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# Accumulative Coliform Population in a Five-Mile Section of the Cibolo Creek

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ACCUMULATIVE COLIFORM POPULATION IN A  
FIVE-MILE SECTION OF THE CIBOLO CREEK

by

Norman Merrill Brown  
" "

A Thesis

Submitted to the Faculty of the Division of Graduate Studies  
of Incarnate Word College in Partial Fulfillment of the  
Requirements for the Degree of  
Master of Science

San Antonio, Texas

August 1974

This Thesis for the Master of Science Degree

by

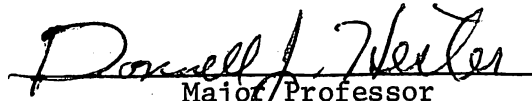
Norman Merrill Brown

has been approved for the

Division of Graduate Studies of

Incarnate Word College

by

  
Major Professor

  
Reader

San Antonio, Texas

August 1974

12 Nov 1974

## PREFACE

The greatest danger associated with water is that it may recently have been contaminated by sewage or by human excrement. Therefore, the protection of public water supplies from intestinal contamination is a necessary obligation of the public health authorities. If there happens to be a break in the protective chain (adequate treatment, disinfection, and protection of water supply), a chance exposure to fecal contamination could trigger an explosive outbreak of disease within a community.

Modern technology and more sophisticated bacteriological methods have made it possible to detect most pathogenic bacteria found in sewage and sewage effluents. However, it is not practicable to isolate these pathogens by any routine procedure. If and when pathogenic organisms are present in feces or sewage, they are always greatly outnumbered by the normal excremental organisms called coliforms. These coliforms are easily detected by a simple bacteriological examination. The presence of one coliform, Escherichia coli, in a sample of water is therefore taken as an index of intestinal pollution.

## INTRODUCTION

The area of study is located near Schertz, Texas on the Cibolo Creek. The dump site of three different sewage plants is found within this section of the creek. (There are plans to build two more sewage plants in the area within the next eighteen months. This will bring the total to five sewage plants within a five-mile area.) The present plants are from Schertz, Universal City, and Randolph AFB.

The Balcones Fault crosses the Cibolo Creek near Boerne, Texas. At this intersection, all the flowing creek water goes underground into the Edwards Aquifer. The result is that the next several miles of the Cibolo Creek is usually dry. However, for short periods after very heavy rains the creek will carry a large volume of water. Normally the Cibolo carries only a trickle of water through the study area. This water is the effluents from the sewage plants. One question to be answered from this study would be "What is the total fecal coliform population as a result of all three sewage plant effluents?"

One additional problem is the dubious habit of sewage plant operators allowing raw sewage to flow freely down the creek during heavy rains. Each plant can handle only a specific volume of water daily. A heavy rain would produce a volume of water many times greater than the plant could satisfactorily process. The probable result is that the flood waters of the Cibolo dilute the raw sewage as it is carried downstream. At the present, this action might be biologically and environmentally plausible because there are no towns, villages, or sewage plants for the next several miles. However, there is a proposed dam to be constructed on the Cibolo Creek in Wilson

County near La Vernia. It is hoped that this would produce a large reservoir and profitable recreational area. These facts raise another question that will be answered from this study. If a large dam and reservoir are built on the Cibolo, would the coliform count of the resulting waters be high enough to be a health hazard to persons utilizing the fresh water recreational facility?

Mitchell Lake was chosen as a site to determine if coliform could survive and accumulate in dammed water. This is a large body of water in south Bexar County that has raw sewage pumped into it as a means of storing sewage before processing it.

#### MATERIALS

A complete list of mechanical and chemical materials used in this study is included in the appendix. The instructions on how to prepare the chemical reagents that were used are also included in the appendix.

#### SAMPLING

Good samples are a critical part of sanitary water testing; therefore, proper sampling techniques are vital. Sample quality will be greatly improved if these general instructions are followed:

- (1) Samples need to accurately represent the selected body of water.
- (2) Collection of an adequate number of samples of adequate sample volume is necessary.
- (3) Preserve aseptic handling of samples; avoid bacterial contamination from skin, clothing, equipment, water, and adjacent surfaces.
- (4) Record necessary sampling data.
- (5) Analyze samples within a permissible time interval after sampling.

This portion of the Cibolo was selected because it contained the terminal points of effluent control as designed by the three sewage plants. With the aid of two junior high students the entire length of the study area was traveled, noting the position of each effluent dump site. Then sampling sites were chosen above and below each merge of creek and effluents. These were designated as Site 1 and Site 2 (Universal City), Site 3 and Site 4 (Randolph AFB), and Site 5 and Site 6 (Schertz). The odd numbers were points above the area of sewage entrance and the even points below.

Site 1 was an extremely rocky creek bed with pools of standing water scattered along its length. Only during heavy rains or severe flooding is there flowing water in this section. Evaporation during the summer months causes a great reduction in pool number, size, and depth. For this reason a rather large, deep pool was chosen as the sample site. The surrounding area is very thinly populated. The majority of the land is wooded with very, very little pasture land. Therefore, the entire area would be rather free of chance contamination by runoff from septic tanks or areas containing domestic animals.

The second site was an area with water flowing rapidly down a narrow trench. The flow was constant during the entire study, stopping only if the effluent flow was halted. The surrounding area was mainly soil covered with a heavy growth of vegetation.

A water-level bridge, at the end of Schertz, was chosen as Site 3. The bridge acted as a dam and retarded the flow of creek water, causing the sample area to be rather wide and deep. This was the only area that had periodic algal blooms with resulting fish kills.

Immediately above the Randolph sewage plant is a large corral where horses are kept. Both are located on the steep bank of the Cibolo; therefore, runoff would be heavy during severe rains. Site 4 was located several yards down from this area. This site also had a bridge across the creek. It was a private bridge that had been built to allow trucks to cross the Cibolo on the way to public roads. This structure also slowed the flow of the creek extremely. The dammed water was deep and cool with a heavy algal growth on the surface. The surrounding area was heavily wooded.

Site 5 was also located in an area covered with trees. Here the water was deep and the flow was constant but very slow.

The last site was also near a low water crossing, approximately one mile from Cibolo, Texas. However, the water flow was quite rapid. The creek bed was once again sandy with a few larger stones.

At Mitchell Lake three sites were chosen at different points along the banks. The lake water level changed very little. The water was very still with an extremely heavy algal growth.

All sample sites were within fifty yards of the road and therefore very easy to reach. Access to Mitchell Lake was gained only by driving through a public dump to the lake's edge.

At each point three 450 ml samples (A, B, C) were taken. The creek flows mainly west to east here. As one would look in the direction of flow, sample A was taken from the right (southern) side; sample B from the middle; and sample C from the left (northern) side.

Each sample was taken by holding the jar near the bottom and plunging it mouth down into the creek. (Any surface scum had previously been swept aside.) The jar was then tilted into the current and held there until



bubbles indicated that it was full. The sample was then withdrawn and .4 ml of a 10% sodium thiosulfate solution added. This solution was used to de-chlorinate the treated samples. The cap was replaced and the jar labeled. After all samples were gathered they were taken to the lab and processed. Total time for collecting and processing was approximately six hours. All glassware was then washed, rinsed with distilled water, and autoclaved at 121°C and 15 psi for fifteen minutes.

### TESTING

Accurate bacterial growth is another critical factor in water analysis. This can be acquired only by using strict aseptic techniques and disciplined laboratory procedures. Any lax attitude or behavior can only result in poor and incorrect results. Obviously, this must be avoided.

The bench or work area should be set up in an ordered manner with a conscious effort to conserve space. This is because work must be rapid as well as efficient. The fecal coliforms can survive only a few hours in a sample bottle at room temperature. If the sample is chilled when collected, the organisms will remain viable up to eighteen hours.

The M-FC broth was prepared as stated in the appendix. It was contained in a sterile Erlenmeyer flask which was kept in a refrigerator. M-FC broth deteriorates after 96 hours; therefore, only the amount of broth that could be used shortly was prepared each time. A series of sterile dilution blanks and the stock buffer solution were prepared and refrigerated.

Before the field work was started the petri dishes were prepared. Twenty-one petri dishes were lined up on the table top. With sterile forceps, a pad was placed in the bottom of each petri dish. Two milliliters of M-FC broth

This caused a large amount of water to flow down the Cibolo. Measurements were taken as dictated by the "volume of flow" formula found in James G. Needham's A Guide to the Study of Fresh-Water Biology. The peak flow measured was on the 26th of June at 13,333.3 feet<sup>3</sup>/second. The volume of flow would have been greater on August 2nd or 3rd because of heavy rains upstream. At this time the plant at Schertz was flooded and suffered severe damage. However, no measurements were taken then.

The air temperature ranged from 27.2°C to 32.7°C with the average temperature 30.4°C. The rain did not have an effect on the air temperature of the sample days.

However, the rain did have a direct effect on the water temperature. Flowing water had an average temperature of 21°C and standing water an average of 24°C. After heavy rains the temperature of all sites dropped to 18°C. The cooler temperatures would persist until flooding ceased. Because of the large volume of water involved at Mitchell Lake, the water temperature changed very little.

### Chemical

The pH ranged from 7.0 to 9.3. The flowing water had a pH of 7.2. This was rather constant. When the flow stopped the pH would change.

The creek bed at Site 1 was covered with large limestone pebbles. When water with large amounts of dissolved carbon dioxide came in contact with the limestone calcium carbonate was formed. This would cause the pH to increase. The pH at Site 1 reached a peak on August 17th at 9.3. The evaporation of the water from the pool caused the percentage of calcium carbonate to increase, thus increasing the pH.

Only one other site had significant change in pH. This was Site 3. This was the point of a low water bridge that stopped the flow of water.

At Site 3 there were periodic algae blooms with resulting fish kills. The algae blooms were created by eutrophication. The effluent from Site 2 added large quantities of nutrients (probably nitrogen, phosphorus, etc). This aquatic fertilizer stimulated the growth of the algae. When the algae died it produced a large mass of decomposing organic matter. This would produce a large amount of  $\text{CO}_2$ . Thus the chemical reaction would occur here also.

The total alkalinity would change with the physical nature of the water. It was low in flowing water (185 ppm, 195 ppm) and high in pool or dammed water (over 400 ppm).

The dissolved oxygen content was just the opposite of the total alkalinity. In large volumes of rapidly flowing water it was 98% saturation, while in standing water the dissolved oxygen was 50% saturation.

### Biological

The criteria used to establish a hazard to health were recommended by the committee on Water Quality Criteria to the U. S. Secretary of the Interior. They suggested an average for all waters not to exceed 2000 fecal coliforms per 100 ml. In waters used for primary contact recreation, the committee recommended 200 per 100 ml with not more than 10% of the samples exceeding 400 per 100 ml. These are permissible values that could be tolerated. However, a more desirable criteria for public surface water would be 20 fecal coliforms per 100 ml.

The only site to reach the desirable values during the study was Site 1. On August 14th, 17th, and 21st Site 1 had no coliforms at all. This was after several days of high temperatures and no rain.

All sites that have effluent water pumped into them do not meet these requirements. On any given day more than 10% of the samples contain more than 400 fecal coliforms per 100 ml.

There is a gradual accumulation of fecal coliforms in the Cibolo Creek in flood waters. In this case the increase after each dump site is quite clear. The average number of coliforms added to the flood waters at each effluent entrance was 33,000 per 100 ml. There was a slight decrease between Site 5 and Site 6. A great deal of vegetation (trees, shrubs, etc.) grows in this area. The distance between these sites is greater than between any of the others. The combination of these two features might act as a filter to remove a portion of the coliform population.

In calm, shallow waters there is no increased accumulation of fecal coliforms. As stated previously there are structures across the Cibolo that act as dams. These are located at Sites 3 and 4. These structures slow the flow of water greatly. Therefore, the coliforms have time to settle out of the creek water. Also because of a heavy algal growth and continuous evaporation the water chemistry changed such that fecal coliforms would be killed (dissolved oxygen at 33% saturation). Either factor (high fecal coliform number and altered water chemistry) are indicators of severe pollution. The hazard to health is especially great during periods of increased water flow.

The study at Mitchell Lake proves that an extremely dense population of coliforms will remain in a large body of dammed water. The average

population was 80,000 coliforms per 100 ml. This number remained rather stable and did not fluctuate. This provides a body of water that is very dangerous to the health of people using the lake for any recreational purpose.

#### CONCLUSION

Sewage polluted water has been the means by which people have been infected with organisms that cause intestinal diseases, for example, cholera, typhoid, and paratyphoid fevers (which are able to survive in polluted water for a week), and gastroenteritis. People swimming in polluted waters might accidentally swallow water containing pathogenic spirochetes and/or viruses.

Cool water aids in pathogen survival while swift water aids in its dispersal. These facts need to be considered by anyone planning to dam the Cibolo at any point below Selma, Texas. Obviously, a reservoir located near La Vernia would not rapidly become a Mitchell Lake. However, because of sporadic flooding, such a lake would be severely polluted periodically. The cost and inconvenience of such pollution would be great.

What is the total fecal coliform population as a result of all three sewage plant effluents? In answer to this proposed question, there is not a gradual bacterial increase. The most probable reason being the lack of flooding water through the study area. However, in rapidly flowing flood waters there is an average coliform increase of 33,000 per 100 ml per dump site.

If a large dam and reservoir are built on the Cibolo, would the coliform count of the resulting waters be high enough to be a health hazard to persons utilizing the fresh water recreational facility? Yes! The cool, swift flood

waters would definitely maintain and transport an extremely large number of fecal coliforms (40,000 per 100 ml) to the proposed dam site. Once there the coliforms and any pathogens present, would pose a health hazard for several days.

Some suggestions that might improve conditions along this portion of the Cibolo might be:

- (1) Eliminate the large number of fecal coliforms presently being dumped into the creek daily.
- (2) Remove the dam effect at two points and improve the creek bed so that the water will flow more freely.
- (3) Improve sewage plant conditions so that flood waters would not interfere with sewage processing.

The Cibolo Creek was once a favorite swimming and fishing spot of people living along its banks. With proper improvements and maintenance it could be an area of sport, recreation, and civic service, aiding all portions of the community.

TABLE 1  
COLIFORMS PER 100 ML

<u>Date</u>	<u>Site</u>	<u>Colonies</u>	<u>Coliforms/100 ML</u>	<u>Average</u>	<u>Logarithm</u>
21 Jun 1973	A11	TNTC			
26 Jun 1973	A11	TNTC			
28 Jun 1973	A11	TNTC			
2 Jul 1973	A11	TNTC			
6 Jul 1973	1a	115	11,500		
	1b	117	11,700		
	1c	100	10,000	11,060	4,04376
	2a	516	51,600		
	2b	520	52,000		
	2c	524	52,400	52,000	4.71600
	3a	855	85,500		
	3b	870	87,000		
	3c	868	86,800	86,430	4.93666
	4a	1052	105,200		
	4b	1080	108,000		
	4c	1067	106,700	106,630	5,0278794
	5a	TNTC			
	5b	TNTC			
	5c	TNTC			
	6a	414	41,400		
	6b	466	46,600		
	6c	435	43,500	43,830	4.64177
	Ma	9*	90,000		
	Mb	10	100,000		
	Mc	8	80,000	90,000	4.95424
7 Aug 1973	1a	14	1,400		
	1b	6	600		
	1c	21	2,100	1,700	3.23045
	2a	490	49,000		
	2b	612	61,200		
	2c	568	56,800	55,660	4,74554

\*The data from Mitchell Lake is based upon a 1-1000 dilution.

TABLE 1  
COLIFORMS PER 100 ML (CONT)

<u>Date</u>	<u>Site</u>	<u>Colonies</u>	<u>Coliforms/100 ML</u>	<u>Average</u>	<u>Logarithm</u>
7 Aug 1973	3a	5	500	600	2.77815
	3b	6	600		
	3c	7	700		
	4a	4	400	366	2.56348
	4b	3	300		
	4c	4	400		
	5a	16	1,600	1,800	3.25527
	5b	20	2,000		
	5c	18	1,800		
	6a	13	1,300	1,566	3.19479
	6b	19	1,900		
	6c	15	1,500		
	Ma	7	70,000	70,000	4.84510
	Mb	8	80,000		
	Mc	6	60,000		
9 Aug 1973	1a	1	100	33.3	1.5224
	1b	0	0		
	1c	0	0		
	2a	53	5,300	6,066	3.78362
	2b	76	7,600		
	2c	53	5,300		
	3a	137	13,700	12,600	4.10037
	3b	94	9,400		
	3c	147	14,700		
	4a	39	3,900	2,930	3.46687
	4b	21	2,100		
	4c	28	2,800		
	5a	20	2,000	2,230	3.34830
	5b	15	1,500		
	5c	32	3,200		



TABLE 1  
COLIFORMS PER 100 ML (CONT)

<u>Date</u>	<u>Site</u>	<u>Colonies</u>	<u>Coliforms/100 ML</u>	<u>Average</u>	<u>Logarithm</u>
9 Aug 1973	6a	4	400	266.6	2,42586
	6b	2	200		
	6c	2	200		
	Ma	9	90,000	90,000	4.95424
	Mb	9	90,000		
	Mc	9	90,000		
14 Aug 1973	1a	0	0	0	
	1b	0	0		
	1c	0	0		
	2a	17	1,700	2,166	3.33566
	2b	28	2,800		
	2c	20	2,000		
	3a	1	100	33.3	1.5224
	3b	0	0		
	3c	0	0		
	4a	4	400	233	2.36736
	4b	2	200		
	4c	1	100		
	5a	1	100	33.3	1.5224
	5b	0	0		
	5c	0	0		
	6a	2	200	166	2.22011
	6b	2	200		
	6c	1	100		
	Ma	800	80,000	80,000	4.90037
	Mb	700	70,000		
	Mc	900	90,000		
17 Aug 1973	1a	0	0	0	
	1b	0	0		
	1c	0	0		

TABLE 1  
COLIFORMS PER 100 ML (CONT)

<u>Date</u>	<u>Site</u>	<u>Colonies</u>	<u>Coliforms/100 ML</u>	<u>Average</u>	<u>Logarithm</u>
17 Aug 1973	2a	6	600		
	2b	6	600		
	2c	0	0	400	2.60206
	3a	9	900		
	3b	4	400		
	3c	7	700	666	2.82347
	4a	1	100		
	4b	1	100		
	4c	3	300	166	2.22011
	5a	0	0		
	5b	0	300		
	5c	0	100	133	2.12385
	6a	0	0		
	6b	1	100		
	6c	1	100	66.6	1.8235
	Ma	800	80,000		
	Mb	700	70,000		
	Mc	900	90,000	80,000	4.90309
21 Aug 1973	1a	0	0		
	1b	0	0		
	1c	0	0	0	
	2a	37	3,700		
	2b	38	3,800		
	2c	41	4,100	3,860	3.58659
	3a	2	200		
	3b	6	600		
	3c	8	800	533.3	2.72697
	4a	3	300		
	4b	4	400		
	4c	0	0	233.3	2.36791

TABLE 1

## COLIFORMS PER 100 ML (CONT)

<u>Date</u>	<u>Site</u>	<u>Colonies</u>	<u>Coliforms/100 ML</u>	<u>Average</u>	<u>Logarithm</u>
21 Aug 1973	5a	37	3,700		
	5b	33	3,300		
	5c	39	3,900	3,860	3.58659
	6a	2	200		
	6b	8	800		
	6c	7	700	566.6	2.75328
	Ma	900	90,000		
	Mb	800	80,000		
	Mc	1000	100,000	100,000	5.00

TABLE 2  
WATER TEMPERATURE

Date	Site 1		Site 2	
	Degrees C	Degrees F	Degrees C	Degrees F
21 Jun 1973	25	77.0	22	71.6
26 Jun 1973	18	64.4	18	64.4
28 Jun 1973	19	66.2	19	66.2
2 Jul 1973	21	69.8	21	69.8
6 Jul 1973	21	69.8	21	69.8
7 Aug 1973	19	66.2	19	66.2
9 Aug 1973	20	68.0	20	68.0
14 Aug 1973	23	73.4	21	69.8
17 Aug 1973	25	77.0	22	71.6
21 Aug 1973	21	69.8	20	68.0

Site 3		Site 4		
21 Jun 1973	22	71.6	21	69.8
26 Jun 1973	18	64.4	18	64.4
28 Jun 1973	19	66.2	19	66.2
2 Jul 1973	21	69.8	21	69.8
6 Jul 1973	21	69.8	21	69.8
7 Aug 1973	19	66.2	19	66.2
9 Aug 1973	20	68.0	20	68.0
14 Aug 1973	21	69.8	20	68.0
17 Aug 1973	22	71.6	21	69.8
21 Aug 1973	20	68.0	19	66.2

Site 5		Site 6		
21 Jun 1973	21	69.8	21	69.8
26 Jun 1973	18	64.4	18	64.4
28 Jun 1973	19	66.2	19	66.2
2 Jul 1973	21	69.8	21	69.8
6 Jul 1973	21	69.8	21	69.8
7 Aug 1973	19	66.2	19	66.2
9 Aug 1973	20	68.0	20	68.0
14 Aug 1973	20	68.0	20	68.0
17 Aug 1973	21	69.8	21	69.8
21 Aug 1973	19	66.2	19	66.2

TABLE 2  
WATER TEMPERATURE (CONT)

<u>Date</u>	<u>Mitchell Lake</u>	
	<u>Degrees C</u>	<u>Degrees F</u>
21 Jun 1973	22	71.6
26 Jun 1973	21	69.8
28 Jun 1973	21	69.8
2 Jul 1973	22	71.6
6 Jul 1973	22	71.6
7 Aug 1973	22	71.6
9 Aug 1973	22	71.6
14 Aug 1973	22	71.6
17 Aug 1973	22	71.6
21 Aug 1973	22	71.6

TABLE 3

WATER pH

Site	Date	Position		
		A	B	C
1	2 Jul 1973	7.2	7.2	7.2
	6 Jul 1973	7.2	7.2	7.2
	7 Aug 1973	7.3	7.3	7.3
	9 Aug 1973	7.6	7.6	7.7
	14 Aug 1973	8.4	8.4	8.5
	17 Aug 1973	9.3	9.3	9.3
	21 Aug 1973	7.7	7.8	7.8
2	2 Jul 1973	7.1	7.1	7.1
	6 Jul 1973	7.1	7.1	7.1
	7 Aug 1973	7.3	7.3	7.3
	9 Aug 1973	7.4	7.4	7.4
	14 Aug 1973	7.4	7.4	7.4
	17 Aug 1973	7.3	7.3	7.3
	21 Aug 1973	7.2	7.2	7.2
3	2 Jul 1973	7.2	7.2	7.2
	6 Jul 1973	7.2	7.1	7.2
	7 Aug 1973	7.3	7.3	7.3
	9 Aug 1973	7.5	7.6	7.6
	14 Aug 1973	8.6	8.5	8.6
	17 Aug 1973	8.0	8.1	8.0
	21 Aug 1973	7.4	7.5	7.5
4	2 Jul 1973	7.1	7.1	7.1
	6 Jul 1973	7.1	7.2	7.2
	7 Aug 1973	7.2	7.2	7.2
	9 Aug 1973	7.3	7.3	7.3
	14 Aug 1973	7.4	7.4	7.4
	17 Aug 1973	7.6	7.6	7.6
	21 Aug 1973	7.2	7.1	7.2
5	2 Jul 1973	7.1	7.1	7.0
	9 Jul 1973	7.1	7.0	7.1
	7 Aug 1973	7.2	7.2	7.2
	9 Aug 1973	7.2	7.3	7.3
	14 Aug 1973	7.2	7.3	7.3
	17 Aug 1973	7.3	7.3	7.3
	21 Aug 1973	7.1	7.2	7.1

TABLE 3  
WATER pH (CONT)

Site	Date	Position		
		A	B	C
6	2 Jul 1973	7.1	7.1	7.1
	6 Jul 1973	7.1	7.0	7.1
	7 Aug 1973	7.2	7.3	7.2
	9 Aug 1973	7.2	7.3	7.2
	14 Aug 1973	7.4	7.5	7.4
	17 Aug 1973	7.5	7.5	7.5
	21 Aug 1973	7.2	7.2	7.2
Mitchell Lake	2 Jul 1973	8.0	7.9	8.0
	6 Jul 1973	8.2	8.1	8.2
	7 Aug 1973	8.2	8.2	8.2
	9 Aug 1973	8.3	8.4	8.4
	14 Aug 1973	8.8	8.9	8.9
	17 Aug 1973	9.2	9.2	9.2
	21 Aug 1973	8.5	8.4	8.5

No data was taken in June because of equipment failure.

TABLE 4

## TOTAL ALKALINITY

Date	Site 1		Site 2	
	Dissolved CO <sub>2</sub>	T.A.	Dissolved CO <sub>2</sub>	T.A.
21 Jun 1973	N/A	N/A	N/A	N/A
26 Jun 1973	N/A	N/A	N/A	N/A
28 Jun 1973	N/A	N/A	N/A	N/A
2 Jul 1973	30	250	30	205
6 Jul 1973	29	245	29	195
7 Aug 1973	25	260	25	260
9 Aug 1973	20	390	20	270
14 Aug 1973	15	400 (over)	20	270
17 Aug 1973	14	400 (over)	19	210
21 Aug 1973	16	380	21	185

Date	Site 3		Site 4	
	Dissolved CO <sub>2</sub>	T.A.	Dissolved CO <sub>2</sub>	T.A.
21 Jun 1973	N/A	N/A	N/A	N/A
26 Jun 1973	N/A	N/A	N/A	N/A
28 Jun 1973	N/A	N/A	N/A	N/A
2 Jul 1973	30	250	30	200
6 Jul 1973	29	240	29	195
7 Aug 1973	25	260	25	210
9 Aug 1973	20	315	20	220
14 Aug 1973	0	400 (over)	18	240
17 Aug 1973	6	400 (over)	14	305
21 Aug 1973	20	330	26	225

Date	Site 5		Site 6	
	Dissolved CO <sub>2</sub>	T.A.	Dissolved CO <sub>2</sub>	T.A.
21 Jun 1973	N/A	N/A*	N/A	N/A
26 Jun 1973	N/A	N/A	N/A	N/A
28 Jun 1973	N/A	N/A	N/A	N/A
2 Jul 1973	30	200	30	200
6 Jul 1973	29	195	29	195
7 Aug 1973	25	215	25	215
9 Aug 1973	25	210	25	210
14 Aug 1973	22	195	22	195
17 Aug 1973	19	205	19	205
21 Aug 1973	31	205	26	220

Mitchell Lake

Cannot be determined because of lack of data.

\*Not available.



TABLE 5  
DISSOLVED OXYGEN

<u>Date</u>	<u>Site 1</u>		<u>Site 2</u>	
	<u>mg/l</u>	<u>% saturation</u>	<u>mg/l</u>	<u>% saturation</u>
21 Jun 1973	4.1	50	5.1	55
26 Jun 1973	9.4	98	9.4	98
28 Jun 1973	9.1	98	9.1	98
2 Jul 1973	8.5	95	8.5	98
6 Jul 1973	8.2	91	8.2	91
7 Aug 1973	6.6	70	6.6	70
9 Aug 1973	6.2	69	6.2	69
14 Aug 1973	4.5	53	4.5	53
17 Aug 1973	4.1	50	4.1	50
21 Aug 1973	5.1	55	5.1	55

<u>Site 3</u>		<u>Site 4</u>		
21 Jun 1973	4.3	51	5.1	56
26 Jun 1973	9.4	98	9.4	98
28 Jun 1973	9.1	98	9.1	98
2 Jul 1973	8.5	95	8.5	95
6 Jul 1973	8.2	95	8.2	91
7 Aug 1973	6.6	70	6.6	70
9 Aug 1973	6.2	69	6.2	69
14 Aug 1973	4.5	50	4.5	49
17 Aug 1973	3.6	37	4.1	45
21 Aug 1973	5.3	59	5.1	55

<u>Site 5</u>		<u>Site 6</u>		
21 Jun 1973	5.1	56	5.1	56
26 Jun 1973	9.4	98	9.4	98
28 Jun 1973	9.1	98	9.1	98
2 Jul 1973	8.5	95	8.5	95
6 Jul 1973	8.2	91	8.2	91
7 Aug 1973	6.6	70	6.6	70
9 Aug 1973	6.2	69	6.2	69
14 Aug 1973	4.5	49	4.5	49
17 Aug 1973	4.1	45	4.1	45
21 Aug 1973	5.1	55	5.1	55

TABLE 5  
DISSOLVED OXYGEN (CONT)

<u>Date</u>	<u>Mitchell Lake</u>	
	<u>mg/l</u>	<u>% saturation</u>
21 Jun 1973	10.4	120
26 Jun 1973	10.4	195
28 Jun 1973	10.4	195
2 Jul 1973	10.4	198
6 Jul 1973	10.5	120
7 Aug 1973	10.4	198
9 Aug 1973	10.4	198
14 Aug 1973	10.5	120
17 Aug 1973	10.5	120
21 Aug 1973	10.5	120

TABLE 6  
WEATHER CONDITIONS

<u>Date</u>	<u>Temperature*</u>		<u>Precipitation (inches)</u>
	<u>Degrees F</u>	<u>Degrees C</u>	
21 Jun 1973	81	27.2	Trace
25 Jun 1973**			3.27
26 Jun 1973	82	27.7	.01
28 Jun 1973	86	30.0	0
2 Jul 1973	87	30.5	0
6 Jul 1973	88	31.1	0
1 Aug 1973**			1.13
7 Aug 1973	88	31.1	0
9 Aug 1973	89	31.6	0
14 Aug 1973	88	31.1	0
17 Aug 1973	87	30.5	Trace
21 Aug 1973	91	32.7	0

\*Temperature was taken between 12:00 PM and 1:00 PM.

\*\*Days that heavy rains occurred.

TABLE 7  
VOLUME OF FLOW

<u>Date</u>	<u>W</u>	<u>D</u>	<u>a</u>	<u>L</u>	<u>T</u> <u>(sec)</u>	<u>R</u> <u>(ft. 3/sec.)</u>
<u>Site 1</u>						
21 Jun 1973	25	5	.8	5	-	-
26 Jun 1973	200	15	.8	10	3	8000
28 Jun 1973	150	12	.8	10	4	3600
2 Jul 1973	100	10	.8	10	5	1600
6 Jul 1973	75	6	.8	10	5	720
7 Aug 1973	75	6	.8	10	5	720
9 Aug 1973	60	4	.8	10	5	384
14 Aug 1973	25	4	.8	10	-	-
17 Aug 1973	20	4	.8	10	-	-
21 Aug 1973	20	4	.8	10	60	10.66
<u>Site 2</u>						
21 Jun 1973	8	.5	.9	10	6	6
26 Jun 1973	125	25	.9	10	3	9375
28 Jun 1973	100	20	.9	10	3	4000
2 Jul 1973	75	15	.9	10	5	2025
6 Jul 1973	50	10	.9	10	5	900
7 Aug 1973	50	10	.9	10	5	2025
9 Aug 1973	40	8	.9	10	5	576
14 Aug 1973	10	.6	.9	10	6	9
17 Aug 1973	6	.5	.9	10	6	4.5
21 Aug 1973	10	.5	.9	10	5	9
<u>Site 3</u>						
21 Jun 1973	30	4	.9	10	-	-
26 Jun 1973	100	30	.9	10	3	9000
28 Jun 1973	75	20	.9	10	3	4500
2 Jul 1973	50	15	.9	10	3	2283.3
6 Jul 1973	30	10	.9	10	3	900
7 Aug 1973	50	15	.9	10	3	2283.3
9 Aug 1973	30	7	.9	10	3	630
14 Aug 1973	30	5	.9	10	-	-
17 Aug 1973	30	4	.9	10	-	-
21 Aug 1973	30	4	.9	10	-	-

TABLE 7  
VOLUME OF FLOW (CONT)

<u>Date</u>	<u>W</u>	<u>D</u>	<u>a</u>	<u>L</u>	<u>T</u> <u>(sec)</u>	<u>R</u> <u>(ft. 3/sec.)</u>
<u>Site 4</u>						
21 Jun 1973	30	10	.9	10	-	-
26 Jun 1973	80	40	.9	10	3	9600
28 Jun 1973	60	30	.9	10	3	5400
2 Jul 1973	40	20	.9	10	3	2600
6 Jul 1973	40	15	.9	10	4	1350
7 Aug 1973	40	20	.9	10	3	2600
9 Aug 1973	30	15	.9	19	-	-
14 Aug 1973	30	12	.9	10	-	-
17 Aug 1973	30	10	.9	10	-	-
18 Aug 1973	30	12	.9	10	-	-
<u>Site 5</u>						
21 Jun 1973	20	6	.9	10	16	37.2
26 Jun 1973	250	30	.9	10	6	11250
28 Jun 1973	200	25	.9	10	7	6427.5
2 Jul 1973	150	15	.9	10	8	2632.1
6 Jul 1973	50	10	.9	10	6	750
7 Aug 1973	150	15	.9	10	8	2632.1
9 Aug 1973	50	8	.9	10	9	400
14 Aug 1973	20	7	.9	10	12	105
17 Aug 1973	20	6	.9	10	14	77.1
21 Aug 1973	20	6	.9	10	14	77.1
<u>Site 6</u>						
21 Jun 1973	10	1.5	.8	10	3	40
26 Jun 1973	200	25	.8	10	3	1333.3
28 Jun 1973	150	20	.8	10	3	8000
2 Jul 1973	100	15	.8	10	4	3000
6 Jul 1973	75	12	.8	10	7	914.2
7 Aug 1973	100	15	.8	10	4	3000
9 Aug 1973	50	11	.8	10	11	438.7
14 Aug 1973	25	4	.8	10	7	118.2
17 Aug 1973	20	4	.8	10	8	80
21 Aug 1973	10	2	.8	10	4	40

## WATER pH

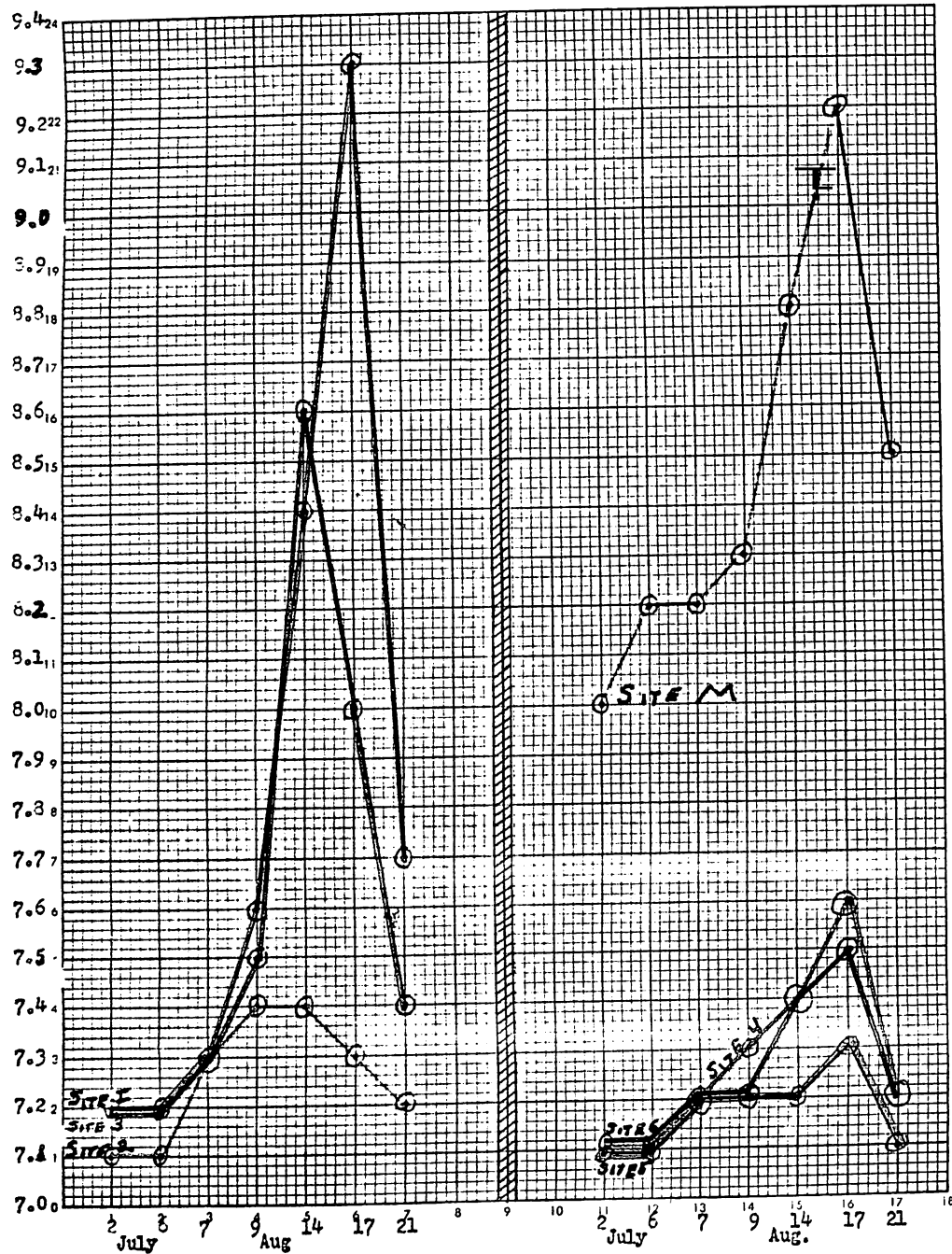
This set of graphs plots the pH at each site versus the date the sample was taken. Each site indicated a gradual increase in the pH from 2 July 1973 until 17 August 1973. Then there was a sharp decrease in the pH during the last sample days. The pH ranged from a low of 7.0 at Site 5 to a high of 9.3 at Site 1.

It is not indicated on the graph but heavy rains caused the pH to drop. This and severe evaporation caused Site 1 to change the most. The site with very narrow pH range was Site 3.

# COLOR KEY TO GRAPHS

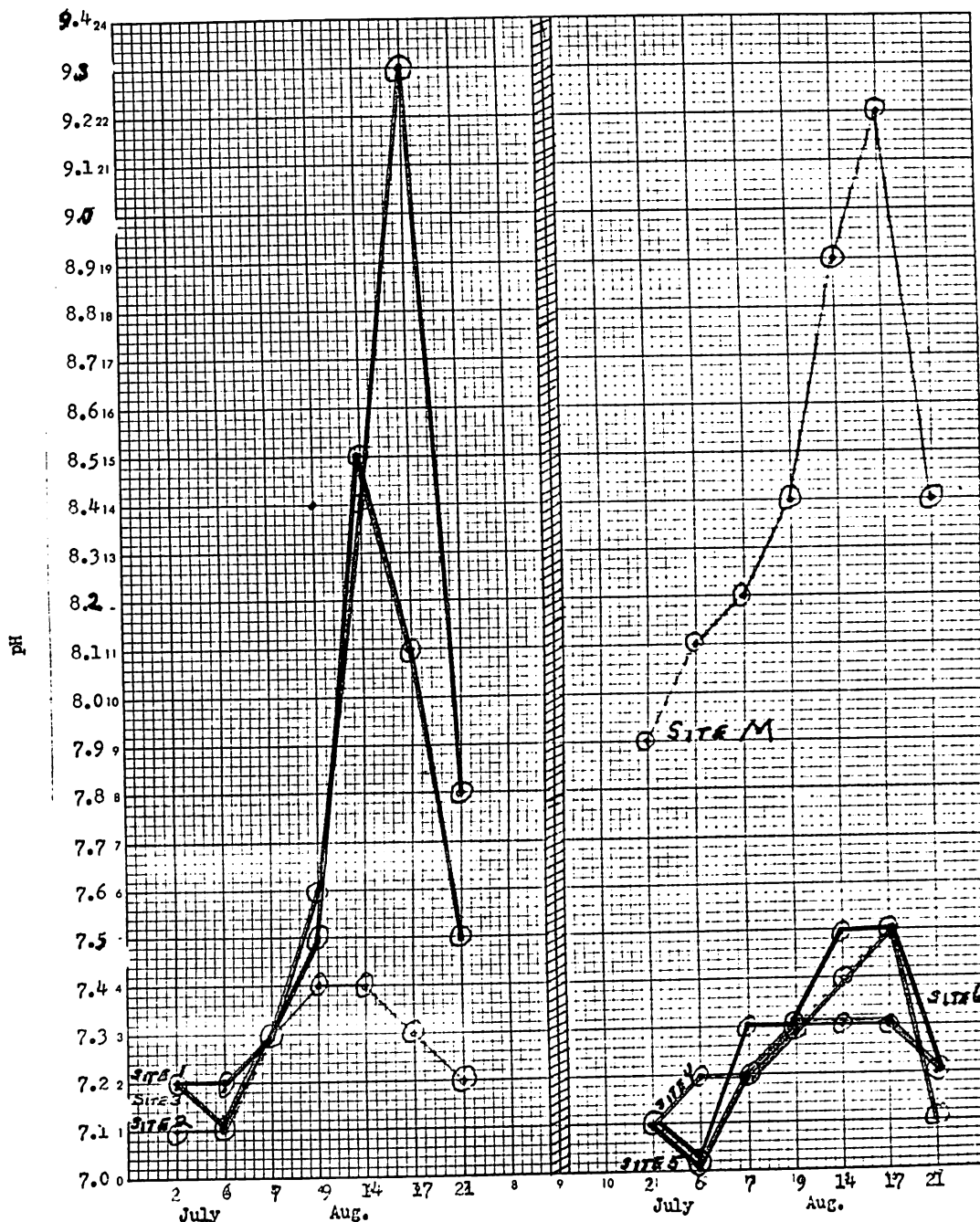
Site 1 \_\_\_\_\_  
Site 2 \_\_\_\_\_  
Site 3 \_\_\_\_\_  
Site 4 \_\_\_\_\_  
Site 5 \_\_\_\_\_  
Site 6 \_\_\_\_\_  
Site M \_\_\_\_\_

WATER pH = SAMPLE A



DATE \_\_\_\_\_

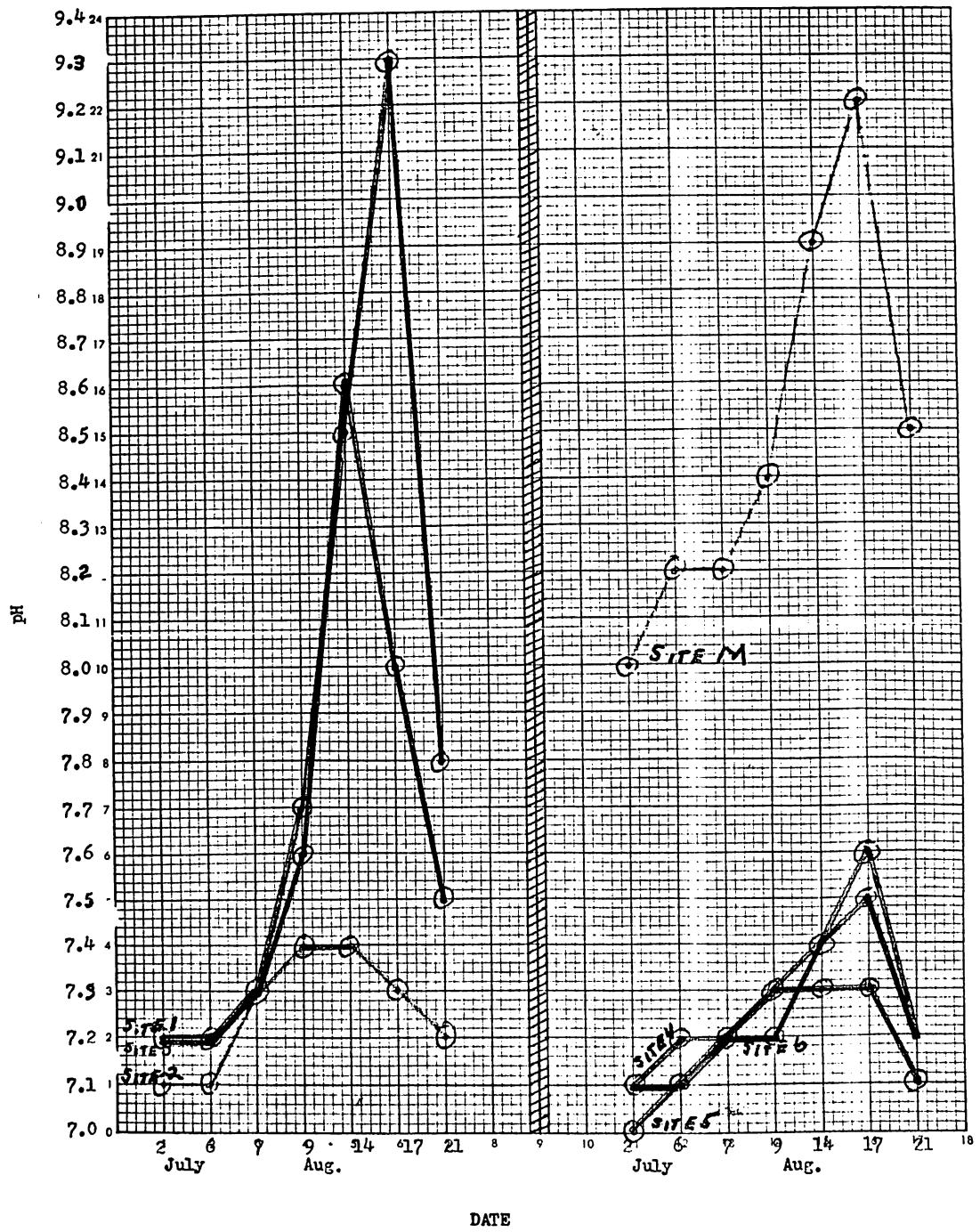
WATER pH- SAMPLE B



DATE \_\_\_\_\_



WATER pH - SAMPLE C



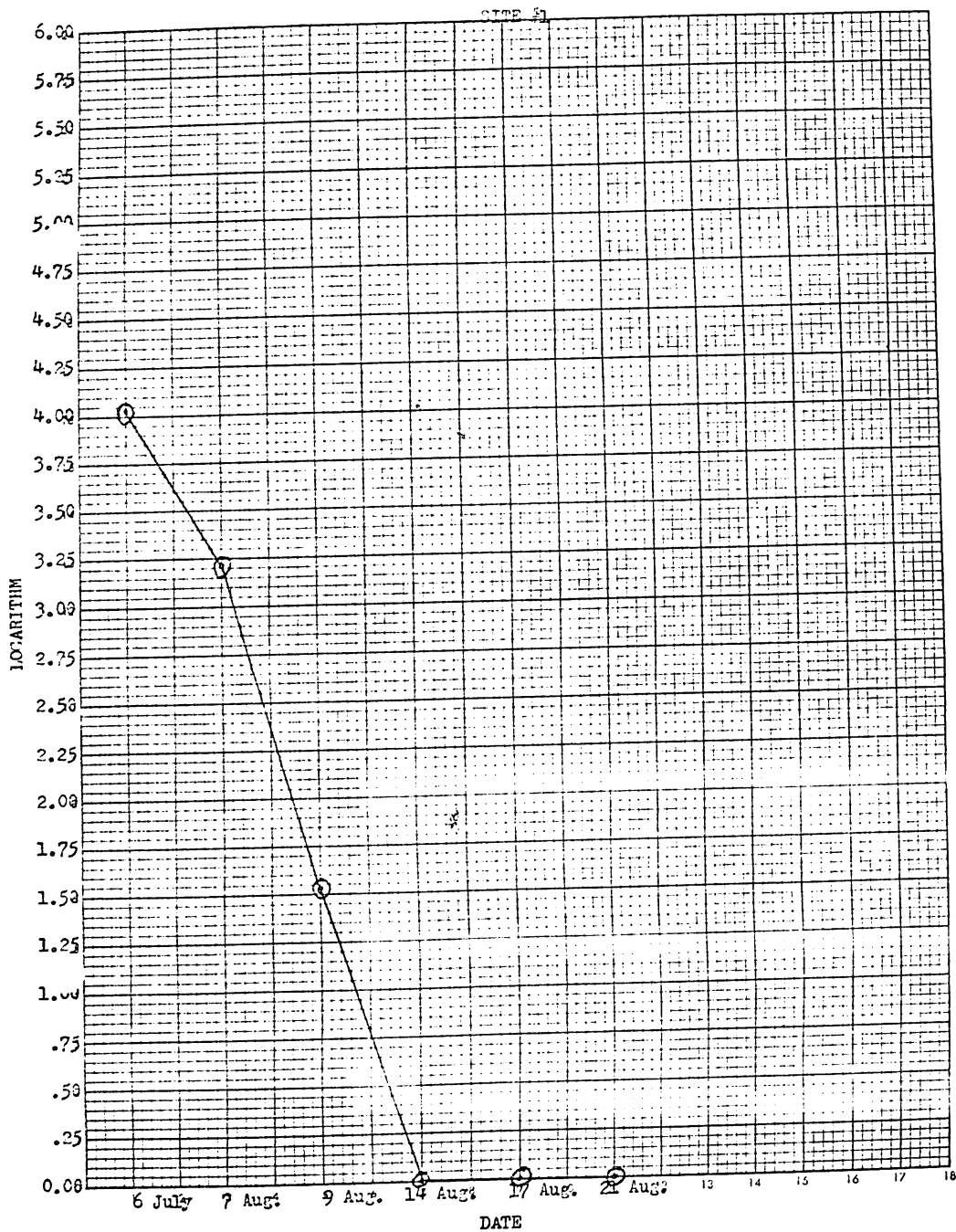
LOGARITHMIC EXPRESSION OF THE AVERAGE NUMBER  
OF COLIFORMS PER 100 ML

The graphs of the coliform average are in two sets. The first has the logarithm plotted against the sample day. The data for each site is plotted per graph. The second set has the logarithm plotted against the site number. Here the data for two days is plotted per graph.

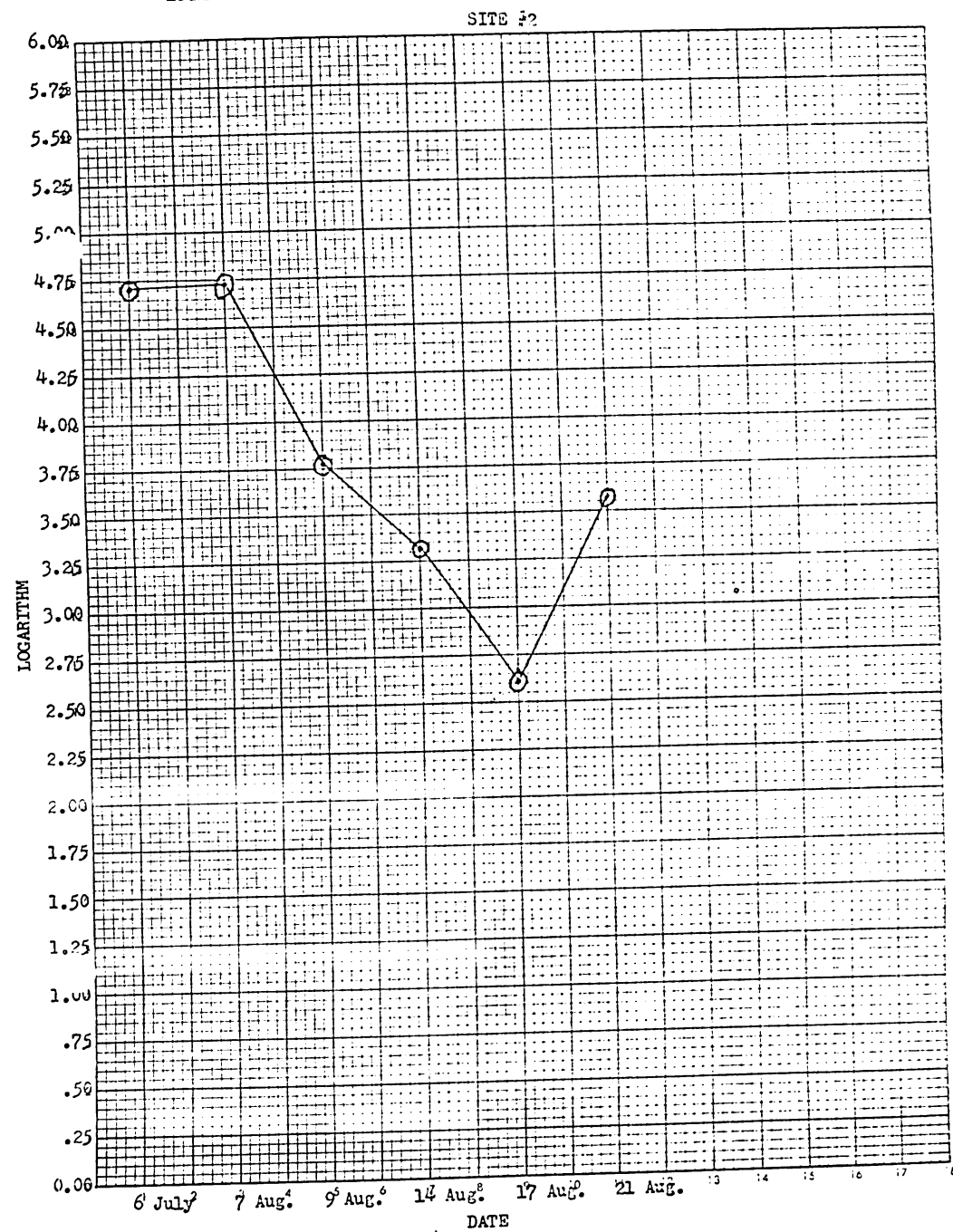
The coliform number fluctuated at each site during the study. Only Site 1 (which decreased to zero and remained there) and Mitchell Lake had little change.

The graphs indicate that there was a gradual increase in total fecal coliforms as each site was passed. The increase was demonstrated to Site 5 then dropped off.

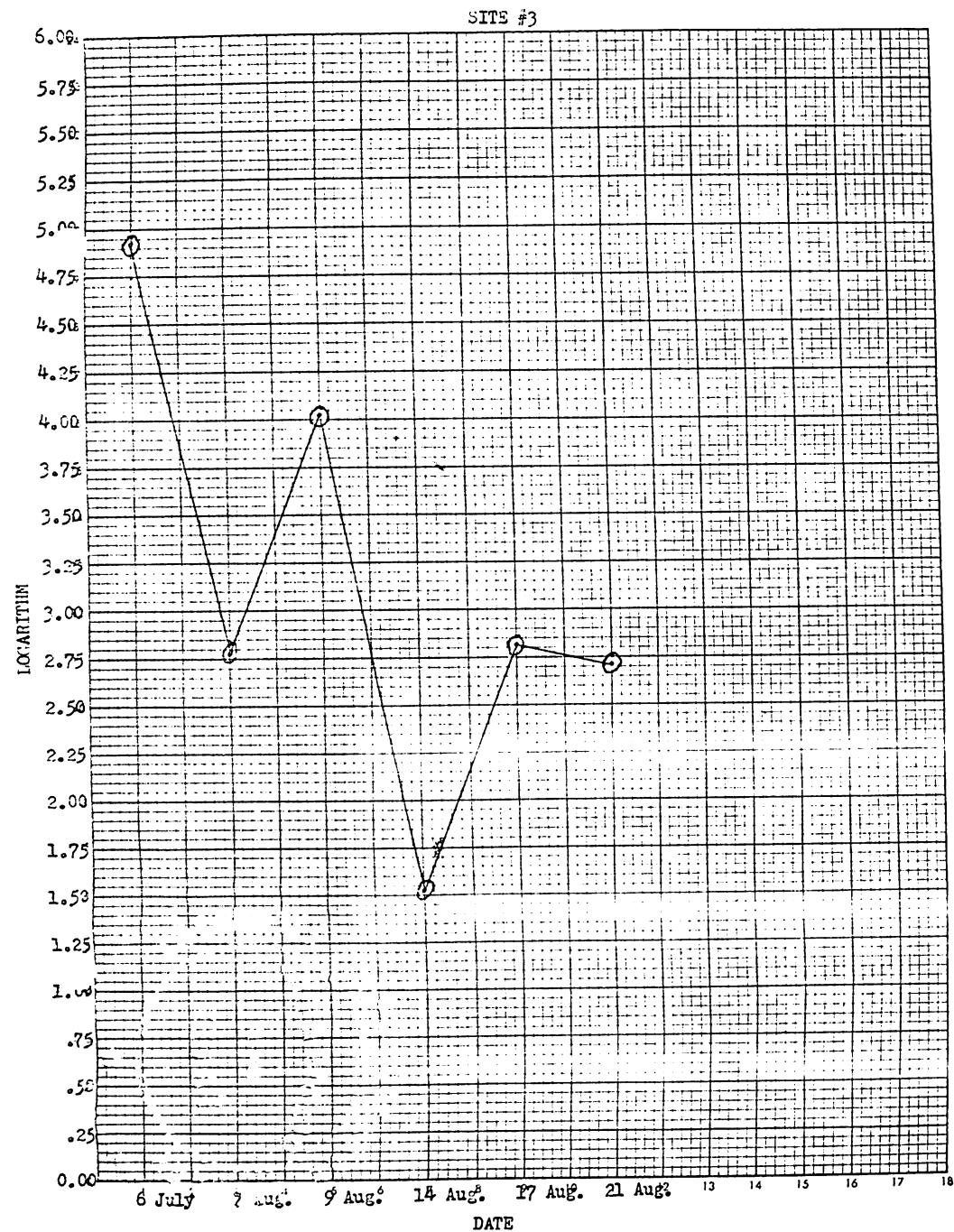
LOGARITHMIC EXPRESSION OF THE AVERAGE NUMBER OF COLIFORMS PER 100 ML



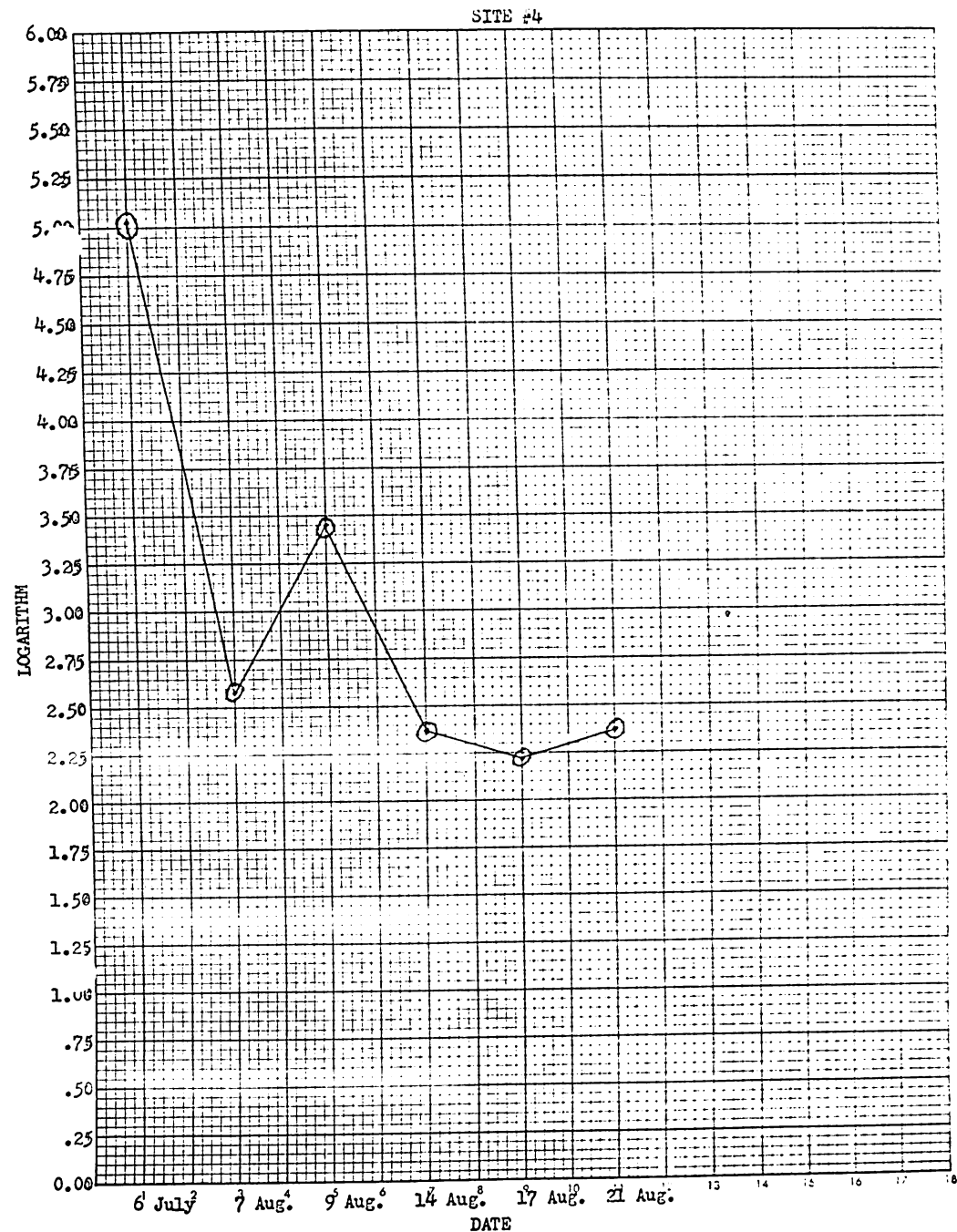
LOGARITHMIC EXPRESSION OF THE AVERAGE NUMBER OF COLIFORMS PER 100 ML



LOGARITHMIC EXPRESSION OF THE AVERAGE NUMBER OF COLIFORMS PER 100 ML

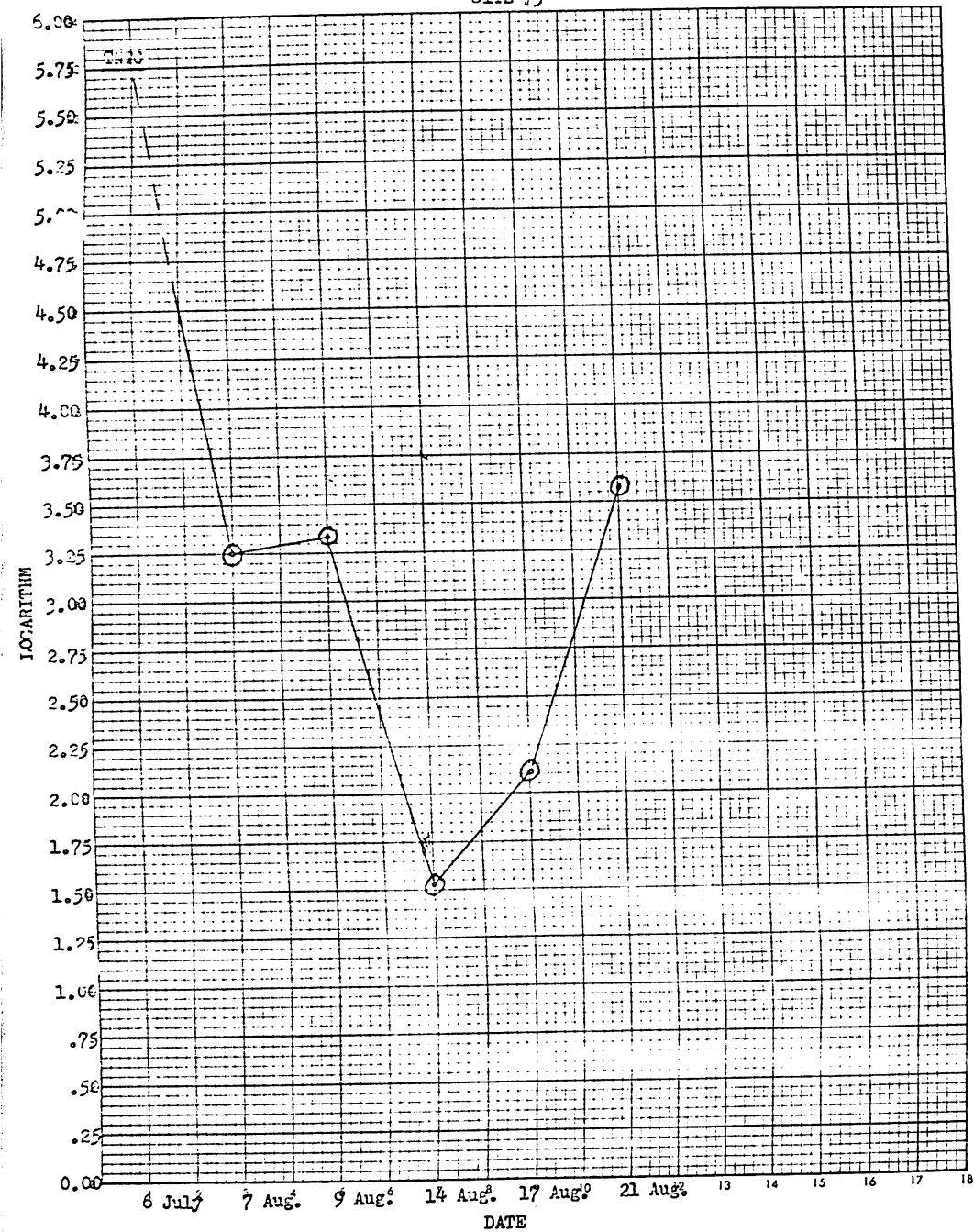


LOGARITHMIC EXPRESSION OF THE AVERAGE NUMBER OF COLIFORMS PER 100 ML



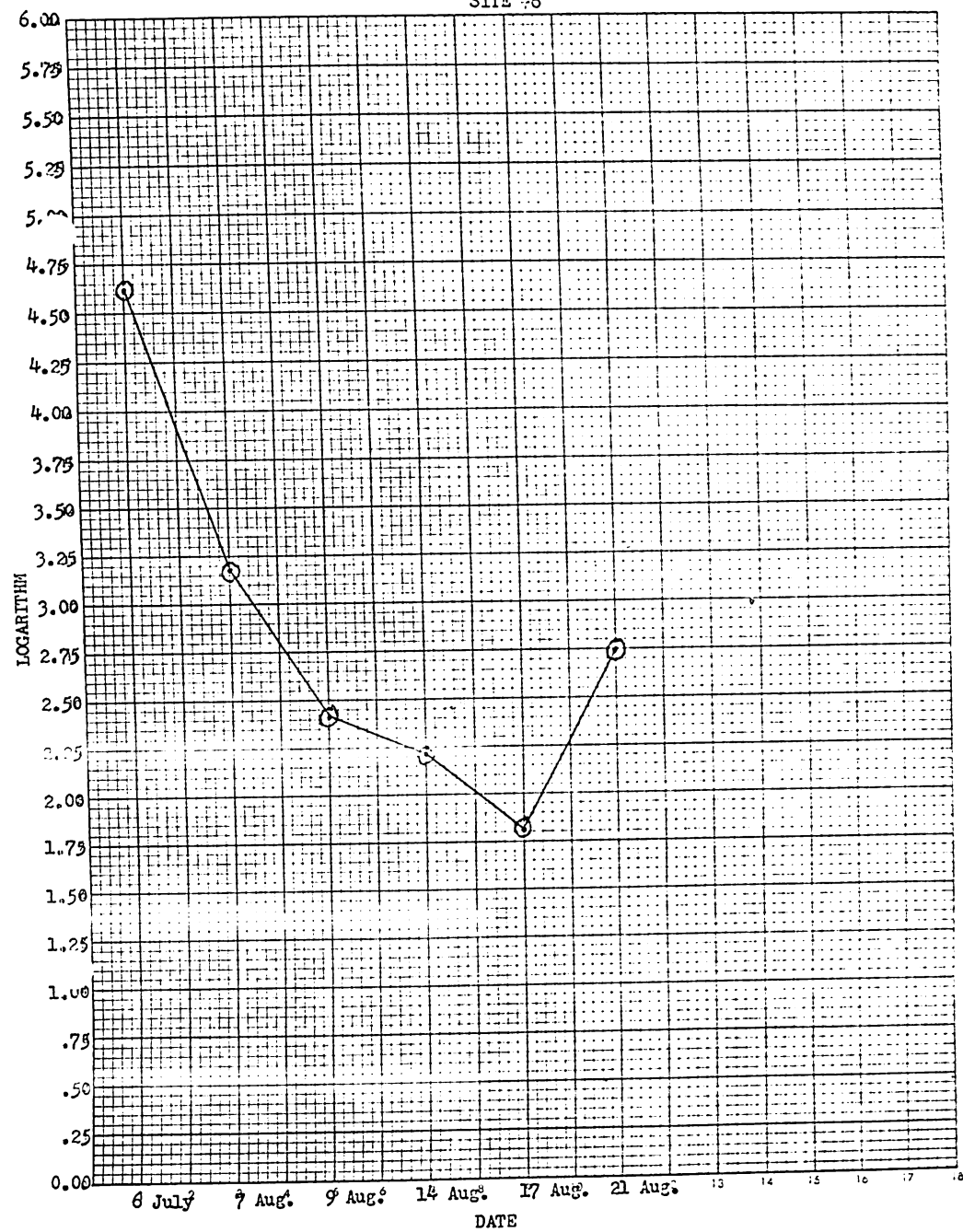
LOGARITHMIC EXPRESSION OF THE AVERAGE NUMBER OF COLIFORMS PER 100 ML

SITE #5



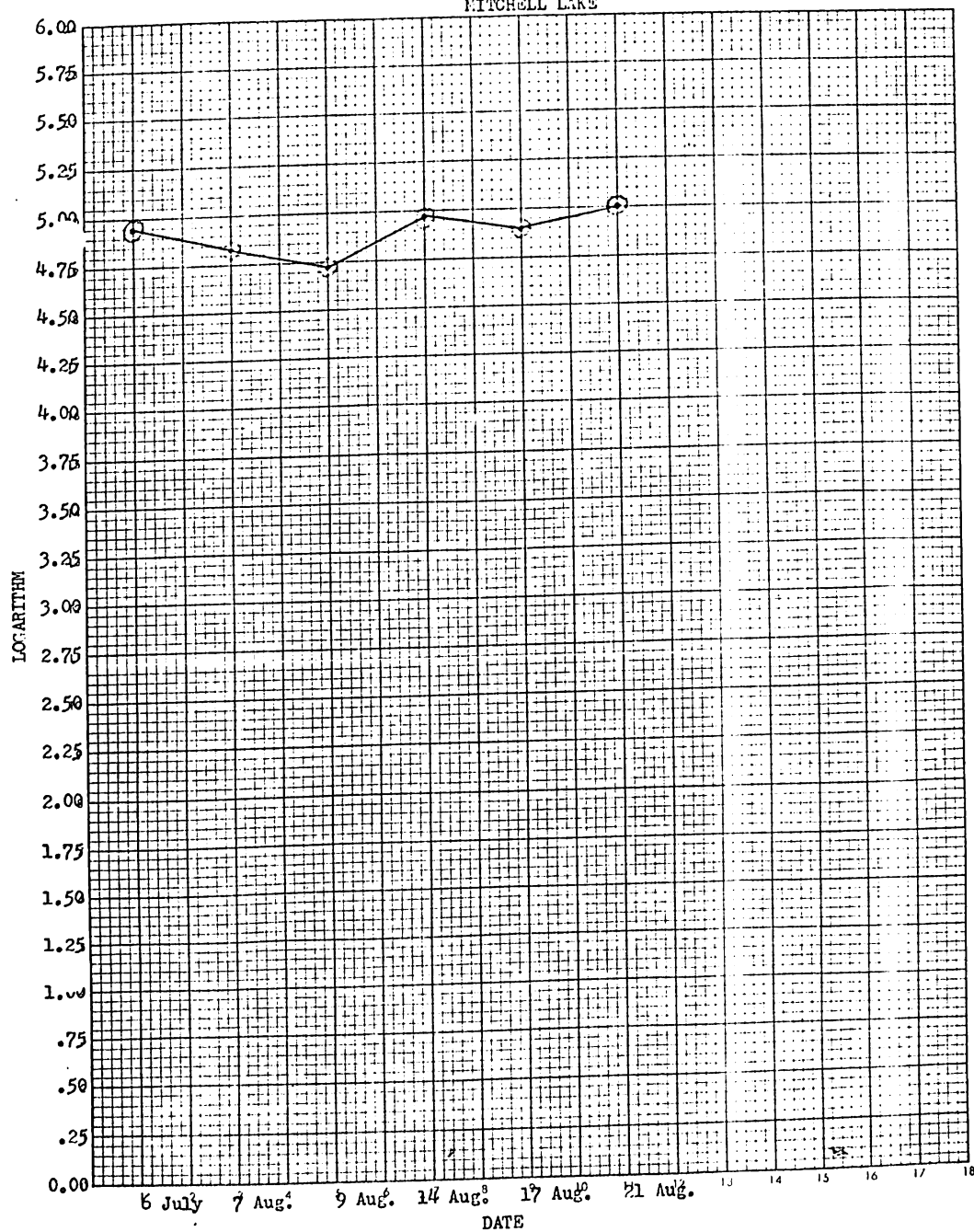
LOGARITHMIC EXPRESSION OF THE AVERAGE NUMBER OF COLIFORMS PER 100 ML

SITE #6



# LOGARITHMIC EXPRESSION OF THE AVERAGE NUMBER OF COLIFORMS PER 100 ML

MITCHELL LAKE

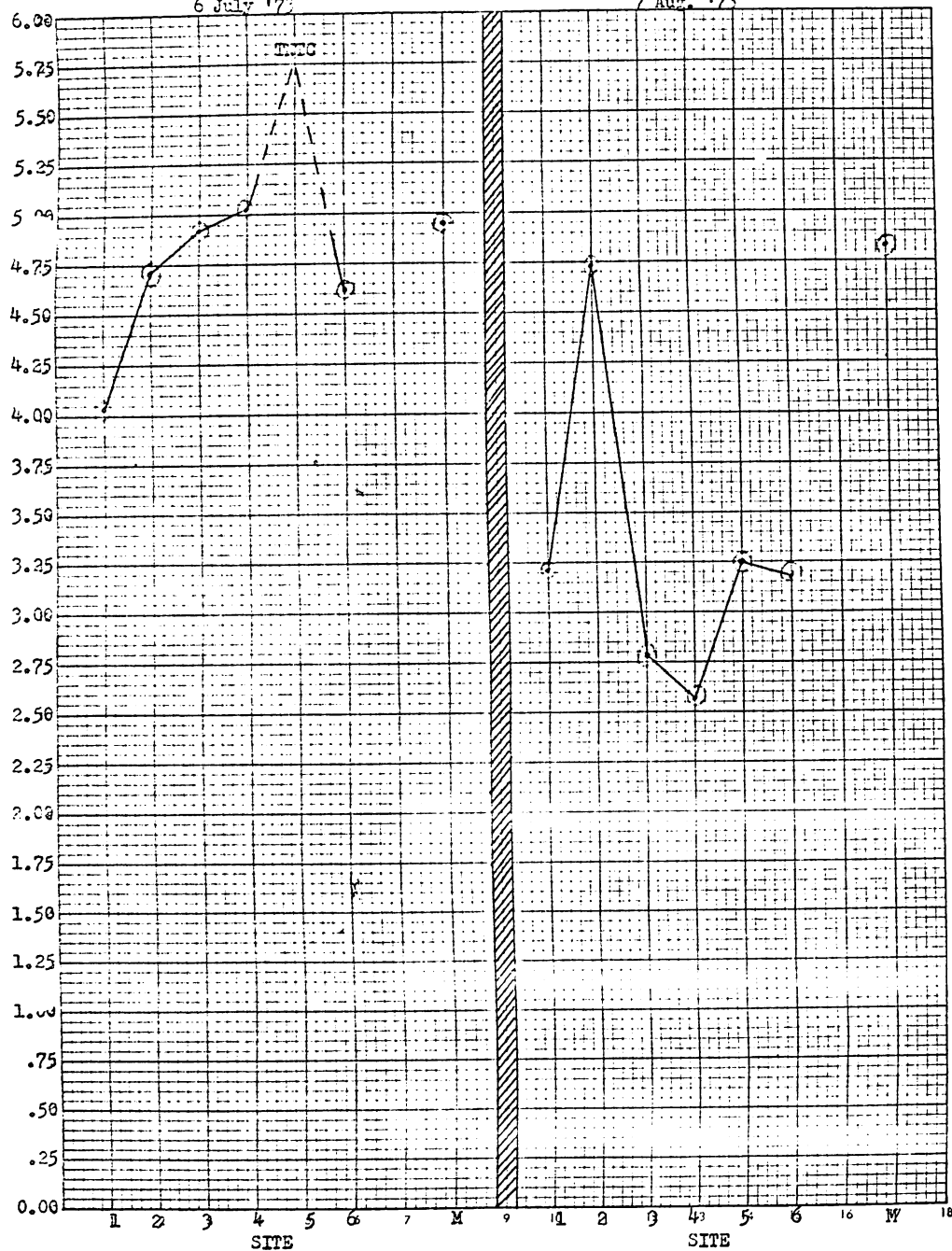




LOGARITHMIC EXPRESSION OF THE AVERAGE NUMBER OF COLIFORMS PER 100 ML

6 July '73

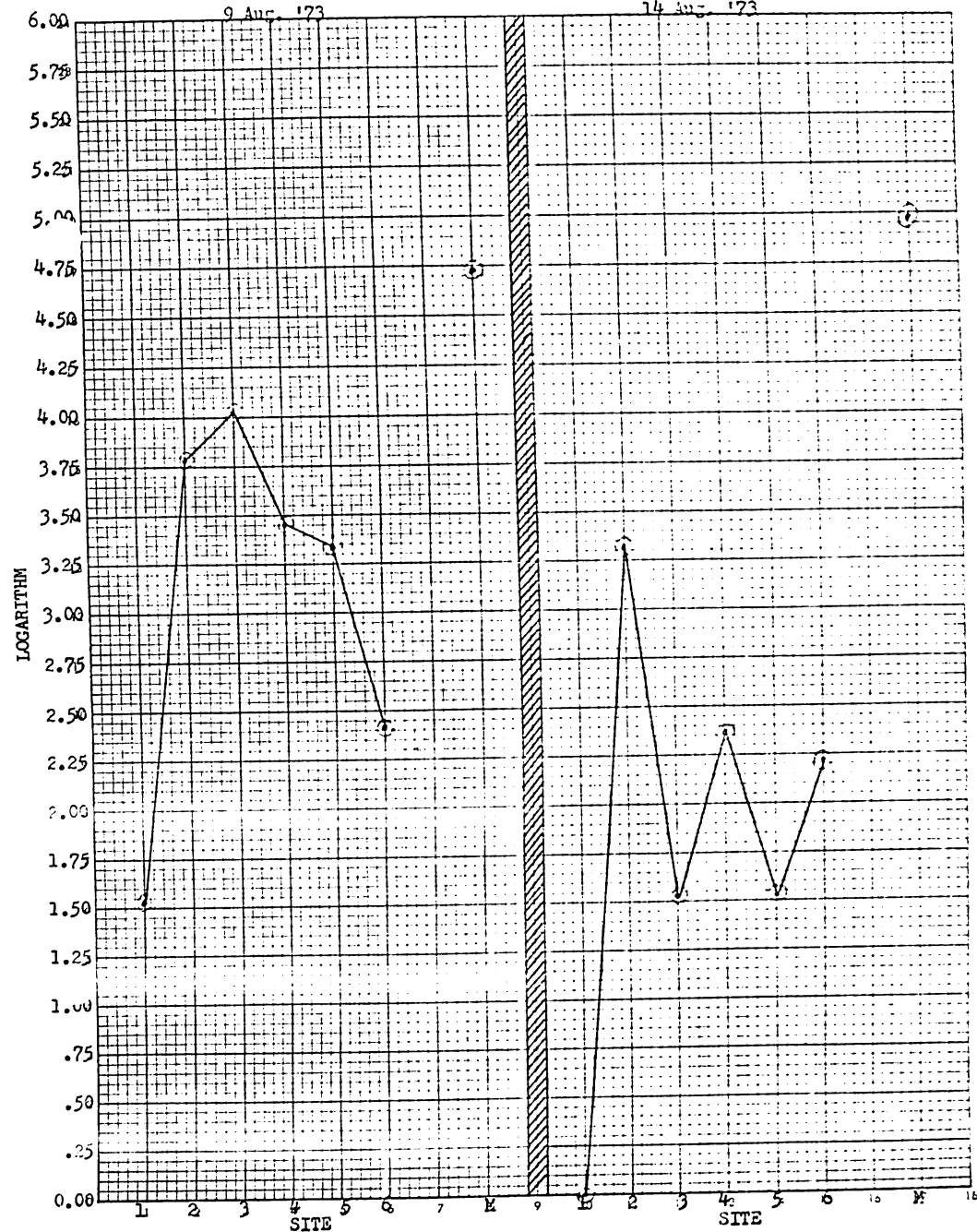
7 Aug. '73



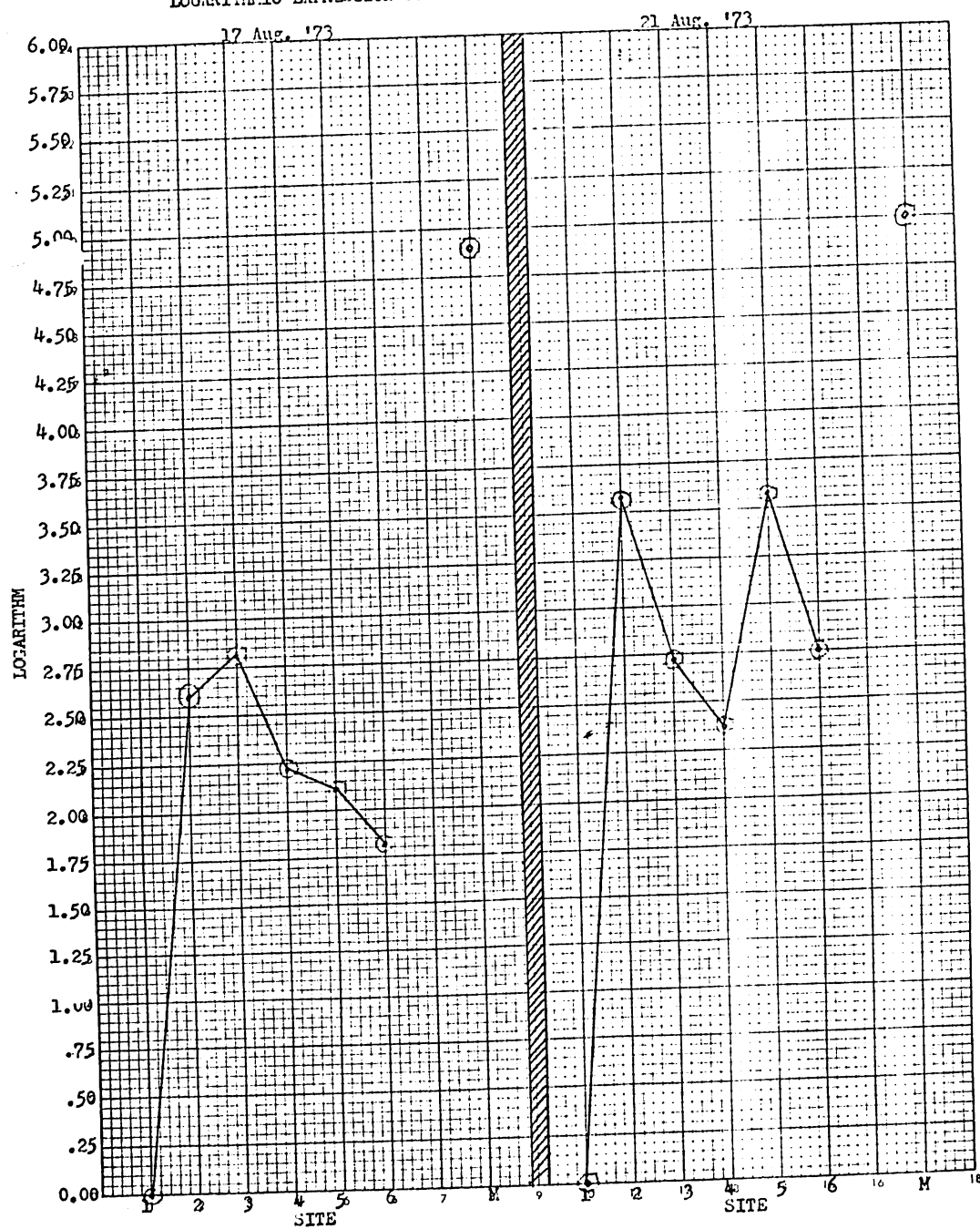
LOGARITHMIC EXPRESSION OF THE AVERAGE NUMBER OF COLIFORMS PER 100 ML

9 Aug. '73

14 Aug. '73

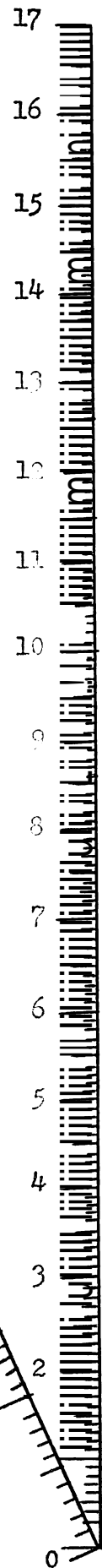


# LOGARITHMIC EXPRESSION OF THE AVERAGE NUMBER OF COLIFORMS PER 100 ML

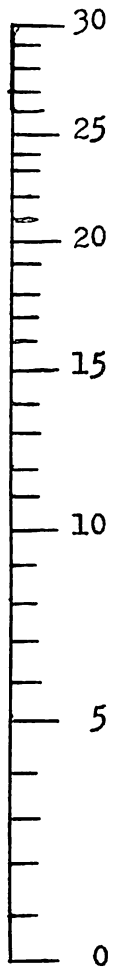




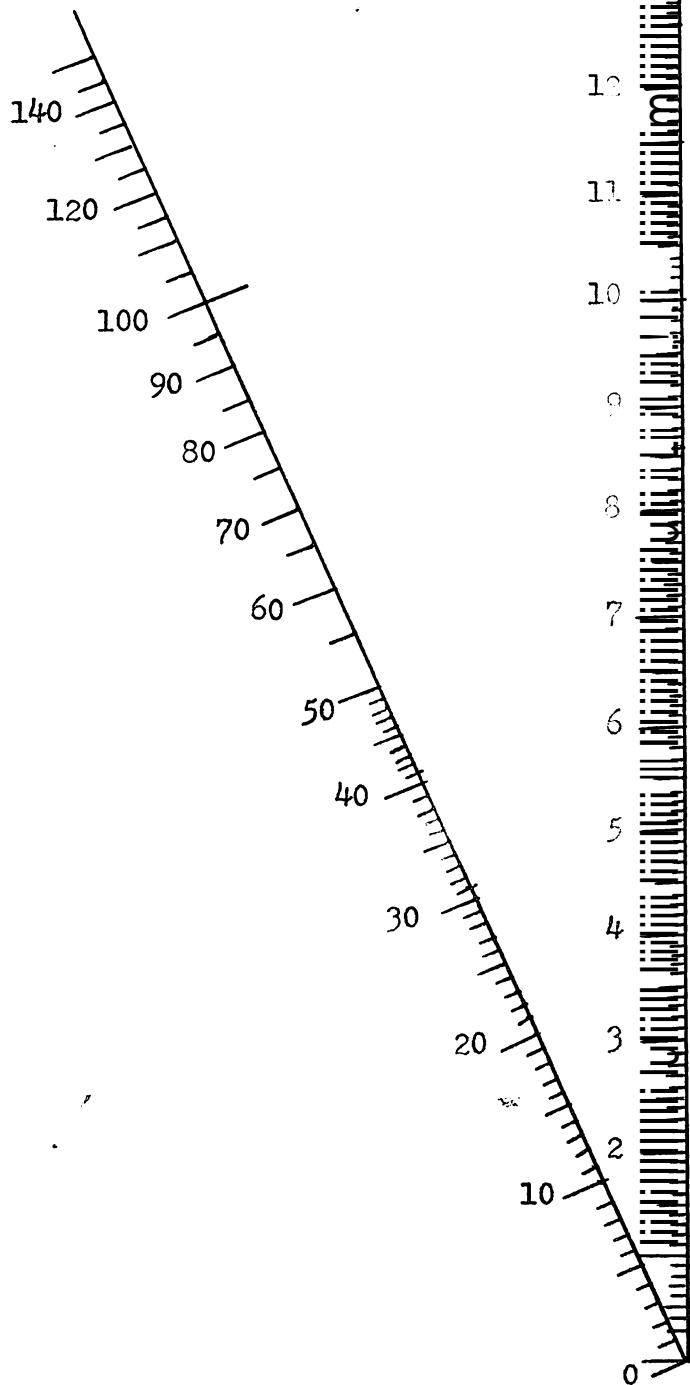
Oxygen  
mg per liter



Water Temperature C°



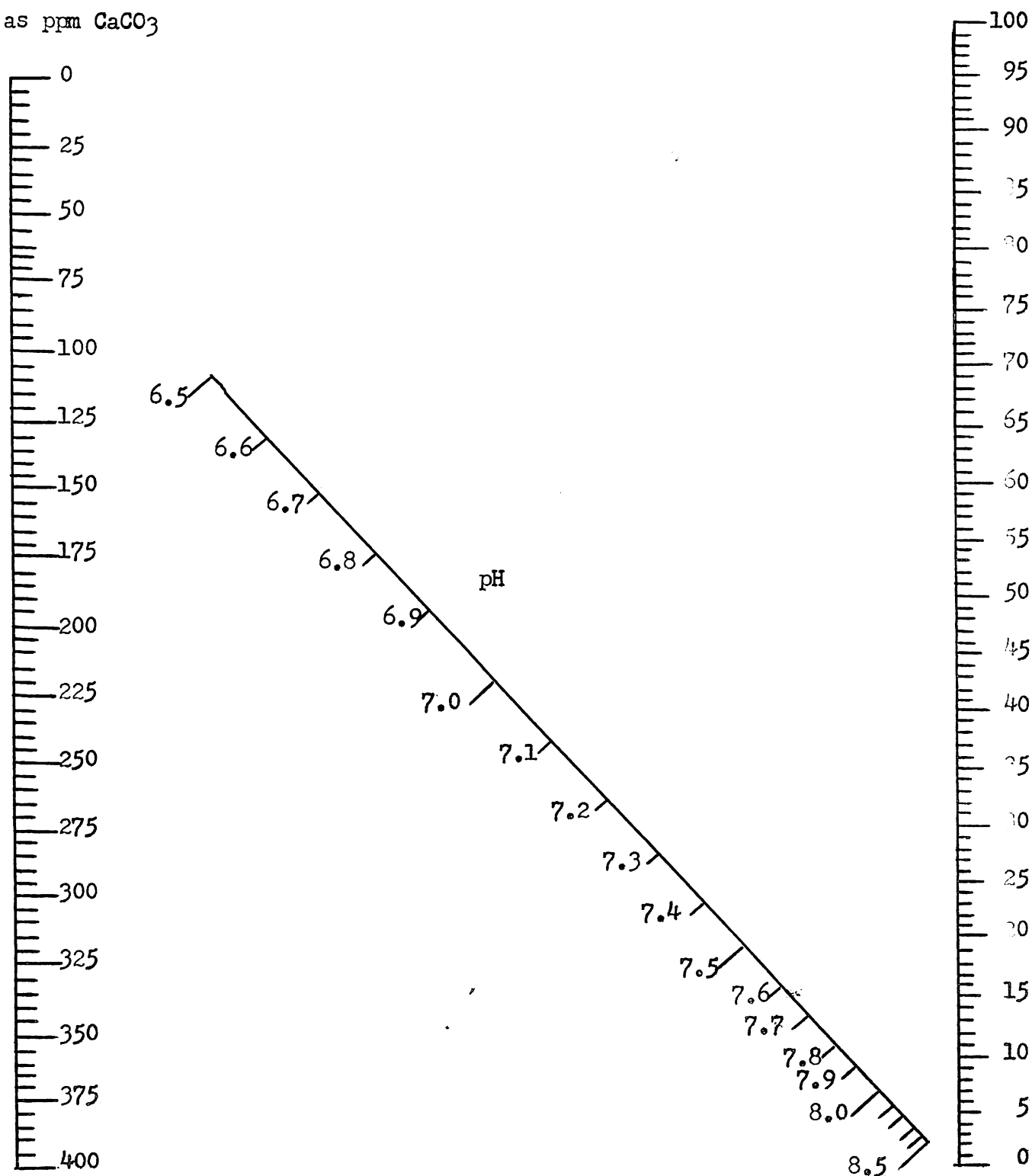
% Saturation



Total Alkalinity,

as ppm  $\text{CaCO}_3$

Free Carbon Dioxide



## APPENDIX A

### CHEMICALS

1. Ethyl alcohol
2. Manganous sulphate
3. Methyl orange indicator
4. M-PC broth
5. Rosolic acid
6. Phenolphthalein indicator
7. Potassium dihydrogen phosphate
8. Potassium iodine
9. Potato starch
10. Sodium hydroxide
11. Sodium thiosulphate
12. Sulphuric acid

# APPENDIX B

## EQUIPMENT

	<u>Name</u>	<u>Number</u>
1.	Asbestos gloves	1 set
2.	Autoclave	1 unit
3.	Bunsen burner	1
4.	Canvas bag	1
5.	Chemical bottle and glass stopper	1
6.	Erlenmeyer flask and glass stopper (250 ml)	2
7.	Forceps	1
8.	Graduated cylinder (100 ml)	2
9.	Laboratory balance	1
10.	Metal racks	4
11.	Millipore disposable petri dishes	3 boxes
12.	Millipore filters and pads (#HAWG 047A0)	4 boxes
13.	Millipore pyrex filter holder	1 unit
14.	pH meter	1 unit
15.	Pipettes	19
	1 ml	2
	10 ml	
16.	Pipette filler	1
17.	Refrigerator	1 unit
18.	Rubber tubing	6 feet
19.	Spatula	1
20.	Test tubes and screwcaps (15 ml)	19
21.	Thermometer (centigrade)	2

	<u>Name</u>	<u>Number</u>
22.	Vacuum pump	1 unit
23.	Water bath	1 unit
24.	Water-proof bags (8 oz. capacity)	1 box
25.	Wax crayon	2
26.	Wide mouth jars and glass covers (400 ml)	21

APPENDIX C  
MATHEMATIC FORMULAS

1. Indicator Organisms per 100 ml

$$\text{I.O./100 ml} = 100 \times \frac{\text{number of indicator colonies}}{\text{number of ml. sample filtered}} \times \frac{\text{dilution}}{1}$$

2. Water Velocity and Flow Measurements

$$R = \frac{W \cdot Da \cdot L}{T}$$

where R = Volume of flow in cubic feet per second

W = Average width of stream in feet

D = Average depth in feet

a = Constant factor for bottom types:

Smooth sand, etc. = 0.9

Rough rocks, etc. = 0.8

L = Length of stream section measured

T = Time in seconds for float to travel the measured distance

APPENDIX D  
SOLUTION PREPARATION

1.     M-FC Broth

- a.     Using a spatula or scoop, weigh out 3.7 grams of dehydrate into a weighing dish on the laboratory balance.
- b.     Pour out 100 ml (0.1 liter) of distilled water into a clean 100 ml graduated cylinder.
- c.     Pour out approximately 20 ml of the distilled water from the graduated cylinder into a clean 250 ml screwcap Erlenmeyer flask without spilling.
- d.     Empty the contents of the weighing dish carefully into the prepared 250 ml Erlenmeyer flask and swirl to disperse the dehydrate.
- e.     Pour the remaining contents of the graduated cylinder into the 250 ml Erlenmeyer flask without spilling.
- f.     Obtain rosolic acid dehydrate from the reagent shelf.
- g.     Weigh out 1 gram of dehydrated rosolic acid on the laboratory balance according to weighing procedure above.
- h.     Measure out 100 ml of 0.2 N sodium hydroxide solution into a clean 100 ml graduated cylinder.
- i.     Pour out approximately 20 ml of sodium hydroxide from the graduated cylinder into a second clean 250 ml screw-cap Erlenmeyer flask without spilling.
- j.     Carefully empty the contents of the weighing dish (rosolic acid dehydrate) into the second prepared 250 ml Erlenmeyer flask and swirl to disperse the dehydrate.

- k. Pour the remaining contents of the graduated cylinder into the second 250 ml Erlenmeyer flask without spilling. This produces a 1% rosolic acid solution.
- l. Pipette out 1 ml of 1% rosolic acid solution.
- m. Dispense 1 ml into the flask containing the dissolved M-FC broth.
- n. Place the flask, loosely covered, in a boiling water bath.
- o. Heat the medium to the boiling point, then remove and cool.
- p. Dispense at room temperature. pH should be 7.4.
- q. Store unused portion at 2-10°C and discard after 96 hours.

2. Preparation of Sterile Dilution Blanks

- a. Obtain either clean standard milk dilution bottles or clean screwcap 15 x 150 mm culture tubes.
- b. Dispense the required amounts of buffer in the appropriate container. The recommended amount for bottles is approximately 102 ml. The recommended amount for tubes is approximately 9.5 ml. Workers are advised to put slightly more dilution water in the container than is required because autoclaving causes some of the solution to evaporate. Experience has shown that the above amounts are appropriate for this procedure.
- c. Autoclave sterilize the dilution water containers, loosely capped, at 121°C for 15 minutes at 15 psi.
- d. After autoclaving, the amounts of water present in each bottle should be  $99 \text{ ml} \pm 2.0 \text{ ml}$ , and the amount of water in each tube should be  $9 \text{ ml} \pm 0.2 \text{ ml}$  at room temperature. Workers may experiment with various preautoclave amounts of solution to determine



exactly how much buffer water is needed prior to autoclaving in order to obtain the required finished amounts within the stated limits.

- e. Store the sterile bottles, tightly covered, on a cool, dark shelf, or refrigerate; store the tubes of water in racks as above for bottles.

### 3. Preparation of Sterile Phosphate Buffer Water

- a. Dissolve 34.0 grams of potassium dihydrogen phosphate,  $\text{KH}_2\text{PO}_4$ , in a clean 1000 ml beaker filled with 500 ml of distilled water.
- b. Adjust the pH to 7.2 with 1N  $\text{NaOH}$  (available commercially).
- c. Dilute to 1000 ml (1 liter) with distilled water to produce stock buffer solution.
- d. Pour the contents of the beaker into a clean Fenwall bottle and label it Stock Buffer Solution.
- e. Place the stopper on the Fenwall bottle and autoclave sterilize it for 15 minutes at  $121^\circ\text{C}$  and 15 psi so that the level of contamination in the stock buffer solution will remain at a minimum.
- f. Allow the stock buffer solution to cool before dispensing it.
- g. Pour out 1 liter portions of distilled water into clean Fenwall bottles, as many as needed.
- h. Add 1.25 ml of sterilized Stock Buffer Solution to each bottle of distilled water, cover each bottle and agitate it to mix the solution.
- i. Replace the stoppers on the Fenwall bottles and autoclave them for 15 minutes at  $121^\circ\text{C}$  and 15 psi. Properly autoclaved bottles produce a "pop" when they are opened for use.

- j. Label each bottle PHOSPHATE BUFFER WATER and store on the shelf until needed.
- k. Store the Stock Buffer Solution at 2-10° or on a cool, dark shelf. Check the pH before each use to make sure it is 7.2.

APPENDIX E  
REAGENT PREPARATION

1. Manganous sulphate: 480 grams  $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$  dissolved in distilled water; filtered and made up to 1 liter.
2. Alkaline-iodide: 500 g. NaOH or 700 g. KOH (these may be used interchangeably) and 135 g. NaI or 150 g. KI in distilled water made up to 1 liter.
3. Concentrated sulphuric acid: sp. gr. 1.83-1.84.
4. Sodium thiosulphate: Use 6.250 g.  $\text{Na}_2\text{S}_2\text{O}_3$  in distilled water; make up to 1 liter. This makes an N/40 solution. Add 5 cc chloroform to preserve it. Use new solution every 3-4 weeks. Standardize occasionally against N/40 potassium dichromate solution as directed below.
5. Starch solution: Dissolve 5 g. of potato starch in small amount of distilled water and make up to 1 liter. Starch solutions, even if preservatives such as chloroform or zinc chloride are added, deteriorate quite rapidly, especially in warm weather. A satisfactory method is to use sterilized solutions in small bottles which are opened as required.
6. Phenolphthalein indicator: Dissolve 2 g. in 400 cc of 50 percent alcohol. Neutralize with N/50 sodium hydroxide. Use boiled distilled water to dilute the alcohol.
7. Sodium hydroxide: N/44 solution preferably made up in some properly equipped chemical laboratory. Since N/44 hydroxide, or stronger solutions, deteriorate once the bottle is opened, this should be restandardized, or a fresh one obtained at frequent intervals. It is helpful to have this reagent supplied in small, tightly corked containers.

8. Methyl orange indicator: Dissolve 0.2 g. in 400 cc of distilled water.
9. Sulphuric acid, 0.2 N or N/50: Make up according to standard specifications.

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