6-1977

Limnological aspects of Lake Mead, Nevada-Arizona

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LIMNOLOGICAL ASPECTS OF LAKE MEAD, NEVADA-ARIZONA

Engineering and Research Center
Bureau of Reclamation

June 1977
Lake Mead is a deep, subtropical, moderately productive, desert impoundment with a negative heterograde oxygen profile occurring during the summer stratification. Investigations of the Boulder Basin of Lake Mead by the University of Nevada were initiated in November 1971. The primary objective of the study was to determine what effects industrial and sewage effluent from the Las Vegas metropolitan area, discharged into Las Vegas Bay, have had on the water quality and limnological conditions of Boulder Basin. Data from the 1975-76 period are presented in detail, with earlier data included in the summaries and discussions. Measurements of water temperature, dissolved oxygen, conductivity, pH, alkalinity, nutrient concentrations, phytoplankton numbers, chlorophyll a, primary productivity, zooplankton concentrations, and coliform bacteria were made monthly or biweekly. Success patterns for both phytoplankton and zooplankton are described.

Physical factors affecting the distribution and deposition of enteric bacteria (including those of special public health importance) in Las Vegas Bay are discussed. The distribution of water and of enteric bacteria of possible fecal origin into Las Vegas Bay from Las Vegas Wash were determined. The unreliability of thoroughly referenced techniques and methods, generally accepted as standard for enteric bacteria, are treated in detail. Water from Las Vegas Wash forms a density current in Las Vegas Bay. The density current is located on the bottom during isothermal conditions and in the metalimnion during summer stratification.

The metalimnetic oxygen minimum was examined in some detail and found to be caused by biological respiration. Estimations of zooplankton and phytoplankton respiration indicate that they could account for the majority of the oxygen lost in the metalimnion

Primary production and algal biomass were higher in Las Vegas Bay because of nutrient enrichment from Las Vegas Wash. The inner portion of Las Vegas Bay would be classified as eutrophic and the outer portion of Las Vegas Bay and Boulder Basin as mesotrophic. Nitrogen is likely to be the most limiting nutrient.

**KEY WORDS AND DOCUMENT ANALYSIS**

- **DESCRIPTORS--** limnology, reservoirs, deserts, water temperature, fish, productivity, zooplankton, bacteria, eutrophication, water quality, dissolved oxygen, water pollution, water chemistry, bibliographies, environmental effects, pumped storage, aquatic environment

- **IDENTIFIERS--** Lake Mead, Colorado River, Nev.-Ariz.

- **COSATI Field/Group** 06F COWRR: 0606

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LIMNOLOGICAL ASPECTS OF LAKE MEAD, NEVADA-ARIZONA

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June 1977
PREFACE

This report is one of a series being sponsored and published by the Bureau of Reclamation's pumped-storage research program. Bureau research on the environmental effects of pumped storage began in 1971 and is continuing. Information on the environmental effects of pumped-storage operations at Twin Lakes, Colo.; Banks Lake, Wash.; and the Salt River Reservoirs, Ariz. is available in this report series.

This report is the result of a 5-year study by personnel of the University of Nevada, Las Vegas, to determine the water quality aspects of Lake Mead. The study has been directed throughout its duration by Dr. James E. Deacon. The Bureau concluded its water quality studies of Lake Mead with reports by Hoffman et al. [1, 2]° of which this report presents an update.

These results are being used by Federal, State, and local agencies in evaluating the degree of "pollution of Lake Mead." In addition, the Bureau has been directed by Congress to determine the feasibility of adding hydroelectric generating units to Hoover Dam to increase its peaking capacity. Alternatives to be studied include additions of conventional generating units, reversible pumped-storage hydroelectric units; upgrading existing units, and variations of these alternatives. The results in this report are essential to accurately evaluate the environmental effects of any proposed alteration of Lake Mead.

The large number of undetected enteric bacteria (with standard procedures), indicates a possible potential public health hazard which, if continued undetected, could reach infectious concentrations. Therefore, at the very least, a critical review of currently accepted standards and procedures should be initiated.

Studies, financed by the Bureau of Reclamation, are continuing on the fisheries and limnological aspects of Lake Mead, and future reports of data obtained from such investigations will be forthcoming.

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° Numbers in brackets refer to items in the Bibliography.
ACKNOWLEDGMENTS

Funding and support for this project were provided by the Las Vegas Valley Water District in 1972 and by Clark County, Nev., in 1974 through 1976. The project is currently being funded by Clark County and the Bureau of Reclamation, Lower Colorado Region, Boulder City, Nev. Research was performed under the direction and supervision of Dr. James E. Deacon, University of Nevada, Las Vegas.

Many people from the University of Nevada, Las Vegas, participated in this project. The authors would like to thank the following personnel who were involved in various aspects of this study:

- Isamu Aoki: Graduate Assistant, Primary Productivity
- Patricia Baugh: Graduate Assistant, Algal Growth Potential
- Peter Calos: Undergraduate Assistant, Algal Counting
- Al Espinosa: Graduate Field Assistant
- Jack Fisher: Graduate Assistant, Algal Counting
- Robert Furtek: Graduate Assistant, Algal Counting
- Karen Harville: Undergraduate Assistant, Algal Counting
- Toni Heiner: Undergraduate Assistant, Algal Counting
- Kathy Kretschmar: Undergraduate Lab Assistant
- Scott Miller: Graduate Field Assistant
- Charles Minckley: Graduate Field Assistant
- Jill Olliffe: Undergraduate Assistant, Algal Counting
- John Priscu: Graduate Assistant, Primary Productivity
- Ed Schmid: Undergraduate Field Assistant
- Paul Serra: Undergraduate Assistant, Microbiology
- Gene Wilde: Undergraduate Field Assistant

The final review of the manuscript was performed by J. M. Tilsley and E. Hunsinger.

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INTRODUCTION

The studies on Lake Mead herein reported were initiated in November 1971, by a contract to UNLV (University of Nevada, Las Vegas) from the LVVWD (Las Vegas Valley Water District). At that time, water quality standards for water flowing into Lake Mead from LVW (Las Vegas Wash) had been set on the basis of a “no discharge” philosophy. Because of the considerable controversy over the best method of cleaning up the “pollution of Lake Mead,” LVVWD felt compelled to develop some objective information regarding the effects of water from LVW on limnological conditions in Lake Mead.

Considerable information had been gathered on the limnological conditions of Lake Mead (USBR [3], Anderson and Pritchard [4], Jones and Summer [5], Thomas [6]). Hoffman et al. [1] established for the first time that a negative heterograde oxygen profile existed in Lake Mead during summer stratification. The FWPCA (Federal Water Pollution Control Administration) [7] showed that algae were most numerous at the head of LVB (Las Vegas Bay) and decreased with distance toward Boulder Basin. This report suggested that phosphorus limitation could be effective in limiting algal growths in LVB. Hoffman et al. [2] again documented the existence of the negative heterograde oxygen profile and described the nature of summer stratification in Lake Mead. Kaufman et al. [8] identified the most significant source of TDS (total dissolved solids) in LVW and traced its flow into LVB as a density current. Koenig et al. [9] described some of the characteristics of the density current as well as other limnological features of Lake Mead. Everett [10] attempted to develop a mathematical model of primary productivity in Lake Mead.

The EPA (Environmental Protection Agency) report [11] indicates that 81.4 percent of the nitrogen and 99.6 percent of the phosphorus flowing into Lake Mead from Las Vegas Valley originate from the sewage treatment plants serving Las Vegas and Clark County. The report states that all waste discharges must eventually be removed from LVW to assure that nuisance algal blooms do not occur in LVB.

Intelligent planning demanded that current information on the trophic status of Lake Mead be developed. Therefore, the purpose for the work reported herein was to meet this need. Results have clearly shown that an extensive 1-year study, while extremely informative, is not sufficient to adequately characterize a body of water as variable as Lake Mead. Over 3 years of monitoring has basically shown that the lake is largely mesotrophic in character with the inner LVB being eutrophic. It appears that as lake level changes, there are important changes in the limnological character and trophic status of the lake. Basically, these involve the appearance of more eutrophic conditions at lower lake levels, probably the result of less dilution of inflowing nutrients. Other possibilities involve changing patterns of nutrient cycling in Lake Mead.

Most importantly, to date these studies have resulted in establishing the concept that Lake Mead is capable of receiving some discharge from LVW without impairing its beneficial uses. The water quality standards have consequently been revised to permit a phosphorus concentration of 0.5 mg/L in the inflowing stream from the LVW. This standard is currently being examined for possible revision. Other standards have also been set, but most have little or no practical influence on the trophic status of Lake Mead. The result of revising the phosphorus standard was that alternative strategies could be examined as possibilities for meeting that standard.

In the course of our studies, many management agencies have presented their problems or requested information on Lake Mead. It is evident that there is much to be learned about Lake Mead, but also that as information accumulates, its application and significance to management interests becomes increasingly obvious. The focus of this work, therefore, is beginning to shift from monitoring the effects of the nutrient-rich waters of LVW to a broader examination of the limnological relationships in Lake Mead and Mojave. It is believed that information of this sort is essential to enlightened management. This report documents the current understanding of the limnology of Boulder Basin of Lake Mead.

SUMMARY

Lake Mead is a deep, subtropical, monomictic lake with surface water temperatures ranging
from 10.5 to 27 °C. Thermal stratification develops in May and June, and a classical thermocline becomes established between a depth of 10 and 15 m in July. A turnover begins in October and the lake is completely destratified in January and February. Turnover is weak due to surface temperature only approaching hypolimnic temperatures.

A negative heterogeneic oxygen profile develops with thermal stratification and is found in all reaches of Lake Mead. The most severe oxygen depletion occurs in LVB as a result of higher eutrophic conditions. Oxygen depletion is always associated with the thermocline and hypolimnic oxygen levels remain high with only minimal oxygen loss during summer stratification.

Data from these studies show that metalimnetic oxygen depletion is the result of biological respiration. This is supported by the vertical distribution of pH which also has minimum values in the metalimnion, indicating higher concentrations of carbon dioxide being produced in this zone. Phytoplankton and zooplankton respiration are the primary causative agents of the metalimnetic oxygen depletion, accounting for 57 to 94 percent of the oxygen lost. The primary factor permitting the negative heterogeneic oxygen profile is the depth of the lake. Only the upper portion of the hypolimnion is affected by metalimnetic oxygen depletion. Oxygen concentrations in the deeper waters remain high and result in the negative heterogeneic oxygen profile.

Phosphorus concentrations throughout LVB, because of nutrient enrichment from LVW, were higher than those found in the Boulder Basin. Phosphorous loading from LVW has increased since 1972 from a mean daily rate of 524 kg/d (1155 lb/d) to 792 kg/d (1746 lb/d) in 1975. The phosphorus loading from LVW is extremely high when compared with the Colorado River, which, with 200 times the volume, discharges only 950 kg/d (2094 lb/d) into Lake Mead. Phosphorus loading for Boulder Basin is in the proposed "dangerous" level. A reduction in the phosphorus loading from LVW to 360 kg/d (794 lb/d) would result in a "permissible" loading rate in Boulder Basin.

Nitrogen appears to be limiting, at least to the numerically dominant dicyclic diatom, *Cyclotella*, during midsummer. This is indicated by the dominance of the blue-green algae, *Anabaena*, a possible nitrogen fixer, between the early summer and autumnal pulse of *Cyclotella*. Nitrate-N is almost completely depleted from the euphotic zone at this time. *Cyclotella* is the most important factor leading to an excess of phosphorus during summer stratification, indicating the possibility of nitrogen limitation.

Although phosphorus inflow has increased, phytoplankton numbers and biomass have decreased since 1972. This may be related to lake elevations. In 1972, the water level was about 6 m lower than it was in 1974 and 1975. The higher water levels increased the volume of the inner LVB alone by approximately 50 x 10⁶ m³. The increased volume would result in a greater dilution of the LWV influent, thereby decreasing the availability of the nutrients to the phytoplankton.

Phytoplankton counts were typically dominated by early summer (June and July) and autumnal (October-December) pulses of diatoms. The blue-green algae, *Anabaena*, is usually dominant in September between the diatom pulses. Numbers are always higher in the inner bay because of nutrient enrichment from LVW. The diatom, *Cyclotella*, is responsible for both the early summer and autumnal pulses. The early summer pulse appears to originate in the inner bay and expands outward in a wavelike manner on succeeding days throughout the middle and outer bay. The autumnal pulse always occurs throughout LVB and Boulder Basin in October and is associated with mixing. The early summer pulse of *Cyclotella* has declined since 1972 and has been accompanied by an increase in the *Anabaena* population. In 1975, there was no apparent early summer pulse in the outer bay and Boulder Basin, apparently because of the complete dominance of *Anabaena*. This may have resulted from the flash flood of July 4, 1975, which damaged the city of Las Vegas sanitation plant and discharged a large quantity of nutrient-rich sediments from the LVW marsh system into LVB. However, the dominance of
Anabaena may be related to other physical or biological factors.

The early summer and autumnal pulses of Cyclotella have relatively little influence on biomass determinations. Cyclotella never comprised more than 15 percent of the total biomass of the phytoplankton based on cell volumes. Anabaena was more important, accounting for 15 to 50 percent. Fragilaria, Glenodinium, Ceratium, and Oocystis were the dominant organisms on the basis of cell volumes. Cyclotella and other diatoms occurring during the autumnal pulse may be important in the taste and odor problems experienced by the Alfred Merritt Smith water treatment plant in the fall.

Primary productivity estimations were extremely high, especially in the inner bay. Lake Mead has been classified as a polluted, eutrophic lake based on primary productivity indices. These indices are based on temperate lakes and do not take into account the extended growing season in a subtropical lake such as Lake Mead. Primary productivity in Boulder Basin is comparable to other tropical or subtropical lakes, and conditions in Boulder Basin are probably not as serious as has been reported. The extremely high primary productivity in LVB is of concern as it is indicative of the enriched conditions which have developed because of LVW. Based on maximum phytoplankton volumes, the inner portion of LVB is highly eutrophic and Boulder Basin is mesotrophic.

The limnetic zooplankton community of Boulder Basin generally shows characteristics similar to typical limnetic communities reported in the literature. Rotifers, cladocerans, and copepods are the main components of the zooplankton community. The total population shows three distinct peak periods of abundance, October/November, January/February, and June/July.

The vertical distribution of the summer zooplankton community is quite different from that seen in winter populations. The summer populations exhibited a preference for the upper layers of the water column. The abundant grazers (copepod nauplii and juvenile instars, and cladocerans) showed a definite affinity for the metalimnion (10 to 25 m). The presence of a large metalimnetic area appears to set up an energy-subsidy system. The density layering caused by the thermal gradient may allow these organisms to remain in this region, expending little energy because of the constant rain of phytoplankton and detritus from the epilimnion.

Coliform populations in LVB were generally low; however, after the flash flood of July 4, 1975, higher concentrations were found. Most of the enteric bacteria at that time were found to be Erwina herbicola and Klebsiella pneumoniae. E. herbicola is generally associated with plant galls and K. pneumoniae is found in association with root systems as well as with fecal material. Because such a large volume of water came down the wash during the flash flood, and because the largest number of enteric organisms found may have been associated with plants, the source of these bacteria may have been from the marsh system and not from the sanitation plants. Salmonella was also detected in LVB after the flash flood.

A laboratory study was conducted to determine the survival of sediment-bound coliforms. Results showed that coliforms do survive for significant periods of time in the sediments and that coliform populations will increase in size in nutrient-rich sediments. Since a large fraction of the entering bacterial load is deposited in the nutrient-rich sediments at the mouth of the wash, resuspended bottom sediments may be a significant source of coliforms in LVB.

Studies of the distribution of water current from LVW into LVB showed that a density current exists just above the mud-water interface for some distance into the lake. Both bacterial tracers and fluorescent dye tracer in water from LVW were detectable in this density current. Arrival time of the dye peak was coincident with the bacterial tracer arrival. The transit time from dye injection at North Shore Road to sample point 1, 1200 m downstream, was 45 min. Transit time from sampling point A to sampling point G, 400 m downstream, was 110 min. Time from G to sampling point 2, a distance of 1000 m, was 245 min.

Oxidase negative bacteria in significant numbers over those already present in the lake have been detected as far into the lake as sampling point 3 under special conditions (i.e., during a period when the sanitation plant was inoperative) but at sampling point 2 on most occasions. In general, the distribution of oxidase negative bacteria
correlates well with the arrival of injected dye peaks at sampling points in LVB.

Difficulty in locating relatively low quantities of dye favors the use of indigenous bacteria as current tracers. This is so because the indigenous bacteria in LVW are injected over a rather long period of time in relatively high concentrations. The “component ratio” concept of bacterial tracing has great promise as a means of tracing water distribution patterns of streams into lakes. The utility of this new concept lies in that it is not dependent on the sporadic occurrence of a unique bacterium that is indigenous to the influent stream and not the lake. Rather, this new concept is dependent on the fact that the relative ratios of bacterial genera in an inflowing stream are maintained as the current from that stream penetrates progressively into the lake. The maintenance of the component ratio makes bacteria occurring in the distinctive current independent of the “noise” created by populations of like bacteria in the lake. Simply, this means that current tracing may be initiated any time the need arises.

For the oxidase negative bacteria, standard methods result in misleading interpretations because the toxicity of inhibitors present in the media results in heavy mortality during incubation of samples. This has proven to be the key barrier to the use of oxidase negative bacteria as tracers of water distribution patterns.

For the oxidase negative bacteria, the multitest scheme for identification provides a source of descriptors once the bacteria have been isolated. The entire array of descriptors is too cumbersome for routine use, as is the sequential treatment of individual single isolates. A single solution to the inhibitory initial isolation and the isolate identification problems has been resolved. This solution involves initial plating on noninhibitory agar followed by serial replica plating onto multidescriptor media, a measure which allows simultaneous evaluation of similar characteristics and percentage distributions of hundreds of bacteria at once rather than one at a time.

APPLICATION

The results of this study will be of interest and use to anyone studying the aquatic biology of desert reservoirs and in particular to those concerned with the management of Lake Mead whether it be the fishery or water supply aspects. Data obtained for this study will be used by the Bureau and others who are planning future power and resource development on and about Lake Mead.

DESCRIPTION OF THE STUDY AREA

Lake Mead was formed by impounding the Colorado River behind Hoover Dam in 1935. The lake is located in the Mohave Desert where maximum temperatures over 40 °C occur regularly in June through September and winds over 30 kilometers per hour are frequent. During recent years, the lake has had a maximum depth of over 130 m. The capacity of the lake, at an elevation of 374 m above sea level, is 36 x 10^9 m^3 with a surface area of 660 km^2 (Lara and Sanders [12]). The average sediment accumulation between 1935 and 1964 was 1.13 x 10^6 cubic meters per year. This has been substantially reduced by the construction of Glen Canyon Dam above Lake Mead (Hoffman and Jonez [13]). Lake Mead consists of four major basins separated by deep narrow canyons. The major reaches in downstream order are Pierce Basin, Iceberg Canyon, Gregg Basin, Virgin Basin, Boulder Canyon, and Boulder Basin. The Moapa and Virgin Rivers, discharging into the Overton Arm of Virgin Basin, and LVW (Las Vegas Wash), discharging into LVB (Las Vegas Bay), a large arm of Boulder Basin, are the only other tributaries to Lake Mead. Investigation of the effects of the Moapa and Virgin Rivers has not been undertaken. LVW is a nutrient-rich stream discharging industrial and sewage effluent into LVB.

LOCATION OF SAMPLING STATIONS

In 1972, 14 stations were located throughout LVB. The midchannel stations were retained in 1974, and three additional stations (stations 6 to 8) were located in the Boulder Basin (fig. 1). In 1975, regular sampling at stations 7 and 8 was discontinued. Additional samples were taken at other locations in the Boulder Basin and in the upper basins at various times.
Samples were taken weekly at each of the 14 regular stations in 1972. In 1974 and 1975, samples were taken monthly during winter and spring or biweekly during the summer and fall (June to October).

**PHYSICAL AND CHEMICAL CHARACTERISTICS OF LAKE MEAD**

**Introduction**

Regular measurements of temperature, D.O. (dissolved oxygen), conductivity, pH, and nutrients were taken in 1972, 1974, and 1975 as part of the Lake Mead Monitoring Program. Sampling was mainly confined to the Boulder Basin. Additional data were obtained from other areas of the lake.

**Materials and Methods**

Temperature, D.O., conductivity, and pH were measured with a model IIIA Water Quality Analyzer (Hydrolab Corporation) at 5-m intervals to a maximum depth of 90 m. Measurements were taken at 1-m intervals through the metalimnion during summer stratification in 1974 and 1975.

Water samples for nutrient analysis were taken at each station at various depths with a 3-L Van
Dorn water sampler throughout 1974 and 1975. The samples were preserved with mercuric chloride and analyzed for total phosphorus, dissolved phosphorus, ammonia nitrogen, nitrite-nitrate nitrogen, and Kjeldahl nitrogen. Nutrient analyses were performed by the Land and Water Monitoring Branch of EPA, Las Vegas, Nev.

Results

Temperature—Water temperatures ranged from 10.5 °C in January and February to 27 °C in July and August (table 1). Thermal stratification developed in May and June with the greatest thermal gradient (about 3 °C change per 5 m) between 5 and 10 m. In July, a classical thermocline (Hutchinson [14]) developed between 10 and 15 m when surface water temperatures reached 26 °C. The thermocline remained at 10 m through September in both 1974 and 1975, although surface water temperatures were slightly cooler. Mixing usually begins in October but this occurred early in 1972 with mixing to a depth of 15 m in September. The lake was completely destratified in January and February when surface water temperatures reached 10.5 °C. Hypolimnetic water temperatures (at 90 m) usually remained constant at 10.5 °C and, therefore, turnover was very weak.

Oxygen.—With the development of thermal stratification there was a loss of metalimnetic oxygen (table 2), resulting in a negative hetrograde oxygen profile (Hutchinson [14]). The loss of metalimnetic oxygen was continuous throughout the period of summer stratification and, therefore, the lowest metalimnetic oxygen levels usually occurred in September just prior to mixing (fig. 2). In 1974 and 1975, the lake remained stratified at 10 m through September resulting in a pronounced narrow zone of low oxygen. This narrow zone did not develop in 1972 due to lower water temperatures and mixing which began in late August and September.

Table 1.—Mean surface water temperatures at station 4

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Table 2.—Temperature and dissolved oxygen at station 4—April through October 1975

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<td>D.O., mg/L</td>
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The negative hetrograde oxygen profile was found in all reaches of the lake (table 3), except in the Gregg and Pierce Basins. LVB and Boulder Basin had the most pronounced metalimnetic oxygen depletion. Metalimnetic oxygen levels below 2.0 mg/L in the Boulder Basin and below 1.0 mg/L in LVB were found in August and September. The zone of depletion was broader in LVB than in Boulder Basin. In 1972, the Overton Arm of Virgin Basin showed a broad zone of depletion similar to LVB, but did not reach comparable low levels.

**pH.** The pH (hydrogen ion concentration) ranged from 7.3 to 8.8. The highest readings were at the surface during the summer and apparently resulted from relatively high phytoplankton production. Vertically, the lowest values were always found within the metalimnion and corresponded with low oxygen levels (fig. 3). This indicates that respiratory activity is important in both decreasing the pH level and reducing the oxygen levels in the metalimnion.

**Conductivity.** Conductivity was generally constant at 1100 μS/cm and did not change vertically except in LVB stations 2 and 3 (table 4). Higher conductivity levels were found at these stations as a result of a density current or cell from LVW. The density current was located along the bottom when the lake was destratified (November-April). In May, thermal stratification developed and water temperatures increased in LVW, changing the density relationships of the two water masses. At that time, the water from LVW was not as dense as the cooler hypolimnetic water and the density current was located in the area of the thermocline. The density current remained within the metalimnion throughout summer stratification (May-September). There was no evidence, as indicated by higher conductivity levels, of the density current reaching the mouth of LVB (station 4). There appeared to be substantial mixing and dilution of the current before it reached this point.

**Nutrients.** Nutrient concentrations for 1975 are presented in appendix I. Phosphorous and nitrogen were higher and more variable in LVB than in Boulder Basin. This was due to nutrient inflow from LVW and was especially evident at stations 2 and 3 within the density current. A complete discussion of nutrient concentrations is included as a separate section of this report.

**Discussion**

Lake Mead can be classified as a deep, subtropical, monomictic lake according to Hutchinson [14]. The lake becomes thermally stratified, although a classical thermocline occurs only for a short period in July. Thermal stratification of a lake is primarily dependent on incident solar radiation, but is also affected by wind and the degree of exposure of the lake. Therefore, thermal stratification in Lake Mead is not pronounced due to the large surface area of the lake and high summer winds which tend to somewhat destratify the lake.

The occurrence of a negative hetrograde oxygen profile in Lake Mead was first reported by Hoffman et al. [1]. A negative hetrograde oxygen profile was evident in 1944 and low metalimnetic oxygen levels probably have occurred since the formation of the lake. The cause of metalimnetic oxygen depletion in Lake Mead appears to be the result of biological respiration. This is supported by the vertical distribution of pH which is a function of carbon

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2 Bureau of Reclamation, unpublished data.
Table 3.—Vertical distribution of dissolved oxygen at various locations in Lake Mead (1972)

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<th>Station 4 August 23</th>
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A further discussion on the causes of the metalimnetic oxygen depletion is presented in the section entitled, “Metalimnetic Oxygen Depletion in Lake Mead.”

STATISTICAL ANALYSIS OF PHYTOPLANKTON COUNTING TECHNIQUES

Introduction

Phytoplankton are sensitive indicators of various limnological factors such as nutrient concentration, light, and temperature. Limnologists therefore estimate one or more phytoplankton parameters (population density, biomass, productivity), then use this information as criteria for judging the severity of water pollution or other water quality perturbations. Defining the extent of such perturbations...
Table 4.—Conducivity at stations 1 to 3—April 1975 through February 1976

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<tr>
<td>30</td>
<td>1050</td>
<td>1050</td>
<td>1050</td>
<td>1050</td>
</tr>
<tr>
<td>35</td>
<td>1050</td>
<td>1050</td>
<td>1050</td>
<td>1050</td>
</tr>
<tr>
<td>40</td>
<td>1050</td>
<td>1050</td>
<td>1050</td>
<td>1050</td>
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<tr>
<td>45</td>
<td>1050</td>
<td>1050</td>
<td>1050</td>
<td>1050</td>
</tr>
</tbody>
</table>

Phytoplankton sampling necessarily involves several stages of subsampling, each stage associated with a degree of variability. The number of subsampling stages depends on the methods employed in a particular study, but generally these stages include: (1) sampling one point in the lake, (2) subsampling the lake sample, (3) counting several fields of the respective subsamples. Error analysis appears complex, but the hierarchical sampling is amenable to statistical procedures which permit simultaneous analysis of variation at each stage of sampling.
The objective in this study was to conduct a series of phytoplankton sampling experiments which could be subjected to rigorous statistical analysis, thus allowing the development of an efficient sampling program for Lake Mead.

Experimental Methods

In this study the membrane filter procedure of phytoplankton enumeration was employed, as described by McNabb [15], modified by Moore [16] for permanent mounting on glass slides, and further revised by Holmes [17] to include dehydration of the filter with ethanol. This procedure involves pouring a known aliquot of water into a filtration apparatus designed to accommodate a micropore filter (47-mm-dia., 0.45-μm pore size). The water is drawn through the filter by vacuum (69 kPa) followed by a series of alcohol rinses. The filter is removed from the apparatus and placed in a petri dish containing cedar oil which clears the filter in approximately 24 hours. After clearing, a portion of the filter is placed on a glass slide with several drops of Permount (a synthetic mounting medium distributed by Fisher Chemical Co.), a cover slip is added, and the slide is set aside for 1 or 2 days to allow the Permount to set. The result is a clear slide ready for microscopic examination.

A method of counting the same portion of a filter in an identical manner for several samples had to be devised for the purposes of these experiments. Sectioning the filter into equal, identifiable quadrats appeared to be a desirable modification. The device used to quarter a filter is merely a replica of a micropore filter (47-mm dia.), subdivided into four equal quadrats, etched into a plexiglass block. Since a micropore filter is unmarked, it was necessary to scribe a mark on the basal part of the filtering apparatus and place a corresponding pencil dot on the filter to guide alignment on the apparatus prior to filtration. This dot, visible after the filter has cleared, permits alignment with a corresponding mark scribed on the plexiglass block. Each quadrant can then be exactly sectioned with a razor blade and mounted on a standard glass slide. Counting is not necessary but is advisable should later reference to a particular slide be desired.

Counting fields were selected by subdividing a quadrat into 0.25-mm squares (the calibrated area of a Whipple disk). Each square was numbered; i.e., 00, 01, 02, . . . n, and counting fields were selected from a table of random numbers (Bliss [18]). This process was repeated for all four quadrats. Orientation to these randomly selected fields was made using the microscope micrometer.

Sampling and Analysis

Three 1-L surface water samples (series I) were collected, with a Kemmerer (1-L) water sampler, in immediate sequence from one location in LVB (Las Vegas Bay) on November 17, 1972. Aliquots of 200 mL were filtered from each sample. The 200-mL volume had been shown (Koening et al. [9]) to be appropriate for achieving a good counting distribution of phytoplankton on the micropore filter at densities occurring in LVB. The predominate genera encountered in this set of samples were: Carteria, Cyclotella, Chlamydomonas, Peridinium, and Mallomonas.

To determine the number of counting fields required to adequately represent a quadrat, an arbitrary 10-percent error limit was set on the mean estimate. A series of 40 random fields were then counted on 1 quadrat and the mean phytoplankton per counting field, standard deviation, and 95 percent confidence interval were computed in cumulative increments of 10 counting fields (table 5).

The 10-percent error limit occurred after 20 fields had been counted. Twenty fields were therefore counted on each quadrat of these three samples.

Having established an adequate counting procedure, it was further considered necessary to evaluate the sampling distribution of organisms on the filters. Researchers frequently employ standard statistical tests without regard for the sampling distribution of a population. The power of a statistical test, however, is greatly enhanced with a knowledge of the sampling distribution. Total phytoplankton from 80 counting fields (20 from each of 4 quadrats) were used to contrast frequency distributions for the three samples. The Chi-square test reveals that the cumulative $\chi^2$ for each of the three samples had probabilities $P(\chi^2) = 0.10-0.50$, within the range of the acceptance region.
Table 5.—Estimates used to determine the adequacy of the counting procedure

<table>
<thead>
<tr>
<th>Counting fields</th>
<th>Mean number phytoplankton per field</th>
<th>Standard deviation</th>
<th>95 percent confidence limits</th>
<th>Error of estimate, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10</td>
<td>20.4</td>
<td>4.45</td>
<td>±2.76</td>
<td>13</td>
</tr>
<tr>
<td>1-20</td>
<td>18.8</td>
<td>4.78</td>
<td>±2.09</td>
<td>11</td>
</tr>
<tr>
<td>1-30</td>
<td>18.3</td>
<td>4.21</td>
<td>±1.50</td>
<td>8</td>
</tr>
<tr>
<td>1-40</td>
<td>18.3</td>
<td>4.52</td>
<td>±1.40</td>
<td>7</td>
</tr>
</tbody>
</table>

(figs. 4a, 4b, and 4c). A satisfactory agreement therefore existed between observed and theoretical normal frequencies, and the normal approximation was an adequate working representation of the data. The approximate normality of the sampling distribution was encouraging because it permitted the use of parametric statistical tests in the subsequent analyses.

A nested, three-factor, random effects analysis of variance (Simpson & Roe [19]) was used to determine the magnitude of variation associated with the sampling. The nested, rather than factorial, design was used because of the hierarchical nature of sampling. In the nested design, the unique effects associated with a factor are restricted to one level within that factor (Winer [20]). Since each factor is treated independently of the others, the nested design with factors B and C nested under factor A, is the desired model.

The computational procedures are similar for the factorial and nested designs; however, they differ in the construction of the AOV (analysis of variance) table. It is possible to obtain a nested design from a factorial by using the fully crossed factorial equivalent outlined by Dixon ([21] p. 504). A method for calculating the nested d.f. (degree of freedom) from a factorial was adopted from Winer [20].

These sources permit use of factorial computer programs, for which most three-way analyses are intended, to obtain valid nested designs.

The results of the nested, three-factor analysis for the data discussed above are outlined in table 6.

The analysis reveals that a significant difference exists between the samples. The methods of treating each sample though are certainly satisfactory as shown by the very homogeneous MS (mean square) and low F-ratio for the quadrat treatments. The implications of the analysis are that only one quadrat need be examined from each filter, thus greatly reducing the processing time for each sample. The remaining problem was to resolve the large variability between samples, and to determine if any difference existed between aliquots of the same sample.

It seems reasonable that if large variability existed between individual samples taken in quick succession at the same lake station, combination of multiple single samples into a large integrated sample would likely reduce that variability. To test this hypothesis, three more samples (series II) were collected from the same location in LVB on December 14, 1972; each of these consisted of three 1-L subsamples pooled to form one larger sample. The predominate genera in these samples were: (1) Anabaena, (2) Chlamydomonas, (3) Cyclotella, (4) Carteria, and (5) Peridinium. The same enumeration procedure was followed, but each sample was subsampled (200-mL aliquots) twice and 20 fields were examined on only 1 quadrat.

Another nested AOV was designed with the following factors: (a) samples (3 levels), (b) subsamples (2 levels) and (c) counts (20 fields). The results of that analysis, summarized in table 7, show no significant F-values in any of the factors.

However, in this AOV a new source of variability was partitioned out; the variability between subsamples within a sample. This variability was included in the between-samples MS of series I. Because of this, the between-samples MS of series I and II are not directly comparable. To compare the results of two AOV sets, they must
Figure 4.—Observed and theoretical normal distribution computed for three lake samples (series I).
Table 6.—Nested AOV for lake samples, quadrats, and counting fields

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>SS (^1)</th>
<th>MS (^4)</th>
<th>F-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake samples</td>
<td>2</td>
<td>577.633</td>
<td>288.817</td>
<td>9.317</td>
</tr>
<tr>
<td>Quadrats</td>
<td>9</td>
<td>279.000</td>
<td>31.000</td>
<td>1.293</td>
</tr>
<tr>
<td>Counting fields</td>
<td>228</td>
<td>5467.302</td>
<td>23.97</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>239</td>
<td>6323.975</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Computed based on total plankton from 20 randomly selected counting fields.
\(^2\) P(F)<0.05.
\(^3\) SS = sum of squares.
\(^4\) MS = mean square.

Table 7.—Nested AOV for lake samples, subsample aliquots, and counting fields

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>SS (^2)</th>
<th>MS (^3)</th>
<th>F = ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake samples</td>
<td>2</td>
<td>46.550</td>
<td>23.275</td>
<td>1.340</td>
</tr>
<tr>
<td>Subsample aliquots</td>
<td>3</td>
<td>52.017</td>
<td>17.330</td>
<td>1.845</td>
</tr>
<tr>
<td>Counting fields</td>
<td>114</td>
<td>1069.750</td>
<td>9.390</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>1168.31</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Computed based on total plankton from 20 randomly selected counting fields.
\(^2\) SS = sum of squares.
\(^3\) MS = mean square.

include the same components. If, however, by lumping samples and quadrat MS of series I and samples and subsamples MS of series II, the new F-ratios become:

\[
\begin{align*}
\text{Series I} & \quad \text{Series II} \\
F_{9228} = \frac{77.87}{23.97} = 3.26 & \quad F_{5114} = \frac{19.71}{9.39} = 2.09 \\
\end{align*}
\]

Series I is still significant at \(P_{0.05}\), but series II is not. These are directly comparable and indicate that pooling of the lake samples is an effective way of reducing variability.

During this statistical evaluation it was discovered that the counting of 20 fields per quadrat was excessively time consuming. This required that the number of counting fields be reduced to handle the number of samples that needed to be analyzed. However, there was some apprehension in adopting a new counting procedure without some statistical basis for accepting it. Sokal and Rohlf [22] describe a procedure which allows one to test what effect reducing the number of counting fields from 20 to 10 would have on the previous statistical analyses. In the nested AOV the expected MS for each factor includes variance components shown in Table 8.

Table 8.—Mean square and degree of freedom estimates for nested AOV

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>((a-1))</td>
<td>(c^2 + na^2 b a + nba^2 A)</td>
</tr>
<tr>
<td>Subsamples</td>
<td>((b-1))</td>
<td>(c^2 + na^2 b a)</td>
</tr>
<tr>
<td>Counts</td>
<td>((a-1))</td>
<td>(a^2)</td>
</tr>
</tbody>
</table>
To portion the variance from each factor, take the difference between corresponding MS and divide by the number of replicates on which it was based. For example, from table 7, the following can be estimated:

\[ \sigma^2_a = \frac{MS(\text{samples}) - MS(\text{subsamples})}{nb} = \frac{23.275 - 17.330}{20 (3)} = 0.099 \]

\[ \sigma^2_b = \frac{MS(\text{subsamples}) - MS(\text{counts})}{n} = \frac{17.33 - 9.39}{20} = 0.397 \]

\[ \sigma^2 = MS \text{ counts} = 9.39 \]

To evaluate what effect reducing the number of counting fields (n) to 10 would have on the previous analysis, the AOV is restructured according to table 9.

Again, the differences are not significant, indicating that reducing the counting fields to 10 would not alter the conclusions of the previous analysis. This is further supported by the analysis of the counting method (table 1). There is only a 2-percent increase of precision by counting 20 versus 10 fields. Therefore, reducing the number of counting fields to 10 seemed justified.

**Discussion**

In evaluating the sampling variability associated with the membrane filter enumeration procedure, it was necessary to assure that each sample was treated identically at all stages of subsampling. This was achieved by sectioning the filters into quadrats and establishing a standard counting procedure. With these modifications, the sampling variability at each stage of subsampling was statistically evaluated. Furthermore, it was possible to adequately reduce field sampling variability at a lake station by increasing the sampling volume.

The membrane filter procedure is highly suited for phytoplankton sampling, since all phases of the procedure are easily manipulated. Therefore, if excessive variation does occur, and can be identified, it can be reduced by making procedural modifications. The sampling program developed from this evaluation required only a few modifications to gain added precision of population estimates. It requires that: (1) three 1-L samples from a single station in the bay be pooled to form one large sample, (2) one 200-mL aliquot be filtered from each sample, and (3) one quadrant be examined from each filter. This program is suitable for sampling the phytoplankton at densities in LVB. It is difficult to foresee what effect a significant change in densities would have on the procedure. However, it seems likely that a uniform distribution can be maintained on the filter simply by controlling the volume of water filtered. This, however, would have to be determined experimentally should higher densities be encountered. By controlling the volume filtered, it is expected that this sampling program could be used effectively to evaluate most phytoplankton communities.

**EFFECTS OF LAS VEGAS WASH, A NUTRIENT-RICH STREAM, ON PHYTOPLANKTON POPULATIONS IN BOULDER BASIN, LAKE MEAD, NEVADA**

**Introduction**

Phytoplankton succession, biomass, and primary productivity were determined to evaluate the effects of nutrient enrichment. LVW (Las Vegas Wash) is a nutrient-rich stream discharging industrial and sewage effluent from the Las Vegas metropolitan area into a narrow estuary at the head of LVB (Las Vegas Bay). LVW is the only external enriched nutrient source of any magnitude discharging into Boulder Basin. Therefore, it was possible to examine the effects
of nutrient enrichment from virtually a single source on a relatively large body of water.

Materials and Methods

Phytoplankton.—A 1-L Van Dorn water sampler was used to collect samples. Each sample was a combination of three separate 1-L subsamples collected from the same site. Samples were usually taken monthly or biweekly at each of the stations. In 1972, the samples were taken weekly. Phytoplankton enumerations were made on samples using a modification of the membrane filter technique (McNabb [15] as described in the previous section.

Cell volumes.—Phytoplankton volumes were estimated by determining the average cell volumes for 20 of the most important organisms. The average cell volumes were multiplied by the cell counts to arrive at a total phytoplankton volume.

Chlorophyll.—Pigment analyses were made on 500- to 1000-mL samples filtered through Watman G F/C filters. The filters were pretreated with MgCO₃ (magnesium carbonate). The chlorophyll was extracted by grinding the filters in 5-mL of acetone. Pigment concentrations were determined from formulas given by Parsons and Strickland [23] using data obtained with a Coleman Junior spectrophotometer.

Primary production.—Estimations of primary productivity were based on carbon-14 techniques (Steeman Nielsen [24]) using modifications of Saunders et al. [25]. Light and dark bottles (pyrex 125-mL) were filled with water collected at the surface and at depths of 1, 3, 5, and 10 m. Each bottle was treated with 0.5 µCi (microcurie) of NaH¹⁴CO₃ (sodium bicarbonate), suspended from a buoy at the depth of collection, and incubated for a period of 4 to 6 hours. After incubation, the samples were returned to the laboratory in a lightproof box. The samples were filtered through 0.45-µm micropore filters and the residue on the filters was washed with 10- to 15-mL of a 0.005N HCl (hydrochloric acid), 5-percent formalin solution. Metricel filters were used in July through October 1975, but were found to reduce counting efficiency by a factor of five times and, subsequently, were not used. The filters were placed in glass or polyethylene scintillation vials and dried in a desiccator for 24 hours. A xylened based liquid scintillation solution was added to the vials and the activity of the samples was determined. The activity of the samples was converted to milligrams of carbon per unit area per day using the methods and tables of Saunders et al. [25]. Daily rates were based on total day length and were not corrected for changing light intensity.

Results

Phytoplankton numbers.—Phytoplankton counts of the most numerous organisms for April 1975 through March 1976 are presented in appendix II. Phytoplankton numbers were always higher in the inner bay (Station 2) due to nutrient enrichment from LVW. Dominant organisms during the 3 years of investigation were similar, especially in the summer. Total numbers have decreased since 1972 and the successional patterns have changed.

Winter and early spring (January-April) phytoplankton populations were generally below 300 organisms/mL except at station 2 where counts over 1000 organisms/mL were found. The phytoplankton populations were usually dominated by diatoms (Cyclotella and Stephanodiscus) in January and chlorophyta (Chlamydomonas, Carteria, Eudorina, Oocystis, and Planktosphaeria) in February through April. The dinoflagellate Glenodinium also became numerous at this time and was dominant at all stations in March 1974.

Phytoplankton populations during May through December were dominated by early summer and autumnal pulses of diatoms. Fragilaria was always dominant in May or June and was succeeded by a Cyclotella pulse in July. In 1972, samples were taken weekly and it was evident that the early summer Fragilaria and Cyclotella pulses originated in the inner bay and expanded outward in a wavelike manner on succeeding days throughout the middle and outer bays. Numbers of organisms declined outward from the inner bay. Navicula, on the other hand, was proportionately more numerous with increased distance from the inner bay and was usually the dominate organism in July or August at stations 4 to 6 in Boulder Basin. The blue-green algae.
Anabaena, increased in numbers throughout July and August (except at station 2 where Cyclotella remained dominant) and was the dominant organism in September after the early summer diatom pulses had declined. An autumnal pulse of Cyclotella always occurred throughout LVB and Boulder Basin in October associated with mixing. The autumnal diatom pulse lasted through December with numbers of Anabaena steadily declining.

The early summer pulse of Cyclotella has declined since 1972, and has been accompanied by an increase in the Anabaena population. There has also been an increase in the autumnal pulse of Cyclotella. In 1972, the early summer pulse of Cyclotella reached a maximum of over 53,000 cells/mL at station 2; there was also a well-defined pulse throughout LVB. In 1974 and 1975, the early summer pulse was much smaller (15,000 cells/mL) and numbers throughout LVB were lower. Total phytoplankton numbers have decreased since 1972, due mainly to the reduction in the early summer pulse of Cyclotella. Numbers of Cyclotella have increased during the autumnal pulse from 1500 in 1972 to 5000 cells/mL at station 4 in 1975. The highest numbers of Cyclotella were found in October and November of 1974 and 1975; whereas, the highest numbers occurred in July 1972. Anabaena has become increasingly more numerous since 1972 in the outer bay and Boulder Basin. In 1975, Anabaena was dominant from July through September at stations 4 to 6. Anabaena was dominant only in September of 1972 and 1974 following the early summer diatom pulses.

Phytoplankton biomass.—Figure 5 shows the seasonal variations in mean chlorophyll a and estimated cell volumes at stations 2 and 6. Station 6 was not sampled in 1972. At station 2, the highest single values for chlorophyll a (55.49 mg/m³) and cell volume (30.1 mL/m³) were found in April and May 1972, respectively. Both chlorophyll a and cell volumes indicate a reduction in the phytoplankton standing crop since 1972, although cell volumes in 1975 were higher than those found in 1974.

Phytoplankton volumes were determined for only the dominant organisms or those organisms that were very large; therefore, total cell volumes were underestimated. Although the volumes were underestimated, they are useful as a comparative index. The cell volumes do reflect the higher eutrophic conditions at station 2 due to nutrient enrichment. The higher cell volumes found in 1972 and 1975 were mainly due to Glenodinium and Fragilaria. Cell volumes in 1974 were low due to the complete absence of Fragilaria and lower numbers of Glenodinium. The early summer and autumnal pulses of Cyclotella had relatively little influence on biomass determinations; Cyclotella is small and never comprised more than 15 percent of the total estimated biomass of the phytoplankton. Anabaena was more important, accounting for 15 to 50 percent of the total estimated biomass. Fragilaria, Glenodinium, Ceratium, and Oocystis were the dominant organisms on the basis of cell volumes.

There were two distinct chlorophyll a peaks. The first occurring in March and April when phytoplankton counts were low and dominated by chlorophyta. The second occurred in October and November during the autumnal Cyclotella pulse when chlorophyta numbers were declining. Chlorophyll a was low in July when Cyclotella numbers were high. There was no evident relationship between chlorophyll a and phytoplankton numbers.

Chlorophyll content per unit cell volume is dependent on a number of factors, such as species type, physiological state of the phytoplankton, and environmental conditions (Vollenweider [26]). High cell volumes corresponded with high chlorophyll a only in the late summer and fall, a situation also described for Lake Erie (Munawar and Burns [27]). The highest chlorophyll a concentrations per unit cell volume occurred in the spring (table 10). The low chlorophyll a concentrations in the summer are possibly due to high light intensity, high water temperatures, and low nutrient concentrations at the surface.

Primary production.—Primary productivity was highest at station 2 (table 11) as were the other phytoplankton parameters. Generally, there was a reduction in productivity outward in LVB with the lowest productivity at station 6 in Boulder Basin. Seasonally, the highest productivity occurred in August and September, ranging from 16,000 to 24,000 (mgC/m²)/d (milligrams of
carbon per square meter per day) at station 2 and from 3000 to 6000 (mgC/m²)/d at station 6. Productivity generally increased from April through September with a substantial reduction in October. During the winter (December-February), productivity remained relatively high, ranging from 780 to 2300 (mgC/m²)/d. Productivity was extremely high at all stations with means ranging from 8300 (gC/m²/d) at station 2 to 2600 (gC/m²)/d at station 6 (table 12).

Nutrients.—Phosphorus, nitrogen, and alkalinity concentrations in LVW, LVB, and Boulder Basin during April 1975 through February 1976 are presented in appendix I. Table 13 summarizes total phosphorus and nitrite plus nitrate nitrogen (nitrate-N) concentrations for 1972, 1974, and 1975. In 1972, nitrate-N was determined only in September and October. LVW discharges approximately 800 kg phosphorus/d and 1100 kg nitrogen/d into LVB. There has been an increase in nitrate-N since 1974.

Total phosphorus levels were extremely variable in LVB and there was not an evident seasonal pattern. In Boulder Basin (station 6), which was not directly influenced by LVW, total phosphorus ranged from approximately 22 μg/L in December and January to 10 μg/L in August and September. Vertically, total phosphorus was uniformly distributed or was slightly lower in the metalimnion. At stations 2 and 3, higher levels were found in the metalimnion due to the density current from LVW. There was never a depletion in epilimnetic concentrations during summer stratification.

Surface nitrate-N at station 6 ranged from approximately 300 μg/L in January and February to 20 μg/L during the summer. At all stations there was a depletion of epilimnetic nitrate-N during summer stratification (table 8). Surface nitrate-N increased in October due to mixing which resulted in distribution of hypolimnetic nitrate-N throughout the water column.

Correlation analysis.—Correlation coefficients for phytoplankton parameters and nutrients (total phosphorus and nitrate-N) are presented in table 14. Cyclotella was significantly correlated with total phosphorus and nitrate-N, and 57 percent of the variance in the Cyclotella population can

Table 10.—Mean chlorophyll "a" concentrations per unit cell volume

<table>
<thead>
<tr>
<th>Month</th>
<th>1972</th>
<th>1974</th>
<th>1975</th>
<th>1976</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan.</td>
<td>7.90</td>
<td></td>
<td></td>
<td>9.41</td>
</tr>
<tr>
<td>Feb.</td>
<td>11.09</td>
<td>16.08</td>
<td></td>
<td>3.58</td>
</tr>
<tr>
<td>Mar.</td>
<td>24.88</td>
<td>17.38</td>
<td>20.99</td>
<td>7.33</td>
</tr>
<tr>
<td>Apr.</td>
<td>0.79</td>
<td>4.70</td>
<td>5.19</td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>0.91</td>
<td>8.86</td>
<td>3.80</td>
<td></td>
</tr>
<tr>
<td>June</td>
<td>1.38</td>
<td>8.41</td>
<td>1.69</td>
<td></td>
</tr>
<tr>
<td>July</td>
<td>1.42</td>
<td>10.51</td>
<td>2.54</td>
<td></td>
</tr>
<tr>
<td>Aug.</td>
<td>1.89</td>
<td>8.89</td>
<td>3.15</td>
<td></td>
</tr>
<tr>
<td>Sept.</td>
<td>2.05</td>
<td>9.45</td>
<td>3.51</td>
<td></td>
</tr>
<tr>
<td>Oct.</td>
<td>3.39</td>
<td>10.36</td>
<td>4.35</td>
<td></td>
</tr>
<tr>
<td>Nov.</td>
<td>7.20</td>
<td></td>
<td>7.87</td>
<td></td>
</tr>
<tr>
<td>Dec.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 11.—Monthly primary productivity in Las Vegas Bay and Boulder Basin, April 1974 through April 1976

<table>
<thead>
<tr>
<th>Month</th>
<th>1974</th>
<th>1975</th>
<th>1976</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Jan.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mar.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apr.</td>
<td>10229</td>
<td>2279</td>
<td>1142</td>
</tr>
<tr>
<td>May</td>
<td>6414</td>
<td>6443</td>
<td>3234</td>
</tr>
<tr>
<td>June</td>
<td>7742</td>
<td>3394</td>
<td>4100</td>
</tr>
<tr>
<td>July</td>
<td>10116</td>
<td>6404</td>
<td>3119</td>
</tr>
<tr>
<td>Aug.</td>
<td>16793</td>
<td>12862</td>
<td>7310</td>
</tr>
<tr>
<td>Sept.</td>
<td>24757</td>
<td>17560</td>
<td>7080</td>
</tr>
<tr>
<td>Oct.</td>
<td>5339</td>
<td>4827</td>
<td>5844</td>
</tr>
<tr>
<td>Nov.</td>
<td>3933</td>
<td>3919</td>
<td>5224</td>
</tr>
<tr>
<td>Dec.</td>
<td>2160</td>
<td>1551</td>
<td>2066</td>
</tr>
</tbody>
</table>
be explained by total phosphorus and nitrate-N. *Anabaena* was negatively correlated with nitrate-N due to its occurrence only in the summer when nitrate-N was depleted from the epilimnion. Chlorophyll *a* and cell volumes were correlated only with total phosphorus. Primary productivity did not correlate with either total phosphorus or nitrate-N. Primary productivity was positively correlated with chlorophyll *a* and cell volume. Primary productivity and standing crop are usually not highly correlated and other investigators have reported inverse relationships between productivity rates and standing crop (Findenegg [28], Goldman et al. [29], Margalef [30], Verduin [31], Vollenweider and Nauwerck [32]).

### Discussion

Early summer and autumnal pulses of diatoms are frequent occurrences in lakes (Hutchinson [33]) and *Cyclotella* is a common organism demonstrating this pattern. Olsen [34] found an early summer and autumnal pulse of *Cyclotella* in Canyon Lake, Ariz., which is similar to Lake Mead in climatic and chemical conditions, although it is much smaller. In Boulder Basin, the early summer *Cyclotella* pulse is restricted to LVB where there is an external nutrient source, LVW. The autumnal pulse is always associated with cooler water temperatures and mixing.

The dominance of *Anabaena*, a possible nitrogen fixer, during late summer would suggest that nitrogen is limiting at that time, although King [35] and Shapiro [36] have shown that this fact alone is not conclusive evidence of nitrogen limitation. The vertical distribution of nutrients involving summer depletion in the epilimnion further suggests that nitrogen may be the most important limiting nutrient. During summer stratification, nitrate-N is depleted from the epilimnion and significantly higher levels occur in the hypolimnion. Therefore, mixing results in increased nitrogen availability in the euphotic zone. This may be the most important factor behind the autumnal pulse of *Cyclotella*. Dissolved silica remained high (> 8 mg/L) throughout the summer (USGS [37]) and is, therefore, not a limiting factor. Phosphorus is uniformly distributed or shows a slight decrease below depths of 10 m and mixing does not result in higher phosphorus levels. If the autumnal pulse is due to higher levels of nutrients, nitrogen would appear to be the controlling factor. The N/P (nitrogen to phosphorus) ratios also show an excess of phosphorus (table 15). The N/P ratios are significant because the ratio in living systems is about 8; therefore, when the N/P ratio exceeds 8, more nitrogen is present than can be utilized and, conversely, when the ratio falls below 8 there is an excessive amount of phosphorus (Verduin [38]). The N/P ratios indicate that nitrogen is in short supply for most of the year in the inner bay and during the summer in the outer bay.

Phosphorus inflow from LVW has increased since 1972 from a mean daily rate of 525 kg/d to 792 kg/d in 1975. The phosphorus inflow from LVW is extremely high when considering the Colorado River, which has 200 times the volume and discharges only 950 kg/d into Lake Mead. Total phosphorus loading for Boulder Basin (1.944 g/m² per year) is in the “dangerous” level proposed by Vollenweider and Dillon [39]. A reduction in the phosphorus inflow from LVW to 360 kg/d would result in a “permissible” loading rate in Boulder Basin. Nitrogen inflow from LVW in 1974 and 1975 was approximately 1100 kg/d. Nitrogen concentrations were not determined for LVW in 1972.

Although nutrient inflow has increased, phytoplankton numbers and biomass have decreased since 1972. This may be directly related to lake elevations. In 1972, the water level was about 6 m lower than it was in 1974 and 1975. The higher water levels increased the volume of the inner LVB alone by approximately 50 x 10⁶ m³. The increased volume would result in...
Table 13.—Mean phosphorus and nitrogen concentrations in Las Vegas Wash, Las Vegas Bay, and Boulder Basin—
1972 through 1975

<table>
<thead>
<tr>
<th>Station No.</th>
<th>Depth, m</th>
<th>1972 May-October</th>
<th>1974 March-December</th>
<th>1975 April-December</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total-P</td>
<td>NO₃</td>
<td>NO₃ *</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>4.236(0.99)</td>
<td>4.156(0.681)</td>
<td>8.54(2.86)</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0.049(0.038)</td>
<td>0.302(0.499)</td>
<td>0.15(0.14)</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0.012(0.006)</td>
<td>0.033(0.008)</td>
<td>0.10(0.09)</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>0.010(0.005)</td>
<td>0.027(0.006)</td>
<td>0.11(0.08)</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0.018(0.008)</td>
<td>0.32(0.09)</td>
<td>0.38(0.07)</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>0.010(0.003)</td>
<td>0.12(0.09)</td>
<td>0.03(0.02)</td>
</tr>
</tbody>
</table>

Total-P = Total phosphorus.
NO₃ = Nitrite plus nitrate nitrogen.
Number in parenthesis = 1 standard deviation.
* Mean values during summer stratification June-September.
Table 14.—Correlation coefficients for Cyclotella, Anabaena, chlorophyll "a," cell volume, and primary productivity

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>Independent Variables</th>
<th>No. of samples</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log₁₀ Cyclotella #†</td>
<td>Total phosphorus</td>
<td>32</td>
<td>0.64</td>
</tr>
<tr>
<td>Log₁₀ Cyclotella #</td>
<td>Nitrate-N</td>
<td>32</td>
<td>0.57</td>
</tr>
<tr>
<td>Log₁₀ Anabaena #</td>
<td>Total phosphorus</td>
<td>32</td>
<td>-0.02</td>
</tr>
<tr>
<td>Log₁₀ Anabaena #</td>
<td>Nitrate-N</td>
<td>32</td>
<td>-0.61</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>Total phosphorus</td>
<td>48</td>
<td>0.55</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>Nitrate-N</td>
<td>48</td>
<td>0.18</td>
</tr>
<tr>
<td>Cell volume</td>
<td>Total phosphorus</td>
<td>48</td>
<td>0.30</td>
</tr>
<tr>
<td>Cell volume</td>
<td>Nitrate-N</td>
<td>48</td>
<td>0.02</td>
</tr>
<tr>
<td>Cell volume</td>
<td>Chlorophyll a</td>
<td>48</td>
<td>0.66</td>
</tr>
<tr>
<td>Primary productivity</td>
<td>Total phosphorus</td>
<td>34</td>
<td>-0.20</td>
</tr>
<tr>
<td>Primary productivity</td>
<td>Nitrate-N</td>
<td>34</td>
<td>0.56</td>
</tr>
<tr>
<td>Primary productivity</td>
<td>Chlorophyll a</td>
<td>34</td>
<td>0.37</td>
</tr>
<tr>
<td>Primary productivity</td>
<td>Cell volume</td>
<td>34</td>
<td></td>
</tr>
</tbody>
</table>

Multiple correlation coefficient

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>Independent Variables</th>
<th>No. of samples</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log₁₀ Cyclotella #</td>
<td>Total phosphorus,</td>
<td>32</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>nitrate-N</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant at the 0.05 level.
† Indicates number.

Table 15.—Nitrogen (nitrate-N and ammonia-N) to phosphorus (dissolved phosphorus) ratios for Las Vegas Bay, stations 2 to 4 and Boulder Basin, station 6, 1975

<table>
<thead>
<tr>
<th>Month</th>
<th>Stations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Apr.</td>
<td>5.9</td>
</tr>
<tr>
<td>May</td>
<td>2.5</td>
</tr>
<tr>
<td>June</td>
<td>1.4</td>
</tr>
<tr>
<td>July</td>
<td>3.0</td>
</tr>
<tr>
<td>Aug.</td>
<td>2.6</td>
</tr>
<tr>
<td>Sept.</td>
<td>4.1</td>
</tr>
<tr>
<td>Oct.</td>
<td>7.1</td>
</tr>
<tr>
<td>Nov.</td>
<td>10.7</td>
</tr>
<tr>
<td>Dec.</td>
<td>9.0</td>
</tr>
<tr>
<td>Jan.</td>
<td>8.1</td>
</tr>
</tbody>
</table>

in a greater dilution of the LVW influent, thereby decreasing the availability of the nutrients to the phytoplankton.

The trophic classification of Lake Mead has received considerable attention in the past few years. Phytoplankton species indices have been discussed by Staker et al. [40]. These indices have given results ranging from polluted eutrophic to oligotrophic. Everett [10], based on Rodhe's [41] primary productivity categories, classified Boulder Basin as a polluted eutrophic body of water. Primary productivity estimates in this study are similar to Everett's and, in addition, show very high production in LVB. Since, Everett's and these primary productivity estimations were based on total day length resulting in an overestimation of primary
Primary productivity and not on daily integrated light intensity, the use of Rodhe's criteria to Boulder Basin is inappropriate. In addition, Rodhe's classification is based on temperate lakes and therefore, is probably not appropriate to conditions in Lake Mead. Not only does this classification not take into account the extended growing season in a subtropical lake but, therefore, is probably not appropriate to conditions in Lake Mead. The higher primary productivity estimates do reveal the extremely fertile conditions that have resulted because of nutrient enrichment from LVW. Primary productivity in Boulder Basin appears to be comparable to other tropical or subtropical lakes (Talling [42], Gannon and Dolliver [43]. Melack and Kammer [44]), and conditions in Boulder Basin are therefore not as serious as Everett stated. Boulder Basin is considered to be mesotrophic based on primary productivity estimations.

Eisenberger [26] proposed the following classification based on maximum phytoplankton volumes:

- oligotrophic: 1 mL/m³
- mesotrophic: 3 to 5 mL/m³
- eutrophic: 10 mL/m³

This classification is also primarily based on temperate lakes and must be used with some reservations when applying it to conditions in Lake Mead. Using this classification the inner portion of LVB is highly eutrophic with the rest of Boulder Basin being in the mesotrophic range (based on primary productivity estimations).

The lack of appropriate indices for subtropical or tropical lakes makes it difficult to classify Lake Mead. In general, Boulder Basin appears to be eutrophic with the inner portion of LVB existing eutrophic conditions.

**Zooplankton Community of Boulder Basin**

**Introduction**

Primary investigations in 1974 showed peak zooplankton concentrations within the metalimnetic oxygen depletion of Lake Washington. This report is the result of a 1-year study of the zooplankton community of Lake Mead. Its purpose was to determine if any unique differences existed between the zooplankton community of Lake Mead and other limnetic communities.

Seasonal succession, species composition, vertical distribution, and specific depth affinities of the zooplankton community were examined. These data were compared to those of other investigators wherever possible.

**Methods and Materials**

Zooplankton samples were collected at approximately 2-week intervals from June through October and at monthly intervals for the remainder of the year. Station 5 was selected as the permanent sampling location for these collections. This station is located in close proximity to the intakes of the Alfred Merrit Smith water treatment plant. The water depth increases sharply from the shore to a depth of 65 m. This allowed for very little contamination of the sample with littoral organisms. Easy access to and from Lake Mead Marina was also favorable to the selection of this station, as many samples were taken over a 24-hour period.

Samples were taken at 5-m intervals from the surface to a depth of 45 m with a portable water pump. Tononli [46] recommended this type of device where large numbers of samples need to be taken in a short period of time. The pump-type sampler has been shown to successfully minimize damage to plankton samples used for identification purposes (Aron [47]). It was also shown to have zooplankton fishing abilities equal to a conventional plankton tow net (Icanberry and Richardson [48]).

Summer samples were collected with a Sears Model No. 563.2692 d-c powered pump having an average flowthrough velocity of 8 L/min. The winter samples were taken using a 1.5-hp gasoline-driven pump with an average velocity of 12.5 L/min. Both pumps were attached to a heavy-duty, reinforced rubber hose. The bottom of the hose was weighted with a plexiglass plate designed to keep the hose vertical and to allow an even draw from a narrow, horizontal band.
From each depth, 40 L of lake water were run through a No. 20-mesh, nylon, plankton net (76-μm mesh). Samples were immediately preserved in a 5-percent formalin solution.

Counting and identification of the plankton was done on a Wetzlar Model No. 6001 12 compound stereo microscope. Taxonomic identifications were based on Coker [49], Davis [50], Edmondson [51], Gurney [52, 53], Marsh [54], and Pennak [55]. All organisms were identified as to genus and species when possible. One-milliliter subsamples were placed in an open Sedgewick Rafter counting cell. All organisms in five such aliquots were tallied. This represented 5 to 10 percent of the summer samples and 10 to 20 percent of the winter samples. With the exception of eggs, organisms less than 50 μm in diameter were not counted (i.e., ciliates and zoo-flagellates).

Results and Discussion

The limnetic zooplankton community of Boulder Basin was dominated by Rotatoria, Cladocera, and Copepoda. Other organisms such as ciliates, zoo-flagellates, insect larvae, and water mites were often found in the samples, but their numbers were usually very low. One species of *Diffugia*, a protozoan, was very abundant in the May 8 samples. The sample was accidentally discarded and verification of the counts cannot be made. Except for a small number of *Diffugia* in the mid-July sample, these organisms have not reappeared.

Rotifers.—The rotifers are represented by 12 genera, 5 of which were classified as major components of the community, and are listed in table 16. While one or more of the following seven genera were often present in the samples, no single genera ever represented more than 5 percent of the rotifer population:

- Trichocerca
- Notholca
- Platyias
- Ploesoma
- Lecane
- Monostyla
- Brachionus

Cladocera.—Three genera of cladocerans were identified from the samples. The first two, *Daphnia* and *Bosmina*, were considered to be truly limnetic organisms, but the third genus, *Alona*, is more typically a littoral organism (Pennak [55]). All *Daphnia* were grouped together, but tentative identification indicates three species may be present. For *Bosmina*, only one species was found, *B. longirostris*.

Copepoda.—The limnetic copepods are represented by four species, two in the order Cyclopoida (*Cyclops bicuspisatus thomasi* and *Mesocyclops edax*) and two in the order Calanoida (*Diaptomus clavipes* and *D. siciloides*).

Seasonal succession.—A review of the literature indicates that zooplankton may have one, two, or three seasonal periods of abundance. These high and low periods may be caused by physical, chemical, and/or biological conditions, with a combination of these usually occurring. Single species show different cycles from lake to lake and may even show differences from year to year in the same lake. These seasonal variations seem to change the community makeup at the species level only. Pennak [56] found that at any given time a typical zooplankton community is dominated by one copepod, one cladoceran, and two to four numerically dominant rotifers.

The zooplankton of Lake Mead show varying patterns of seasonal succession. Table 17 shows the number of organisms of each major group occurring in the samples from May 1975 through April 1976. The population of each group shows relatively high numbers of individuals during June/July, October/November, and January/February. Although three peak periods occur in the lake, analysis of the community by each species shows that the majority of the organisms are either dicyclic or monocyclic.

As mentioned earlier, the rotifer population of Boulder Basin was best represented by five genera, each of which was the dominant rotifer on one or more of the dates sampled (table 16). *Asplanchna* was dicyclic, being abundant in June and January. The number of organisms in the water column was 320,000 and 150,000/m² of surface area, respectively. The counts for January may be conservative due to the presence of *Syncheata*, which was very abundant. *Syncheata* was present in all stages of development from newly hatched individuals to
Table 16.—Major rotifers in the water column at station 5

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Asplanchna Syncheata</td>
<td>50</td>
<td>320</td>
<td>98</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>20</td>
<td>150</td>
<td>35</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Polyarthra</td>
<td>260</td>
<td>75</td>
<td>92</td>
<td>19</td>
<td>41</td>
<td>65</td>
<td>43</td>
<td>16</td>
<td>340</td>
<td>8</td>
<td>5</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Collotheca</td>
<td>0</td>
<td>25</td>
<td>240</td>
<td>27</td>
<td>15</td>
<td>25</td>
<td>0</td>
<td>15</td>
<td>18</td>
<td>20</td>
<td>13</td>
<td>40</td>
<td>16</td>
</tr>
<tr>
<td>Keratella*</td>
<td>821</td>
<td>159</td>
<td>53</td>
<td>94</td>
<td>9</td>
<td>5</td>
<td>11</td>
<td>11</td>
<td>64</td>
<td>13</td>
<td>3</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>All others</td>
<td>10</td>
<td>10</td>
<td>25</td>
<td>18</td>
<td>0</td>
<td>8</td>
<td>3</td>
<td>25</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1141</td>
<td>591</td>
<td>614</td>
<td>327</td>
<td>46</td>
<td>124</td>
<td>149</td>
<td>726</td>
<td>483</td>
<td>5528</td>
<td>239</td>
<td>58</td>
<td>221</td>
</tr>
</tbody>
</table>

* Keratella—Total of K. quadrata and K. cochlearis.

Table 17.—Number of organisms in each major group in the upper 45 meters of the water column

<table>
<thead>
<tr>
<th></th>
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<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Copepods (CI-CV Adult)</td>
<td>259</td>
<td>861</td>
<td>766</td>
<td>817</td>
<td>351</td>
<td>905</td>
<td>944</td>
<td>177</td>
<td>172</td>
<td>369</td>
<td>345</td>
<td>576</td>
</tr>
<tr>
<td>Copepod Nauplli</td>
<td>952</td>
<td>1220</td>
<td>2150</td>
<td>1430</td>
<td>211</td>
<td>200</td>
<td>1040</td>
<td>195</td>
<td>318</td>
<td>1307</td>
<td>830</td>
<td>1250</td>
</tr>
<tr>
<td>Total Cladocera</td>
<td>25</td>
<td>192</td>
<td>46</td>
<td>106</td>
<td>160</td>
<td>63</td>
<td>491</td>
<td>376</td>
<td>162</td>
<td>464</td>
<td>226</td>
<td>140</td>
</tr>
<tr>
<td>Total Rotiferfa</td>
<td>1120</td>
<td>591</td>
<td>327</td>
<td>46</td>
<td>124</td>
<td>149</td>
<td>726</td>
<td>483</td>
<td>5528</td>
<td>239</td>
<td>58</td>
<td>221</td>
</tr>
<tr>
<td>Total</td>
<td>2356</td>
<td>2864</td>
<td>3289</td>
<td>2399</td>
<td>846</td>
<td>1317</td>
<td>3201</td>
<td>1231</td>
<td>6180</td>
<td>2369</td>
<td>1459</td>
<td>2187</td>
</tr>
</tbody>
</table>
large saclike adults. This latter form is very similar to a small saccate form of *Asplanchna*. The majority of *Asplanchna* were of a very large campanulate form (twice the size of adult *Syncheata*), but a quantitative reexamination of January samples showed a small number of saccate *Asplanchna* to be present. It is interesting to note that the June population also contained a large campanulate form of *Asplanchna*. Gilbert [57] reports that *Asplanchna sieboldii* will greatly increase in size in the presence of any large prey species. High numbers of *Syncheata* in January and of *Keratella* and *Polyarthra* in May and June represent an adequate food for the *Asplanchna* and may have given rise to this large form.

*Syncheata* was abundant in January and reached a density of 320 organisms/L at the 10-m depth. While this seems to be a very high figure, Pennak [55] reports finding single rotifer species at densities in excess of 5000/L. *Syncheata* was also abundant in July and November, suggesting that the species may be tricyclic. However, the November peak results from hatching of resting-stage eggs. This generation then goes on to reproduce amictically, reaching its peak in January. Therefore, the apparent tricyclic occurrence of *Syncheata* is actually dicyclic. The phenomenon of diapause or resting stages is discussed in detail in the subsection on Diapause.

Two species of *Keratella* were identified from the samples. *Keratella cochlearis* was the dominant species, being present in all of the samples and responsible for the peaks in May and December. *Keratella quadrata* was present in lower numbers, and was found only during periods of peak zooplankton abundance (June/July, October, and January).

*Collotheca* seems to be dicyclic, having peaks in March and July. *Polyarthra* also appears to be dicyclic, although its peaks occur in January and May. It is difficult to determine if the fluctuations in the *Polyarthra* population between June and December are truly cyclic responses. A third peak may have occurred in October, although numbers were much lower than those found in January and May.

Of the five genera of rotifers discussed, none seem to have both of their dominant periods in the same 2 months. There was some overlap in midwinter, but the late spring or early summer populations were spread over a 3-month period (table 18).

The two limnetic cladocerans were monocyclic with their peak populations being out-of-phase (table 19). *Daphnia* had its maximum abundance in June and July, while *Bosmina* was most abundant in November, December, and February. It is common practice when counting organisms for quantitative analysis to neglect fragments or incomplete specimens. Ephippial stages of *Daphnia* were extremely abundant in the surface film starting in February and continuing into May. This stage contains resting eggs attached to the valve or carapace of the adult. The valve separates from the rest of the organism at the next molt. The ephippia then float to the surface and concentrate in windrows as a result of surface winds and Langmuir circulation. This makes it difficult to get a quantitative estimate of their numbers. It was noted that zooplankton samples from March and April contained a large number of *Daphnia* heads. Assuming that each head represented an adult *Daphnia* that had given rise to an ephippium, one could add these counts to the population and possibly obtain a better estimate of the actual population density of *Daphnia*. There were 67,000 heads/m² in the water column in March. The total *Daphnia* in the water column was estimated as 48,000 individuals/m². Summing these two figures, the total *Daphnia* population for March appears closer to 115,000 individuals/m². For April, the heads totaled 112,500/m² and the *Daphnia* totaled 121,000/m². This would give an adjusted population estimate of 233,500 individuals/m². These adjusted population estimates for *Daphnia* appear to be more accurate than those listed for March and April (table 19).

Table 18.—Periods of peak abundance of the major rotifers at station 5, Boulder Basin

<table>
<thead>
<tr>
<th>Organism</th>
<th>Peak months</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Asplanchna</em></td>
<td>June, January</td>
</tr>
<tr>
<td><em>Syncheata</em></td>
<td>July, January</td>
</tr>
<tr>
<td><em>Polyarthra</em></td>
<td>May, January</td>
</tr>
<tr>
<td><em>Keratella</em></td>
<td>May, December</td>
</tr>
<tr>
<td><em>Collotheca</em></td>
<td>July, March</td>
</tr>
</tbody>
</table>
### Table 19. Cladocerans in the upper 45 meters of water column, station 5

<table>
<thead>
<tr>
<th></th>
<th></th>
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<td>32</td>
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<td>376</td>
<td>162</td>
<td>464</td>
<td>226</td>
<td>140</td>
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</tbody>
</table>

Numbers of cladocerans/m$^2 \times 10^3$

### Table 20. Copepods in the upper 45 meters of the water column at station 5

<table>
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<tr>
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<tbody>
<tr>
<td>Cyclops bicuspidatus thomasi</td>
<td>106</td>
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<td>Mesocyclops edax</td>
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<tr>
<td>Diaptomus siciloides</td>
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<td>77</td>
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<td>29</td>
</tr>
<tr>
<td>Diaptomid copepods</td>
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<td>993</td>
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<td>843</td>
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<td>200</td>
<td>1040</td>
<td>195</td>
<td>318</td>
<td>1307</td>
<td>830</td>
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</tbody>
</table>

Numbers of copepods/m$^2 \times 10^3$
The copepod population had three periods of abundance. The summer period was marked by 3 months of relatively high numbers (table 20). This was due to an exchange of dominance between the two cyclopoid copepods. The other two dominant periods occurred in October/November and March/April. All three periods had a different species composition.

The predaceous cyclopoid, *Cyclops bicuspidatus thomasi*, (McQueen [58]) was monocyclic, remaining dominant from February into July. During the month of July it was replaced by another predatory species, *Mesocyclops edax* (Confer [59]). *M. edax* remained the dominant cyclopoid from July through December.

The two species of *Diaptomus*, *D. clavipes* and *D. siciloides*, never obtained the numerical dominance of the cyclopoids. *D. siciloides* reached its highest population in November, when it represented over 30 percent of the adult copepod community. This was more than five times the numbers reached by either *Cyclops bicuspidatus thomasi* or *D. clavipes*.

*D. siciloides* was dicyclic, first appearing in July and reaching its peak population in November; another peak appeared in March. The samples for June 16 and July 31 will be reexamined to determine what percent of the *Diaptomus* population was actually *D. siciloides*. This will also allow for a better interpretation of the *D. clavipes* population, which at this time appears to have no real period of abundance.

In general, copepods have six nauplii instars, five juvenile instars (copepodids), and a single adult stage. The *Diaptomus* nauplii and the *Cyclopoid* nauplii were grouped together in the counts.

**Vertical migration.**—Diurnal vertical migration of zooplankton is a well-known and well-documented phenomenon. Hutchinson [33] describes three types of vertical migration: (1) nocturnal migration, with a single maximum in the upper stratum at night, (2) twilight migration, with a maximum at dawn and dusk, and (3) reverse migration, with one maximum at the surface at midday. Possible explanations for the causes of the migrations include light, temperature, pH, conductivity, and predator avoidance. Pennak [60] concluded light to be the single most important factor.

The vertical range of the migration varies from species to species and from lake to lake. Campbell [61] found that physical and chemical factors in Douglas Lake were able to restrict the range of migration. He noticed that as the zone of hypolimnetic oxygen depletion increased during the summer, the rotifer population was constricted into the upper 15 m of the water column.

The rotifer population of Boulder Basin undergoes vertical migration during both thermally stratified and unstratified periods. Staker [62] found the average range of *Keratella cochlearis* and *Polyarthra* in Boulder Basin during January to be 4.8 and 2.6 m, respectively. These amplitudes of vertical migration agree with those discussed by Pennak [55]. He states that amplitudes of 1 to 3 m are common, with amplitudes of 8 to 10 m being very unusual. George's and Fernando's [63] results disagree with Pennak's average. Their report covered a 1-year period and showed the vertical amplitudes to vary between summer and winter. The data they present shows *Polyarthra vulgaris* to migrate 1.8 to 4.8 m in February, 3.0 to 4.1 m in April, 7.0 to 10.0 m in June, 3.0 to 8.6 m in July, and 4.8 to 8.4 m in August.

The Lake Mead population of *Polyarthra* shows similar patterns to those listed above. Figure 6 shows the vertical profiles of *Polyarthra* for three consecutive samples. The vertical amplitudes in May and June were about 5 to 10 m and similar to the results reported by George and Fernando [63]. In July, when thermal stratification was well established and an oxygen minimum was present in the metalimnion, *Polyarthra* was restricted to the upper 20 m of the lake, possibly because of the low oxygen concentrations.

Thermal stratification appears to have an effect on the vertical distribution of other rotifers in Boulder Basin (fig. 7). Both winter and summer *Asplanchna* populations had high concentrations in the upper 10 m of the water column. The summer population was more or less restricted to this region, but the winter population extended down to a depth of 35 m. *Collotheca*
also showed a different profile for winter and summer. While summer populations of Collotheca showed highest concentrations in the top 10 m, it was rare to find any of this genera above 10 m during the winter. This rotifer is encased in a mucilaginous sheath and is considered to be a poor swimmer. Winter populations appeared to have a considerable amount of detritus and particulate matter adhered to this sheath, more so than that found on the summer organisms. Syncheata showed vertical distribution similar to that of Asplanchna. Organisms were concentrated in the top 10 m, extending down to 20 m in the summer. Winter populations ranged from 5 to 45 m, with the concentrations being highest at 15 m.

Vertical profiles for the cladocerans are shown in figure 8. Accurate values for the vertical amplitude are difficult to obtain with samples taken at 5-m intervals. Staker [62] reported that Bosmina migrated 4.7 m. While figure 8 shows some possible migration, the bulk of the population remains between 10 and 20 m. The sunrise sample indicates that the bulk of the population may have been between sampling depths. Both cladocerans showed differences in summer and winter profiles (fig. 9) similar to the rotifers.

The vertical amplitudes of migration for the copepods is generally believed to be much greater than that of the rotifers and about equal to that of the cladocerans. Staker concluded that the copepods of Boulder Basin did not migrate during the winter. His data did not report any breakdown of instars of these copepods. Marshall and Orr [64] report that the juvenile instars of the marine copepod, Calanus finmarchicus, showed different vertical ranges and patterns from those of the adults for both winter and summer populations. This was evident in the summer populations of copepods from Lake Mead. A comparison of copepod nauplii, cyclopoid copepodids I-V, and C. b. thomas from early July showed the vertical migration to be increasing with each group, respectively, with the nauplii undergoing the least amount of vertical change (fig. 10).

Data from the July 31 sample shows a different pattern. C. b. thomas had its highest density for the sunset sample at a depth of 15 m. Mesocyclops edax had its greatest
Figure 8.-Vertical migration of cladocerans at station 5, Boulder Basin.

Figure 9.-Vertical distribution winter and summer cladoceran populations, sunset samples, station 5, Boulder Basin.

Figure 10.-Vertical profiles of copepod nauplii, cyclopoid copepodids, and Cyclops bicuspidatus thomasi at station 5, Boulder Basin, July 1975.
concentrations at the surface at this time. The cyclopoid copepods showed a peak at each of these depths, indicating different patterns for these two species (table 21).

Diaptomus populations were generally low, but a comparison of the winter and summer profiles indicated that a more confined distribution exists during thermal stratification (fig. 11). Comita and Anderson [65] report a similar observation. They found more diaptomids in the epilimnion than in the hypolimnion of Lake Washington during times of thermal stratification.

**Depth affinities.**—Figures 6 through 11 depict vertical distribution relative to vertical migrations, but they also illustrate the preference of these organisms for specific zones of the water column. The rotifers showed summer concentrations restricted to the upper 20 m. The cladocerans and copepods showed a preference for the 10- to 25-m layer during summer stratification. Marshall and Orr [64] attempted to explain this preference as it applies to copepods which migrate into and through this layer. They suggest that vertical migration is a means by which the animal can sample fresh layers of water, and these organisms may be able to stop at layers where optimal conditions exist (e.g., food and light).

In general, the herbivorous members of the zooplankton community show a definite affinity for the metalimnion during summer stratification (fig. 12). These data show that the zooplankton do maintain a substantial population within the metalimnion during thermal stratification.

**Diapause.**—Brief mention of resting stages has been made in this report. The occurrence of this phenomenon is known for a variety of common freshwater zooplankters. Some organisms develop resting eggs, while others may have cystlike stages similar to pupal stages of insects. The ability of an aquatic organism to exist in a dormant state is generally considered to be a successful adaptation to its environment. This would allow the species to withstand periods of stress such as summer drought, anoxic water conditions, and cold winter periods of low food availability. Species from each major group of zooplankton from Boulder Basin have been found to exhibit some type of resting stage.

In the rotifers, both *Syncheata* and *Polyarthra* were found hatching from resting stage eggs. Resting eggs of *Syncheata* first appeared in the October 30 samples, with higher numbers being found in the November 19 samples. Vertical oxygen profiles showed that rapid mixing occurred during the October/November period. The water column was completely mixed from surface to a depth of 16 m on October 1, to 20 m on October 15, to 29 m on October 30, and to 40 m on November 19. This mixing resuspended these eggs into the water column where they developed into the adult forms. The resting egg is different from the summer egg of *Syncheata*, having a thicker outer wall and numerous spines. Pennak [55] gives diagrams of common rotifer resting eggs, illustrating these characteristics.

*Polyarthra* first indicated the presence of a resting stage on July 1, when an apterous form without the paddlelike appendages was found. Edmonson [51] reported this form to be *Polyarthra* developed from a resting egg. It is so unique that taxonomists had originally separated it into a separate genus, *Anarthra*. Many resting eggs and the above-described apterous forms were found in the April samples. These resting eggs of *Polyarthra* also show a thicker wall and numerous shell spines.

Resting eggs from other rotifers in Boulder Basin have not been found.

*Daphnia* resting stages are commonly called ephippia, named so for the resemblance to a saddle. After formation, the ephippia separates from the adult with the next molt. These ephippia usually contain one to three resting eggs (usually two). Stross [66] reports both photoperiod and water temperature to be controlling factors for the development and release of diapause in *Daphnia*. *Daphnia* ephippia were found in the May 1975 and the February through April 1976 samples.

*Bosmina* did not show a resting stage in the samples, but diapause may play an important role in its life cycle. *Bosmina* and *Daphnia* populations occur at different times of the year in Boulder Basin. The fact that *Daphnia* goes into a dormant state may allow the *Bosmina*
population to become abundant. Hammer and Sawchyn [67] concluded that this type of reproductive segregation permits the use and exploitation of an environment by organisms that could not coexist if their reproductive cycles were the same.

Reproductive segregation due to diapause also occurs in the copepods. *Cyclops bicuspidatus thomasi* and *Mesocyclops edax* occur at different times of the year, similar to the cladocerans. *C. b. thomasi* goes into a cyst stage in its fourth instar or copepodid. Cysts of this species were first recorded by Birge and Juday in 1908. Cole [68] found these cysts in anoxic waters rich in H₂S (hydrogen sulphide) and showed them to be insensitive to various respiratory poisons (NaCN (sodium cyanide), NaN₃ (sodium nitrate), and idoacetic acid).

It is not known if *Mesocyclops edax* has an encysted stage, but Smyly [69] reports cysts of the fifth instar of *Mesocyclops leuckarti*. Coker [49] reports these two species to be closely related, with *M. edax* being the most common North American representative of the

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**Table 21.** Cyclopoid copepods at station 5, Boulder Basin, sunset samples

<table>
<thead>
<tr>
<th>Species</th>
<th>Depth, m</th>
<th>July 1</th>
<th>July 31</th>
<th>Aug. 27</th>
<th>Sept. 17</th>
<th>Oct. 15</th>
<th>Nov. 19</th>
<th>Dec. 23</th>
<th>Jan. 29</th>
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<tr>
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Figure 11.-Vertical distribution of early copepod stage, winter and summer populations, sunset samples, station 5, Boulder Basin.

Figure 12.-Depth of maximum density for zooplankton grazers at station 5, Boulder Basin, May 1975 to March 1976.

genus and *M. leuckerti* the generic representative for European waters.

Cole [70] concluded that the resting stage of *Cyclops bicuspidatus thomasi* may make it possible for it to coexist in the same lakes with *Mesocyclops edax*. They commonly occur together in North America, with *M. edax* usually abundant in the summer plankton, while *C. b. thomasi* cysts are abundant in the bottom sediments.

Resting eggs have been found for a few species of *Diaptomus*, but no resting cysts have been reported (Cooley [71]). *Diaptomus clavipes* and *Diaptomus siciloides* were first reported from Lake Mead by Mildred S. Wilson in 1955 (Cole [70]). This association is known from other lakes in this region. Cole reports finding these two species together in large impoundments of the Salt and Gila Rivers of Arizona. He also reports them from a small, newly impounded stock tank in that State. Resting eggs have not been found for either species of *Diaptomus* from Lake Mead.
METALIMNETIC OXYGEN DEPLETION IN LAKE MEAD

Introduction

Metalimnetic waters of Lake Mead show a reduction in D.O. (dissolved oxygen), resulting in a negative heterograde oxygen profile. This condition in Lake Mead was first reported by Hoffman et al. [1]. A negative heterograde oxygen profile was evident in 1944 and low metalimnetic oxygen levels probably have occurred since the formation of the lake. Data collected have shown that: (1) metalimnetic oxygen depletion has been a regular occurrence since 1972, (2) oxygen depletion is always associated with the thermocline, (3) depletion begins in May and continues through September, and (4) hypolimnetic oxygen levels remain high with only minimal oxygen loss during summer stratification.

Midwater oxygen minimums have been related to three possible causes (Shapiro [45]): (1) horizontal movement of low oxygenated water due to sediment uptake from a midwater shelf, (2) density current flows low in oxygen, and (3) oxygen consumption in situ due to biological respiration. The first of these probably does not occur in Lake Mead. There is not an obvious shelf present and the oxygen minimum always develops at the same depth independent of the lake elevation. Density currents have been reported for Lake Mead (Smith et al. [72]). These currents were usually found in the hypolimnion and not in the metalimnion where the oxygen minimum occurs. Hypolimnetic density currents in Lake Mead, if high in D.O., may increase the oxygen levels in the hypolimnion, but do not directly cause the midwater oxygen minimum as Ellis [73] concluded for Elephant Butte Reservoir, N. Mex. Midwater oxygen minimums due to biological respiration have been attributed to seston layering, bacteria, and zooplankton. The vertical distribution of bacteria was examined in 1974. Bacterial counts were low and could not account for the oxygen depletion in the metalimnion. A preliminary investigation of the zooplankton in 1974 revealed high concentrations in the metalimnion. Phytoplankton respiration was also suspected as a possible cause. The vertical distribution of both zooplankton and phytoplankton was examined to determine whether their respiration could account for the metalimnetic oxygen depletion.

Methods

The methods for the collection and counting of zooplankton and phytoplankton have been given in the "Experimental Methods" and "Methods and Materials" sections of this report.

To obtain the total amount of oxygen lost from this area, it was necessary to determine both the amount of oxygen "visibly" lost and the amount of oxygen being transported into the metalimnion through eddy currents. The amount of oxygen being transported into the metalimnion through eddy diffusivity was computed from the following transport equation (Ruttner [74]):

\[ Q_{at} = \frac{D_e (C_1 - C_2)}{L} \]

where,

- \( Q_{at} \) = the quantity of oxygen transported across a given area \( a \) of a horizontal plant (12.5 m) in some period of time \( t \).
- \( D_e \) = the eddy diffusivity or mixing rate.
- \( C_1 - C_2 \) = the difference in oxygen concentrations across this plane.
- \( L \) = the distance between \( C_1 \) and \( C_2 \).

(A practical application of this method is given in Verduin [75]).

Eddy diffusivity in cm²/s was determined from the above transport equation using temperature data, where \( Q_{at} \) was the amount of heat transported across this plane. The observed or "visible" oxygen lost was computed as follows:

\[ \text{Visible oxygen lost} = \frac{\text{mean loss of oxygen for some period}}{\text{number of days in the period}} \]

The quantity of oxygen "visibly" lost plus the quantity of oxygen transported into the
metalimnion equaled the total amount of oxygen to be accounted for.

Calculations for the oxygen consumption of phytoplankton and zooplankton were computed for the metalimnetic populations (depths from 10 to 30 m) based on reported respiration rates for these organisms. Daily respiration for the major components of the zooplankton community (table 22) were determined from Comita [76], Baudouin and Ravera [77], Shapiro [45], Bishop [78], Vollenweider and Ravera [79], Richman [80], Scherbakoff [81], Marshall and Orr [64], Schindler and Noven [82], and Kibby [83]. Phytoplankton respiration was determined for estimated cell volumes. A respiration rate of 74 g/L of algae per day, calculated from data given in Verduin [75], was used for phytoplankton respiration.

Results and Discussion

The vertical distribution of zooplankton has been discussed in the previous section of this report. High concentrations of copepods and cladocerans were always found in the zone of oxygen depletion as shown in figure 13 and total numbers always declined below 30 m. In general, peak zooplankton concentrations remained within the metalimnion over a 24-hour period. This occurred for all species and their juvenile stages, except for Mesocyclops edax, which nocturnally migrated to the surface waters during July and August. Respiration rates were determined from the evening samples (sunset) which represented resident populations in the metalimnion.

Phytoplankton numbers were highest in the upper 10 m and declined with depth. There was never an evident seston layer as indicated by phytoplankton counts. Mean phytoplankton volumes between the 10- and 30-m depths on June 16, July 18, August 20, and August 28, 1975, were 0.81, 0.89, 1.33, and 1.03 mL/m³, respectively.

Oxygen transport ($Q_{at}$) and oxygen "visibly" lost in the metalimnion during summer stratification were calculated from the data presented in table 23. Estimated zooplankton and phytoplankton respiration accounted for 57 to 94 percent of the total amount of oxygen lost during this period (table 24). Overall, zooplankton accounted for approximately 31 percent and phytoplankton accounted for approximately 43 percent of the oxygen lost.

Shapiro [45] reported that copepods alone accounted for 23 to 110 percent of the oxygen lost in the metalimnion of Lake Washington and concluded that copepods were responsible for the metalimnetic oxygen minimum in that lake. The authors are in general agreement with Shapiro's findings, except for his estimated percentage of oxygen consumption by the copepods. Shapiro considered mixing to be minimal and did not determine oxygen transport into the metalimnion. In Lake Mead, mixing results in a substantial amount of oxygen being transported into the metalimnion. For some periods, zooplankton respiration accounted for over 200 percent of the oxygen "visibly" lost. Adding the amount of oxygen transported into the metalimnion to the computations reduced this to a realistic value of 31 percent.

Zooplankton respiration rates were conservative estimates and these rates may be higher than those reported. This may have little effect on the results since Verduin⁴ feels that the mixing rates used in this study are also conservative because

Table 22.—Calculated respiration rates for limnetic zooplankton in Lake Mead at 20 °C

<table>
<thead>
<tr>
<th>Organism</th>
<th>µg oxygen/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copepod nauplii</td>
<td>0.25</td>
</tr>
<tr>
<td>Copepod copepodites (instars I–IV)</td>
<td>0.80</td>
</tr>
<tr>
<td>Cyclops bicuspidatus thomasi</td>
<td>1.25</td>
</tr>
<tr>
<td>(instar V &amp; adult)</td>
<td></td>
</tr>
<tr>
<td>Mesocyclops edax</td>
<td>1.35</td>
</tr>
<tr>
<td>(instar V &amp; adult)</td>
<td></td>
</tr>
<tr>
<td>Diaptomus siciloides (instar V)</td>
<td>1.50</td>
</tr>
<tr>
<td>(adult)</td>
<td></td>
</tr>
<tr>
<td>Diaptomus clavipes (instar V)</td>
<td>4.50</td>
</tr>
<tr>
<td>(adult)</td>
<td></td>
</tr>
<tr>
<td>Daphnia spp.</td>
<td>4.10</td>
</tr>
<tr>
<td>Bosmina longirostris</td>
<td>0.86</td>
</tr>
</tbody>
</table>

⁴ J. Verduin, personal communication.
Figure 13.—Vertical distribution of zooplankton and dissolved oxygen at station 5, July 1975.

Table 23.—Temperature and dissolved oxygen at station 5—June 17 through September 17, 1975

<table>
<thead>
<tr>
<th>Depth, m</th>
<th>June 17</th>
<th>July 2</th>
<th>July 18</th>
<th>Aug. 1</th>
<th>Aug. 28</th>
<th>Sept. 17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temp., °C</td>
<td>D.O., mg/L</td>
<td>Temp., °C</td>
<td>D.O., mg/L</td>
<td>Temp., °C</td>
<td>D.O., mg/L</td>
</tr>
<tr>
<td>0</td>
<td>22.5</td>
<td>10.1</td>
<td>22.5</td>
<td>9.9</td>
<td>24.5</td>
<td>7.4</td>
</tr>
<tr>
<td>5</td>
<td>21.5</td>
<td>10.4</td>
<td>22.5</td>
<td>9.9</td>
<td>24.5</td>
<td>7.4</td>
</tr>
<tr>
<td>10</td>
<td>20.0</td>
<td>9.4</td>
<td>21.5</td>
<td>9.4</td>
<td>23.5</td>
<td>7.2</td>
</tr>
<tr>
<td>15</td>
<td>17.0</td>
<td>7.7</td>
<td>20.0</td>
<td>8.1</td>
<td>20.5</td>
<td>5.9</td>
</tr>
<tr>
<td>20</td>
<td>15.0</td>
<td>7.4</td>
<td>16.5</td>
<td>7.2</td>
<td>17.5</td>
<td>5.1</td>
</tr>
<tr>
<td>25</td>
<td>14.0</td>
<td>7.4</td>
<td>15.0</td>
<td>6.9</td>
<td>15.5</td>
<td>5.0</td>
</tr>
<tr>
<td>30</td>
<td>13.0</td>
<td>7.9</td>
<td>13.5</td>
<td>7.0</td>
<td>14.5</td>
<td>5.3</td>
</tr>
<tr>
<td>35</td>
<td>12.5</td>
<td>8.0</td>
<td>13.0</td>
<td>7.2</td>
<td>13.5</td>
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<td>40</td>
<td>12.0</td>
<td>8.0</td>
<td>12.5</td>
<td>7.5</td>
<td>13.0</td>
<td>6.0</td>
</tr>
<tr>
<td>45</td>
<td>11.5</td>
<td>8.0</td>
<td>12.0</td>
<td>7.7</td>
<td>12.0</td>
<td>6.3</td>
</tr>
<tr>
<td>50</td>
<td>11.5</td>
<td>7.9</td>
<td>11.5</td>
<td>7.7</td>
<td>12.0</td>
<td>6.3</td>
</tr>
<tr>
<td>55</td>
<td>11.5</td>
<td>7.9</td>
<td>11.5</td>
<td>7.7</td>
<td>11.5</td>
<td>5.8</td>
</tr>
<tr>
<td>60</td>
<td>11.0</td>
<td>7.9</td>
<td>11.0</td>
<td>7.7</td>
<td>11.5</td>
<td>5.8</td>
</tr>
</tbody>
</table>
Table 24.—Total amount of oxygen lost (oxygen transported in plus oxygen "visibly" lost) and respiration rates for zooplankton and phytoplankton within the metalimnion

<table>
<thead>
<tr>
<th>Date</th>
<th>1975</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>June 17 to July 2</td>
</tr>
<tr>
<td>Oxygen transports ($Q_{at}$)</td>
<td>118</td>
</tr>
<tr>
<td>Oxygen &quot;visibly&quot; lost</td>
<td>23</td>
</tr>
<tr>
<td>Total oxygen lost</td>
<td>141</td>
</tr>
<tr>
<td>Estimated zooplankton respiration</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td><em>(42%)</em></td>
</tr>
<tr>
<td>Estimated phytoplankton respiration</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td><em>(41%)</em></td>
</tr>
</tbody>
</table>

* Numbers in parenthesis equal the percentage of oxygen possibly consumed due to zooplankton and phytoplankton respiration.

they were computed for biweekly periods. Mixing rates or eddy diffusivity computed on a daily basis are usually higher than those computed over an extended time interval.

Other organisms were found within the metalimnion, and their respiration could possibly account for the remaining amount of total oxygen lost. Respiration rates for the rotifer were not determined because peak concentrations generally were found above 10 m, but individuals did occur within the metalimnion in low concentrations (table 25). Echograms showed large concentrations of shad within the metalimnion during the early morning (fig. 14). The shad were dispersed throughout the epilimnion in the afternoon and were concentrated in a narrow band at the surface nocturnally. While these organisms were present within the metalimnion either in low numbers or for short periods of time, their respiration would result in further loss of oxygen from this area. The results indicated that biological respiration could account for the metalimnetic oxygen depletion and that phytoplankton and zooplankton respiration are the primary causative agents.

Phytoplankton and zooplankton communities in Lake Mead are not strikingly different from other lakes. The primary factor resulting in the negative heterograde oxygen profile is the deep hypolimnion and minimal oxygen uptake of the mud-water interface. Oxygen depletion in the metalimnion affects only the upper portion of the hypolimnion because of reduced turbulence during summer stratification. At station 3, which has a maximum depth of 45 m, and at other shallow areas, a clinograde oxygen curve does develop. If Lake Mead were more shallow, a typical clinograde oxygen profile would probably occur.

**COLIFORM BACTERIA IN LAS VEGAS WASH AND LAS VEGAS BAY**

**Introduction**

The coliform group of bacteria have been generally accepted as indicators of sanitary quality and as standards for general use. These indicator organisms are assumed to indicate the degree of fecal pollution in water.

Standards for coliforms in potable and recreational water have been established by various agencies. Counts of these bacteria may be interpreted to indicate compliance or noncompliance with these standards.

Enforcement of coliform water quality standards depends on the validity of the methods employed for coliform enumeration. This investigation incorporated organism identification (Cowan [84]) and media comparisons to assure reliable estimates of coliform concentration.
Table 25.—Rotifers at station 5, Boulder Basin, July 1, 1975

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1530 HOURS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotifers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asplanchna</td>
<td>5</td>
<td>3.5</td>
<td>11.0</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyarthra</td>
<td>8.0</td>
<td>3.0</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collotheca</td>
<td>4.0</td>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Keratella cochlearis</td>
<td>0.5</td>
<td>3.5</td>
<td>2.0</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keratella quadrata</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syncheta</td>
<td>11.0</td>
<td>10.0</td>
<td>5.0</td>
<td>1.0</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>2030 HOURS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotifers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asplanchna</td>
<td>1.0</td>
<td>3.5</td>
<td>14.0</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyarthra</td>
<td>2.0</td>
<td>10.0</td>
<td>3.0</td>
<td>4.0</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collotheca</td>
<td>1.5</td>
<td>19.0</td>
<td>8.0</td>
<td>5.0</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keratella cochlearis</td>
<td>0.5</td>
<td>3.0</td>
<td>4.0</td>
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<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Keratella quadrata</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syncheta</td>
<td>2.5</td>
<td>18.0</td>
<td>0.5</td>
<td></td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Methods**

*Coliform enumeration.*—Water samples were collected at stations 1 to 3 in a 3-L Van Dorn bottle previously sanitized with methyl alcohol. Samples were transferred to a screwcap, glass bottle, placed on ice, and delivered to the laboratory for enumeration of fecal and total coliforms by the membrane filter procedure (American Public Health Association [85]).

*Coliform survival in sediments.*—Four gallons of water were collected, at a point just above the convergence of LVW (Las Vegas Wash) with LVB (Las Vegas Bay), in previously sanitized glass collection bottles, placed on ice, and delivered to the laboratory. All four samples were combined and 1 L was placed in each of four Imhoff funnels. The sediments were allowed to settle and incubated at 25 °C. A fifth aliquot was shaken and evaluated for total coliforms by the membrane filter procedure. Two days after collection, two Imhoff funnels were evaluated for coliform concentrations in supernatant and sediment by the membrane filter procedure. Fifteen days after collection, the remaining Imhoff funnel was enumerated for total coliforms in both sediment and supernatant by the membrane filter procedure.

**Results and Discussion**

Both fecal and total coliform populations were low in April 1975. An increase in their incidence was noticed in May and continued through June (table 26).

During July 1975, a flash flood damaged the city of Las Vegas sanitation plant. The flood resulted in greatly increased coliform concentrations in LVW and LVB. However, during this period higher concentrations of enteric bacteria were found to be *Erwinea herbicola* and *Klebsiella pneumoniae*. *E. herbicola* is generally associated with plant galls, while *K. pneumoniae* is found in association with root systems along with fecal material (Cowan [84]). Because such a large volume of water came down the Wash during this period, and because the largest number of enteric organisms found may have been associated with plants, the source of these bacteria may have been from the marsh system and not from the sanitation plant. However, this observation is conjecture because sampling was not continuous during the breakdown of the sanitation plant.

Prior to the July 4, 1975 flash flood, lake drawdown caused erosion and redistribution of
sediments. It was felt that sediment redistribution may resuspend sediment-bound organisms of coliforms entering LVB via LVW. However, the persistent effects from the flood curtailed continued examination.

In September, coliform counts were low with the exception of station 2 at 10-m depth. Relatively high counts were obtained in October and November. Counts were low in December but an increase was noted in January 1976. However, February and March 1976 coliform counts were low and comparable with April 1975.

Normally, the FC/TC (fecal to total coliform) ratio is less than one; however, FC/TC ratios of greater than one were found at various times and locations during the year (table 26). Causes for the noted anomalies have not been determined. Technical procedures have been eliminated as a causative factor. Possible inhibitory and toxic effects of plating media (type m-Endo m-FC) have not been eliminated conclusively and, therefore, are still implicated.

Data from the current study and from the 1974-75 monitoring program indicate that a significantly greater survival rate for K. pneumoniae than E. coli occurred on these media. A comparison between type m-Endo and type m-Endo-LES (table 27) indicates a significantly greater survival of coliforms plated on Endo-LES. Obviously, further investigation is required to determine which of the recommended coliform media is least detrimental to coliform survival.

Samples collected in LVW from Sunrise power station to the convergence of LVW with LVB resulted in relatively low fecal and total coliform counts (table 28). However, samples were collected between 7 and 10 a.m. and may not
Table 26.—Fecal and total coliform bacteria in Las Vegas Wash and Las Vegas Bay

<table>
<thead>
<tr>
<th>Station</th>
<th>Depth, m</th>
<th>April 28, 1975</th>
<th>May 16, 1975</th>
<th>May 18, 1975</th>
<th>June 18, 1975</th>
<th>July 22, 1975</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fecal</td>
<td>Total</td>
<td>Fecal</td>
<td>Total</td>
<td>Fecal</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>60</td>
<td>80</td>
<td>800</td>
<td>*230</td>
<td>300</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td>*2</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>40</td>
<td>*30</td>
<td>0</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
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<td>10</td>
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<td>20</td>
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<td>40</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>July 31, 1975</td>
<td>1700</td>
<td>1900</td>
<td>700</td>
<td>2000</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Aug. 19, 1975</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>*2</td>
<td>30</td>
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<tr>
<td></td>
<td>Aug. 28, 1975</td>
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<td>0</td>
<td>7</td>
<td>25</td>
<td>10</td>
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<td></td>
<td>Sept. 17, 1975</td>
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<td>0</td>
<td>8</td>
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<td>150</td>
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<tr>
<td></td>
<td>Oct. 16, 1975</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
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<td>Nov. 24, 1975</td>
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<td>0</td>
<td>40</td>
<td>0</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>Dec. 22, 1975</td>
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<td>120</td>
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<tr>
<td></td>
<td>Jan. 30, 1976</td>
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<td>0</td>
<td>0</td>
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<td>10</td>
</tr>
<tr>
<td></td>
<td>Feb. 19, 1976</td>
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<td>0</td>
<td>0</td>
<td>1</td>
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<tr>
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<td>Mar. 30, 1976</td>
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<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

* Indicates fecal coliforms greater than total coliform bacteria.
Table 27.—Media comparison of type m-Endo, m-Endo-LES, and m-FC

<table>
<thead>
<tr>
<th>Depth, m</th>
<th>Station 1</th>
<th>Station 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m-Endo</td>
<td>m-Endo-LES</td>
</tr>
<tr>
<td>0</td>
<td>2.19 x 10³</td>
<td>7.6 x 10³</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 28.—Total and fecal coliforms in Las Vegas Wash

<table>
<thead>
<tr>
<th>Location</th>
<th>Total coliforms</th>
<th>Fecal coliforms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Numbers/100 mL</td>
<td></td>
</tr>
<tr>
<td>Sunrise power station</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Las Vegas sewage plant</td>
<td>48</td>
<td>18</td>
</tr>
<tr>
<td>Clark County sewage plant</td>
<td>37.5</td>
<td>20</td>
</tr>
<tr>
<td>Pabco road</td>
<td>84</td>
<td>24</td>
</tr>
<tr>
<td>Above gravel pit</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gravel pit</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Las Vegas Bay estuary</td>
<td>46</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 29.—Survival of sediment bound coliforms from Las Vegas Wash

<table>
<thead>
<tr>
<th>Days</th>
<th>Supernatant</th>
<th>Sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Numbers/100 mL</td>
<td>Numbers/100 mL</td>
</tr>
<tr>
<td>0</td>
<td>520</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>140</td>
<td>800</td>
</tr>
<tr>
<td>15</td>
<td>9.2</td>
<td>6.72 x 10³</td>
</tr>
</tbody>
</table>

have been during peak flow periods from any of the sanitation plants. Further sampling of LVW should be conducted during peak flow periods and extended over a period of several months to determine the major sources of coliform introduction to LVW. Sample points should also include the contribution of marsh areas as well as other streams flowing into LVW.

The incidence of *Salmonella* and *Shigella* in LVW and the inner bay of LVB was determined during the July 1975 flash flood. Although *Salmonella* and *Shigella* cannot be determined quantitatively, their presence or absence can be determined. *Salmonella* was detected at stations 1 and 2.

The survival of sediment bound coliforms was significant (table 29). Although results from the laboratory study performed cannot be applied directly to a lake situation, certain empirical conclusions may be reached: (1) Coliforms survive significantly longer in sediments, (2) the initial coliform population will increase in size in nutrient-rich sediments, and (3) resuspended bottom sediments may be a cause for increased coliform densities in LVB.

**DISTRIBUTION OF ENTERIC BACTERIA IN LAS VEGAS BAY**

**Introduction**

The utility of oxidase positive bacteria for tracing water distribution patterns has been established (Tew et al. [86]). Although these bacteria are a large component of the bacterial population, there is another component of the population composed of oxidase negative bacteria, among which are included those *Enterobacteriaceae* of special medical significance (*Salmonella, Shigella, Klebsiella, Yersinia*).

Also, media used for the isolation of oxidase negative bacteria are inhibitory and usually result in low population estimates, while in fact these bacteria may be present in numbers much higher than one would expect.

For these reasons it was decided to look at the oxidase negative bacteria as a means of determining water distribution patterns. Data concerning their deposition in the lake could perhaps be obtained simultaneously.
Previous students of water distribution patterns did not use a confirmed method to substantiate their results (Hoganson and Elliot [87], Losane et al. [88], McFeters et al. [89], Storey et al. [90]). However, a study conducted in 1899 (Jordan [91]), to determine the extent of natural purification of fecal bacteria by lakes and streams, did utilize chlorine and fluorescein dye as a confirming technique in conjunction with bacterial sampling. Although the supporting methods did not correlate directly with bacterial concentrations, they did indicate water distribution patterns from a specific sewage source.

Fluorescent dyes, because of their relatively high fluorometric detectability, should provide an excellent precursor for bacterial sample collections. An instantaneous or "slug" injection into a moving body of water should produce a well-defined dye peak that could be easily monitored by fluorometric methods as the dye moves away from the point of injection (St. John [92] and Storey et al. [90]). The dye peak would indicate water velocity, dilution rate, and provide a signal to assure sampling of a specific bacterial population as it progresses away from an initial sampling point.

Inhibitory effects of differential media on oxidase negative bacteria have been discussed extensively in the literature (Bascomb et al. [93], Dufour and Cabell [94], Hartman et al. [95], Hoganson and Elliot [87], McCoy and Seidler [96], and Ray and Speck [97]). The results of these investigations indicate that utilization of noninhibitory media may be instrumental in isolation of far greater numbers of oxidase negative bacteria than could be achieved with differential media. Obviously the use of noninhibitory and nondifferential media would make characterization of the enteric genera more difficult, but replica plating methods (Lederberg and Lederberg [98]) and biochemical characterization on initial isolation plates (Dufour and Cabell [94]) might facilitate characterization greatly. Difficulties in enteric identification created by the use of noninhibitory media would be far outweighed by the increased harvest of these bacteria.

The distance to which bacterial tracing can be accomplished with confidence will depend on the ITP (initial tracer population) amplitude and duration. Past data have indicated that ITP may last from a few minutes to several days. Also, the concentration of enteric bacteria may vary from 0/100 mL (Las Vegas Wash sample) to as high as 10^4/100 mL. Previous studies (Tew et al. [86]) have demonstrated that individual tracer bacteria concentrations of greater than 10^4/mL over a finite period of time are required for extensive tracing of water distribution patterns in LVB (Las Vegas Bay); i.e., to overcome the noise created by a given count of indigenous lake bacteria, and to obviate sedimentation and biological decay with specific reference to enteric bacteria. Other precepts were that tracer bacteria should not be indigenous to the lake and should appear in LVW (Las Vegas Wash) periodically. Regarding the latter there is also the possibility that resuspension of sediment bound organisms may perturb, to some extent, ratios of organisms found (Cook et al. [99], Grimes [100], Hendricks and Morrison [101], and Hendricks [102, 103]).

Previous studies conducted in Lake Mead indicate that there may be residual population of these organisms present in the lake at all times. Assuming this is true, the entering enteric population from LVW must be in concentrations high enough to overcome the noise created by the residual enteric populations for any extensive tracing in LVB.

Enumeration of the individual genera and species in the population may serve as a second confirmatory means to assure that the same incident population is being sampled at all times. Also, this is an excellent way of studying the concept that tracers need not be intermittent to be used if two or more of them vary in relative numbers consistently over a given period of time (Component Ratio Concept). For example, if organism A is usually twice as numerous as B, then suddenly, for two weeks or so becomes half as numerous as B, then the change in ratio constitutes a traceable situation.

Materials and Methods

Sampling locations were selected from midstream of LVW to a point approximately 1400 m below the convergence of the wash with LVB. The sampling locations are illustrated on figure 15.

Dye injection and detection. - The 3.785 L of Rhodamine WT, which was determined to be the
Figure 15.—Sample point locations in Las Vegas Wash and Las Vegas Bay.
same density as the water in the Wash (1.025 g/mL), was placed in a glass container, suspended in midstream of LVW at sampling point 1 and allowed to equilibrate for 30 min. The container was then broken and an instantaneous dye injection was achieved.

Because of the shallow water at sampling point A (less than 0.3 m), the boat containing the fluorometric detection equipment could not be used for continuous sampling of the dye peak. Grab samples were collected at 5-min. intervals and returned to the boat for analysis. Samples were collected until 15 minutes after the dye peak passed the sampling point. Detection of the dye was accomplished using a Turner Model No. 111 Fluorometer, the output of which was recorded on a stripchart recorder. At sampling points G and 2, the complete dye envelope was monitored and recorded. This was accomplished by suspending a hose into the water column at density current depth, a depth established by conductivity readings and pilot dye studies. The hose was connected to a d-c self-priming pump, the output of which was connected by 9.5-mm tygon tubing to the flowthrough cell of the fluorometer.

Bacterial sample collection.-Bacterial samples were collected at the dye injection point 15 minutes after dye injection and 15 minutes after dye peak arrival at all other sampling points. The samples were collected in this manner to ensure against the possible toxic effects of the dye on the organisms. Also, this method theoretically would assure continuous sampling of a discrete bacterial population at each sampling point.

Samples collected at sampling points 1 and A were collected using a 3-L Van Dorn bottle previously sanitized with methyl alcohol. Because of the shallowness of the water at 1 and A and because of the rapid flow, only one subsurface grab sample was taken. Samples collected at sampling points G and 2 were collected with a previously sanitized 3-L Van Dorn bottle. Samples were collected at the surface and at a depth of 3.5 m at G, and at the surface and at a depth of 10.5 m at 2 (samples collected at depths of 3.5 and 10.5 m had been previously determined as the vertical center on the density current at these sampling points). Immediately upon collection, the bacterial samples were transferred to glass screwcap bottles, placed on ice, and transported to the laboratory for analysis. Note that the time from collection to initial isolation of the bacterial samples did not exceed 3 hours.

Bacterial isolation.-Initial isolation of the samples was by the membrane filter method (American Public Health Association [85]). Sample volumes filtered were 100 to 0.0001 mL in dilutions of 10. All dilution volumes less than 1 mL were obtained by diluting 10:1 in 9 mL peptone water dilution blanks. The samples were filtered in two sets of five replicates for each dilution.

One set of filtered samples was placed on pads saturated with m-Endo media and then incubated at 35 °C for 24 hours. The remaining set of filtered samples was placed on pads saturated with NB (nutrient broth) and incubated at 27 °C for 48 hours (Dufour and Cabell [94]). After 24 hours, the membranes incubated on m-Endo were enumerated for typical coliform colonies of dilutions containing 7 to 70 colonies per plate. Typical metallic sheened colonies were isolated onto type EMB (eosine methylene blue) agar plates for tentative identification and culture purification. All colonies demonstrating typical characteristics were then identified as to species. The second set of plates was enumerated for total bacterial growth at the end of incubation, replica plated onto VRB (violet red bile) agar (Lederberg and Lederberg [99]), and incubated at 35 °C for an additional 24 hours. After incubation, the plates were enumerated for typical colonies. All typical colonies were isolated onto EMB agar for colonial purification. Both typical and atypical colonies were then identified as to species.

Type m-Endo media was included in this experiment as a standard referenced method. Also, note that identification of isolates from each of the media would result in information concerning its specificity, or inhibitory action, for certain tribes or specific genera of the enteric group.

Identification of isolates.-All isolates were first evaluated for their oxidase reaction. Colonies growing on filters incubated on sugar-free NB were subjected to the oxidase test of Daubner and Mayer (Dufour and Cabell [94]), with the test reagent being applied directly to absorbent pads and placing the filter on the pad. All colonies demonstrating oxidase negative reactions were
enumerated and their corresponding position on the replica plate was recorded. Isolates from m-Endo were transferred to duplicate NB slants and oxidase reactions were performed in the slant only. Pure cultures of isolates from VRB were also transferred to NB slants and oxidase reactions were performed in the slant only. Pure cultures of isolates from VRB were also transferred to NB slants and the oxidase test was reconfirmed. All isolates were tested for gram reactions and their ability to ferment dextrose. All isolates exhibiting oxidase negative, gram negative, and dextrose fermentation characteristics were subjected to the multimedia identification scheme.

Results

The dye peak was easily monitored; its transit time from initial injection to sampling point A was 45 minutes. The distance from sampling point 1 to A is 1200 m; thus, water velocity from 1 to A was 0.444 m/s.

From sampling point A to G dye transit time was 110 min. The distance from sampling point A to G is 400 m and the water velocity is $6.06 \times 10^{-2}$ m/s. The force required for this deceleration, assuming the density of the water is 1.025 g/mL, is calculated by the formula $F = \Delta v / \Delta t \cdot m$, is $5.8 \times 10^{-8}$ newton.

The water column temperature at G is isothermal from surface to bottom. Obviously, if the energy of deceleration is not given up as heat, it must be conserved as turbulence resulting in redistribution of bottom sediments.

Transit time of the dye from sampling point G to 2 was 4 hours 5 min, representing a distance of 1000 m and a water velocity of $6.06 \times 10^{-2}$ m/s. Between points G and 2, the density current encounters a significantly larger volume of water than was encountered prior to reaching point G; yet in the 1000 m between points G and 2, there was no decrease in water velocity. This would indicate that the turbulent water is not mixing as it penetrates further into the lake, but acts as though it is being held in a tight column, a situation not unlike that in a pipe. (An attempt was made to trace the dye peak further into LVB, but it was never detected beyond sampling point 2).

No attempt was made to determine the dye concentration at sampling point A, but concentrations at sampling points G and 2 were 1.035 and 0.44 mg/L, respectively. The dye dilution factor indicated at sampling point G was $9.13 \times 10^{11}$ to 1 while at point 2 the dilution factor was $2.28 \times 10^{12}$ to 1. The increase of dilution from point G to 2 was only a factor of approximately 2:1, which appears to substantiate the theory that the density current is relatively unaffected as it proceeds from G to 2. The leading edge of the dye envelope was much sharper at point G than at point 2 (fig. 16). The sharp peak at G and the decreased slope of the envelope at 2 indicates decreasing turbulence from G to 2. Note that the dye duration is approximately 4 min shorter at 2 than at G.

Because of the high background fluorescence in the lake, the actual leading and trailing edges could not be determined with a great deal of accuracy. The dye envelope may in fact be significantly longer than indicated fluorometrically. The shape of the dye peaks indicate that there is more turbulent flow at sampling point G than at 2.

The apparent dye peak broadening would seem to preclude the coincident sampling of any incident population initially correlated with dye in the stream. In fact, bacterial samples collected at sampling points did not reflect the same dilution pattern as the dye (table 30). Between sampling points A and G, there was a decrease in bacterial concentrations (both total bacteria and enteric bacteria), and between points G and 2 there was an increase in the bacterial populations.

Surface samples collected at sampling points G and 2 contained bacterial concentrations of the same order of magnitude, which indicates that there may be a residual population in the lake at all times. Prior to this investigation a bacterial study was conducted between sampling points A and G (table 31) during a period when enteric concentrations were approximately $1.4 \times 10^4/100$ mL (determinations were on the basis of m-Endo isolation media) and during this time, the surface concentrations were of the same order of magnitude as when incoming concentrations of enterics were at $5.6 \times 10^2/100$ mL. There was, however, a
change in the ratios of the component species in the population.

Total bacterial concentrations were consistently higher than enteric concentrations isolated on either VRB or m-Endo. Enteric concentrations were consistently higher on replica-plated VRB than on m-Endo. There was a slight error in the enumeration of oxidase negative bacteria based on the results of the oxidase reaction on colonies grown on NB media. A significant number of the oxidase negative colonies were actually very weak oxidase positive bacteria. Therefore, the differential count of total bacteria versus enteric bacteria was similarly affected.

The comparison between m-Endo and VRB media (to determine the extent to which m-Endo was inhibitory to enterics) was a complete success. Data concerning total numbers of enterics isolated on each of the media confirmed literature conclusions that VRB was less inhibitory. However, data on the specific genera affected to the greatest extent were not determined because of the choice of EMB as a purification medium. Although widely used as a recommended medium for this purpose, EMB turned out to be as inhibitory as m-Endo to most of the isolates from VRB and resulted in a markedly reduced percentage of identified colonies. This is another example of the unreliability of thoroughly referenced methods. However, subsequent identification procedures using standard multimedia techniques were eminently satisfactory.

Discussion

Optimal tracing of bacterial distributions depends primarily on the detection of a unique population component present over a finite period of time consistent with requirements predicted from the systems analysis.

An intensive search revealed no unique component in the bacterial population. Because greater than 500 isolates were identified, this conclusion is eminently justified. The results did indicate that varying ratios of consistently present species might be equally useful.

The results also provided an excellent picture of the enteric bacteria actually present, a result quite significant for public health evaluation.

For example, consider the high numbers of *Klebsiella pneumoniae*, a known pathogen.

**Table 30.—Counts of total bacteria versus enteric bacteria at sample points A, G, and 2.**

<table>
<thead>
<tr>
<th>Sample point</th>
<th>Depth, m</th>
<th>Total bacteria per 100 mL (NB)</th>
<th>Enteric bacteria per 100 mL (VRB)</th>
<th>Enteric bacteria per 100 mL (m-Endo)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(\bar{x}) (\delta)</td>
<td>(\bar{x}) (\delta)</td>
<td>(\bar{x}) (\delta)</td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td>(3.73 \times 10^7) (2.82 \times 10^6)</td>
<td>(1.04 \times 10^6) (2.79 \times 10^5)</td>
<td>(5.6 \times 10^2) (5.5 \times 10^1)</td>
</tr>
<tr>
<td>G</td>
<td>0</td>
<td>(9.5 \times 10^4) (2.13 \times 10^4)</td>
<td>(6.8 \times 10^3) (4.9 \times 10^3)</td>
<td>(0) (0)</td>
</tr>
<tr>
<td>G</td>
<td>3.5</td>
<td>(6.7 \times 10^5) (8.52 \times 10^4)</td>
<td>(8.8 \times 10^4) (1.67 \times 10^4)</td>
<td>(78) (16)</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>(8.8 \times 10^4) (5.5 \times 10^3)</td>
<td>(7.0 \times 10^3) (3.3 \times 1.0^5)</td>
<td>(3.4) (1.14)</td>
</tr>
<tr>
<td>2</td>
<td>10.5</td>
<td>(1.06 \times 10^7) (5.45 \times 10^5)</td>
<td>(5.8 \times 10^5) (1.79 \times 10^5)</td>
<td>(24) (11.4)</td>
</tr>
</tbody>
</table>
Table 31.—Counts of enteric bacteria at sample points A and G during a period of high concentration in Las Vegas Wash

<table>
<thead>
<tr>
<th>Sample point</th>
<th>Depth, m</th>
<th>Enteric bacteria per 100 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>$1.12 \times 10^4$</td>
</tr>
<tr>
<td>G</td>
<td>0</td>
<td>$22.5$</td>
</tr>
<tr>
<td>G</td>
<td>3.7</td>
<td>$5.44 \times 10^3$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$1.68 \times 10^3$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$3.6$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$4.3 \times 10^2$</td>
</tr>
</tbody>
</table>

Present in the Wash (fig. 17). From a public health point of view, it is fortunate that greater than 50 percent of these organisms are sedimented out at the convergence of LVW with LVB. Also it is equally important that large numbers of dysentery (Shigella sp.) and enteric fever (Salmonella sp.) bacteria were not found, although they have been demonstrated to be present in numbers far less than an infectious dose ($10^6$) per unit volume of water. Additional work concerning the fate of sedimented pathogens is strongly advised.

Returning to the basic discussion of water and bacterial distributions, it is felt that differential count of total numbers of LVW enterics versus oxidase positive bacteria, and the proportionate distribution of constituents comprising LVW and residual lake enteric populations provided a valid tracing procedure.

The preceding conclusion was fortified by coincident substantiating experiments conducted with Rhodamine WT. Both the bacteria and the dye (1) went to the same place, (2) were influenced by similar factors, turbulence and sedimentation (biological decay was an additional factor affecting bacteria only), and (3) both were reduced in their respective concentrations. However, the bacteria were affected to a greater extent than Rhodamine WT. The results, however, demonstrate that this unfavorable relative reduction could be more than compensated for by the use of bacteriological procedures of much greater sensitivity.

Inhibitory effects of media.—It is abundantly obvious that the inhibitory nature of the standard media totally counterindicates their further use and also thoroughly justifies the concern of this study with optimized techniques.

The inhibitory effects of m-Endo to bacteria have been well documented (Bissonnette et al. [104], Cook [99], Dufour and Cabell [94], Klein and Wu [105], Knittel [106], Scheusner et al. [107], and Warneck et al. [108]). VRB, on the other hand, has demonstrated a capability of supporting the growth of much greater numbers of enteric bacteria than m-Endo. A comparison of counts of enterics obtained from both m-Endo and VRB is clearly indicative of the inhibitory effect of m-Endo. The inhibitory aspect of the media appears to be associated with dye concentrations in the formulation.

Dye concentrations of $1:100,000$ will inhibit gram-positive bacteria while allowing gram-negative organisms to grow. Obviously the $1.05 \text{ g/L} (105 \text{ parts per 100,000})$ of basic fuchsin would be highly selective and severely limit the survival of enteric bacteria that do not readily ferment lactose. VRB, however, contains only $0.2 \text{ part per 100,000}$ of crystal violet, a dye concentration that would be much less inhibitory to slow and nonlactose fermenting enteric genera.

When the dilution volumes of VRB and m-Endo plates selected for enteric enumeration are examined, it is apparent that dilution volumes were much smaller on VRB, yet this medium resulted in a significantly much higher count of enteric organisms. This also indicates that there may be far greater numbers of slow or nonlactose fermenting enteric tracers than there are lactose fermenting tracers. These results may also indicate that there may be more stressed or damaged enterics in the environment than are detectable with methods and media generally accepted as standard.

The results show that significantly fewer transfers of isolates from VRB grew on EMB than those transferred from m-Endo. In the case of EMB the high concentration ($65 \text{ parts per 100,000}$) of methylene blue dye is implicated. The few organisms already growing in the poisonous environment of Endo could withstand the similar concentration of dye in EMB.
The inhibitory effects of EMB on isolates taken from VRB may have been amplified during incubation. The environment of the incubator may have had a tendency to dehydrate the media. Dehydration would increase the dye concentration and thus increase its inhibitory action toward slow or nonlactose fermenting enterics.

Public health implications. Public health aspects relative to these results are clear. The large number of undetected enteric bacteria (with standard procedures) indicates a possible potential public health hazard which if continued undetected could reach infectious concentrations. This indicates that at the very least a critical review of currently accepted standards and procedures should be initiated.

Dilution effects. Examination of the dye envelope (fig. 16) indicates an overall broadening and amplitude attenuation as it proceeds from sampling point G to sampling point 2. It seemed that bacterial numbers and component distributions should follow the same trend. The lengthening and attenuation of bacterial numbers would have the same effect as dilution in decreasing the numbers of an incident population. Therefore, decreases in enteric tracers may not be caused by broadening.

Component ratio concept. The graphic display (fig. 18) of the component genera of the lactose fermenting enteric tracers suggests that there could be no tracing of these bacteria much beyond sampling point G, where tracer concentrations are a factor of 10 times greater than background. Sampling point 2 appears to be approaching the limit of detectability for the tracers utilized. At this point, while still a detectable entity, they are of the same order of magnitude as background concentrations.

In applying the component ratio concept, it was found that it held to an amazing degree to G. This is especially significant in view of the enormous energy change, decreases in velocity, and dilution effects found in this region. This new concept may have great potential for future application with more sensitive methods.

Limits of the component ratio concept. Enteric tracer bacteria entering the lake via LVW are also
indigenous to the lake and a residual population may be present at all times with some variation in the concentrations of the component genera. Therefore, to utilize enteric bacteria as tracers, lower numerical limits must be preestablished for detection and also for the entering concentration of tracer. The precept that individual genera or ratios must be intermittent should be applied. For example, the data collected during this investigation indicate in one instance an entering tracer population whose component genera are in concentrations of 1 and 2 x 10^2/100 mL and a background population of 3 and 7/100 mL in lake water. Thus, tracing beyond sampling point G could not be accomplished with any confidence because tracer concentrations were diluted or broadened to the same order of magnitude as the residual population of the lake.

Although there is no control of the entering bacterial population limit, lower detection limits can be based on the residual population component concentrations. Figure 16 demonstrates that the detection cutoff for enteric tracers is reached when the individual components of the tracer fall below 3 x 10^3/100 mL (for an acceptable degree of confidence). Figure 18 also illustrates that extensive bacterial tracing in LVB would require a tremendous concentration of enteric organisms for them to be detectable above the residual population of the lake. If, however, the enteric tracer is not indigenous to the lake and present intermittently in LVW, tracing could be accomplished with significantly lower enteric tracer concentrations.

During a period when very high concentrations of enteric bacteria were present in LVW (table 31), the background (lake) concentrations of enteric tracer (Enterobacter colacae and Erwinea herbicolae) were of the same order of magnitude as they were at the time of the dye study. Comparison of the component genera in the background population (figs. 17 and 18) demonstrates the variability of the

Figure 18.—Component enteric tracer bacteria detected in the density current during a period when their numbers were very high in Las Vegas Wash.
concentrations of the component genera within this population. This supports the hypothesis that total numbers of residual enteric bacteria remain relatively constant in the lake while numbers of component genera of the population show significant variability, an observation weighing against the Component Ratio tracing procedure. Note that in both cases the data were based on isolates taken from m-Endo media and the number of isolates per sample were not as numerous as was desired. However, they did indicate the feasibility of utilizing component enteric genera to determine water distribution patterns. Obviously, isolation and identification of much greater numbers of enteric bacteria would bestow a greater degree of confidence on this kind of tracing, especially with the new technical concepts presented in this study.

The problems inherent in using enteric bacteria tracers are basically isolation of large numbers of enteric bacteria and rapid identification of those isolates. Both problems may be resolved by careful selection of media and refining present techniques.

Initial isolation and culture purification.—The feasibility of initially isolating enteric bacteria on NB and replica plating to VRB has been demonstrated.

Table 30 indicates that the enteric population represent approximately 3 percent of the total bacterial population; however, the actual mean number of enterics in the population may be significantly greater. Note that the mean of the enteric population is significantly lower than the standard deviation of the total bacteria count. This indicates that a fortified NB or TGY (tryptone glucose yeast) extract medium may result in higher counts of oxidase negative organisms. The results also indicate that VRB is capable of supporting the growth of a much greater number of enteric bacteria than m-Endo. This suggests that VRB may be a very good medium for both initial isolation and purification of enteric bacteria collected from natural environments.

Initial isolation of enteric organisms on nonselective media and incubating at temperatures below 30 °C would have the advantage of allowing stressed and damaged bacteria time to repair themselves before being replica plated to differential media. The disadvantage of this procedure lies in the dilution volumes required for competent colony counting. For example, if the total bacterial concentration is 10³/mL, a dilution volume of sample of approximately 0.001 mL would be required to achieve a countable number of colonies per plate. Dilutions to this extent may eliminate all the enterics associated with fecal pollution and a significant number of the total enteric population. The results, however, indicate that the total enteric population would remain in traceable concentrations.

Identification of enteric bacteria.—The Enterobacteriaceae are a family with closely related genera. Only a few colony descriptors are available; with the exception of certain strains of Serratia marcescens, there is a complete lack of chromogenesis within the family. Therefore, descriptors for the component genera of the population must be based on the results of several biochemical tests.

Differentiation between Enterobacter aerogenes and Klebsiella pneumoniae is based on the lack of motility of K. pneumoniae and decarboxylation of ornithine by E. aerogenes. The reliability of these two characteristics is suspect because it has been demonstrated that some strains of E. aerogenes are nonmotile and some strains are ornithine decarboxylase negative (American Public Health Association [85], Grimes[100]).

In the majority of instances, species identification of any member of the enteric group requires 11 or more biochemical descriptors (Edwards and Ewing [109], Johnson et al. [110], McCoy and Seidler [96], Painter and Isenberg [111], and Tomfohrde et al. [112]), and in the case of Salmonella, specification can be achieved by specific antibody reactions only.

Identification of large numbers of enteric isolates would be very time consuming and costly if each individual isolate is treated separately. A possible alternative to this procedure would be replica plating a multidescriptor array from initial isolation plates. A method such as this would facilitate the simultaneous identification of a large number of enteric bacteria.
Replica plating a multidescriptor array poses certain technical problems that must first be resolved if it is to become a standard procedure. The primary problem is concerned with the transfer of discrete colonies to a series of plates containing the descriptive media. The method generally used incorporates short nap velvet disks cut to the same dimensions as the isolation plates used (Lederberg and Lederberg [98]). This method, although satisfactory for two to three transfers, has a tendency to distort the individual colonies and, in some instances, is responsible for cross contamination with adjacent colonies. These effects appear to be caused by the bending of the velvet nap. It appears that to continuously achieve discrete colonies on a series of plates, a more rigid transfer applicator such as a densely bristled metal or nylon brush would be desirable.

Another problem involved in the transfer and maintenance of discrete colonies in a series of replica plates is the dryness of medium surfaces. A medium surface must be dry enough to inhibit the motility of the isolates, yet not so dry as to increase the concentration of medium constituents to the point where they become inhibitory or toxic to individual isolates.

Baseline grouping and a few confirmed identifications can be achieved with eight media (Bascomb et al. [92], Cook et al. [99], Edwards and Ewing [109], McCoy and Seidler [96], Painter and Isenberg [111], and Storey et al. [90]). Further description beyond baseline grouping would be dictated by individual groups, and media would be made as needed.

IDENTIFICATION OF ENTERIC BACTERIA IN WATER SUPPLIES

Introduction

The Standard Methods for the Examination of Water and Wastewater (American Public Health Association [85]) lists only two methods for presumptive and one method for confirmed identification of coliform bacteria. Both presumptive methods, membrane filter, and most probable numbers, rely on lactose as the differential sugar. According to Cowan [84], lactose fermentation is quite variable among the Enterobacteriaceae. Thus, colonies counted and isolated may not be coliforms, but other enteric bacteria. For confirmation, Standard Methods utilizes the IMViC tests, which are actually directed toward the characterization only of Escherichia coli, Enterobacter aerogenes, and Citrobacter freundii, or, 3 of the 11 enteric genera.

To test the extent and significance of the problem with presumptive procedures, a program of isolation and identification was initiated with environmental samples [Lake Mead and LVW (Las Vegas Wash)] known to contain large numbers of enteric bacteria. To underscore the limitations of Standard Methods for confirmation, a slightly modified version of the multittest procedure advocated by Martin [113] was applied to the same isolates subjected to IMViC testing.

Materials and Methods

Water samples were collected in a 3-L Van Dorn bottle sanitized with methyl alcohol. The water column was sampled at 10-m increments from surface to bottom at five sampling locations (stations 1 to 5). The samples were placed on ice and transported to the laboratory for analysis. Duplicate dilutions of 100, 10, and 1 mL were filtered on membrane filters, treated with media, incubated, and counted according to Standard Methods procedures for fecal and total coliforms (American Public Health Association [85]).

Typical colonies were randomly selected from the filters, streaked on EMB agar, and incubated at 35 °C until well-defined colonies appeared. Both typical and atypical coliform colonies on EMB were transferred to maintenance slants. When substantial growth was obtained on each of the slants, the individual isolates were gram-stained, inoculated into dextrose broth, and tested for exodase and catalase capability. The organisms demonstrating characteristics of the Enterobacteriaceae as defined by Cowan [84] were introduced to the IMViC and multittest procedures. ROCHE Improved Enterotubes were used for the latter.

After all biochemical tests were complete, the organisms were identified according to schemes for both procedures. No additional tests were performed on organisms not identified by the IMViC method. Rhamnose and raffinose
fermentations were required in some instances to supplement the 11 biochemical tests of the multitest procedure.

Results

The results of the comparison demonstrate the inadequacy of the IMViC method of identification, which resulted in relatively low numbers of identified organisms (table 32). On the other hand, the multitest procedure resulted in 100 percent identification of all organisms introduced into the system.

For example, isolate 1, identified as *Escherichia coli* Variety I by the IMViC procedure, demonstrated characteristics that were actually those of *Klebsiella* spp, as indicated by biochemical reactions, lack of motility, and the presence of a capsule. The results of the reactions of the IMViC tests, together with the multitest procedure, indicated that the organism was either *Klebsiella pneumoniae*, type 3, or *Klebsiella ozaenae*.

Isolates 2, 4, and 10 were identified as *Enterobacter aerogenes* Variety I by IMViC, but the multitest procedure confirmed their identity as *Klebsiella* spp. The presence of ornithine decarboxylase was the only biochemical test differentiating *E. aerogenes* and *K. pneumoniae*. The lack of motility may be another identifying characteristic, but according to Johnson, et al. [110], these characteristics are not sufficient to differentiate genera, and because they are so closely related, they should be combined into a single genus that would accommodate both motile and nonmotile forms.

Isolates 7, 12, and 18 were identified as *E. aerogenes* Varieties I, I, and II, respectively, by IMViC. On the basis of rhamnose fermentation and lack of raffinose fermentation, the multitest indicated that *E. aerogenes* Varieties I and II were actually *E. cloacae*, a different species.

Of the 18 isolates used in this comparison, only 50 percent were identified by the Standard Methods procedure (IMViC), and of those identified by this method, only two were correct by current standards for nomenclature. The multitest procedure identified all the isolates to a great degree of confidence with the exception of isolate 1. This particular organism would have required a much more detailed biochemical series of tests to establish whether it was *K. pneumoniae* or *K. ozaenae*. Table 32 contains organism identification for both the IMViC and multimedia systems.

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Discussion

The multitest procedure employed in this study greatly facilitated the identification of the isolated enterics. This was pointed out by Leers [114], who stated that more than 92 percent of the enteric bacteria could be identified within 24 hours without additional tests. Actually, it was found that all the isolates could be identified to genus within 24 hours, but further tests were required to apply epithets to species of *Klebsiella*, *Enterobacter*, *Citrobacter*, *Shigella*, and *Salmonella*. Although not shown in the rapid screening chart, *Yersinia spp* can also be identified by using this multitest method of identification.

In all instances, Cowan's [84] methods should be utilized in conjunction with the rapid screening chart because isolates do not always demonstrate a classic identification profile, and unless the technician examines all possibilities, the probability of misidentification is always present.

The multitest system, which perhaps is not the ultimate answer for enteric identification, appears to be the best interim procedure known at this time. Similar methods have been used in clinical bacteriology, computer assisted taxonomy, and numerical taxonomy (Finlayson and Gibbs [115], Friedman et al. [116], Johnson et al. [110], Martin [113], Oliver and Cowell [117], and Shewane et al. [118]).

Standard Methods procedures seem burdened with inherent possibilities for error, with lactose fermentation producing gas within 24 ± 2 hours at 44.5 °C being the basic problem. Only certain biotypes of *E. coli* and *C. freundii* exhibit the capability. Also, there is some disagreement concerning *E. aerogenes*. It has been shown that *E. aerogenes*, which is capable of fermenting lactose with gas production at 44.5 °C, appears to be from soil or vegetation free from fecal contamination, while the other biotype, negative for this reaction, appears to be of fecal origin (Cowan [84]). This is in direct contradiction with the Standard Methods.

For the above reason, it is the opinion of the authors that the multitest method of identification should be considered for the next revision of the Standard Methods. This would allow substitution of identification of the enteric bacteria actually present for the rather suspect current "coliform" procedures. Obviously, lactose in present presumptive media would have to be replaced by dextrose, the sugar common to all *Enterobacteriaceae*.

BIBLIOGRAPHY

References Cited


Additional References


Appendix I.—Nutrient concentrations in mg/L at stations 1 to 6, 1975-76

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Total-P = total phosphorus; Diss-P = dissolved phosphorus; NH₃-N = ammonia nitrogen; NO₂+NO₃ = nitrite plus nitrate nitrogen; TKN = total kjeldahl nitrogen; Total-ALK = total alkalinity.
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**Additional Notes:**
- Represents counts per milliliter (No./mL) for each species.
- Data is organized by station and date.
- Species include Chlamydomonas, Cyclotella, Carteria, Stephanodiscus, Oocystis, Navicula, Eudorina, Fragilaria, and Stephanodiscus.
### Appendix II B.—Dominant phytoplankton in Las Vegas Bay and Boulder Basin 1975-76 by depth

#### STATION 5

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83
Lake Mead is a deep, subtropical, moderately productive, desert impoundment with a negative heterograde oxygen profile occurring during the summer stratification. Investigations of the Boulder Basin of Lake Mead by the University of Nevada were initiated in November 1971. The primary objective of the study was to determine what effects industrial and sewage effluent from the Las Vegas metropolitan area, discharged into Las Vegas Bay, have had on the water quality and limnological conditions of Boulder Basin. Data from the 1975-76 period are presented in detail, with earlier data included in the summaries and discussions.

Measurements of water temperature, dissolved oxygen, conductivity, pH, alkalinity, nutrient concentrations, phytoplankton numbers, chlorophyll a, primary productivity, zooplankton concentrations, and coliform bacteria were made monthly or biweekly. Success patterns for both phytoplankton and zooplankton are described.

Physical factors affecting the distribution and deposition of enteric bacteria (including those of special public health importance) in Las Vegas Bay are discussed. The distribution of water and of enteric bacteria of possible fecal origin into Las Vegas Bay from Las Vegas Wash were determined. The unreliability of thoroughly referenced techniques and methods, generally accepted as standard for enteric bacteria, are treated in detail. Water from Las Vegas Wash forms a density current in Las Vegas Bay. The density current is located on the bottom during isothermal conditions and in the metalimnion during summer stratification.

The metalimnetic oxygen minimum was examined in some detail and found to be caused by biological respiration. Estimations of zooplankton and phytoplankton respiration indicate that they could account for the majority of the oxygen lost in the metalimnion.

Primary production and algal biomass were higher in Las Vegas Bay because of nutrient enrichment from Las Vegas Wash. The inner portion of Las Vegas Bay would be classified as eutrophic and the outer portion of Las Vegas Bay and Boulder Basin as mesotrophic. Nitrogen is likely to be the most limiting nutrient.