

OBSERVATIONS ON THE EFFECTS OF OIL FIELD BRINES  
ON THE FISH AND BENTHIC MACRO-INVERTEBRATE  
POPULATIONS OF A CENTRAL OKLAHOMA  
INTERMITTENT STREAM

By

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## PREFACE

This study contrasts populations of fish and benthic macro-invertebrates in an oil field brine contaminated stream with those in a nearby, uncontaminated stream. The streams were selected on the basis of similar morphometric and hydrologic characteristics.

Sampling frequency was impacted by drought. Significant differences in the community structures of the streams were evident despite the low sample numbers. The study was funded by a grant from The U.S. Fish and Wildlife Service.

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## CHAPTER I

### THE RESEARCH PROBLEM

In recent years, the increasing price of foreign oil has sparked new interest in oil exploration and development within the continental United States. The higher price of crude oil has made it feasible to re-open abandoned oil fields and exploit the smaller, less productive oil pockets that are plentiful in the Midwest. It is estimated that 5 to 10 barrels of salt water are produced for each barrel of crude oil obtained (Hinje, 1970). On this basis, in 1974 the United States was producing 20 to 30 million barrels of brine per day (EPA, 1974).

Past indiscriminant brine disposal practices, most of which are now illegal, have led to severe cases of surface and groundwater pollution. Disposal methods commonly employed included direct surface discharge, controlled release into streams during periods of high flow, and impoundment in unlined evaporation/ seepage pits (EPA, 1974). Surface waters and aquifers in many areas have been rendered unusable due to salt water contamination resulting from these practices. Salt water injection wells have also been major sources of groundwater contamination in cases where well casings have corroded or cracked, or where casings did not extend far enough to sufficiently separate the brines from freshwater aquifers. Once aquifers are contaminated with brines, it may take years, decades, or even millennia for them to return to their original state since natural flushing depends on several

hydrogeologic factors as well as the amount of rain and the extent of contamination (Pettyjohn, 1971).

Impact upon the aquatic ecosystem is dependent on the concentration and chemical constitution of the brines. Mild cases of brine contamination have resulted in increased biomass and species diversity (Mathis and Dorris, 1968). High concentrations of brines have reduced species diversity and abundance and occasionally eliminated all benthic macroinvertebrates and higher organisms.

Oil production is presently increasing in many parts of the state and lobbyists are pressing for relaxation of environmental constraints, it is therefore essential that accurate assessments of the impacts of oil production activities on fish and wildlife populations be attained. While the scope of this project is rather limited, it is hoped that information obtained will prove useful in the assembly of a more comprehensive data base and perhaps in directing future investigators.

## CHAPTER II

### REVIEW OF THE LITERATURE

#### Introduction

Sodium chloride is seldom regarded as a toxic compound, yet it affects water uses and aquatic ecosystems to a much greater extent than more acutely toxic environmental contaminants such as heavy metals and pesticides. While acutely toxic effects of sodium chloride are seldom observed, hypertonic solutions of sodium chloride can induce stress in virtually all freshwater species by the creation of a severe cellular or tissue water imbalance. Effects can range from plasmolysis in algae and some vascular plants, to dehydration, impairment of kidney function, and a breakdown of the blood-brain barrier in higher organisms.

The natural salinity of inland waters varies widely among geographic locations and is influenced by numerous factors, including the geologic and hydrologic conditions. The world average salinity for river water is 198.8 mg/l (Livingstone, 1963). Natural surface water salinities in Oklahoma in 1975 ranged from 22.3 mg/l in the Kiamichi River near Big Cedar to 20,616 mg/l in the Cimarron River near Freedom. Consequently the terms "fresh" and "saline" are rather subjective and their interpretation is often dependent upon water availability. The United States Environmental Protection Agency (EPA) and The United States Public Health Service (USPHS) suggest a maximum concentration of 250 mg/l of chlorides and sulfates for municipal water sources (EPA,

1976; USPHS, 1962).

Robinore, Langford and Brookhart (1958) proposed the following water classification scheme based on total dissolved solids (TDS):

	<u>TDS. (mg/l)</u>
Fresh	1,000
Slightly-saline	1,000-3,000
Moderately-saline	3,000-10,000
Very-saline	10,000-35,000
Briny	35,000

#### Effects of Brines on Water Quality

One of the most common anthropogenic sources of sodium chloride contamination in inland areas is oil field development. Brines associated with crude oil production vary in their chemical constitution but typically contain sodium, chloride, and sulfate in concentrations higher than those found in seawater (Table I).

Improper brine disposal can increase the salinity, conductivity, and concentrations of the major ions in surface and groundwaters dramatically. In the case of some brines, one barrel could cause over seven hundred barrels of fresh water to exceed the USPHS chloride limit (Wright, 1957).

Oil field brines also affect the ratios in which the dominant cations and anions occur. In hard-water areas of the temperate zone, the major ions exist in the following order of predominance (Wetzel, 1975):

Cations: Ca>Mg>Na>K

Anions: CO<sub>3</sub>>SO<sub>4</sub>>Cl

The order of predominance typical in oil-field brines is (EPA, 1974):

Cations: Na>Ca>Mg>K

Anions: Cl>SO<sub>4</sub>>HCO<sub>3</sub>

Brine polluted streams typically contain very high concentrations of sodium and chloride. Calcium and magnesium concentrations are elevated to a lesser extent, while sulfate and bicarbonate concentrations usually are not significantly affected. The high concentrations of positively charged ions (Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>) in brines can cause precipitation of negatively charged colloidal clay particles (Sawyer and McCarty, 1978). Keeton (1959) used this principle to reduce the turbidity of farm ponds. Keeton observed increases in specific conductance, and sodium, calcium, and chloride concentrations in two farm ponds following the addition of oil field brines. Bicarbonate alkalinity, pH, and potassium concentrations were not affected by the brine additions.

In Cardington, Ohio, several producing oil wells were drilled in the vicinity of four municipal water wells in 1964. The brines were disposed of in pits or an excavated ditch within fifty feet of the water wells. By January 1965 chloride concentrations had increased from 10 mg/l to 3,750 mg/l in two of the wells. Eventually the entire well field had to be abandoned. In the same county, chloride concentrations in two intermittent streams increased from less than 10 mg/l to as high as 1,350 mg/l (Pettyjohn, 1971). Pressure injection of brines in Kanawha and Roane counties, West Virginia, increased chloride concentrations in some area wells from less than 100 mg/l to as much as 1,000 mg/l in a few months (Wilmoth, 1970). Mathis and Dorris (1968) attributed variations in conductivity from 231 to 16,000 umhos/cm<sup>2</sup> in Black Bear Creek near Pawnee, Oklahoma to oil field brine disposal.

## Effects of Brines on Community Structure

Community structure effects are dependent upon the concentration and chemical constitution of the brines. Keeton (1959) used oil field brines to reduce turbidity in farm ponds and observed increased production of plankton, some benthic macroinvertebrates, and aquatic plants. Mathis and Dorris (1968) found maximum numbers of taxa and benthic biomass in sections of Black Bear Creek, near Pawnee Oklahoma, containing moderate concentrations of brines (conductivity 2,000 to 4,000  $\mu\text{mhos}/\text{cm}^2$ ).

Higher concentrations of brines can adversely affect aquatic ecosystems. Remane and Schlieper (1971) found that the numbers of species inhabiting brackish water are much smaller than those inhabiting either fresh or marine waters with similar habitat. The minimum number of species occurs at salinities of 5,000 to 7,000 mg/l. Largemouth bass (Micropterus salmoides) and bluegill (Lepomis macrochirus) survive in salinities of 8,000 mg/l and thrive at salinities near 5,000 mg/l but reproduction ceases at 2,500 to 3000 mg/l (Neely, 1969). Channel catfish (Ictalurus punctatus) and blue catfish (Ictalurus furcatus) tolerate salinities of 11,000 mg/l, but similarly, reproduction is unsuccessful above 2,000 mg/l (Perry and Avault, 1968). In 96 hour bioassays, Clemens and Jones (1955) found median toxicity thresholds (MTT) in terms of mg/l chloride in brine solutions varied from 4,883 mg/l for fathead minnows to 12,717 mg/l for plains killifish (Table 2). In seawater bioassays conducted by Renfro (1959), Gambusia affinis was the most tolerant species tested with median toxicity occurring at 15,000 to 24,000 mg/l salinity. The MTT for largemouth bass was 9,000 to 15,000 mg/l salinity.

Thomas (1970) found that areas in Wilson Reservoir, on the Saline River, with conductivity of 5,760 ohms/cm<sup>2</sup> (chloride and sulfate concentrations of 3,840 and 990 mg/l respectively) supported no benthic organisms. In contrast, eight taxa with a mean density of 28 individuals/ft<sup>2</sup> were taken in areas where the conductivity had decreased to 1,700 ohms/cm<sup>2</sup>. Rawson and Moore (1944) found only one species in Little Manitov Lake, Saskatchewan, where the total dissolved solids (TDS) concentration was 120,000 mg/l. Walker (1973) saw similar results in Lake Werowrap, Victoria, where salinity ranged from 23,000 to 56,000 mg/l. In a study of Black Bear Creek, a tributary of the Arkansas River, Mathis and Dorris (1968) found that specific conductance ranged from 231 to 16,000 umhos/cm<sup>2</sup>. They observed 55 species of benthic invertebrates where brine concentrations were relatively low and only 31 species immediately below a brine outfall. Clemens and Finnell (1955) found the following correlation between chloride concentration and mean number of aquatic taxa.

<u>Chlorides(mg/l)</u>	<u>Mean</u>	<u>Numberof Taxa</u>
16,000 - 20,000		4.0
11,000 - 16,000		4.8
8,000 - 11,000		6.5
4,000 - 8,000		8.5
1,000 - 4,000		9.3
100 - 1,000		13.0

## CHAPTER III

### THE STUDY AREA

#### INTRODUCTION

Two intermittent streams, Wewoka Creek and Snake Creek, tributaries of the North Canadian River in Seminole County, Oklahoma, were chosen for this study. Five sampling sites were established on each of the streams (figure 1). The study area is centered over the Bowlegs formation, a layer of oil producing sandstone that fueled a major oil boom in the 1920's and 30's and is still one of the largest zones of production in Oklahoma today. In this area, brine disposal methods previously included evaporation and direct discharge into nearby streams. Wewoka Creek, near Seminole, Oklahoma is one stream where direct discharge previously occurred and area residents allege may still occur during periods of high flow. This contention was not verified during the study, but at least one large crude oil spill occurred, resulting in the water being covered with a layer of oil up to 5 mm thick.

#### Wewoka Creek

The section of Wewoka Creek studied extends from the fourth section road east of Hwy. 99 to the first section road east of Hwy. 99, near Seminole, Oklahoma. Wewoka Creek is a low gradient, sand bottomed stream, draining an area that is predominantly pasture with sparse isolated stands of post oak and black jack oak. The drainage basin



contains several active wells and storage tanks, and drilling activities are presently increasing. Site 1 contains two relatively large pools, with maximum depths of about 1.5 m. The two pools are connected by a short riffle area. This site is the only one in the study area with substantial quantities of gravel and rubble substrate. Site 2 is a pool with a maximum depth of about 0.75m and a substrate of sandstone and sand. Sites 3, 4 and 5 contain shallow pools, of variable depth which appeared as the result of shifting sand substrate. On April 2, 1980, an extensive crude oil slick was observed near site 3.

#### Snake Creek

The section of Snake Creek studied extends from the first section road south of Hwy. 99A to the first section road south of interstate Hwy. 40. The drainage basin of Snake Creek contains some pasture and a few small agricultural areas but is characterized primarily by a sparse post oak and black jack oak forest. Sites 1 and 2 have substrate similar to sites 3, 4, and 5 on Wewoka Creek and the substrate of site 3 is similar to that of sites 1 and 2 in Wewoka Creek. However, site 3 on Snake Creek contains virtually no pools. The confluence of Gar Creek with Snake Creek is above site 4 and flow rates at sites 4 and 5 were generally higher than at sites 1, 2, and 3. However, the water quality parameters were similar to those of upstream sites during periods of normal flow. Site 4 on Snake Creek contained more gravel and rubble than site 5 and was similar in substrate to site 1 of Wewoka Creek.

Site 5 on Snake Creek was different from other sites in that it carried a higher silt load and contained several large pools. This site contained the largest volume of water but the flow rate was generally very low (approximately 0.1 cfs).

## CHAPTER IV

### METHODS AND MATERIALS

#### Introduction

In order to determine the extent to which brine contamination had altered the water chemistry of Wewoka Creek, it was necessary to determine what range of values for certain parameters might have been expected in an unpolluted condition. This was accomplished by comparing conductivity, chloride, fluoride, hardness, sodium, potassium, magnesium, calcium, and iron data obtained from Wewoka Creek, with those for Snake Creek, a relatively uncontaminated stream with similar substrate and flow characteristics.

Benthic organisms were selected as in situ monitors because they are particularly vulnerable to environmental contaminants. Benthos are relatively immobile and long lived, thus populations reflect conditions of the recent past as well as the present. This attribute is particularly useful in assessing the effects of sporadic or intermittent discharges such as those often resulting from oil production activities. While fish are often less useful as indicators of water quality because of their higher mobility, they are undoubtedly of greater public concern. The section of Wewoka Creek studied has probably never provided much sport fishing, but some area residents maintain that the lower reaches of the creek sustained good populations of channel

catfish in the past. Catches have decreased in recent years.

#### Water Quality Parameters

Water samples were collected from March 1980 to August 1980 and temperature, conductivity and dissolved oxygen readings were measured in situ with a Yellow Springs Instrument (YSI) Model 33 Specific Conductance Meter and YSI Model 51B Dissolved Oxygen Meter. Chloride and fluoride ion concentrations were determined in the lab with an Orion expanded mV scale pH meter and ion selective electrodes. Hardness (expressed as mg/l CaCO<sub>3</sub>) estimates were obtained by titration with disodium ethylenediamine tetraacetate (Na<sub>2</sub>EDTA). Samples were preserved for heavy metal analyses by acidification to pH 2.0 or below with 3 ml of concentrated nitric acid (HNO<sub>3</sub>). The samples were evaporated to approximately 3 ml and then reflux digested with concentrated HNO<sub>3</sub> to oxidize organic compounds (EPA, 1976). The prepared samples were then analyzed with a Perkin Elmer Model 5000 Atomic Absorption Spectrophotometer. The concentrations of sodium, calcium, potassium, magnesium and iron were determined by flame atomic absorption. Water samples were also collected following an oil spill and extracted with methylene chloride (CH<sub>2</sub>Cl<sub>2</sub>) for hydrocarbon analysis using a Hewlett Packard Model 5992B Combination Gas Chromatograph/Mass Spectrophotometer.

#### Benthic Macroinvertebrates

On March 7, 1980, five multiple-plate artificial substrate type sampling devices were placed at each of the 10 study sites. However, high water washed most of these samplers away and artificial substrate

samplers were replaced by circular depletion samples collected from natural substrate (Carle, 1976). Three substations were sampled at each of the 10 sites and three equal units of effort were expended at each substation.

### Fish

Electroshocking proved to be ineffective in Wewoka Creek due to the high specific conductance of the water, therefore a 20-foot minnow seine was used to make fish collections. The same seine was used in Snake Creek to avoid sampling bias. At each of the sites a 100-foot section of stream was blocked off and each area was sampled with an approximately equal amount of effort.

### Statistical Methods

Benthic macroinvertebrate population estimates and species diversity ( $\bar{d}$ ) were calculated on The Oklahoma State University's IBM Model 370 digital computer (see Appendix C for program). Species diversity ( $d$ ) was calculated by the formula:

$$\bar{d} = - \sum_{i=1}^k \frac{n_i}{n} \log_2 \frac{n_i}{n}$$

Where  $n$  is the total number of individuals in the sample,  $n_i$  is the number of individuals per taxa, and  $k$  is the number of taxa per sample. Statistical comparisons of  $\bar{d}$  values for stations or substations were made using the Statistical Analysis Systems, Inc. (SAS) computer programs (Barr et al. 1976). Analyses of variance were performed using the General Linear Models procedure. When an analysis of variance indicated significant difference at the 95% confidence level, Duncan's new multiple range test was performed to identify the source(s) of

variation.

The percentage community similarity index (Psc) was calculated by the formula:

$$Psc=100-0.5k \ a-b$$

Where a and b are for a given species, percentages of the total samples A and B which that species represents. The absolute value of their difference is summed over all species, k.

## CHAPTER V

### RESULTS

#### Water Quality Parameters

The estimated flow rate of both streams varied considerably during the study period with a maximum of 10-15 cfs observed on May 20 and complete cessation of flow occurring sometime between July 2 and July 29. Due to extended drought and unprecedented high temperatures, flow never resumed. However, stagnant pools remained at sites 1 and 2 of Wewoka Creek and sites 4 and 5 of Snake Creek throughout the remainder of the study period.

Increases in the total iron (Fe) content of both streams coincided with the occurrence of heavy rainfall and elevated flow rates. Hardness, conductivity, and Na, K, Mg, Ca, and Cl concentrations fluctuated inversely with the flow rate. Patterns of fluctuation were very similar for most parameters in the two streams, although actual concentrations varied as much as a hundredfold. (Figures 1 and 2)

Organic compounds identified by combination gas chromatography/mass spectrometry included naphthalene (Figure 7), dibutyl phthalate (Figure 8), aliphatic hydrocarbons (Figure 9), and bis (2-ethylhexyl) phthalate (Figure 10).

#### Benthic Macroinvertebrates

Benthic macroinvertebrate population structures differed greatly in

the two streams. The pooled species diversity index ( $\bar{d}$ ) ranged from 0.14 to 1.29 in Wewoka Creek and 2.23 to 3.12 in Snake Creek (Tables 4 and 5). The grand mean  $\bar{d}$  was significantly lower in Wewoka Creek than in Snake Creek ( $P > .001$ ). Duncan's new multiple range test (Figure 12) indicated that no significant difference existed between mean  $\bar{d}$  values for stations within each stream ( $P > .05$ ). Mean  $\bar{d}$  for station 1 of Snake Creek was not significantly different from those for stations 1, 2, 4, and 5 of Wewoka Creek ( $P > .05$ ). Mean  $\bar{d}$  values for all other stations on Snake Creek were significantly different from those for stations on Wewoka Creek ( $P > .05$ ). The contrast is particularly striking when the representatives of the family Chironomidae are considered separately. Wewoka Creek was dominated by one species of Cricotopus, which for the purpose of this study was labeled species "C" (Table 5). Three other taxa collected were represented by only one individual each. Snake Creek, however, was represented by 17 species and not dominated by any particular one (Table 4). Although two species of Cricotopus were present, species "C" was completely absent from this stream. While further study would be necessary, these findings could warrant consideration of Cricotopus sp. "C" as an indicator species. Percentage community similarity (Psc) values were higher for all pairs of stations within streams than for pairs of stations between streams. Several stations on Wewoka Creek had no taxa in common with stations on Snake Creek, resulting in Psc values of 0.00 (table 15).



## Fish

The effect of brines on fish populations in Wewoka Creek was equally significant. As the water level dropped during the summer, fish became entrapped in pools at sites 1 and 2 where they were subjected to continuously increasing brine concentrations. On June 9, eight species were collected with Gambusia affinis being the most abundant (Table 12). Dead and moribund sunfish were observed from June 25 to August 5, when only the prolific mosquito fish (Gambusia affinis) remained at site 2 (Table 14). No fish were found at site 1. In contrast, Snake Creek still contained 7 of the 11 species originally present at sites 4 and 5, plus one species (Dorosoma cepedianum) not previously collected. The number of individuals, however, was considerably reduced (Tables 13 and 14). The mean  $\bar{d}$  was significantly lower in Wewoka Creek than in Snake Creek ( $P > .0001$ ) on both sampling occasions. Percentage community similarity (Psc) values were higher for all pairs of stations within streams than for pairs of stations between streams (Table 16). Several stations on Wewoka Creek had no taxa in common with stations in Snake Creek, resulting in Psc values of 0.00.

## CHAPTER VI

### DISCUSSION

#### Water Quality

Concentrations of sodium, calcium, magnesium, potassium, and chloride ions in Wewoka Creek are indicative of oilfield contamination. High concentrations of these ions persisted throughout the study period and fluctuated inversely with observed rainfall and flow rate. This relationship suggests that the probable source of the ions was contaminated groundwater. Concentrations of the ions were not the same at all sites but were consistently higher at sites 1 and 2, than at the farther downstream sites (Tables 3, 4, 5, 7, 8). The differential concentrations may indicate that sites 1 and 2 were in closer proximity to a contaminated area than were sites farther downstream. Pettyjohn (1971) reported similar results of groundwater contamination in Morrow Co., Ohio. In those cases chloride concentrations in area streams also reached maxima in contaminated areas and then slowly decreased downstream, due to dilution.

The similarity of the fluctuation patterns of the major ions in Snake Creek and Wewoka Creek (Figure 3 and 4) indicates that both streams were being contaminated with brines, but Snake Creek to a much lesser extent than Wewoka Creek. It is possible that both streams were affected by the same contamination source, but verification would

require drilling a series of observation wells between the two stream basins.

Contrary to expectations, mean concentrations of dissolved minerals decreased in both streams in August in spite of lack of measurable precipitation. One would expect that during August evaporative water loss would have increased mineral concentrations. Hulman (1969) observed fluctuations in chloride concentrations in groundwater in a contaminated area and attributed the changes to salt crystals formed within the zone of aeration in the soil following the previous infiltration of highly concentrated brines. These crystals were solubilized by infiltrated rainfall following storm events.

A similar situation could exist in the study area. Indiscriminant brine disposal could have resulted in the formation of salt crystals in the area soils. During periods of rainfall, these crystals would be solubilized, but the effect would not be immediately observed in streams due to dilution from runoff. As the flowrate decreased and the percentage contribution of groundwater increased, the dissolved mineral concentrations would rise, as was observed from late May to early June. When the water table receded below the zone of aeration, the dissolved mineral concentrations would decrease. Flushing by uncontaminated groundwater would then result in decreased concentrations in surface waters as was observed in August.

In addition to groundwater contamination, subsurface injection of brines could also be responsible for high ion levels. On at least one occasion, brines erupted at the surface near an abandoned well in a small wheat field approximately 10 miles south of the Wewoka Creek study area. While it was beyond the scope of this study to determine the

extent to which subsurface injection has influenced the area water quality, it must be considered a possible source of contamination. The presence of naphthalene in the water sample collected at the site of the crude oil spill on April 2 indicated that the spill probably occurred after March 30. Aliphatic hydrocarbons are associated with crude oil and most petroleum derivatives, but are not as toxic as the more volatile naphthalene. The phthalates are common environmental contaminants, although bis (2-ethylhexyl) phthalate is included in the Environmental Protection Agency's list of priority pollutants (Federal Register, 1980).

#### Benthic Macroinvertebrates

The benthic macroinvertebrate community structure indicated substantial differences in the water quality of the two streams. The low Psc values for station comparisons between streams were indicative of the observed differences in community structures (Table 15). The low species diversity ( $\bar{d}$ ) and numbers of taxa observed in Wewoka Creek indicate highly stressful conditions. In addition, some of the taxa from Wewoka Creek, notably the Ephydriidae (salt flies), Palpomyia sp., and Cricotopus sp. C. are adapted to high salinity environments. The salt flies (Ephydra spp.) are characteristic of very saline waters (Claassen, 1926; Thorpe, 1931). Roback (1974) found Palpomyia spp in areas with chloride and magnesium concentrations above 2,000 mg/l and 150 mg/l, respectively, but not in areas with chloride concentrations below 1,000 mg/l and total hardness concentrations above 300 mg/l. Cricotopus sp. "C" was the dominant taxon in Wewoka Creek but completely absent in Snake Creek. The presence of these species would seem to further indicate the presence of a persistent source of contamination.

## Fish

Fish populations on June 9 were represented by a larger number of individuals but fewer species in Wewoka Creek than in Snake Creek. Discretion must be used in making this comparison, however, because a large percentage of the populations of both streams was probably composed of immigrants, resulting from the recent flood. The low species diversity ( $\bar{d}$ ) and small number of taxa encountered at sites 1 and 3 of Snake Creek, and 3 and 5 of Wewoka Creek could have resulted from lack of suitable habitat (see site descriptions) and low flow rates.

No such limitations of the data were present on August 5 when it was obvious that deteriorating water quality had eliminated all species at site 1 and all but one species (Gambusia affinis) at site 2 in Wewoka Creek. Reproduction in that species was unhampered by the high brine concentrations. Clemens and Jones (1955) conducted a series of tolerance tests of selected species of fish to varying oil field brine concentrations (Table 2) and found Gambusia affinis to be the second most tolerant species tested. Lepomis cyanellus and L. macrochirus, also fairly tolerant species, were among those most frequently encountered in Wewoka Creek on June 9. In addition, it should be noted that the chloride MTT (analogous to LC 50) values reported for Gambusia affinis by Clemens and Jones (1955) and Renfro (1959) is considerably lower than the chloride concentrations observed when the fish were found in Wewoka Creek. The discrepancy may have resulted from insufficient acclimation periods in the studies of Clemens and Jones (1955) and Renfro (1959), or to brine-resistant local populations.

Wewoka Creek currently does not provide any recreational fishing

because of brine contamination. However, nearby Snake Creek, of similar size, provides much fishing for area residents and on several occasions land owners have expressed concern over the possible threat of contamination. Wewoka Creek is considered a dead creek by area residents and little consideration or public concern exists over its condition. If Wewoka Creek were an isolated occurrence in the oil fields one might understand this disregard for stream losses. However, the extent of similar conditions is unknown in Oklahoma and we know of no effort to obtain this information.

## CHAPTER VII

## SUMMARY AND CONCLUSIONS

1. This study was undertaken to assess the impacts of oil field brines on the community structure of fish and benthic macroinvertebrate populations. Wewoka Creek and Snake Creek, intermittent tributaries of the North Canadian River, were chosen for study. Water samples, fish, and benthic macroinvertebrates were collected from 5 sites on each stream.

2. Wewoka Creek was contaminated with oil field brines. Concentrations of the major ions contributing to salinity were often 100 times greater in Wewoka Creek than in relatively uncontaminated Snake Creek. Brine concentrations fluctuated inversely with the estimated flow rate. The probable source of the brines was contaminated groundwater.

3. The species diversity ( $\bar{d}$ ) of fish and benthic macroinvertebrate populations was significantly lower ( $P > .001$ ) in Wewoka Creek than in Snake Creek.

4. The low percentage community similarity indices for station comparisons between Snake Creek and Wewoka Creek indicate substantial differences in the benthic community structures of the streams.

tolerant species (Tables 2, 12, 14). Snake Creek contained relatively brine sensitive species in addition to brine tolerant species (Tables 2, 13, 14).



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APPENDIXES

APPENDIX A

FAUNAL LISTS AND WATER QUALITY DATA

TABLE I  
COMPARISON OF SEAWATER AND OILFIELD BRINE \*

	Seawater (mg/l)	Oilfield Brine (mg/l)
sodium	10,600	12,000 - 150,000
potassium	400	30 - 4,000
calcium	400	1,000 - 120,000
magnesium	1,300	500 - 25,000
chloride	19,000	20,000 - 250,000
bromide	65	50 - 5,000
iodide	0.5	1 - 300
bicarbonate	?	0 - 3,600
sulfate	2,700	0 - 3,600

<sup>a</sup> from EPA-430/9-73-013.

TABLE II  
 COMPARISONS OF MEDIAN TOXICITY THRESHOLDS (MTT) FOR  
 FISH IN OIL FIELD BRINE AND SODIUM CHLORIDE

Fish	Brine Wastes			Sodium chloride (ppm NaCl)
	MTT (percentage)	MTT (ppm Cl)	MTT (ppm hypothe- tical NaCl)	
Plains killfish	11.2	12,717	18,783	16,000
Gambusia	7.4	8,592	12,690	10,670
White Crappie	6.1	7,068	10,439	---
Bluegill	5.5	6,330	9,349	---
Green Sunfish	5.5	6,330	9,349	---
Channel Catfish	5.4	6,308	9,317	---
Red Shiner	5.1	5,960	8,803	9,513
Black Bullhead	5.0	5,802	8,569	7,994
Largemouth Bass	4.6	5,380	7,346	---
Fathead Minnow	4.3	4,883	7,212	8,718

TABLE III  
PHYSICOCHEMICAL PARAMETERS OF WEWOKA CREEK

Date	Station	Temp C°	Parameter				
			Cond. umhos/cm	D.O. ppm	Cl <sup>-</sup> ppm	F <sup>-</sup> ppm	Hardness (mg/l CaCO <sub>3</sub> )
3/19/80	1	19	41,500	12.7	11,200	0.120	3,260
	2	19	38,000	14.5	11,800	0.110	3,400
	3	21.5	16,000	13.0	9,200	0.141	2,460
	4	19	16,000	13.0	8,400	0.141	---
	5	17	17,500	14.0	8,700	0.120	1,620
4/02/80	1	17	13,000	--	5,900	0.177	1,860
	2	17	13,000	--	6,000	0.172	1,880
	3	--	---	--	4,100	0.203	1,440
	4	--	---	--	---	---	---
	5	19	10,000	--	4,700	0.187	1,620
5/20/80	1	19	6,000	13.0	2,200	0.240	940
	2	18	5,500	13.6	2,200	0.240	940
	3	19	2,600	12.8	890	0.296	580
	4	19	2,500	13.1	890	0.286	660
	5	20	2,500	13.2	910	0.296	540
6/25/80	1	29	15,500	--	6,200	0.260	2,620
	2	30	20,000	--	6,500	0.255	2,680
	3	34	14,000	--	3,750	0.285	1,660
	4	34	13,500	--	3,400	0.285	1,580
	5	34	7,500	--	---	0.260	1,020



TABLE III (continued)

Date	Station	Temp C°	Parameter				
			Cond. umhos/cm	D.O. ppm	Cl <sup>-</sup> ppm	F <sup>-</sup> ppm	Hardness (mg/l CaCO <sub>3</sub> )
7/02/80	1	36	31,500	--	10,000	---	3,360
	2	39	29,000	--	10,000	---	3,520
	3	24	24,000	--	9,900	---	3,220
	4	24	24,500	--	10,050	---	3,260
	5	22	24,000	--	12,000	---	3,240
7/29/80	1	31	40,000	--	16,400	---	5,500
	2	30	31,000	--	14,400	---	4,500
8/05/80	1	32	42,000	7.9	49,000	---	---
	2	29	30,500	8.1	31,000	---	---
8/18/80	1	28.5	45,000	--	50,500	---	---
	2	28.5	20,000	--	19,500	---	---

TABLE IV  
PHYSICOCHEMICAL PARAMETERS OF SNAKE CREEK

Date	Station	Temp C°	Parameter				
			Cond. umhos/cm	D.O. ppm	Cl <sup>-</sup> ppm	F <sup>-</sup> ppm	Hardness (mg/1 CaCO <sub>3</sub> )
3/19/80	1	13	750	11.0	165	0.203	460
	2	11	770	13.0	220	0.193	1,100
	3	14	700	10.0	150	0.203	480
	4	9	320	10.4	72	0.136	340
	5	10	390	11.2	105	0.120	320
4/02/80	1	18	650	--	110	0.208	360
	2	16	750	--	185	0.198	280
	3	16.5	650	--	150	0.198	200
	4	16	385	--	85	0.146	260
	5	16	420	--	98	0.136	220
5/20/80	1	15	420	9.9	43	0.198	420
	2	15.5	450	14.0	79	0.167	450
	3	15.5	415	10.2	62	0.172	415
	4	17	230	11.0	35	0.120	250
	5	17	255	11.6	39	0.136	255
6/25/80	1	34	1,450	--	320	---	600
	2	31	1,150	--	215	---	360
	3	32	850	--	130	---	440
	4	32	850	--	105	---	460
	5	34	1,100	--	190	---	440

TABLE IV (continued)

Date	Station	Temp C°	Cond. umhos/cm	Parameter		F <sup>-</sup> ppm	Hardness (mg/l CaCO <sub>3</sub> )
				D.O. ppm	Cl <sup>-</sup> ppm		
7/02/80	1	23	2,900	--	820	---	---
	2	24.5	1,350	--	260	---	---
	3	--	---	--	---	---	---
	4	28	700	--	85	---	---
	5	30	950	--	180	---	---
7/29/80	4	26	440	--	360	---	---
	5	30	900	--	360	---	---
8/05/80	4	26	200	8.3	53	---	---
	5	30	352	7.8	190	---	---
8/18/80	4	30	600	--	46	---	---
	5	30	850	--	195	---	---

TABLE V  
TOTAL SODIUM (Na) CONTENT (mg/l)

Date	Wewoka Creek					Station	Snake Creek				
	1	2	3	4	5		1	2	3	4	5
03/19/80	4,831	4,884	3,306	3,296	3,467		56.8	64.1	52.0	27.7	40.1
04/02/80	2,631	2,660	1,585	--	1,726		42.8	57.7	47.7	31.3	47.7
05/20/80	985	940	383	403	39		21.3	30.5	24.0	15.1	20.5
06/25/80	3,492	3,522	1,958	1,864	917		15.8	72.7	49.4	40.8	70.3
07/02/80	4,487*										
07/02/80	4,647	4,502	3,931	4,432	5,104		270.0	93.9	--	40.2	75.2
07/29/80	8,912	8,122	--	--	--		--	--	--	21.0	60.1
08/05/80	8,877	4,899	--	--	--		--	--	--	19.4	51.7
08/18/80	8,257	2,764	--	--	--		--	--	--	20.1	48.0

\*Sample taken from the bottom of a pool approximately 1.5m in depth. All other samples were collected 0.1m below surface of water.

TABLE VI  
TOTAL POTASSIUM (K) CONTENT (mg/l)

Date	Wewoka Creek					Station	Snake Creek				
	1	2	3	4	5		1	2	3	4	5
03/19/80	29.56	28.51	27.47	26.96	27.10		2.69	2.49	2.83	3.29	4.33
04/02/80	22.42	22.97	17.51	--	18.72		2.50	3.02	2.75	3.30	3.25
05/20/80	13.20	13.59	9.07	8.77	2.26		4.09	4.07	4.17	4.03	4.16
06/25/80	24.44	24.83	20.95	20.65	13.76		6.41	3.73	3.79	4.05	4.93
07/02/80	26.17	26.82	28.86	28.58	30.01		7.15	10.70	--	4.89	5.31
07/29/80	28.37	27.24	--	--	--		--	--	--	2.23	5.72
08/05/80	27.75	27.34	--	--	--		--	--	--	2.29	6.52
08/18/80	27.67	22.52	--	--	--		--	--	--	3.22	6.59

TABLE VII  
TOTAL CALCIUM (Ca) CONTENT (mg/l)

Date	Wewoka Creek					Station	Snake Creek				
	1	2	3	4	5		1	2	3	4	5
03/19/80	909	976	608	615	642		70.62	84.58	79.19	37.38	41.06
04/02/80	507	488	329	--	385		64.60	76.96	72.06	41.17	40.10
05/20/80	253	243	95	102	61		23.29	74.44	42.25	24.87	22.49
06/25/80	630	645	396	379	210		96.76	80.00	66.53	55.24	67.07
07/02/80	917*										
07/02/80	809	828	752	765	1,024		153.76	89.04	--	51.50	72.06
07/29/80	1,311	1,288	--	--	--		--	--	--	38.94	58.82
08/05/80	1,392	972	--	--	--		--	--	--	31.18	59.73
08/18/80	1,620	591	--	--	--		--	--	--	37.68	--

\*Sample taken from the bottom of a pool approximately 1.5m in depth. All other samples were collected 0.1m below surface of water.

TABLE VIII  
TOTAL MAGNESIUM (Mg) CONTENT (mg/l)

Date	Wewoka Creek					Station	Snake Creek				
	1	2	3	4	5		1	2	3	4	5
03/19/80	257.9	268.7	195.7	202.7	211.6		24.68	28.61	27.12	15.92	16.70
04/02/80	152.3	152.2	110.0	--	131.6		22.21	25.93	24.31	17.28	16.83
05/20/80	72.3	71.4	33.1	36.1	--		11.61	19.07	17.44	12.71	12.03
06/25/80	--	--	131.1	127.7	70.3		26.64	25.18	22.40	19.53	21.98
07/02/80	241.3	298.6	254.0	224.2	226.7		45.51	31.75	--	20.98	22.25
07/29/80	425.0	455.6	--	--	--		--	--	--	16.06	20.52
08/05/80	481.0	335.7	--	--	--		--	--	--	15.79	19.40
08/18/80	513.0	215.5	--	--	--		--	--	--	16.71	--

TABLE IX  
TOTAL IRON (Fe) CONTENT (mg/l)

Date	Wewoka Creek					Station	Snake Creek				
	1	2	3	4	5		1	2	3	4	5
03/19/80	0.14	0.34	0.10	0.10	0.23		0.61	0.51	0.44	1.96	8.10
04/02/80	0.10	0.06	0.14	--	0.34		0.55	0.35	0.19	1.16	1.18
05/20/80	1.11	1.36	2.36	2.32	2.53		2.73	2.78	2.27	2.52	2.77
06/25/80	2.61	0.26	0.21	0.21	0.30		0.77	--	0.16	0.26	0.36
07/02/80	0.24*										
07/02/80	1.68	2.02	0.94	0.64	0.24		1.85	2.72	--	0.08	0.49
07/29/80	0.45	0.32	--	--	--		0.58	1.05	--	--	--
08/05/80	0.38	3.91	--	--	--		1.46	1.15	--	--	--
08/18/80	0.31	0.43	--	--	--		0.02	1.06	--	--	--

\*Sample taken from the bottom of a pool approximately 1.5m in depth. All other samples collected 0.1m below surface of water.



TABLE X

BENTHIC MACROINVERTEBRATES COLLECTED FROM WEWOKA CREEK ON MAY 7, 1980

Taxa	Station														
	1			2			3			4			5		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
<b>Annelida</b>															
Oligochaeta				1								2	2		
<b>Decapoda</b>															
Astacidae				3									1		
<b>Insecta</b>															
<b>Odonata</b>															
<u>Progomphus</u> sp.											1			1	1
<u>Ischnura</u> sp.															1
<b>Hemiptera</b>															
Unidentified Corixidae			1												
<b>Diptera</b>															
<u>Palpomyia</u> sp.	20	49	5									3			
Unidentified Ephydriidae		3		2	1		1								
Unidentified Eriopterini			1												
<u>Cricotopus</u> sp. C	1	14	106	31	6	4	39	11	11	8	14	3	10	2	
<u>Dicrotendipes modestus</u>														1	
<u>Paratendipes</u> sp.								1							
<u>Potthastia</u> sp.													1		

TABLE X (continued)

	Station														
	1			2			3			4			5		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Total No. Individuals	20	53	25	106	33	7	4	40	12	12	10	20	3	14	2
Total No. Taxa	1	3	6	1	2	2	1	2	2	2	2	4	2	5	1
Species Diversity( $\bar{d}$ )	0.00	0.45	1.86	0.00	0.33	0.59	0.00	0.17	0.41	0.41	0.72	1.32	0.81	1.43	0.00
Pooled species Diversity ( $\bar{d}$ )	1.23			0.14			0.26			1.13			1.29		

TABLE XI

BENTHIC MACROINVERTEBRATES COLLECTED FROM SNAKE CREEK ON MAY 7, 1980

Taxa	Station														
	1			2			3			4			5		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Annelida															
Oligochaeta			4	12	16	40	8	17	1	28	7	35	1		1
Molluska															
Pelecypoda															
<u>Musculium</u> sp.												2			
Crustacea															
Isopoda															
<u>Asellus</u> sp.	1			1				4				1			
Decapoda															
Astacidae			1							1		1			
Insecta															
Ephemeroptera															
<u>Baetis</u> sp.				1	1							1			1
<u>Brachycercus</u> sp.								3		3		4			
<u>Pseudocloeon</u> sp.							4	1		12	7	28			
Odonata															
<u>Progomphus</u> sp.	2	2	3	4									1		4
<u>Erpetogomphus</u> sp.															
<u>Ischnura</u> sp.															
Plecoptera															
<u>Perlestra</u> sp.				2			2		1	5	3	13			
Hemiptera															
Unidentified Corixidae															
Trichoptera															
Unidentified Psychomyiidae								1							

TABLE XI (continued)

Taxa	Station														
	1			2			3			4			5		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
<b>Coleoptera</b>															
<u>Dryops</u> sp.								1							
<u>Cylleopus</u> sp.										2	1	1			
<u>Agabus</u> sp.												1			
<u>Dineutus</u> sp.												2			
<u>Hydroporus</u> sp.	1														
<b>Diptera</b>															
<u>Palpomyia</u> sp.										4					
<u>Chrysozona</u> sp.										1					
<u>Chrysops</u> sp.						1		3		1					
Unidentified Simuliidae				3	6										
Unidentified Ephydriidae															
Unidentified Eriopterini															
<u>Cladotanytarsus</u>				5		2	1								
<u>Cricotopus</u> sp. B				4	1	4	1						1		
<u>Cricotopus</u> sp. F				8	1	2	1						1		
<u>Cryptochironomus</u>				1											
<u>Larsia</u>				2											
<u>Micropsectra</u>														1	
<u>Orthocladus</u>						1									
<u>Paralauterborniella</u> sp.				3		3		3				1	1	2	
<u>Paratendipes</u> sp.			4	11		5	2	6		1		9	1	6	4
<u>Polypedilum</u> sp. A			11	13		4	8	20		3	15	47	1	7	3
<u>Polypedilum</u> sp. B								1							
<u>Potthastia</u> sp.	2	2	2	24		5	3					2			

TABLE XI (continued)

Taxa	Station														
	1			2			3			4			5		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
<u>Stictichironomus</u> sp.															4
<u>Tanytarsus</u> sp.				1		2									
Total No. Individuals	4	4	24	93	27	70	31	58	2	62	33	143	8	15	20
Total No. Taxa	3	2	6	15	6	11	10	9	2	12	5	15	8	3	6
Species Diversity ( $\bar{d}$ )	1.50	1.00	2.24	3.29	1.74	2.29	2.87	2.52	1.00	2.59	1.93	2.75	3.00	1.43	2.24
Pooled species diversity ( $\bar{d}$ )			2.23			3.12			2.95			2.81			2.67

TABLE XII

FISH COLLECTED FROM WEWOKA CREEK ON JUNE 9, 1980

Taxa	Station				
	1	2	3	4	5
Cyprinidae					
<u>Notropis lutrensis</u>		8	1	7	1
<u>Notropis stramineus</u>		2	1	2	
Ictaluridae					
<u>Ictalurus melas</u>		2			
Poeciliidae					
<u>Gambusia affinis</u>		35	34	17	75
Centrarchidae					
<u>Lepomis cyanellus</u>	26	1			
<u>Lepomis macrochirus</u>	2	19			
<u>Micropterus salmoides</u>					2
<u>Pomoxis annularis</u>	6	2			
<hr/>					
Total Number Individuals	34	69	36	26	78
<hr/>					
Total Number Taxa	3	7	3	3	3
<hr/>					
Species Diversity ( $\bar{d}$ )	0.98	1.90	0.37	1.20	0.27

TABLE XIII

FISH COLLECTED FROM SNAKE CREEK ON JUNE 9, 1980

Taxa	Station				
	1	2	3	4	5
Cyprinidae					
<u>Campostoma anomalum</u>	8	1	10	6	
<u>Notropis lutrensis</u>		16	3	18	19
<u>Notropis stramineus</u>		2		5	8
<u>Pimephales notatus</u>				4	2
<u>Pimephales vigilax</u>					2
Poeciliidae					
<u>Gambusia affinis</u>	1				
Centrarchidae					
<u>Lepomis cyanellus</u>		4		1	
<u>Lepomis macrochirus</u>		6		5	14
<u>Lepomis megalotis</u>				1	4
<u>Lepomis auritus</u>				1	1
<u>Micropterus salmoides</u>		3		2	7
Percidae					
<u>Etheostoma radiosum</u>				7	
Total Number Individuals	9	32	13	50	57
Total Number Taxa	2	6	2	10	8
Species Diversity ( $\bar{d}$ )	0.50	2.06	0.78	2.78	2.51

TABLE XIV  
FISH COLLECTED ON AUGUST 5, 1980

Taxa	Wewoka Cr.		Snake Cr.	
	1	2	4	5
Clupeidae				
<u>Dorosoma cepedianum</u>				1
Cyprinidae				
<u>Notropis lutrensis</u>			27	
<u>Notropis stramineus</u>			4	
Centrarchidae				
<u>Lepomis cyanellus</u>			4	5
<u>Lepomis macrochirus</u>			10	5
<u>Lepomis megalotis</u>				2
<u>Micropterus salmoides</u>			1	3
Poeciliidae				
<u>Gambusia affinis</u>		6,549		7
Total No. Individuals		6,549	46	23
Total No. Taxa		1	5	6
Species Diversity ( $\bar{d}$ )		0	1.66	2.37



APPENDIX B

GRAPHIC PRESENTATION OF COMMUNITY SIMILARITY INDICES,  
DUNCAN'S NEW MULTIPLE RANGE TEST,  
SALINITY, AND MASS SPECTRA

TABLE XV  
 PERCENTAGE COMMUNITY SIMILARITY OF BENTHIC MACROINVERTEBRATES  
 FOR ALL PAIRS OF STATIONS

		Wewoka Creek					Snake Creek				
		1	2	3	4	5	1	2	3	4	5
Wewoka Creek	1										
	2	17.36									
	3	17.09	98.21								
	4	28.23	78.57	78.57							
	5	15.31	75.00	75.00	77.38						
Snake Creek	1	2.52	0.00	0.22	12.72	8.44					
	2	0.79	0.00	2.05	11.89	1.58	43.70				
	3	7.03	0.0	1.79	9.52	0.00	55.82	58.06			
	4	2.49	0.00	0.95	10.99	0.00	37.04	43.24	74.22		
	5	3.99	0.00	4.11	10.52	13.49	56.28	38.23	48.01	41.52	

TABLE XVI  
 PERCENTAGE COMMUNITY SIMILARITY OF FISH  
 FOR ALL PAIRS OF STREAMS

		Wewoka Creek					Snake Creek				
		1	2	3	4	5	1	2	3	4	5
Wewoka Creek	1										
	2	9.5									
	3	0.0	33.0								
	4	0.0	65.5	42.0							
	5	0.0	49.5	95.0	66.0						
Snake Creek	1	0.0	21.0	6.5	38.0	12.0					
	2	19.0	24.5	4.5	9.0	4.0	1.5				
	3	0.0	16.5	3.0	27.0	1.0	51.0	26.0			
	4	7.5	25.5	6.0	20.0	4.0	6.0	61.0	35.0		
	5	6.0	27.0	4.0	7.5	2.5	0.0	66.5	22.5	64.5	

Figure 1  
Map of Study Area

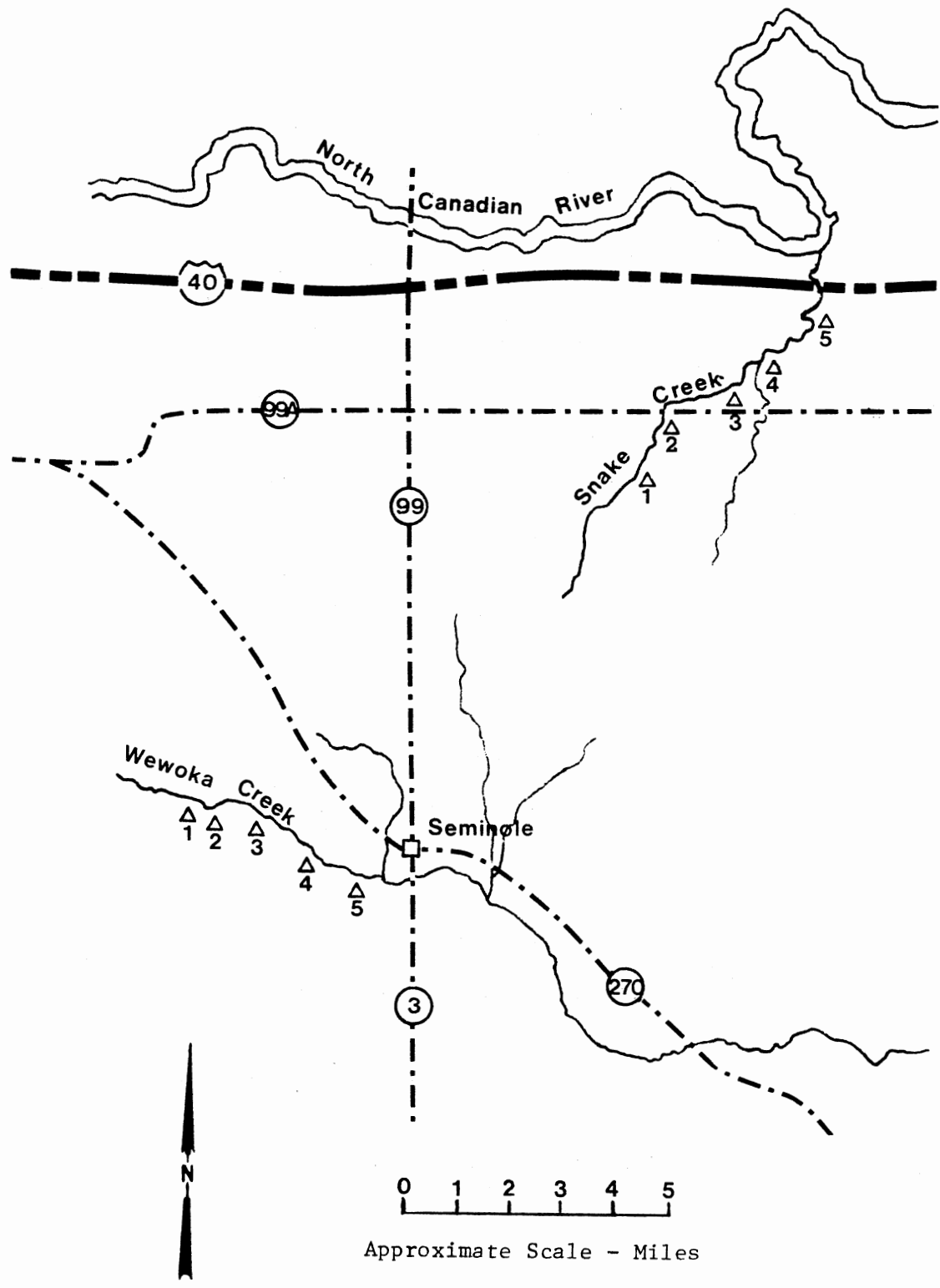


Figure 2

Duncan's New Multiple Range Test for Mean  $\bar{d}$   
of Benthic Macroinvertebrates

DUNCAN'S NEW MULTIPLE RANGE TEST

	Snake Creek					Wewoka Creek				
Station	2	4	5	3	1	4	1	5	2	3
Mean $\bar{d}$	2.44	2.42	2.22	2.13	1.58	0.82	0.77	0.75	0.31	0.19
	_____					_____				
	_____									

Any two mean  $\bar{d}$  values underscored by the same line are not significantly different (95% confidence level). Mean  $\bar{d}$  values not underscored by the same line are significantly different.

Figure 3

Mean Total Sodium Concentrations



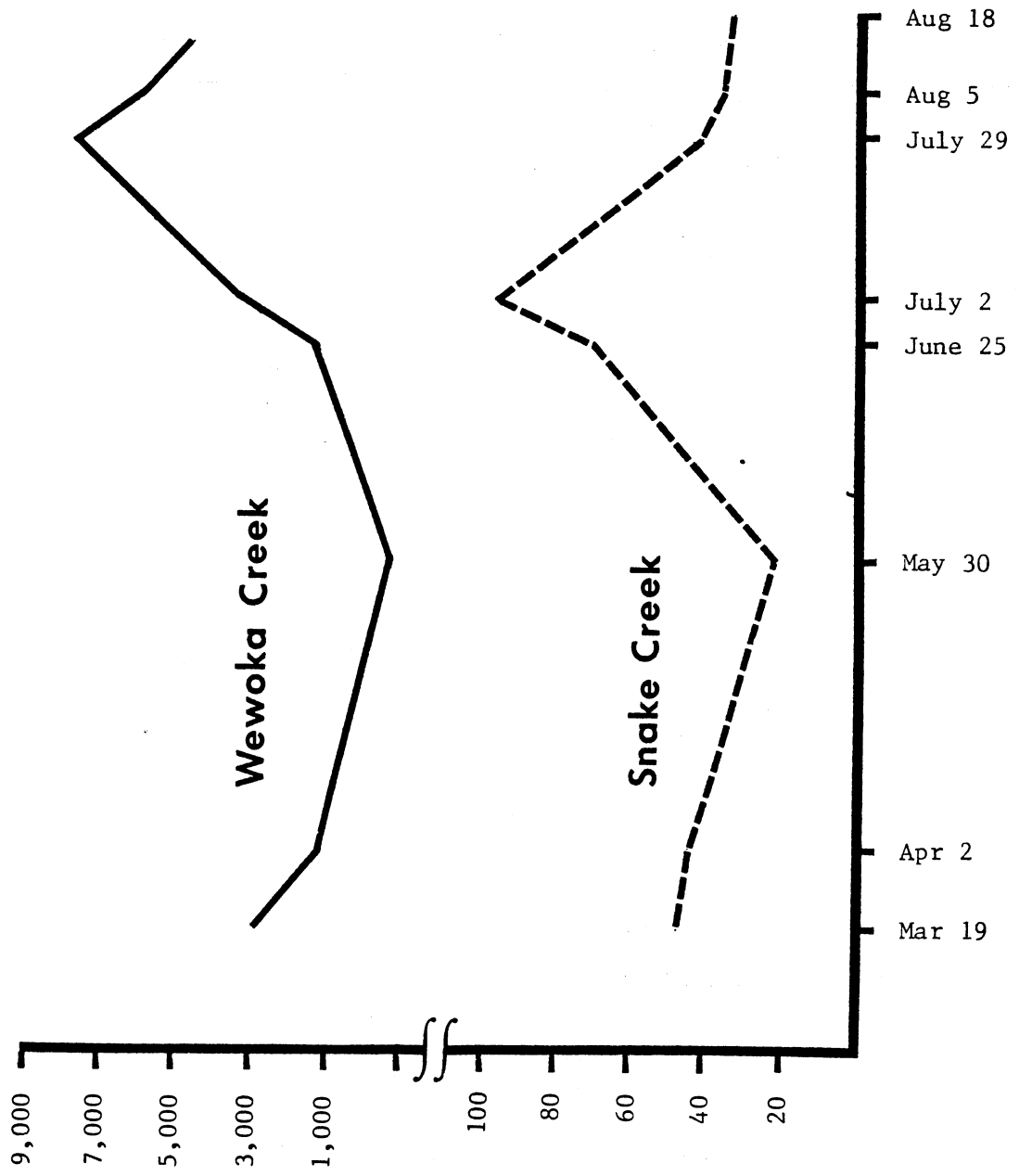


Figure 4

Mean Chloride Concentrations

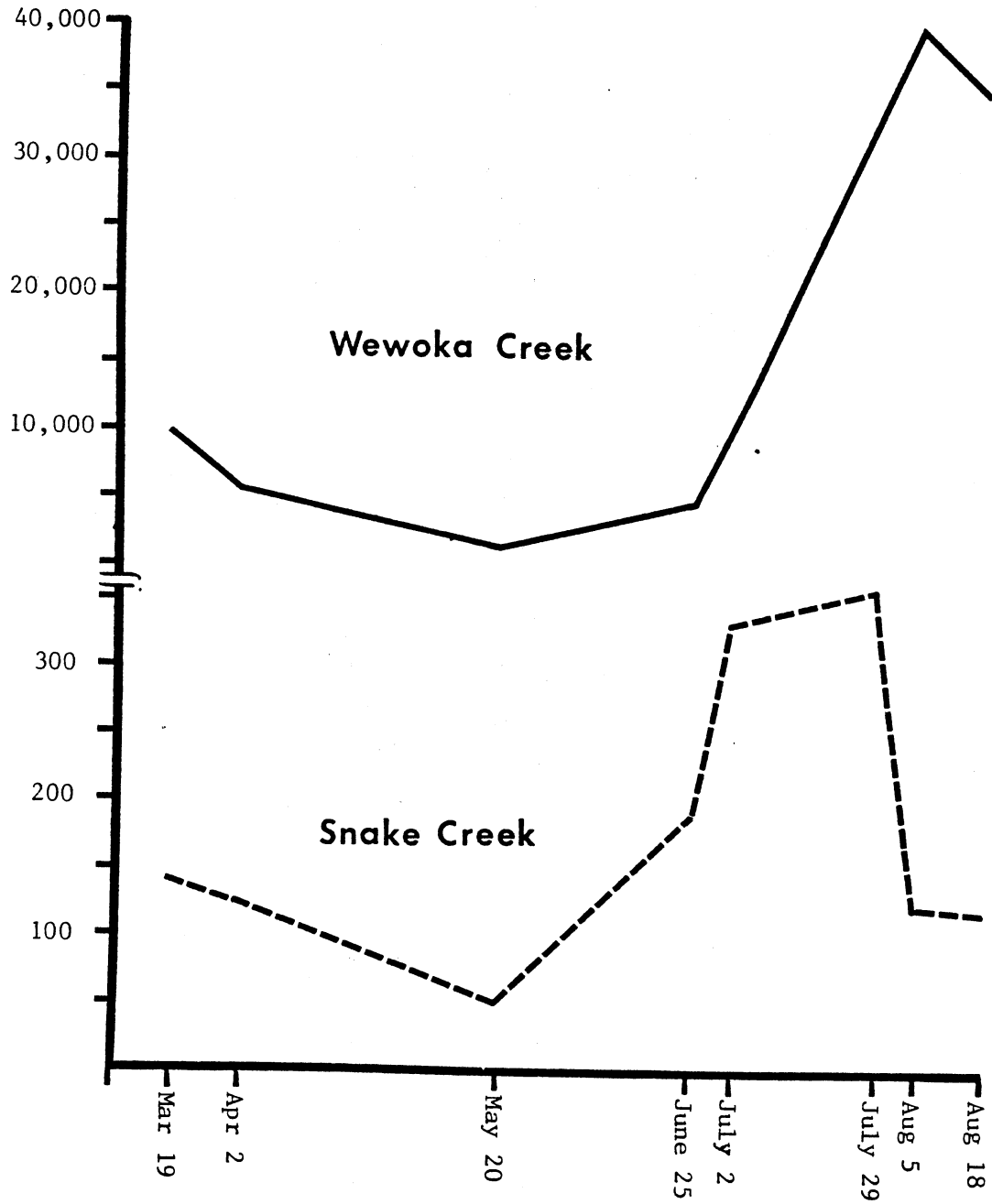


Figure 5

Total Ion Fragmentation Current

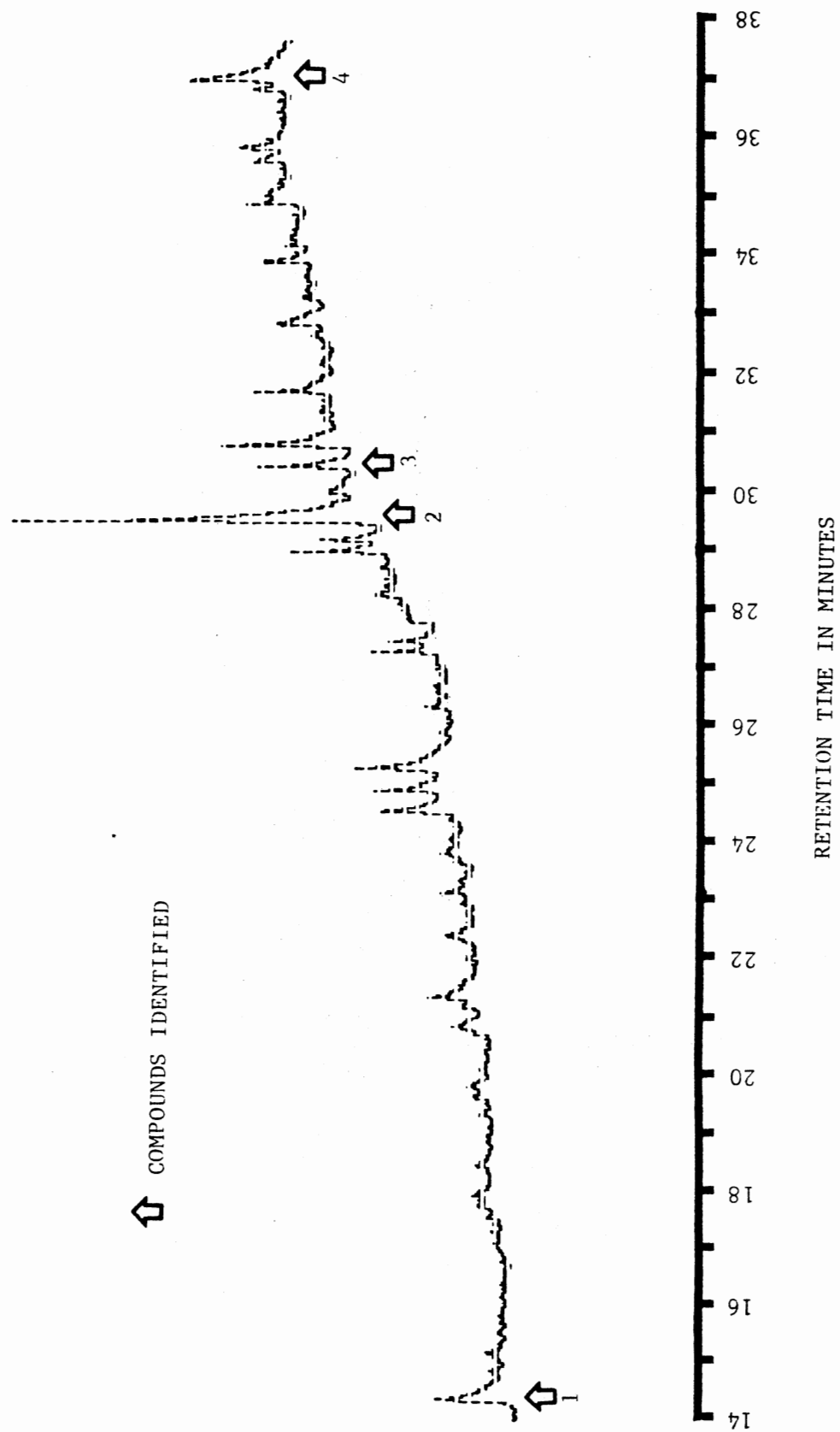
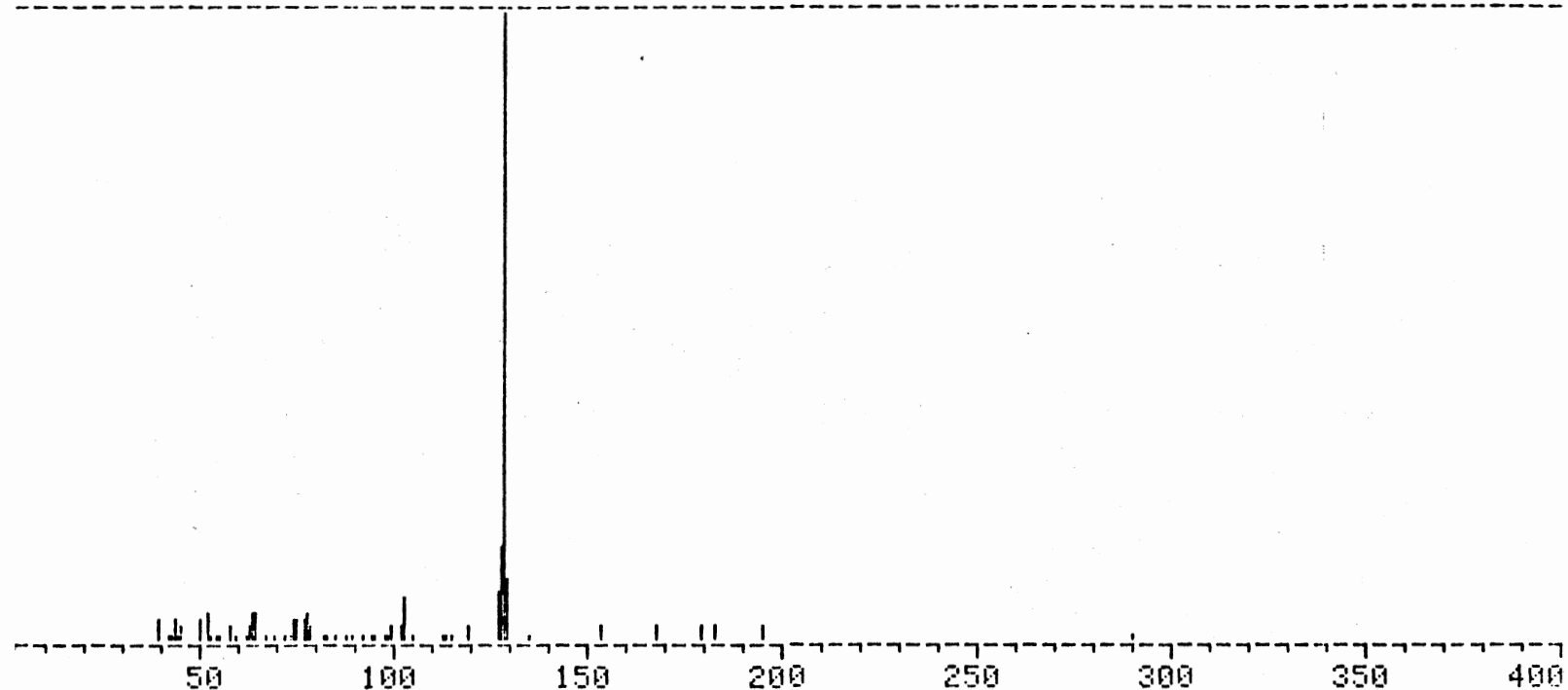


Figure 6

Mass Spectrum 1: Napthalene

Base Peak = 128.00 Base Peak Abundance = 88 Total Abundance = 209



Lower Abundance Cutoff Level = 5.0%

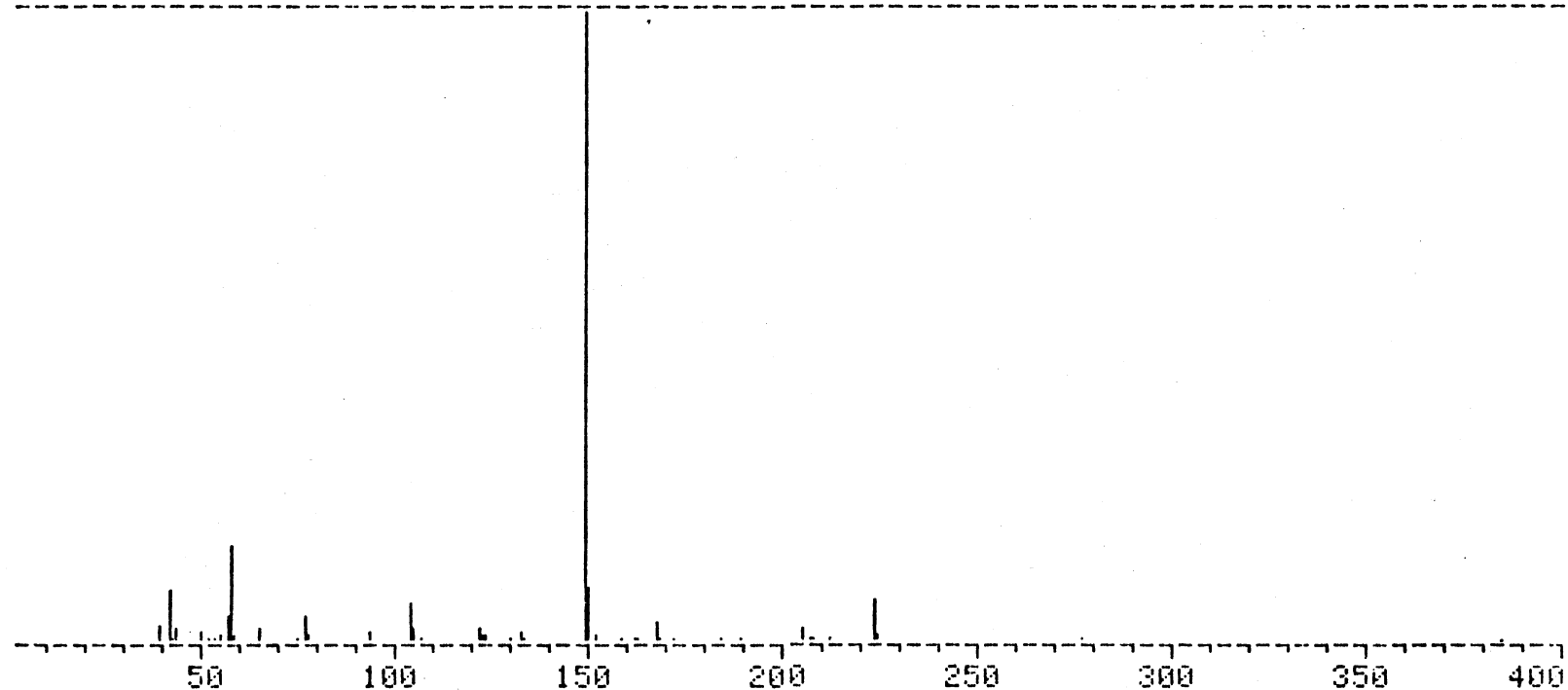
MASS	ABUNDANCE (%)	MASS	ABUNDANCE (%)	MASS	ABUNDANCE (%)
102.00	6.8	127.00	14.8	129.00	10.2
126.00	8.0	128.00	100.0		

Figure 7

Mass Spectrum 2: Dibutylphthalate



Base Peak = 149.05 Base Peak Abundance = 473 Total Abundance = 922



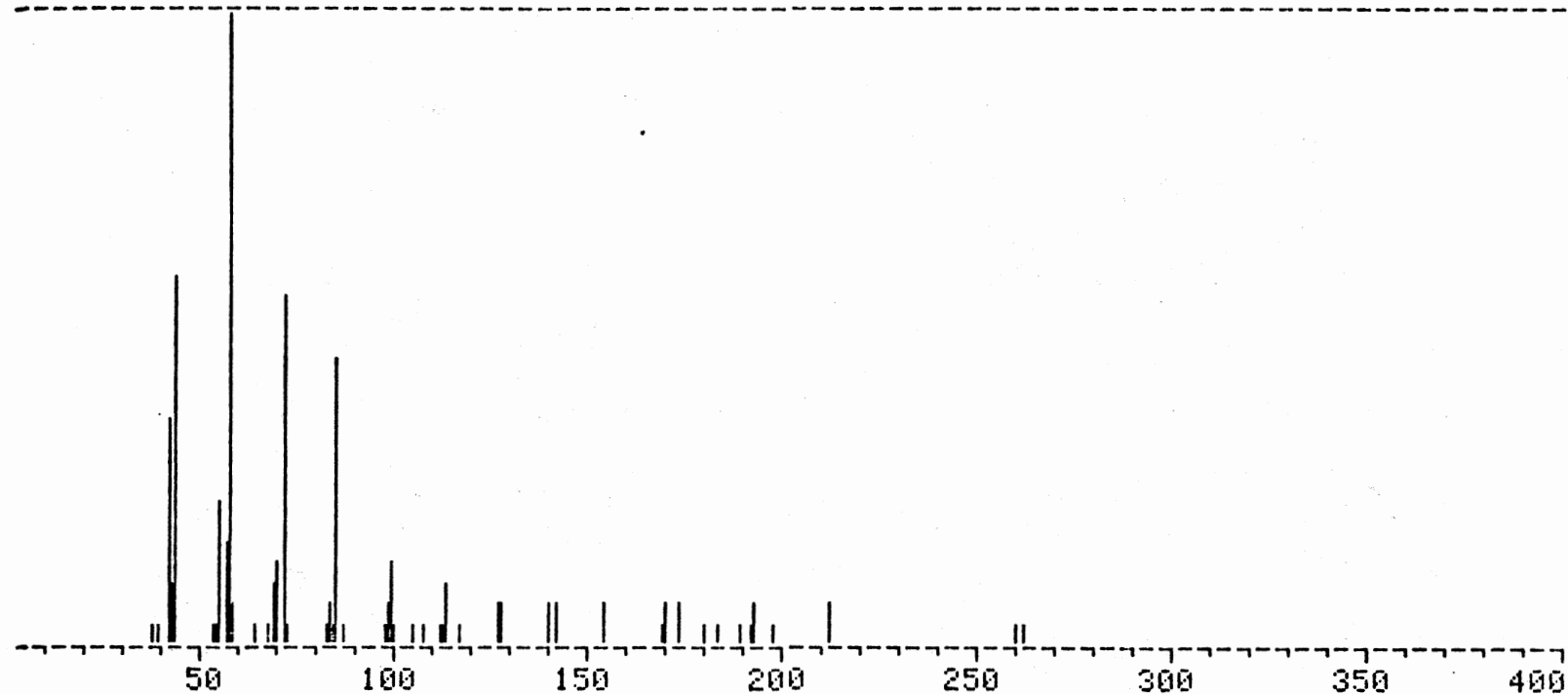
Lower Abundance Cutoff Level = 5.0%

MASS	ABUNDANCE (%)	MASS	ABUNDANCE (%)	MASS	ABUNDANCE (%)
41.10	8.0	104.10	5.9	150.05	8.5
57.10	15.0	149.05	100.0	223.15	6.3

Figure 8

Mass Spectrum 3: Unidentified aliphatic hydrocarbon

Base Peak = 57.10 Base Peak Abundance = 31 Total Abundance = 169



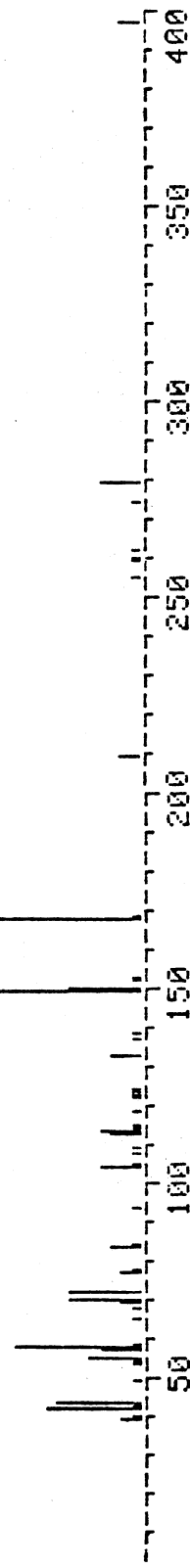
Lower Abundance Cutoff Level = 5.0%

MASS	ABUNDANCE (%)	MASS	ABUNDANCE (%)	MASS	ABUNDANCE (%)
41.10	35.5	70.10	12.9	127.10	6.5
42.00	9.7	71.10	54.8	140.00	6.5
43.10	58.1	83.10	6.5	141.00	6.5
55.10	22.6	85.10	45.2	154.05	6.5
56.10	16.1	98.10	6.5	170.05	6.5
57.10	100.0	99.10	12.9	173.05	6.5
58.10	6.5	113.00	9.7	192.05	6.5
69.10	9.7	126.00	6.5	211.15	6.5

Figure 9

Mass Spectrum 4: Bis(2-ethylhexyl) phthalate

Base Peak = 149.05 Base Peak Abundance = 60 Total Abundance = 199



Lower Abundance Cutoff Level = 5.0%

MASS	ABUNDANCE (%)	MASS	ABUNDANCE (%)
41.10	15.0	132.00	5.0
43.10	13.3	149.05	100.0
55.10	8.3	150.05	11.7
56.10	6.7	167.05	30.0
57.10	20.0	279.15	6.7
70.10	11.7		
71.10	5.0		
83.10	5.0		
104.10	6.7		
112.10	5.0		
113.10	6.7		

APPENDIX C

"DEplete" COMPUTER PROGRAM

Figure 10

"Deplete" Computer Program

```

REAL D(20,100),RO(20),E(200),H(20),SR(200)
INTEGER KR(20),C(3),ST,SP
DO 2 I=1,20
  H(I)=0.
  DO 1 J=1,200
1  D(I,J)=0.
2  CONTINUE
  READ,RN,NS
3  READ,ST,SP,C
  IF(ST.EQ.-1) GO TO 4
  CALL MAXA(C,RN,EST)
  D(ST,SP)=EST
  GO TO 3
4  DO 8 ST=1,NS
  PRINT 5,ST
5  FORMAT('1',10X,'STATION',1X,I2//1X,'(ESTIMATES BY THE METHOD OF
#CARLE 1976)'/10X.'SPECIES',5X,'ESTIMATE'//)
  DO 7 SP=1,200
  IF(D(ST,SP).EQ..) GO TO 7
  PRINT 6,SP D(ST,SP)
6  FORMAT(/11X,I3,8X,F7.1)
7  CONTINUE
8  CONTINUE
  PRINT 9,(I,I=1,NS)
9  FORMAT('1',45X,'EVENNESS DIVERSITY(1-REDUNDANCY)'/1X,'ITRS',45X,
#'NUMBER OF SAMPLES POOLED'/1X,20(5X,I2))
  IX=13133
  DO 15 IT=1,50
  DO 14 J=1,10
  DO 10 I=1,NS
  CALL RANDU(IX,IY,R)
  IX=IY
10 RO(I)=R
  CALL RANK(RO,NS,KR)
  DO 11 I=1,200
  SR(I)=0
11 E(I)=0.
  DO 13 I=1,NS
  S=0.
  ST=KR(I)
  DO 12 SP=1,200
  IF(D(ST,SP).GT.0.) SR(SP)=1.
  S=S+SR(SP)
12 E(SP)=E(SP+D(ST,SP))
  CALL R NDEX(E,S,A)
13 H(I)=H(I)=A
14 CONTINUE
15 PRINT 16,IT*10,(H(I)/IT*.1,I=1,NS)
16 FORMAT(/1X,I3,20(IX,F5.3))
  STOP
  END

```



VITA

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