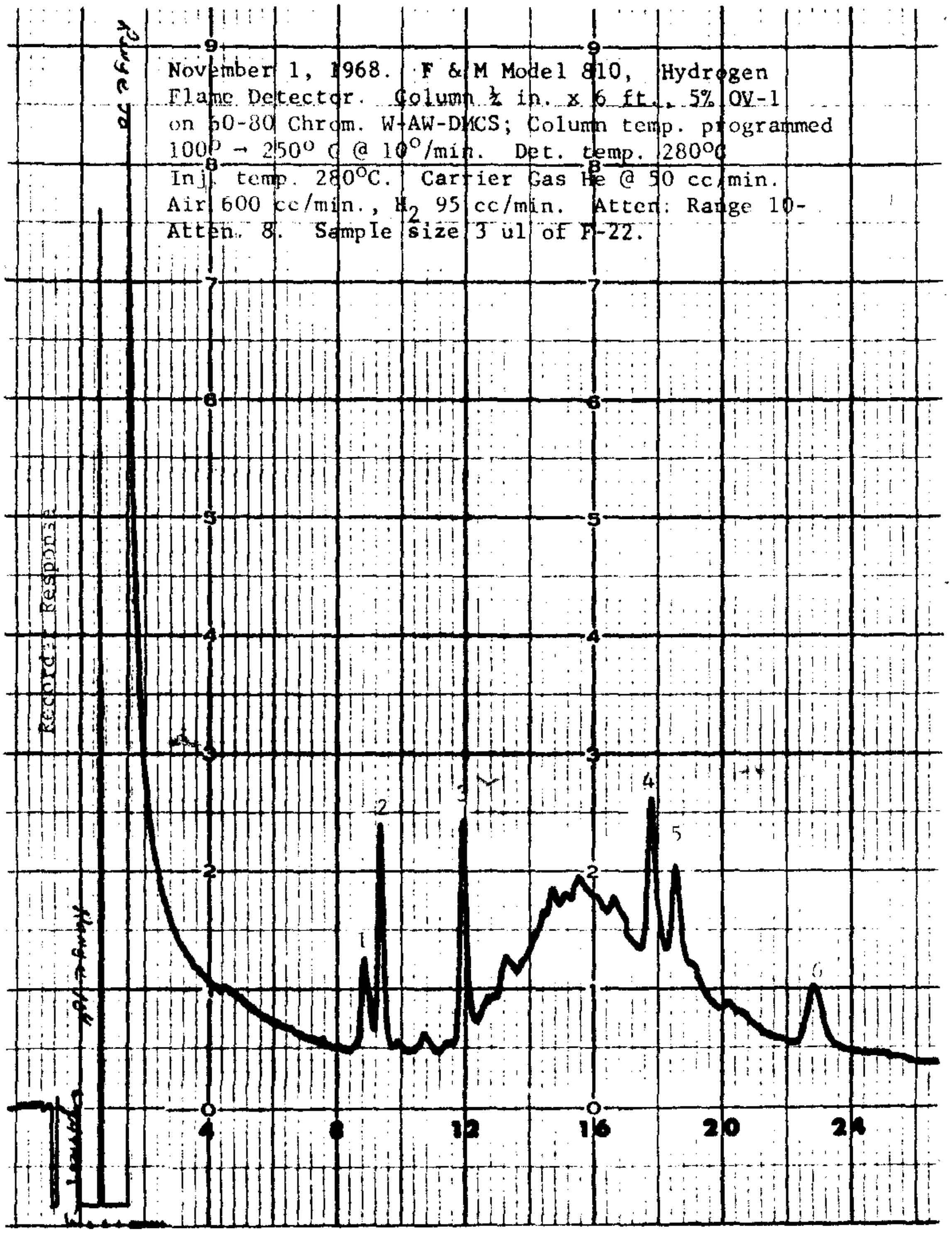
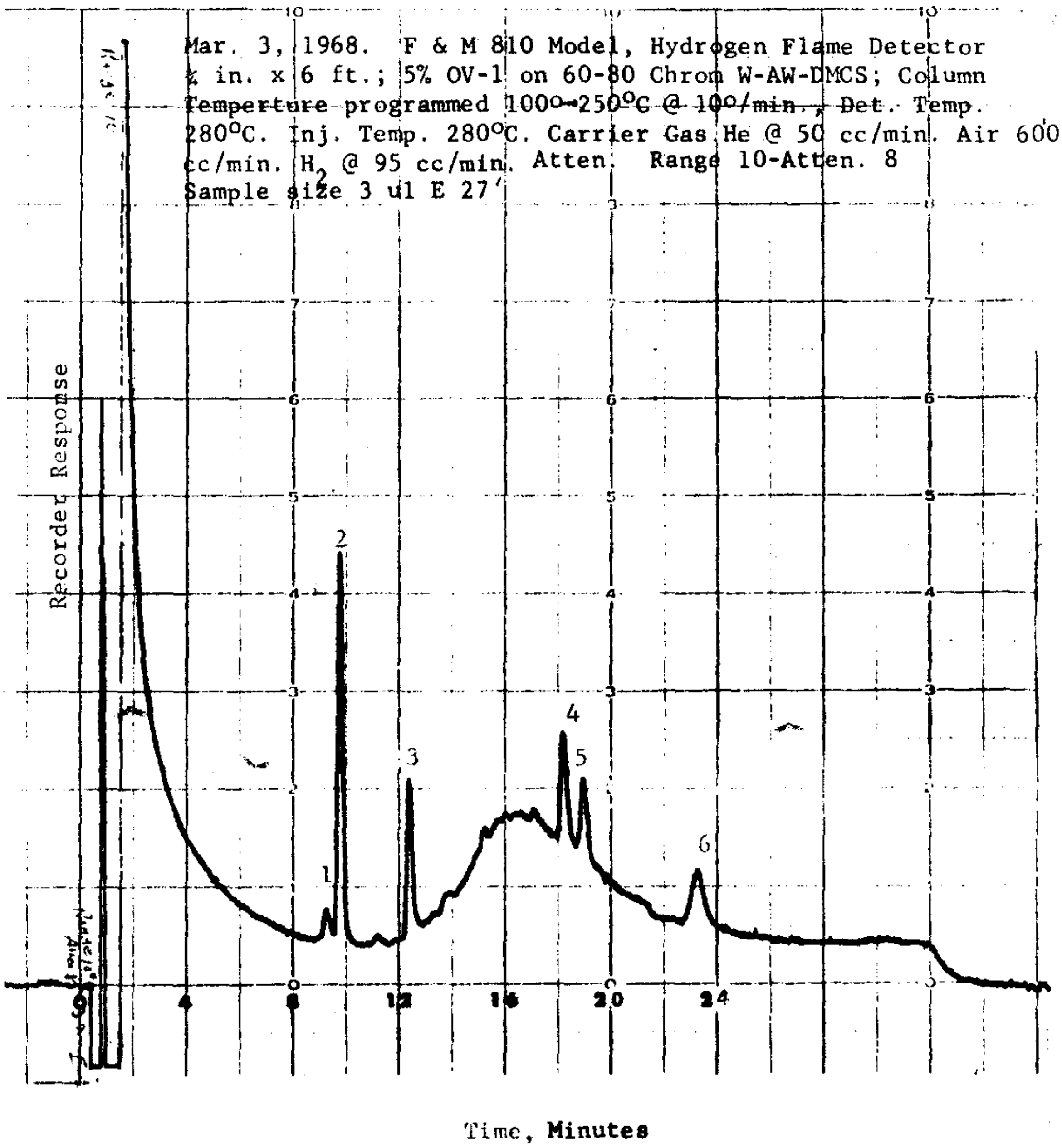


Figure 7. Concentration of Carbon Chloroform Extract (Diagonal) and Carbon Alcohol Extract (Stippled) Collected from (F) Upper End of Arkansas River Arm of Keystone Reservoir.



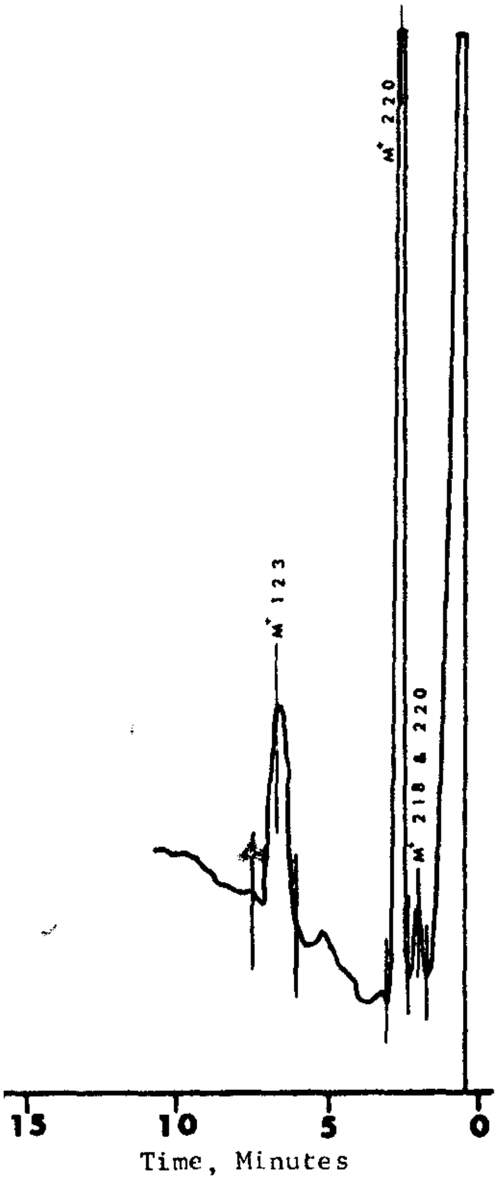
November 1, 1968. F & M Model 810, Hydrogen Flame Detector. Column ½ in. x 6 ft., 5% OV-1 on 60-80 Chrom. W-AW-DMCS; Column temp. programmed 100° → 250° C @ 10°/min. Det. temp. 280°C Inj. temp. 280°C. Carrier Gas He @ 50 cc/min. Air 600 cc/min., H_2 95 cc/min. Atten: Range 10-Atten. 8. Sample size 3 ul of F-22.

Figure 8. **Chromatogram of Compounds Collected from** Upper **Arkansas River** Arm of Keystone Reservoir.



Mar. 3, 1968. F & M 810 Model, Hydrogen Flame Detector
¼ in. x 6 ft.; 5% OV-1 on 60-80 Chrom W-AW-DMCS; Column
Temperture programmed 100°→250°C @ 10°/min., Det. Temp.
280°C. Inj. Temp. 280°C. Carrier Gas He @ 50 cc/min. Air 600
cc/min. H_2 @ 95 cc/min. Atten. Range 10-Atten. 8
Sample size 3 ul E 27'

Figure 9. Chromatogram of Compounds Collected from Lower Arkansas River Arm of Keystone Reservoir.



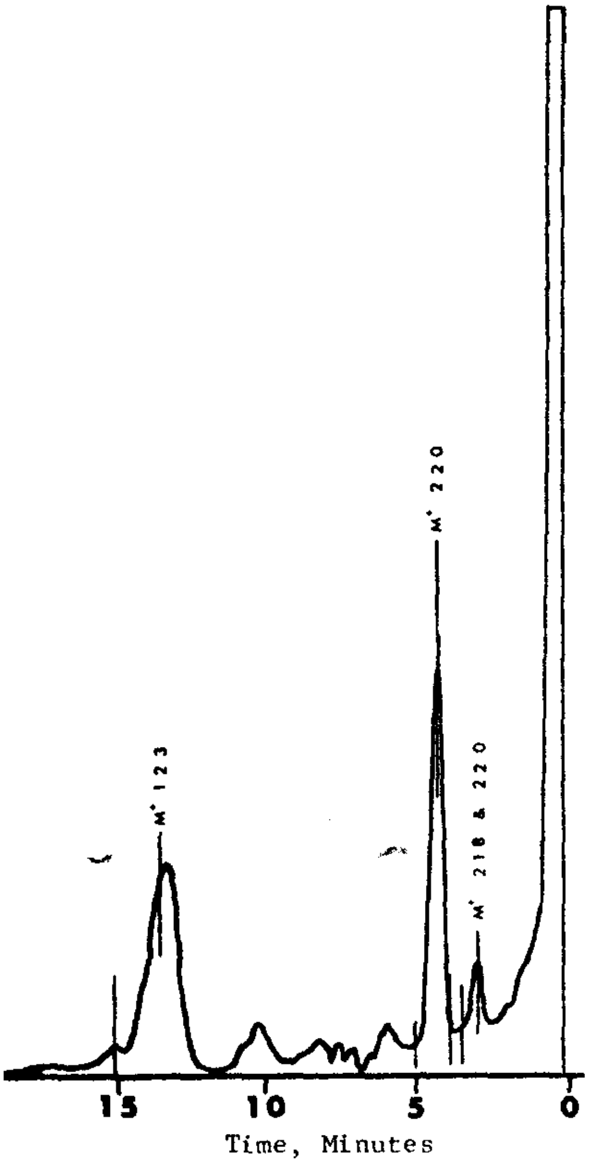


Figure 10. GC-MS Chromatogram of
1.0 µl of Sample E-27
Column temp. 150° C

Figure 11. GC-MS Chromatogram of
4 µl of Sample F-22
Column temp. 130° C

GLC conditions for Figures 10 and 11: LKB 9000 ionization detector, 6 ft by 1/4 in glass, 5% Apiezon L on Chrom W-AW-DMCS, Mol. Sep. temp. 200° C; Inj. temp. 190° C; Car. Gas He @ 21 cc/min.

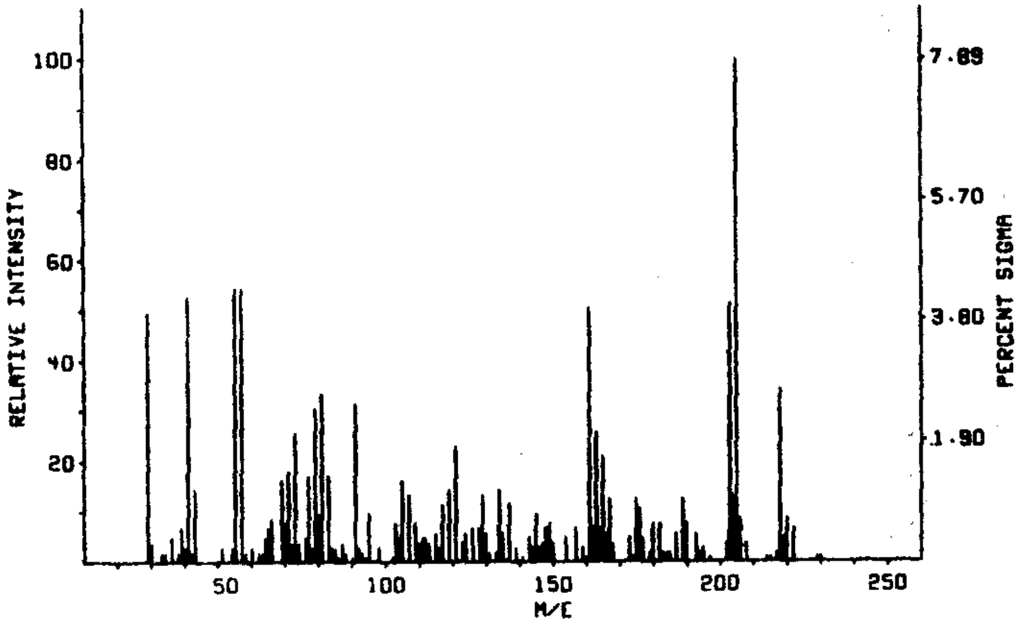


Figure 12. Mass Spectrum of GLC Peak #1 Collected from Lower Arkansas River Arm of Keystone Reservoir, Introduced via Gas Chromatograph.

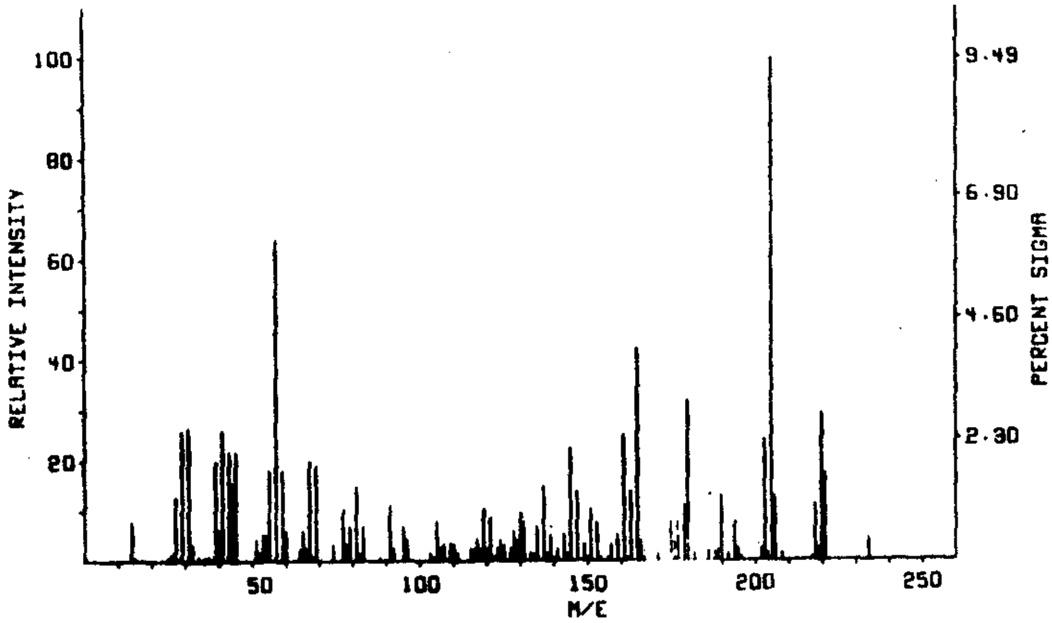


Figure 13. M.S. of GLC Peak #1 Collected from Upper Arkansas River Arm of Keystone Reservoir, Introduced via Gas Chromatograph.

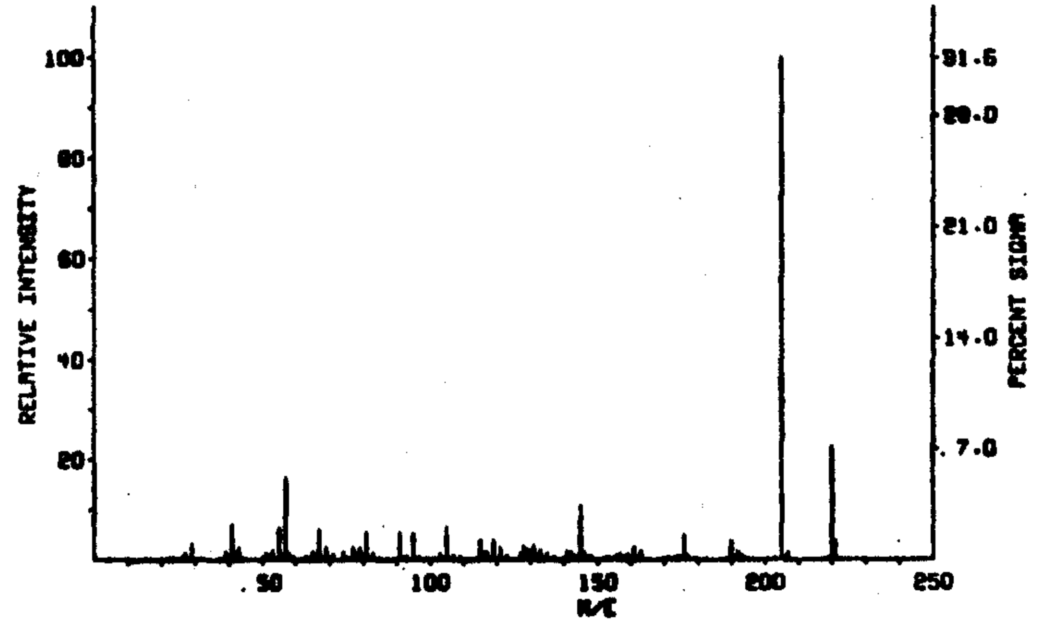


Figure 14. M.S. of Peak #2 Collected from Lower Arkansas River Arm of Keystone Reservoir, Introduced via Gas Chromatograph.

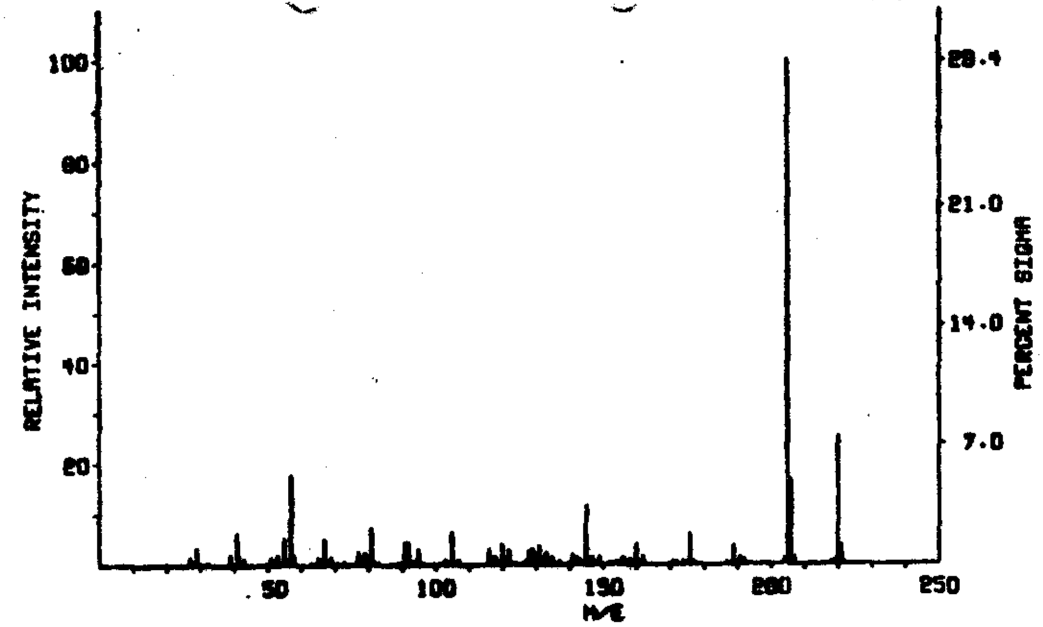
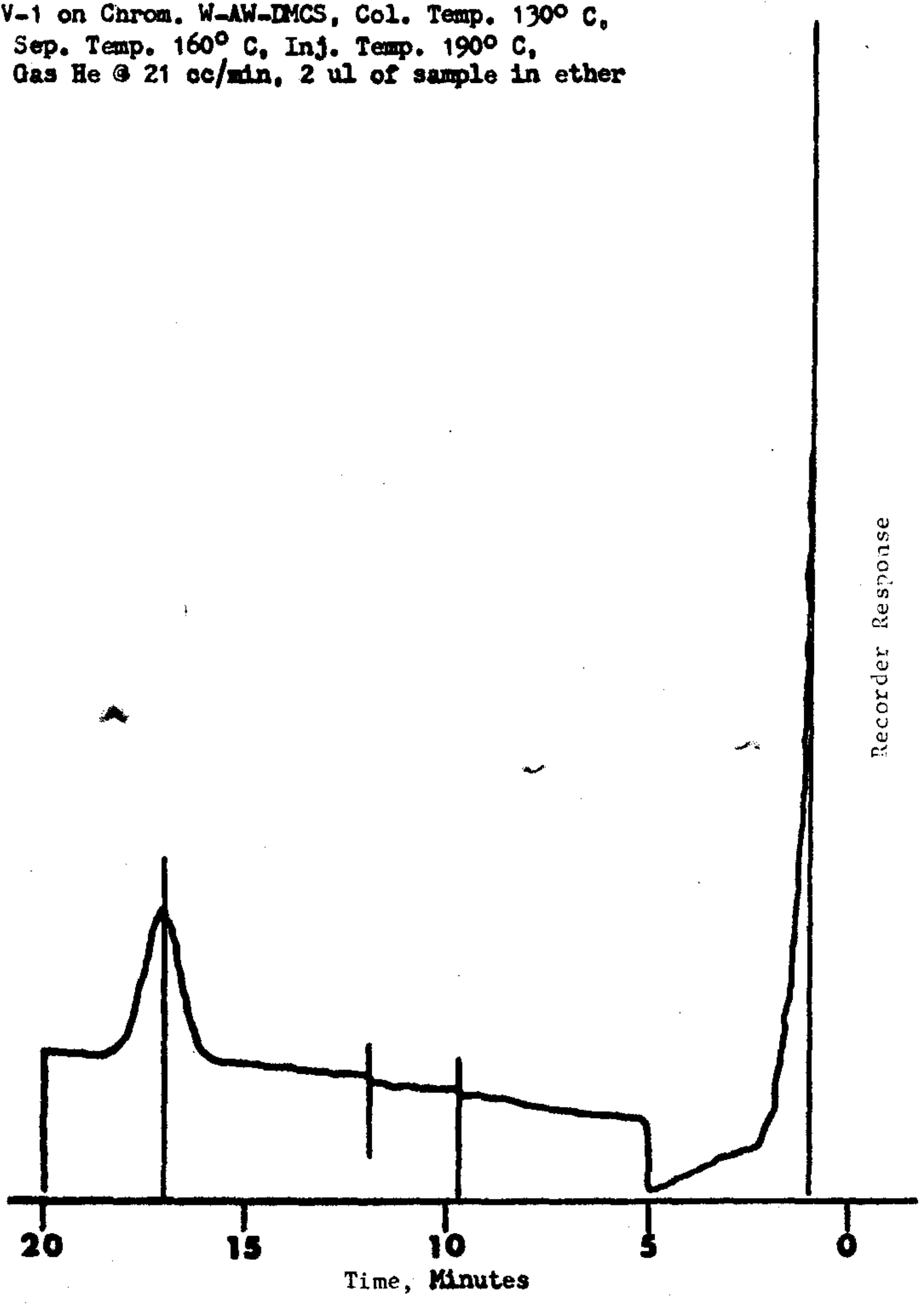


Figure 15. M.S. of Peak #2 Collected from Upper Arkansas River Arm of Keystone Reservoir, Introduced via Gas Chromatograph.



LKB-9000 ionization detector, 6 ft x ⅛ in. glass,
5% OV-1 on Chrom. W-AW-DMCS, Col. Temp. 130° C,
Mol. Sep. Temp. 160° C, Inj. Temp. 190° C,
Car. Gas He @ 21 cc/min, 2 ul of sample in ether

ure 16. Gas Chromatogram of Reagent 2,6-di-tert-butyl-4-methylphenol. Vertical Lines Indicate Mass Spectral Scans.

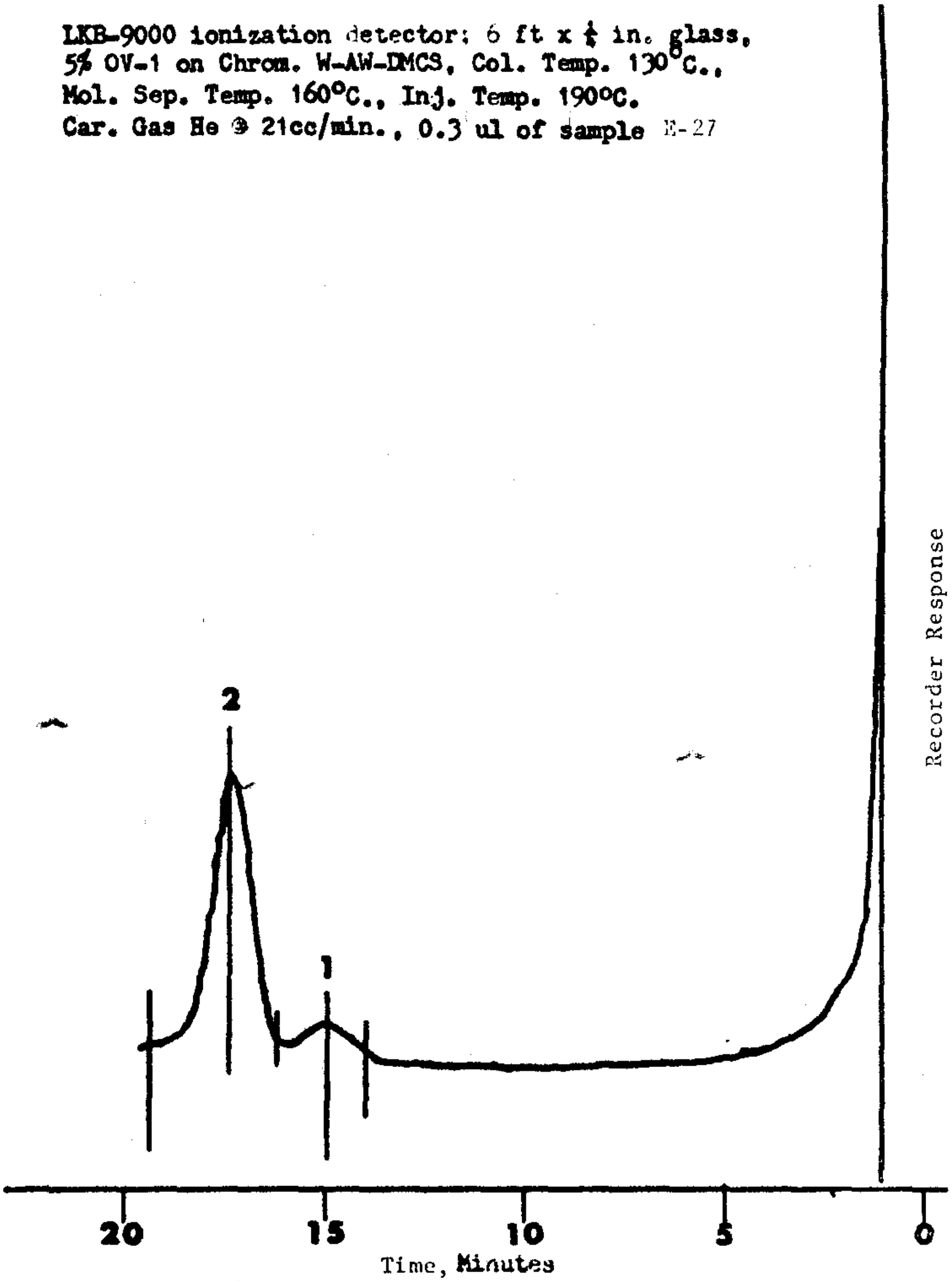


Figure 17. Partial Gas Chromatogram of Compounds Collected from the Lower End of the Arkansas River Arm of Keystone. Vertical Lines Indicate Mass Spectral Scans.

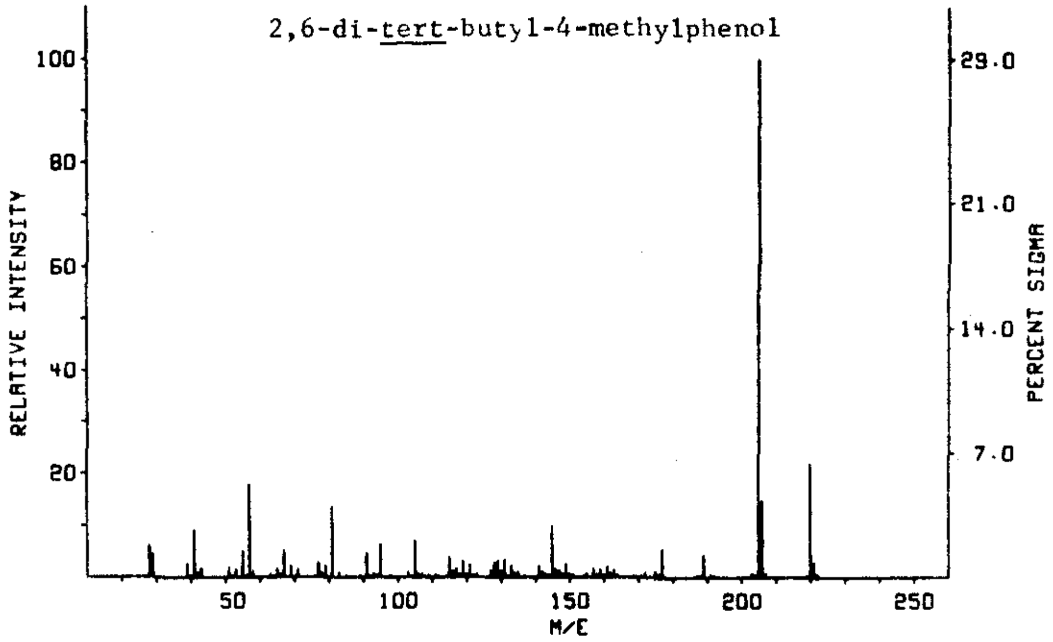


Figure 18. Mass Spectrum of Standard 2,6-di-*tert*-butyl-4-methylphenol, Introduced via Gas Chromatograph.

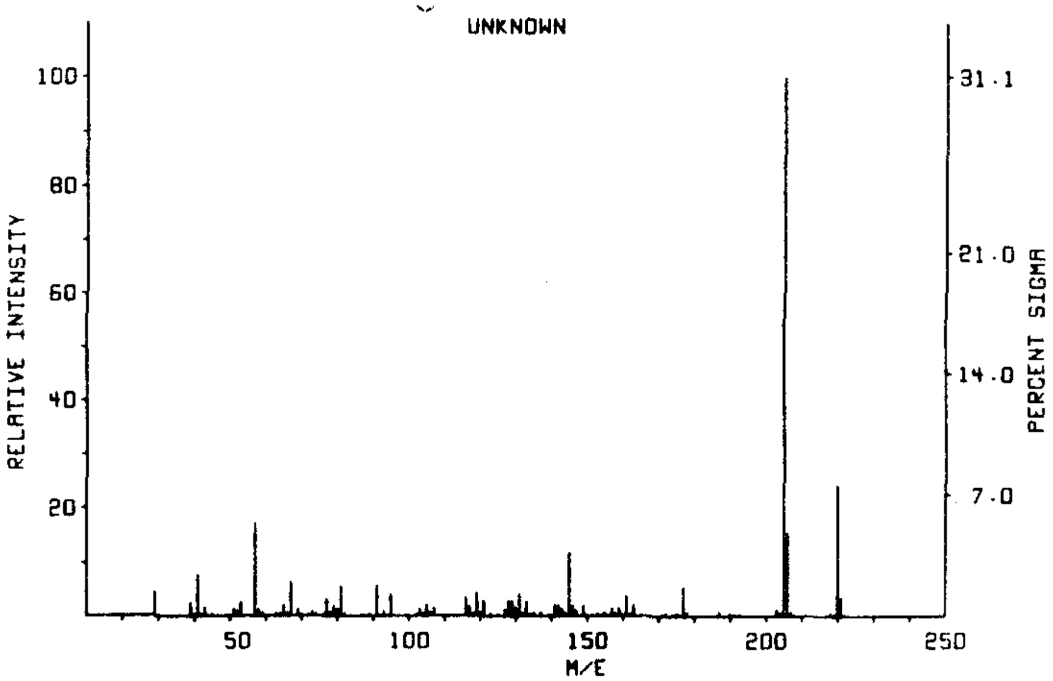
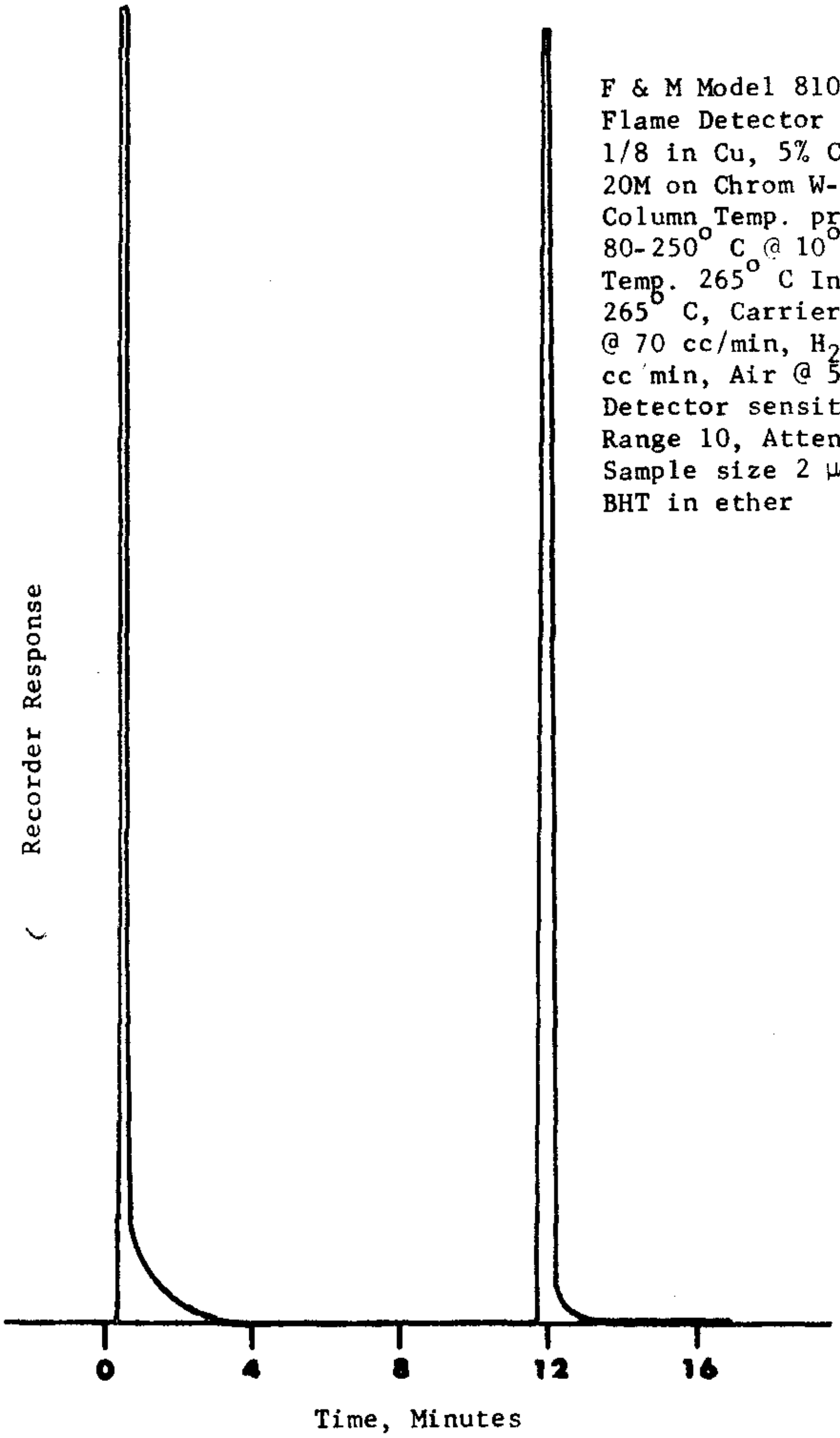


Figure 19. Mass Spectrum of Unknown Collected from Lower Arkansas River Arm of Keystone Reservoir, Introduced via Gas Chromatograph.



F & M Model 810 Hydrogen
Flame Detector 12 ft x
1/8 in Cu, 5% Carbowax
20M on Chrom W-AW-DMSC,
Column Temp. programmed
80-250° C @ 10°/min, Det.
Temp. 265° C Inj. Temp.
265° C, Carrier Gas He
@ 70 cc/min, H_2 @ 95
cc/min, Air @ 580 cc/m
Detector sensitivity,
Range 10, Attenuation 256,
Sample size 2 μl reag.
BHT in ether

Figure 20. Gas Chromatogram of Standard 2,6-di-tert-butyl-4-methylphenol.

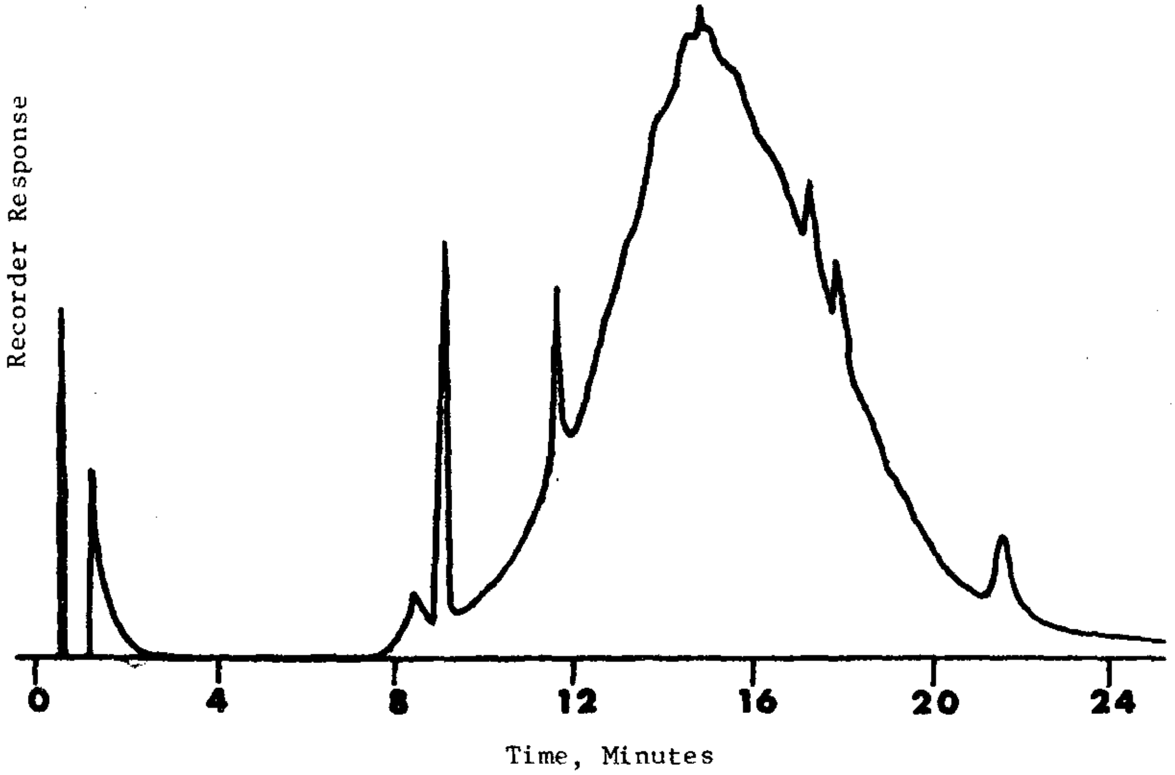
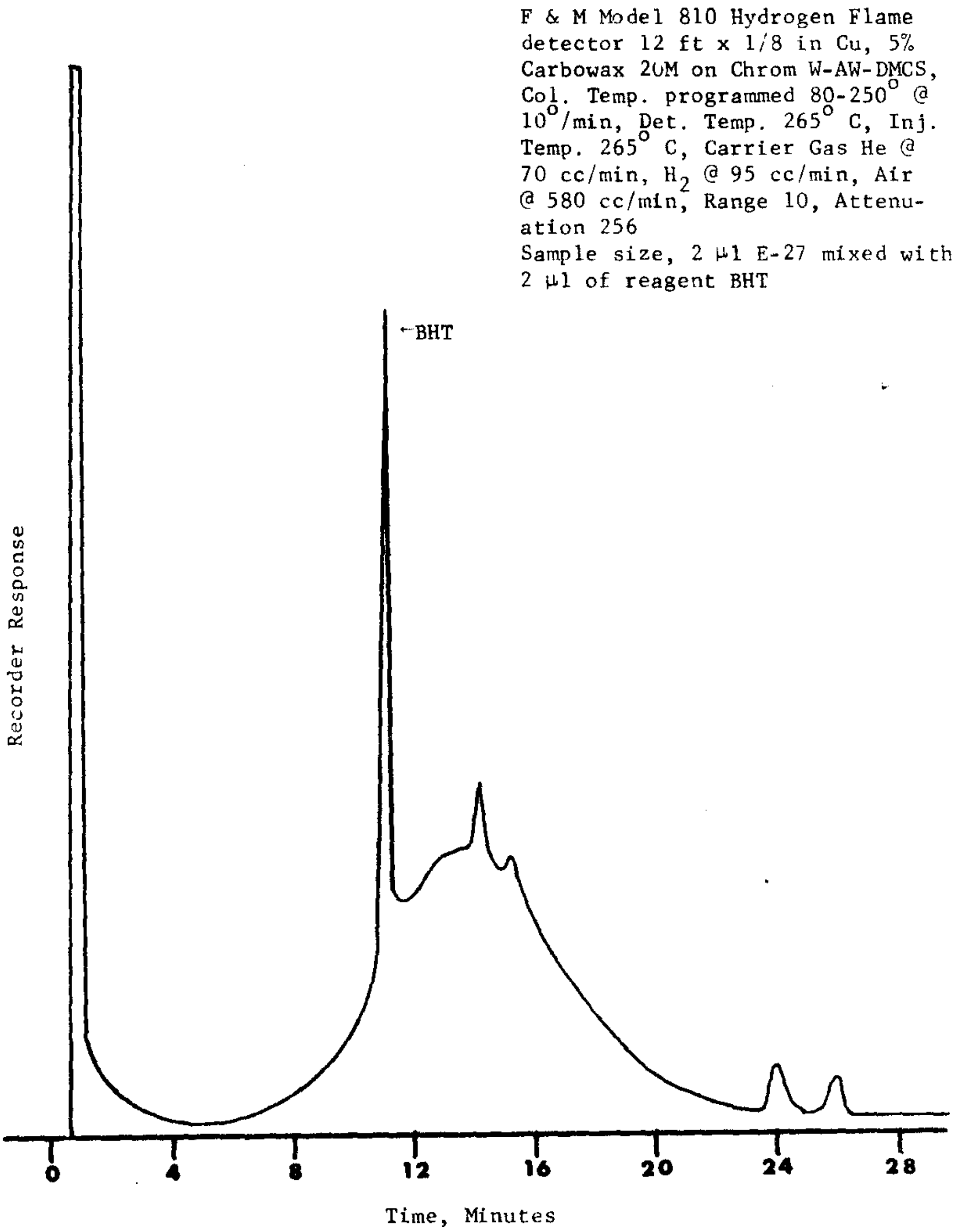


Figure 21. Gas Chromatogram of Sample E-27 Collected from Lower Arkansas River Arm of Keystone Reservoir.



F & M Model 810 Hydrogen Flame detector 12 ft x 1/8 in Cu, 5% Carbowax 20M on Chrom W-AW-DMCS, Col. Temp. programmed 80-250° @ 10°/min, Det. Temp. 265° C, Inj. Temp. 265° C, Carrier Gas He @ 70 cc/min, H_2 @ 95 cc/min, Air @ 580 cc/min, Range 10, Attenuation 256

Sample size, 2 μl E-27 mixed with 2 μl of reagent BHT

Figure 22. Chromatogram of Standard BHT and Steam Volatile Compounds Collected from E.

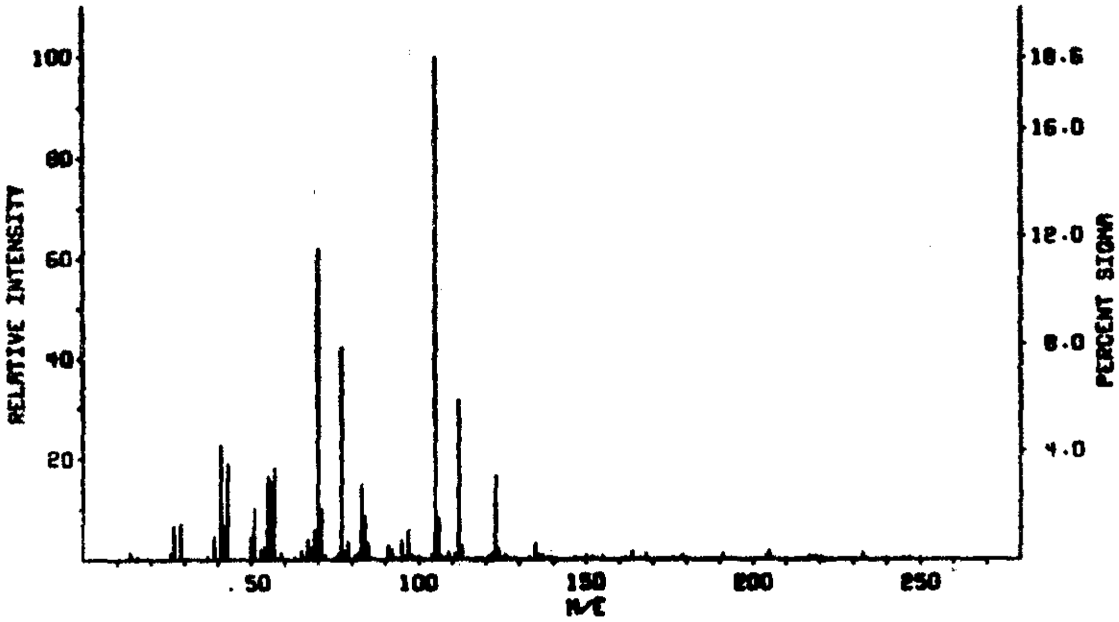


Figure 23. M.S. of Peak #3 Collected from Lower Arkansas River Arm of Keystone Reservoir, Introduced via Gas Chromatograph.

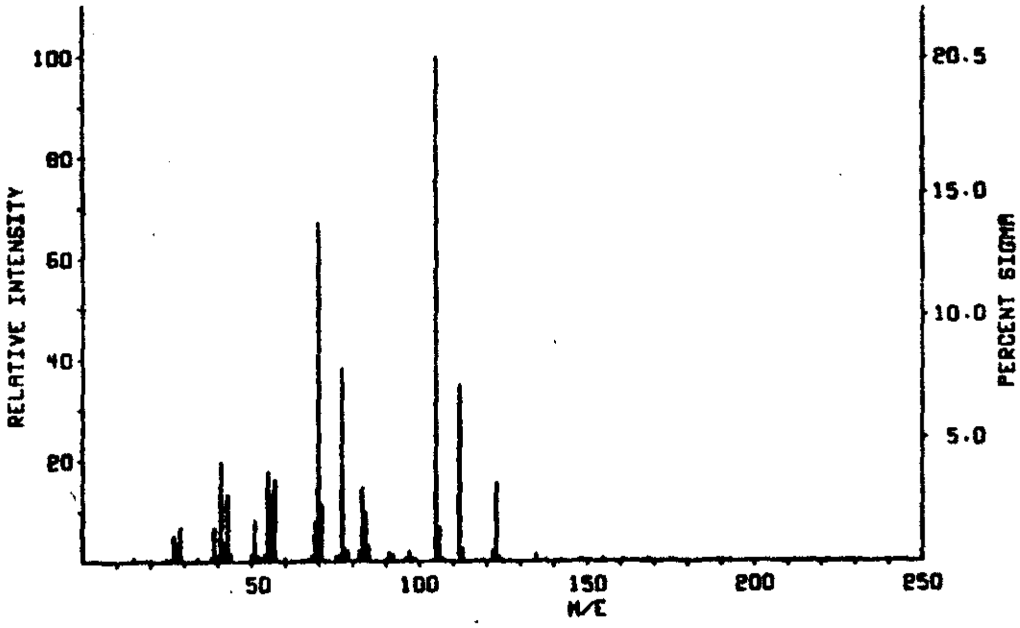


Figure 24. M.S. of Peak #3 Collected from Upper Arkansas River Arm of Keystone Reservoir, Introduced via Gas Chromatograph.

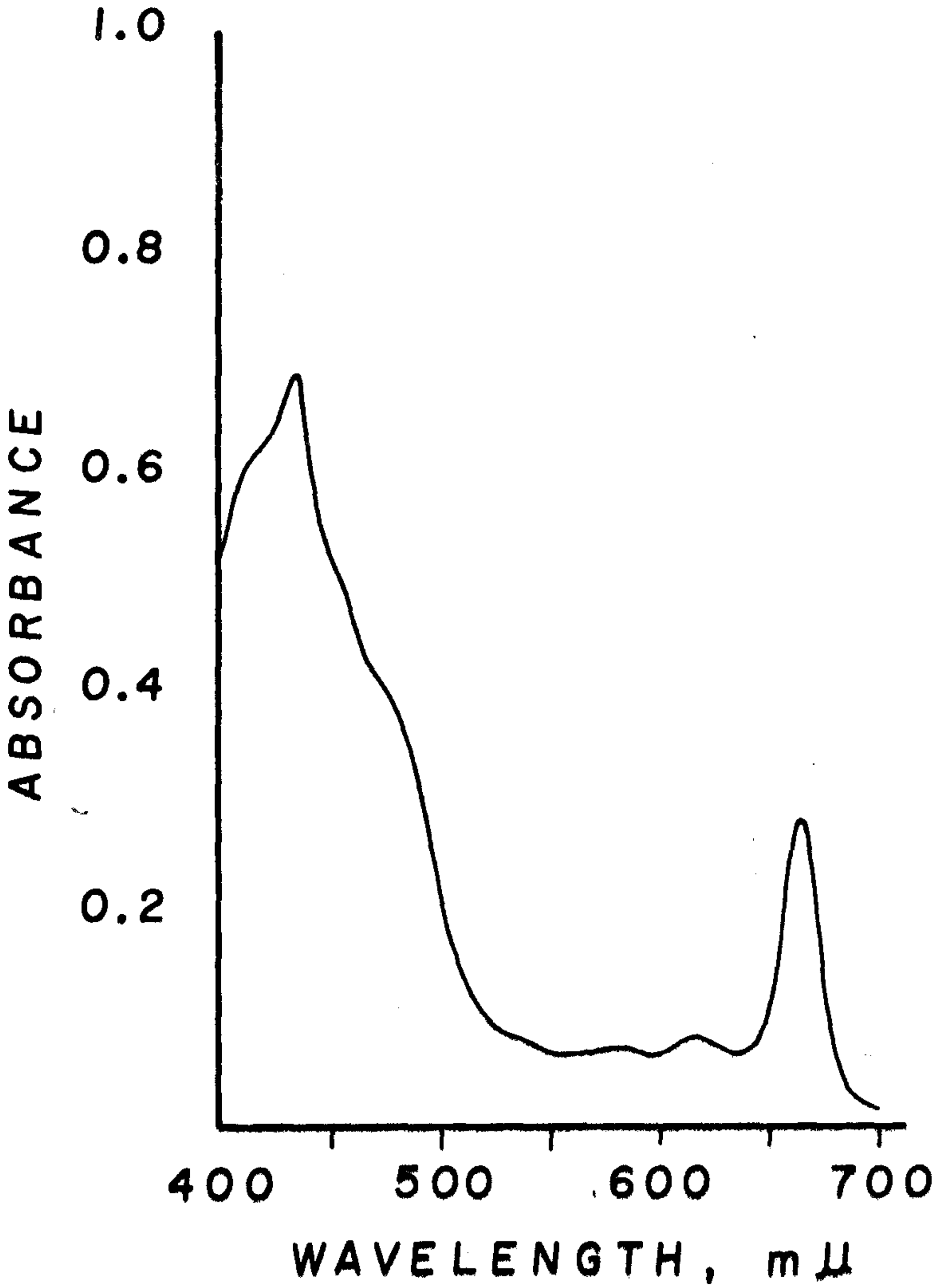


Figure 25. Example of an Absorption Curve of a 90 Per Cent Acetone Extract from the Natural Phytoplankton Population of Keystone Reservoir. Absorbancies Are Read at Wavelengths of 430, 480, 510, 635, 645, and 665 mμ.

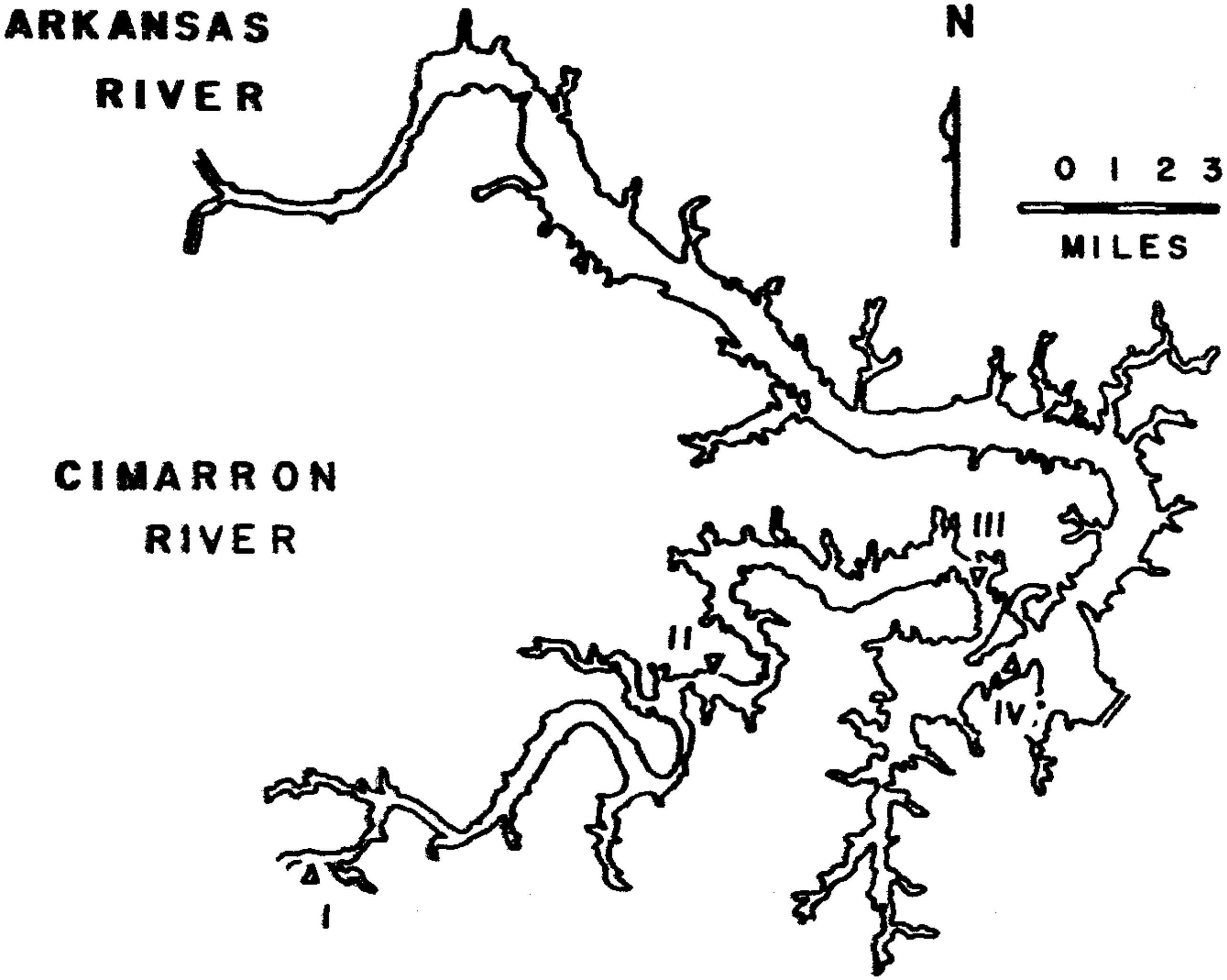

ARKANSAS RIVER
N
0 1 2 3
MILES
CIMARRON RIVER
III
II
IV
I

Figure 26. Keystone Reservoir, Tulsa, Oklahoma. Roman Numerals Indicate Locations of the Sampling Stations.

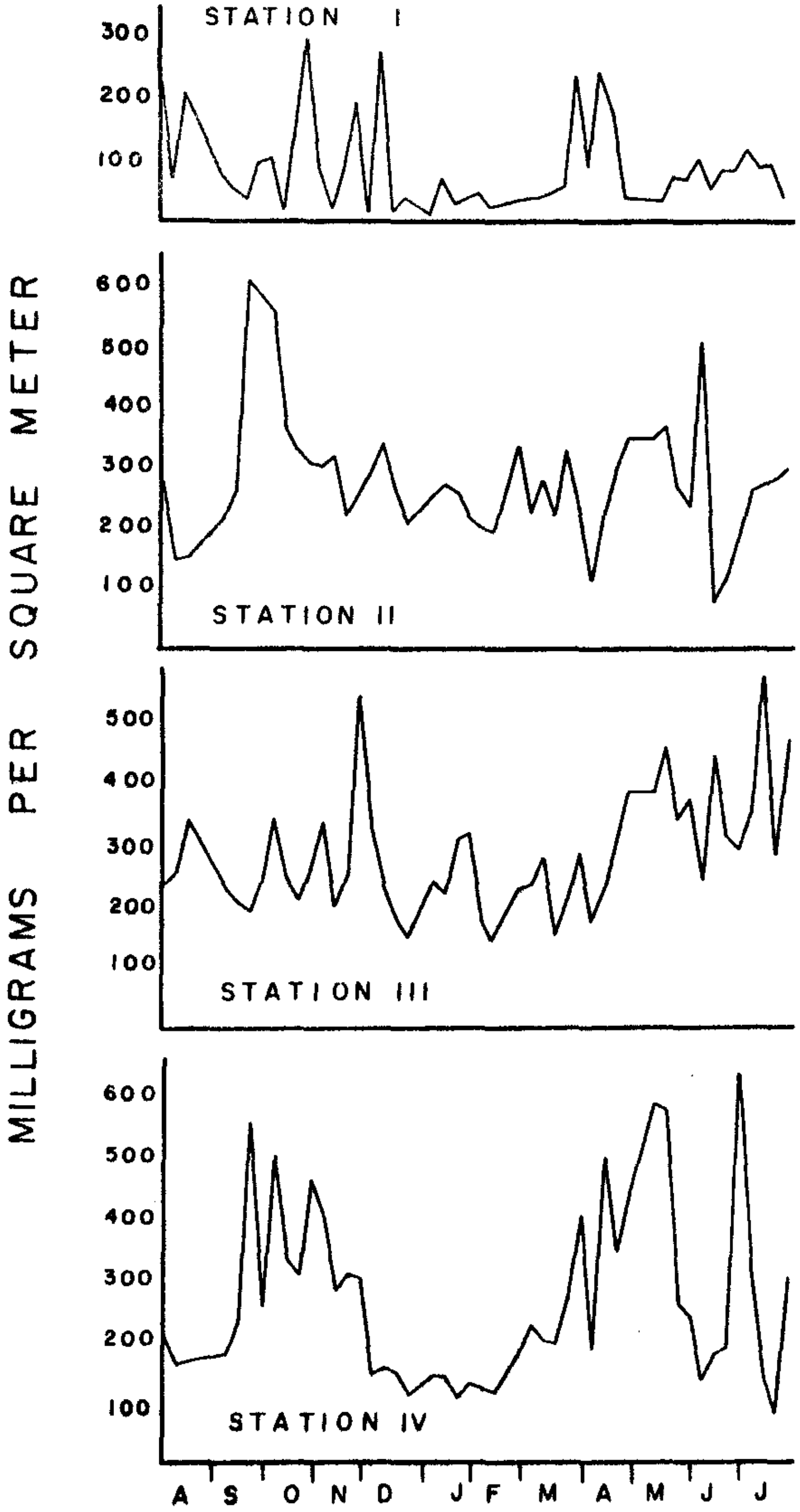


Figure 27. Temporal Variation in Amounts of Chlorophyll a in a Water Column One Meter Square.

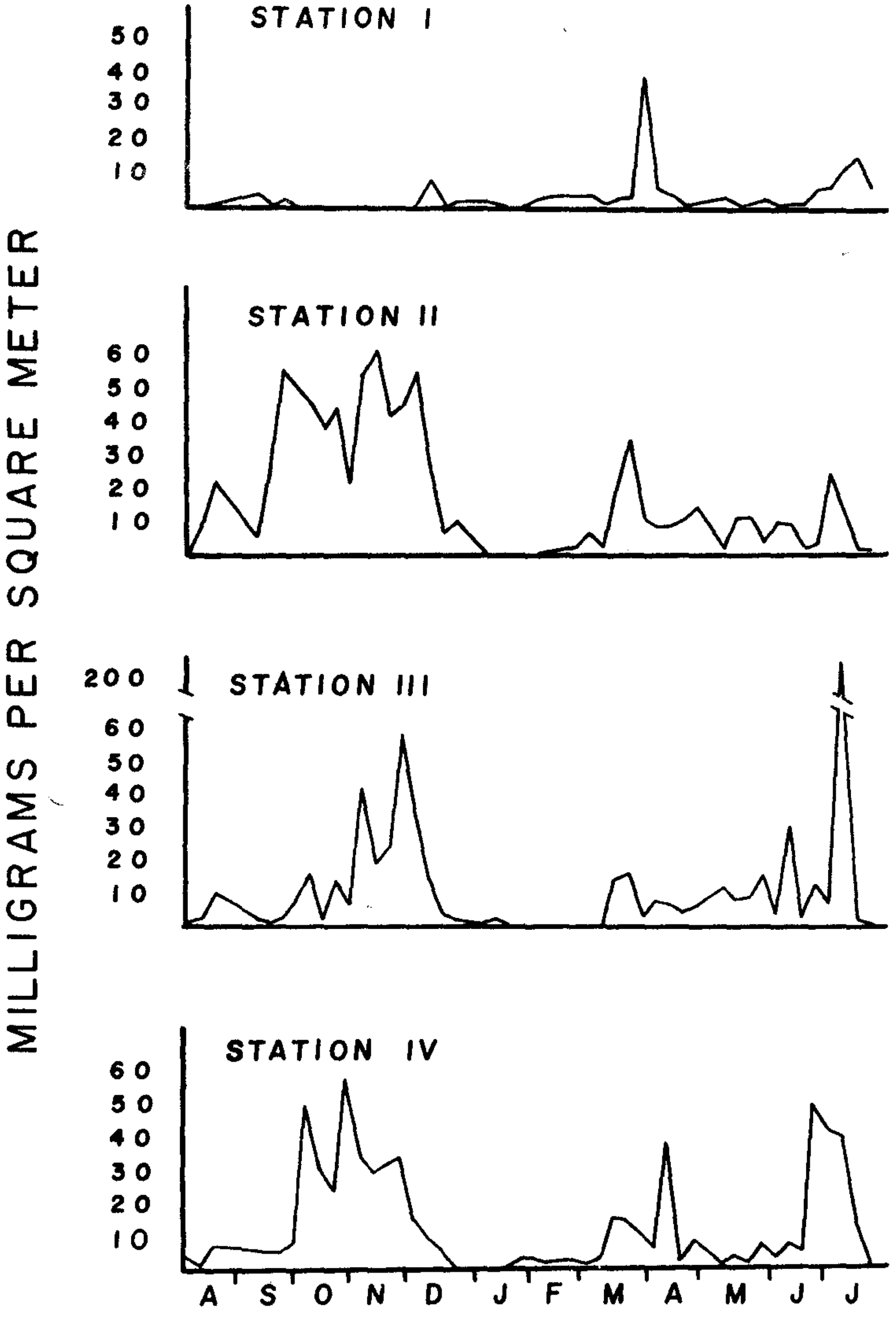


Figure 28. Temporal Variation in Amounts of Chlorophyll b in a Water Column One Meter Square.

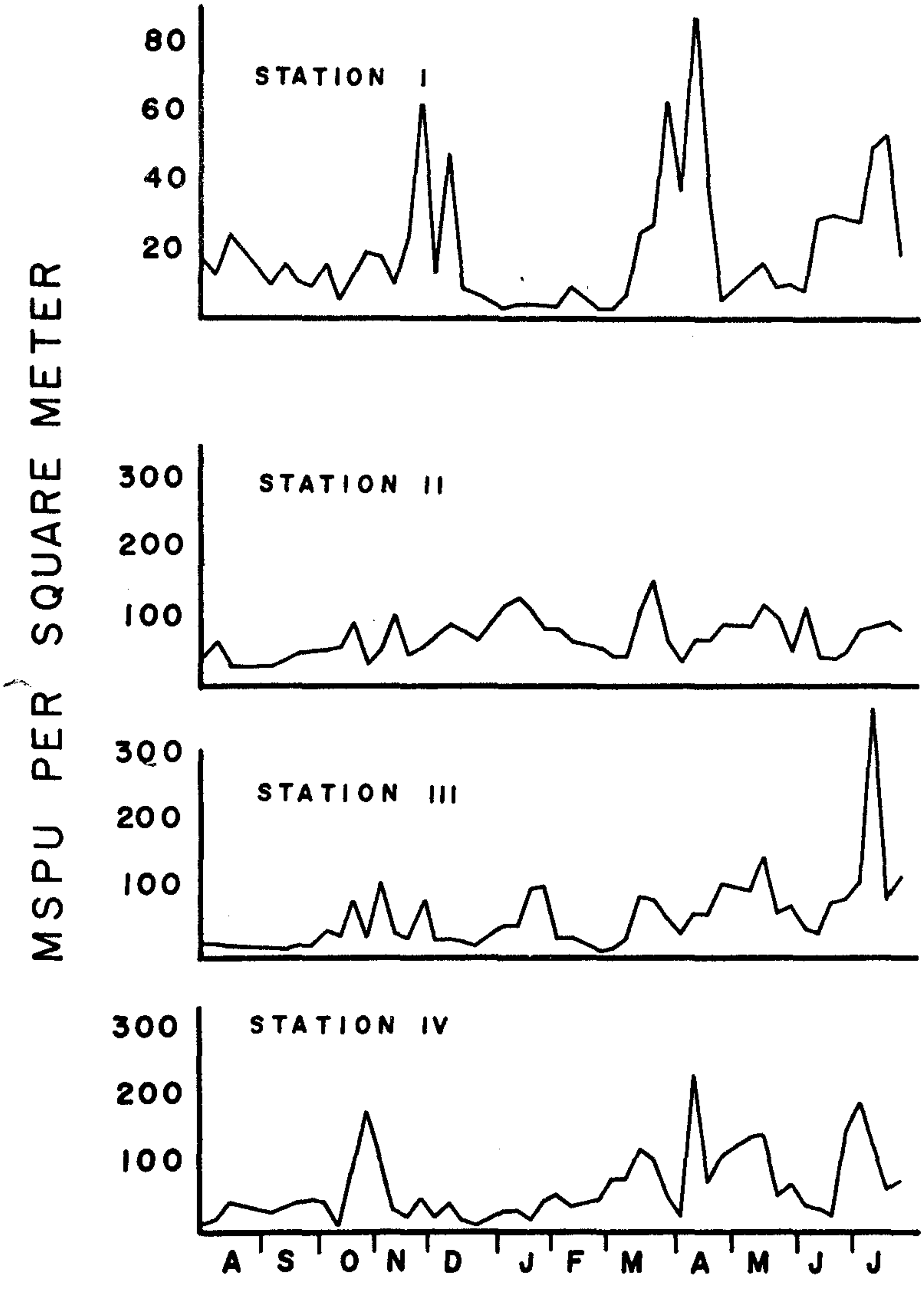


Figure 29. Temporal Variation in Amounts of Chlorophyll c in a Water Column One Meter Square.

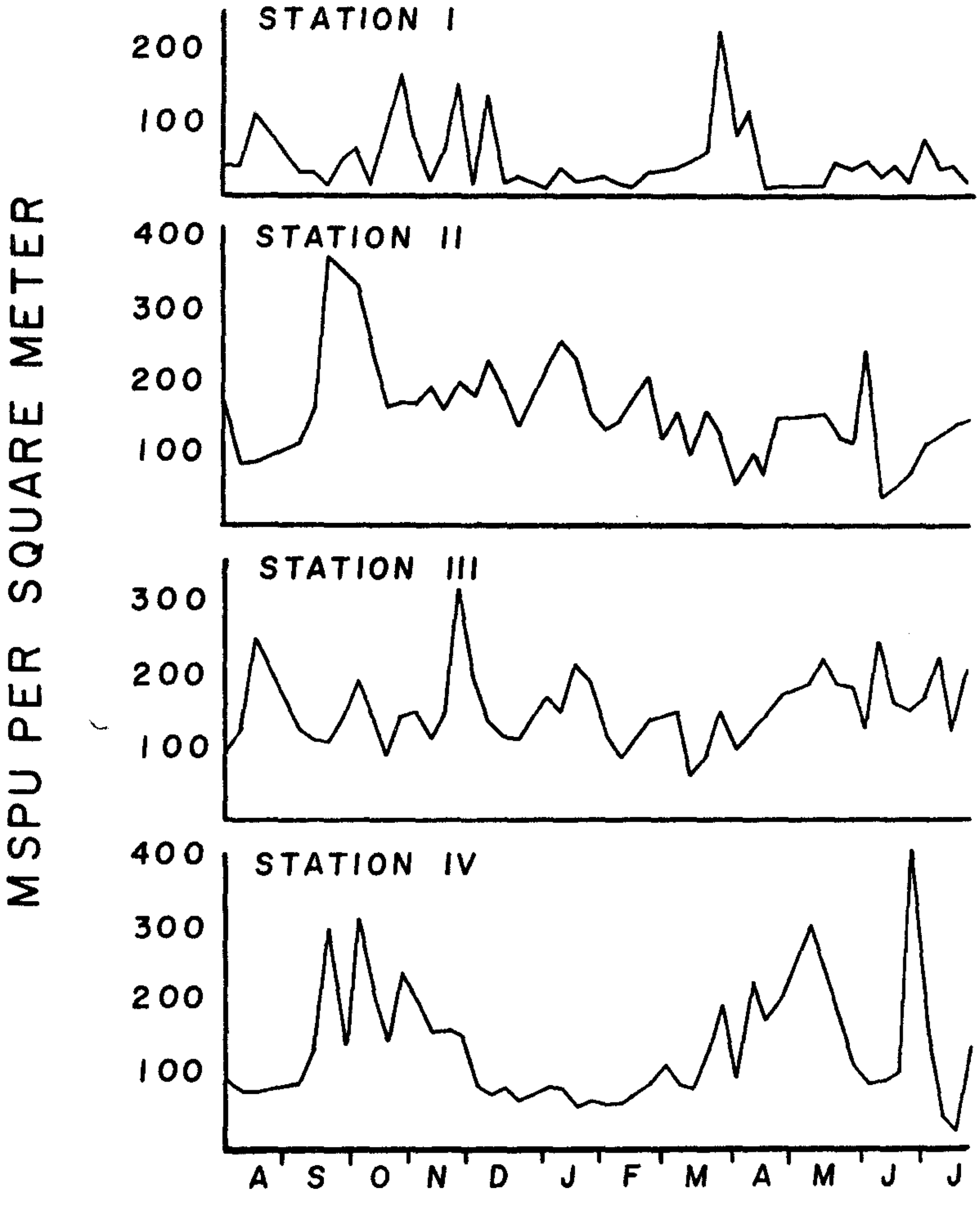


Figure 30. Temporal Variation in Amounts of Non-Astacin Carotenoids in a Water Column One Meter Square.

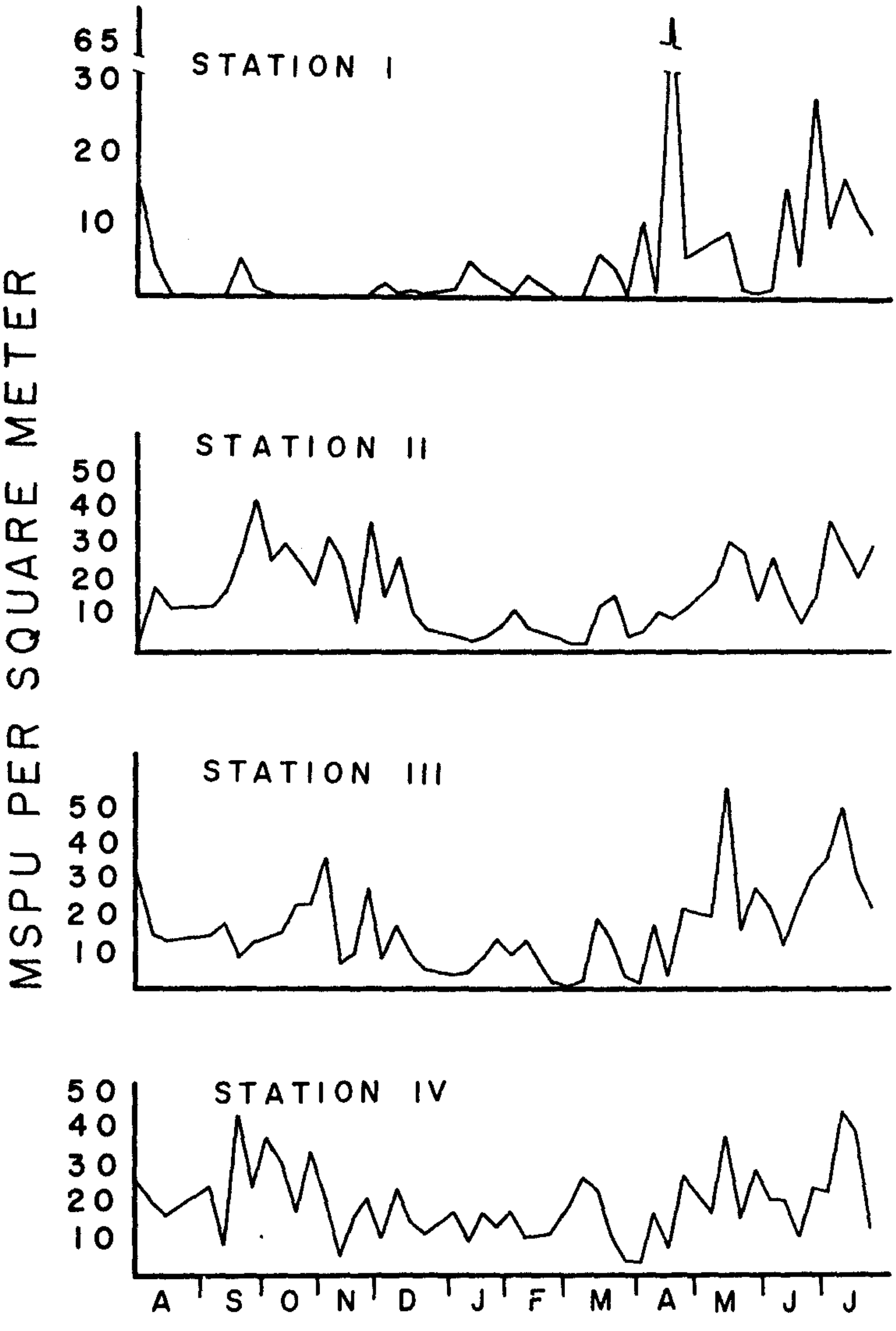


Figure 31. Temporal Variation in Amounts of Astacin-Type Carotenoids in a Water Column One Meter Square.

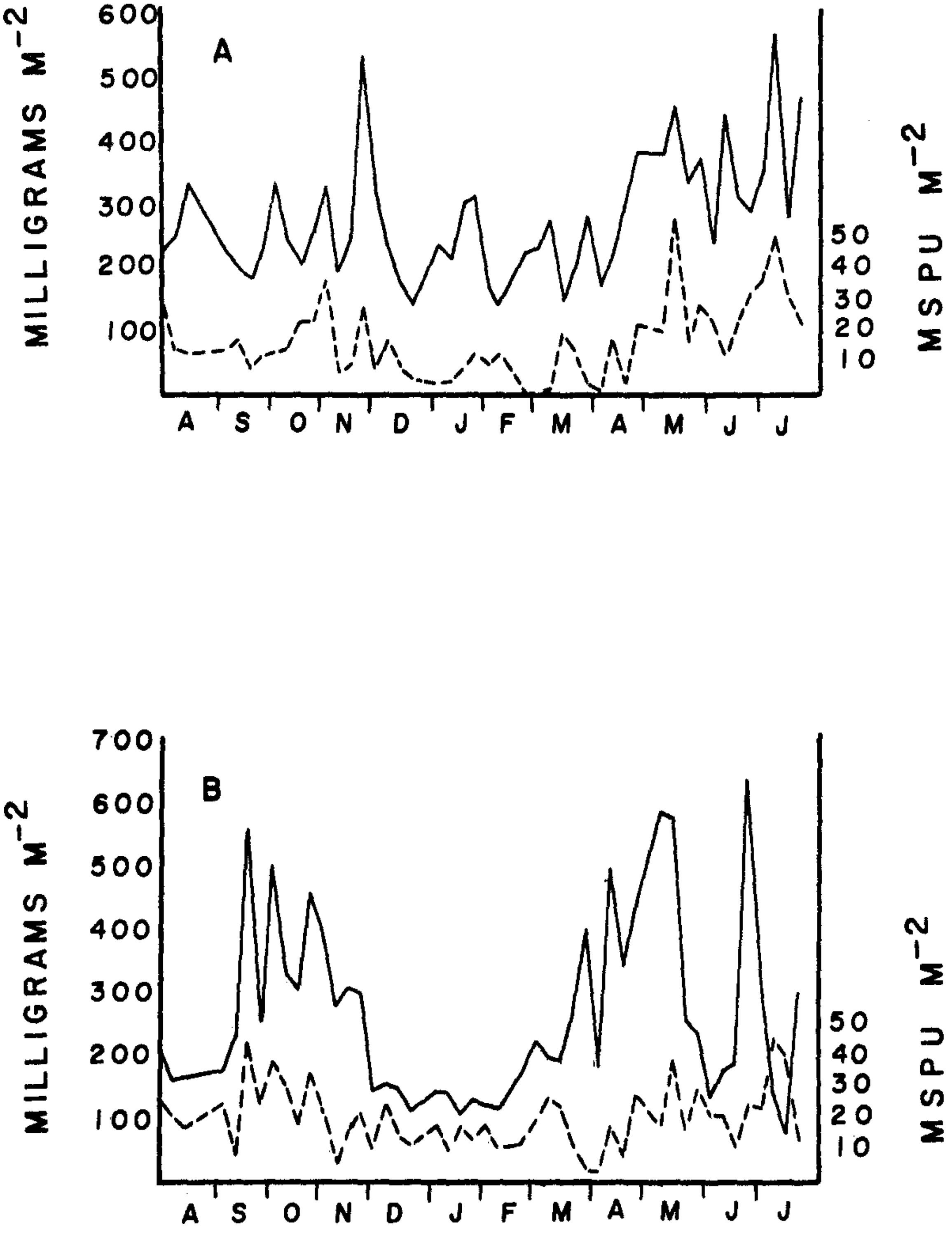


Figure 32. Temporal Variation in Chlorophyll a (———) and Astacin-Type Carotenoids (- - -) at A--Station III and at B--Station IV.

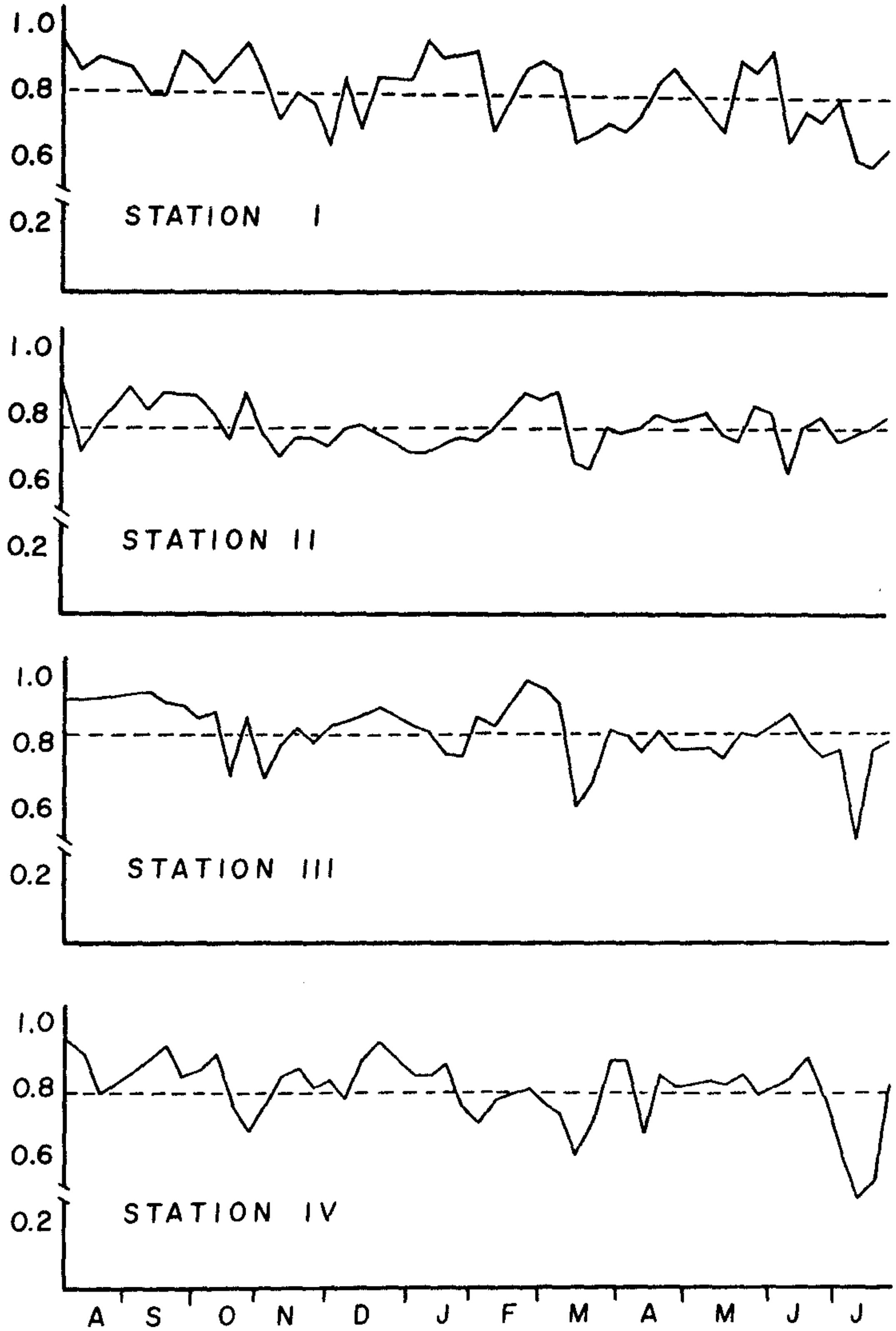


Figure 33. Temporal Variation in Chlorophyll a Fraction (Amount of Chlorophyll a Relative to Total Chlorophyll Amount) in a Column of Water.

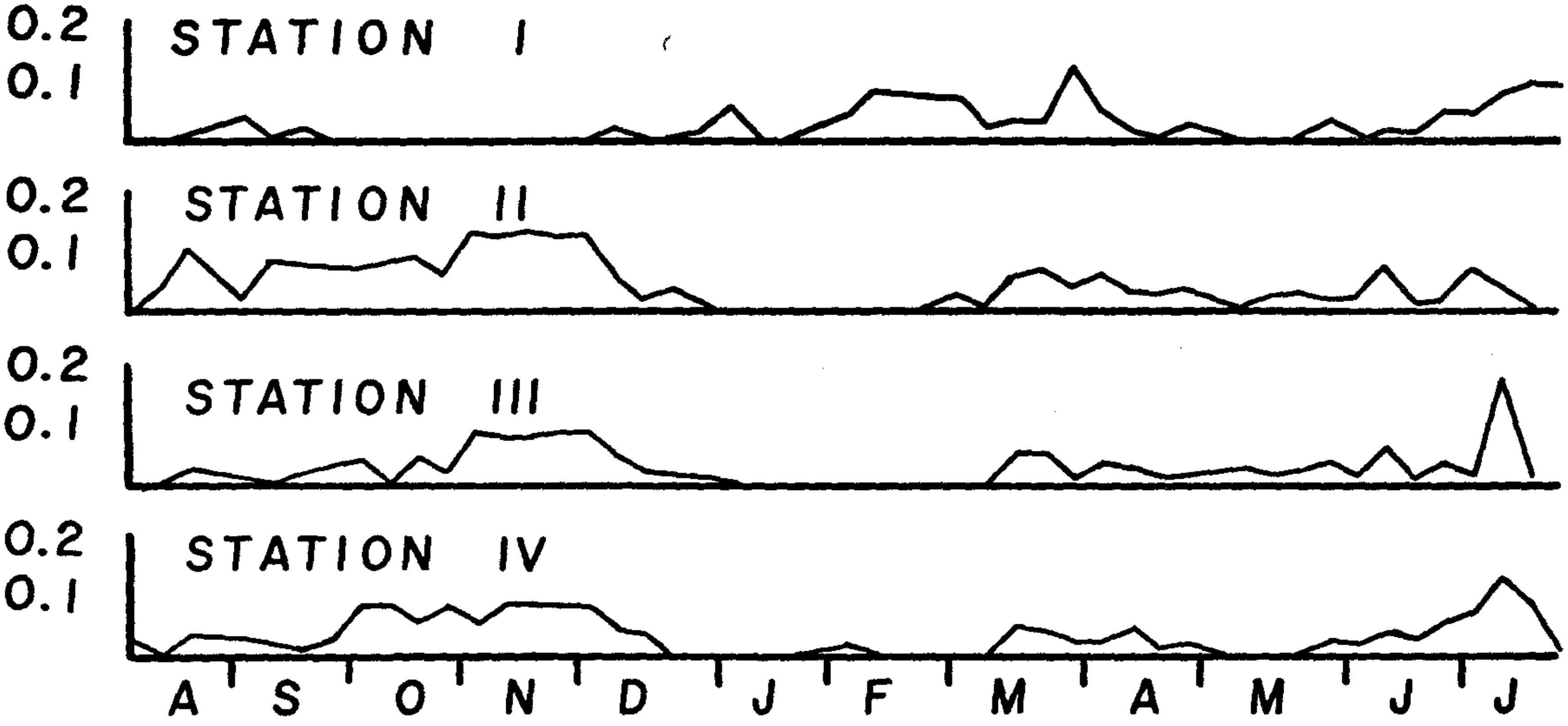


Figure 34. Temporal Variation in Chlorophyll b Fraction (Amount of Chlorophyll b Relative to Total Amount of Chlorophyll) in a Column of Water.

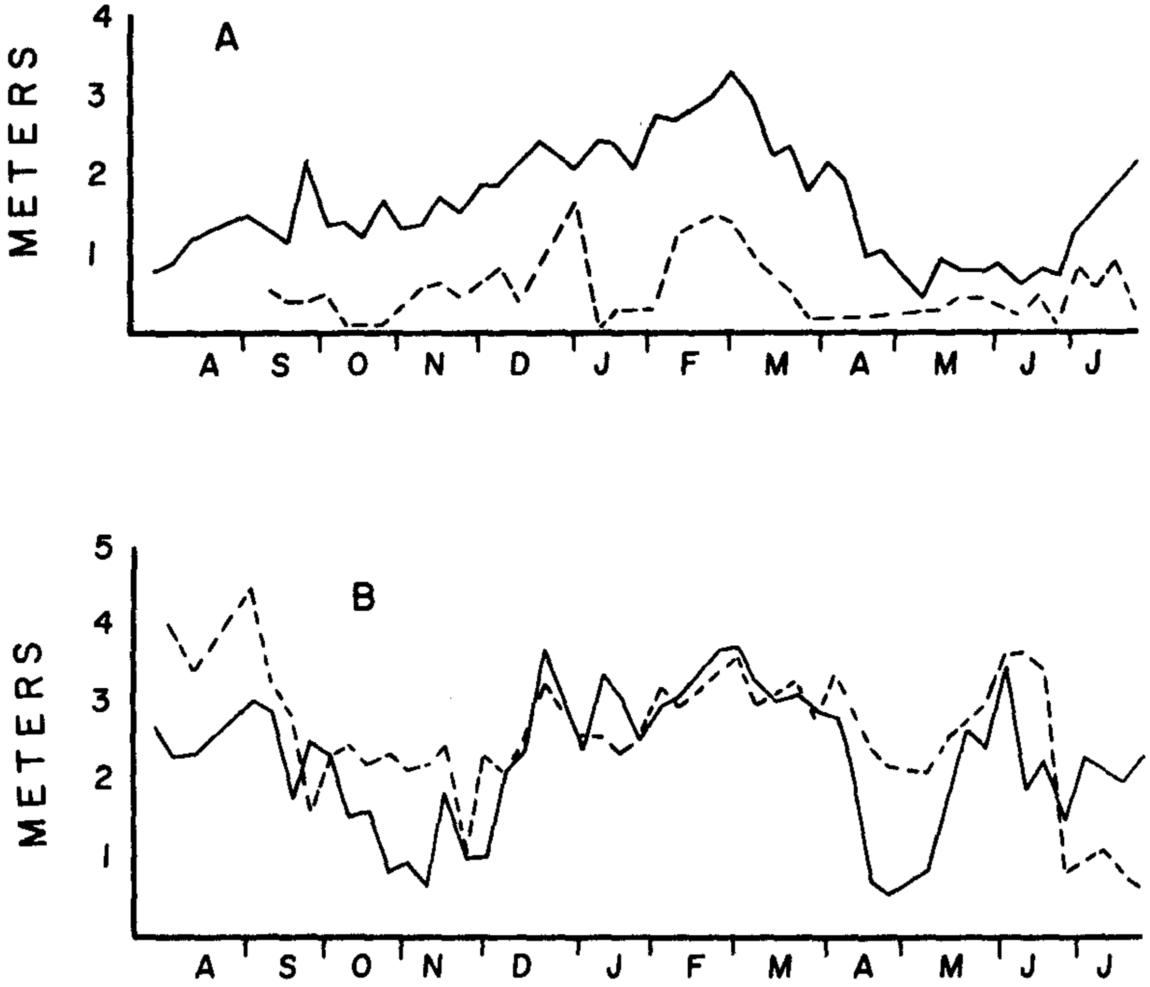


Figure 35. Temporal Variation in Euphotic Zone Depth at A--Station I (- - -) and Station II (———), and at B--Station III (———) and Station IV (- - -).

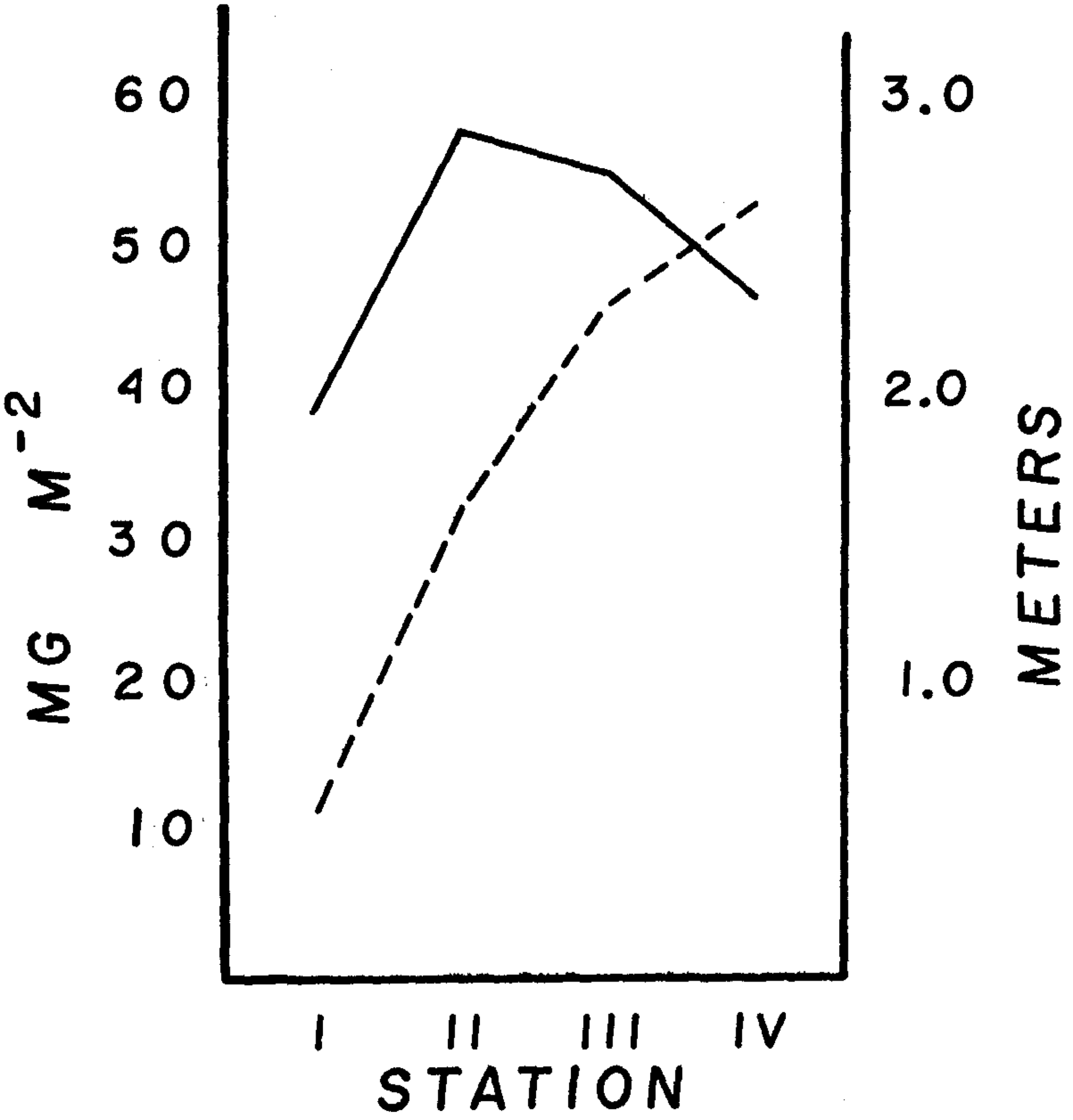


Figure 36. Mean Annual Euphotic Zone Depth in Meters (- - -) and Mean Annual Chlorophyll a Content in mg Per Square Meter of Euphotic Zone (——).

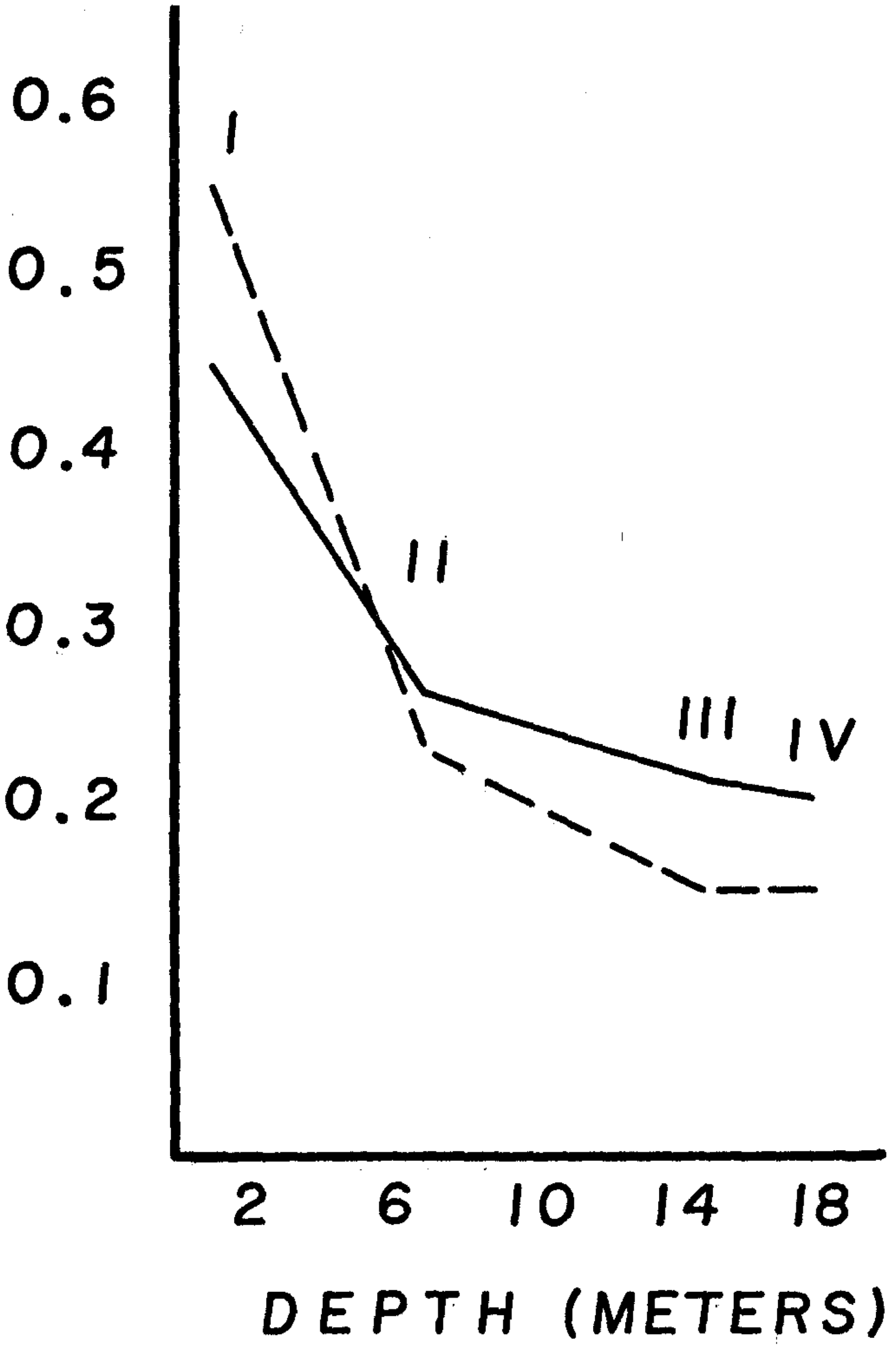


Figure 37. Amount of Chlorophyll a in the Euphotic Zone as a Fraction of the Total Amount of Chlorophyll a in the Water Column (———). Depth of the Euphotic Zone as a Fraction of the Total Depth of the Water Column (- - -).

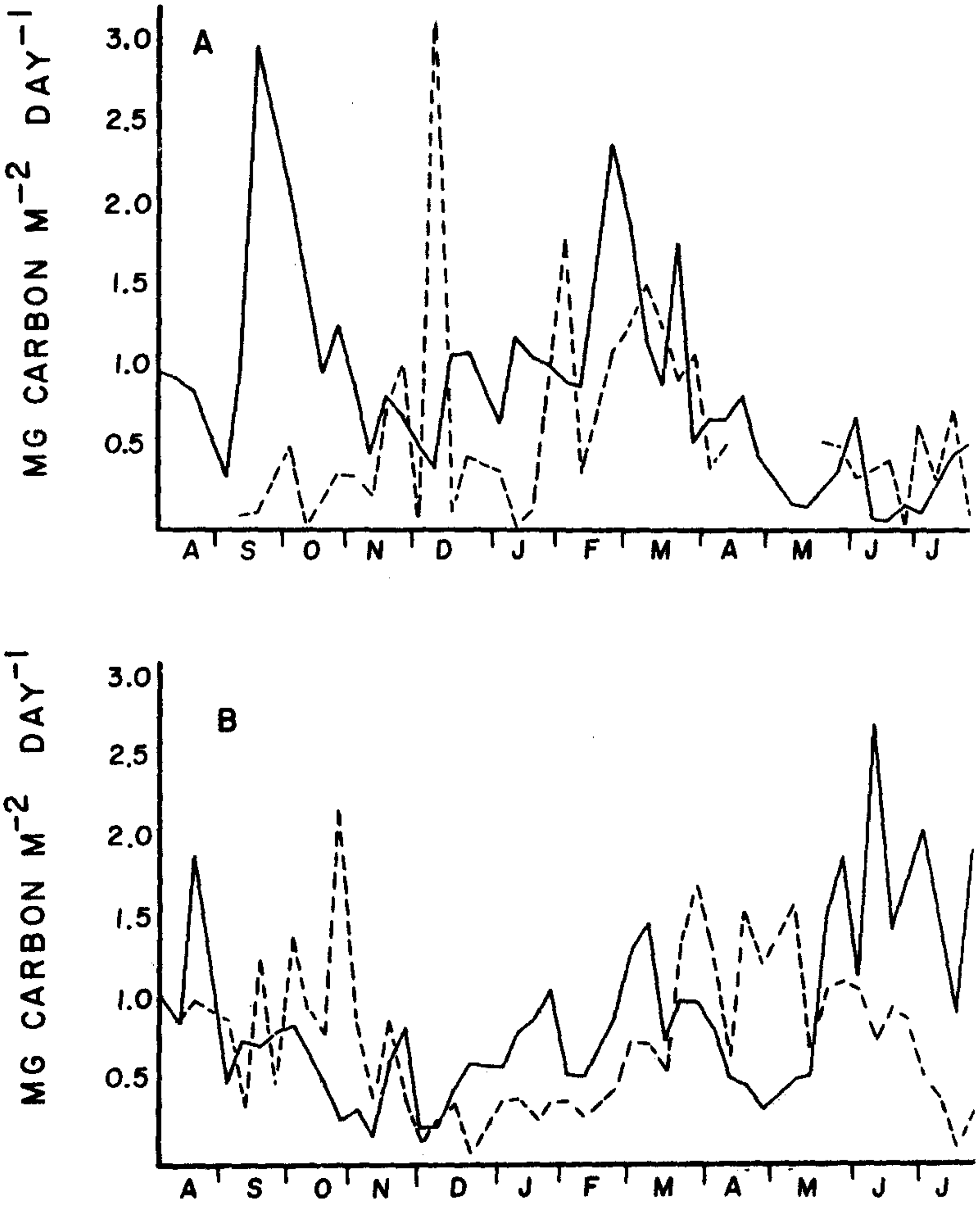


Figure 38. Temporal Variation in Rate of Gross Production as Calculated from Chlorophyll and Light Data. A--Station I (- -- -) and Station II (———). B--Station III (———) and Station IV (- - -).

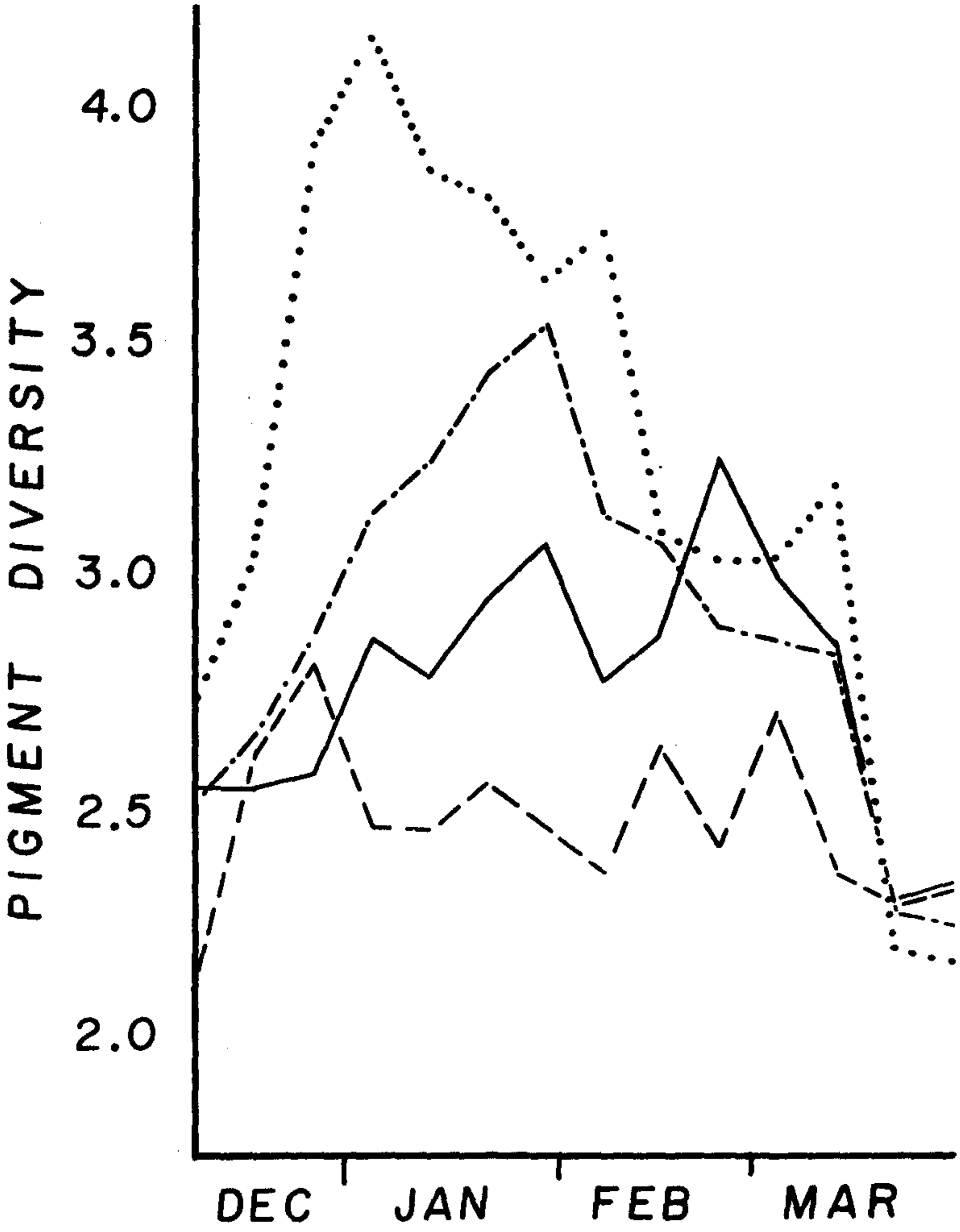


Figure 39. Pigment Diversity (D_{430}/D_{665}) During the Cold Months. Station I (- - -), Station II (· · ·), Station III (-·-·-), Station IV (———).

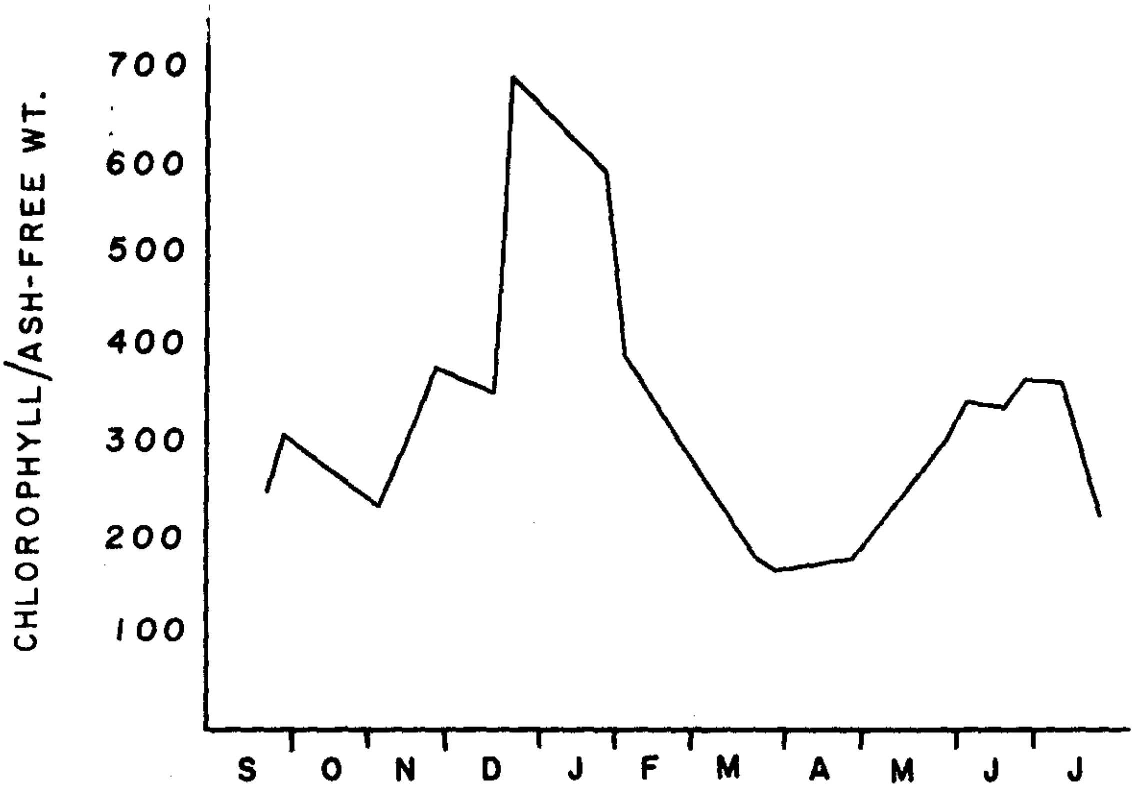


Figure 40. The Ratio of Chlorophyll to Ash-Free Weight at Station IV.

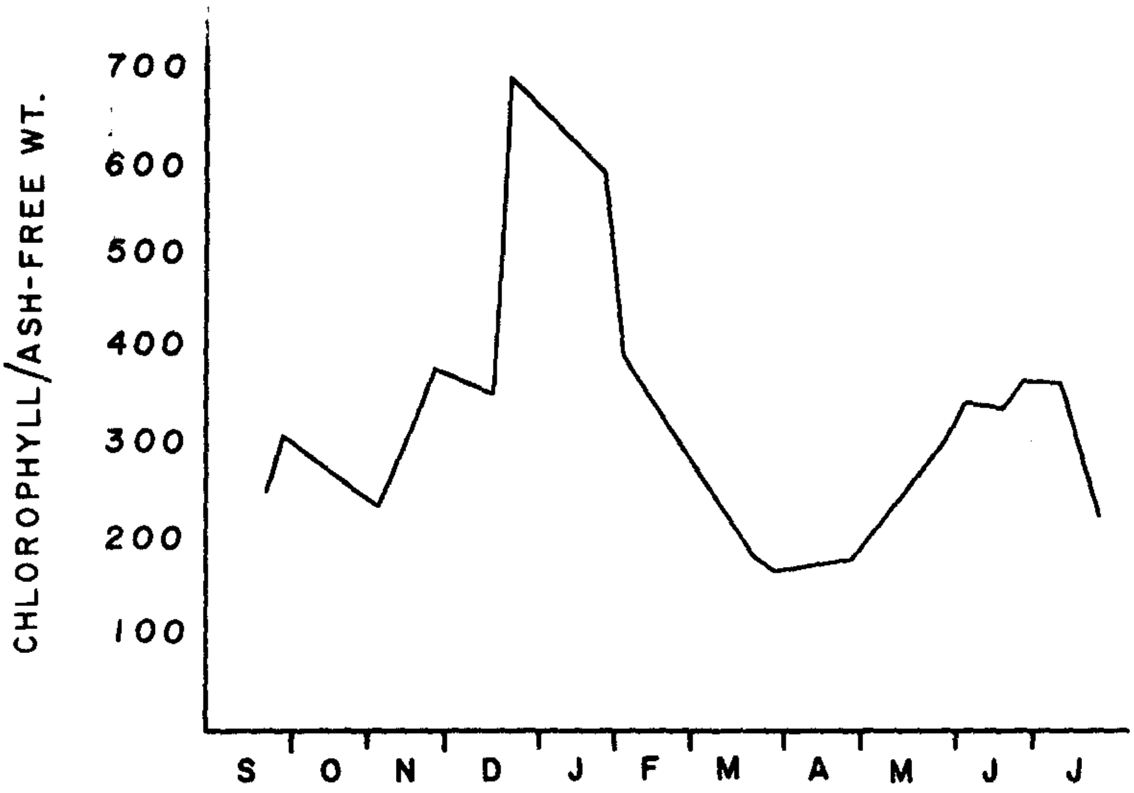


Figure 40. The Ratio of Chlorophyll to Ash-Free Weight at Station IV.

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