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Seasonal fluctuations of thermophilic and psychrophilic bacteria in Westhampton Lake, Richmond, Virginia

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SEASONAL FLUCTUATIONS OF THERMOPHILIC
AND PSYCHROPHILIC BACTERIA IN WESTHAMPTON LAKE,
RICHMOND, VIRGINIA

By

CHUKWUKERE GODWIN DURUNNA

B.S. Arkansas State University, 1982

A Thesis

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in

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AND PSYCHROPHILIC BACTERIA IN WESTHAMPTON LAKE,
RICHMOND, VIRGINIA

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TABLE OF CONTENTS

	<u>Page</u>
List of Tables	iv
List of Figures	v
Acknowledgements	vi
Abstract	vii
Introduction	1
Materials and Method	3
Results	6
Discussion	8
Tables	11
Figures	17
References Cited	35
Vita	38

LIST OF TABLES

<u>Number</u>	<u>Page</u>
1. Data collected from sampling station A, (0.6 m deep)	11
2. Data collected from sampling station B, (1.9 m deep) 0.3 m from the water surface	12
3. Data collected from sampling B, (1.9 m deep) 0.3 m from the bottom of the lake	13
4. Data collected from sampling station C, (3.7 m deep) 0.3 m from the surface of the lake	14
5. Data collected from sampling station C, (3.7 m deep) 0.3 m from the bottom of the lake	15
6. Chart of spore stain and Gramstain characteristics	16

LIST OF FIGURES

<u>Figure #</u>		<u>Page</u>
Fig. 1.	Watershed of Westhampton lake	17
Fig. 2.	Morphometric chart of Westhampton lake	19
Fig. 3.	CFU/ml of bacteria isolated at 10 ⁰ C against sample number	21
Fig. 4.	CFU/ml isolated at 55 ⁰ C incubation temperature against sample number	23
Fig. 5.	Number of bacteria (CFU/ml) isolated at 10 ⁰ C incubation temperature against temperature of lake	25
Fig. 6.	CFU/ml isolated at 55 ⁰ C incubation temperature against temperature of lake	27
Fig. 7.	Number of bacteria CFU/ml isolated at 10 ⁰ C against O ₂ concentration of lake	29
Fig. 8.	Number of bacteria CFU/ml isolated at 55 ⁰ C against oxygen concentration	31
Fig. 9.	Relationship of temperature of the lake with oxygen concentration (PPM) of the lake at time of sampling . .	33

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ABSTRACT

This study was based on the hypothesis that the populations of thermophilic, mesophilic, and psychrophilic bacteria in Westhampton Lake vary seasonally and are related to prevailing temperatures. It may be inferred that the kinds of bacteria present at a given season are those most directly involved in decomposition and recycling activities in the lake.

Water samples were collected biweekly from September of 1986 through mid- August 1987 at three stations in Westhampton Lake. At each station, sample depths ranged from near the surface to near the bottom. Serial dilutions were plated in duplicate on nutrient agar and were incubated at 10, 37, and 55 C. The number of CFU (colony forming units)/ml were calculated. Pure isolated colonies were subcultured as stock on nutrient agar slants. Experimentally, subcultures from the 10 C (psychrophilic) and 55 C (thermophilic) conditions were incubated at 37 C to check for facultative tendencies. Other subcultures were incubated under strictly anaerobic conditions to determine oxygen relationships. Standard staining methods were used to determine the gram staining reaction and the presence or absence of endospores for each isolate.

Psychrophilic populations reached their maximum levels in winter during periods of minimum water temperatures while thermophile populations were at their maximum in the summer. No obligately thermophilic species were isolated, although several isolates of gram positive spore-forming rods were found that grew well at 55 C as well as

at 37 C. Approximately 40-50% of the isolates originally obtained from plates cultured at 10 C proved to be obligately psychrophilic; of these, approximately two-thirds were gram-positive spore-forming bacilli. No strictly anaerobic forms were isolated.

It is suggested that the diluting effect of rainfall and snow may affect both the temperature and the dissolved oxygen of the lake, resulting in immediately detectable effects on bacterial populations.

INTRODUCTION

Bacteria of many different species contribute to decomposition and recycling of essential elements in lakes. The interactions of the species present determine the extent and nature of the decomposition and recycling that can occur in a given place at a given time.

The present work is based on the hypothesis that the seasonal temperature cycle would be a major factor determining population dynamics in the major thermal groups of bacteria in a lake and that population fluctuations may influence rates of decomposition and recycling of organic substances. No studies of seasonal fluctuations in populations of the various thermal groups could be found in the literature reviewed. While other ecological aspects of Westhampton Lake have been studied, no bacteriological investigations have been conducted.

Bacteria can be categorized into three major thermal groups (e. g. Wistreich and Lechtman, 1984) with temperature ranges (minimum and maximum for reproduction) as follows: psychrophiles, <0-20; mesophiles, 20-45, and thermophiles, 45->90 C. In the present research species with optimum temperatures of 55 C are regarded as thermophiles while the psychrophiles have optima of 10 C. The modifier, "facultative", as applied to either major group refers to species that grow at 37 C while "strict" or "obligate" designates those that do not. Since oxygen content of water is related to temperature, the oxygen requirements of the organisms were also taken into consideration, and the terms "strict" and "facultative", refer to those that survive with and without oxygen respectively.

Bishop (1982) and Newlin (1981) found the lake to be moderately to highly productive of phytoplankton and Slater and Witmore (1988) found that the fish population was comparable to those of nearby lakes. Within the lake itself, major contributors of decomposable materials are algae, fishes, amphibians, a few reptiles, and waterfowl. During late summer and early fall, algal masses cover the lake surface. Runoff from the surrounding areas in periods of high precipitation also contributes materials subject to bacterial degradation (Bishop, 1982). Except in periods of high rainfall the current in the lake is slow, and in periods of drought, nonexistent; thus, there is usually little removal of nutrients from the lake by outflowing water.

MATERIALS AND METHODS

Sampling Site

Westhampton Lake is located in the Piedmont plateau in the City of Richmond, Virginia. It is surrounded by the campus of the University of Richmond; the area immediately to the North of the campus, and draining into the lake, is occupied by single-family dwellings (Fig. 1). When last measured, the lake had a total surface area of about 5.9 Km²; the mean and maximum depths were 2.8 and 7.8 m respectively (Bishop, 1982). Due to silting from construction, the lake was drained and dredged beginning in June, 1976.

Westhampton Creek, with a length of about 1970 m and a slope of 1%, is the main tributary and enters the lake on the northeast after passing through a residential area (Fig. 1, Bishop, 1982); The lake empties over a dam and the spillage water continues as Westham Creek into the Kanawha Canal, which in turn drains into the James River.

Sampling

Three sampling stations were established on Westhampton Lake (Fig. 2). Water samples were collected biweekly from September, 1986 to August, 1987. The sampling device consisted of a 250 ml erlemeyer flask with a two-holed rubber stopper equipped with capillary tubes. The entire assembly was housed in a metal container attached to a rope. Preliminary testing showed that the flasks would admit water only from the desired depth. Samplers were autoclaved at 121 C for 15 minutes prior to use.

The same procedure was followed each time samples were collected. At station A, located near the edge of the lake, (0.6 m total depth) one sample was collected at a depth of approximately 0.3 m, which was about half-way between the surface and the bottom. The water depth at station B was about 1.9 m; one sample was collected 0.3 m from the bottom and another 0.3 m from the surface. The water was 3.7 m deep at station C and was sampled as for station B. Samples were placed in an ice chest for transportation to the laboratory.

Using an electronic meter, the concentration of dissolved O₂ (ppm) was determined for each station at each collection depth; also, water temperature was measured for each sampling depth.

From June 7 through August 16, the water level of the lake was lowered to accommodate a construction project nearby on campus; this resulted in the loss of sampling station A and a reduction in the depth of the remaining sampling stations.

Sample Processing

Samples were returned to the laboratory and processed within 30 minutes following collection. Standard procedures were used to prepare serial decimal dilutions through 10⁻⁴ and for plating out samples using nutrient agar as the medium for enumeration and isolation of pure cultures (ALPHA, 1985). Plates were prepared in duplicate for each dilution and incubation temperature (10, 37 and 55 C, $\pm 1^{\circ}$ C. For a sterility check, uninoculated nutrient agar plates were incubated along with the samples.

Because psychrophiles generally grow more slowly than mesophiles or thermophiles (Stokes, 1963), the plates at 10 C were incubated for a

minimum of seven days while the other groups were evaluated after 24-48 hours. No attempt was made to isolate obligate anaerobes because the oxygen content of the lake water never was low enough (Fig. 9) to permit their reproduction in the habitat (Morris, 1976.)

Colonies were counted with the aid of a Quebec counter. Generally, only plates having 30 - 300 colonies were used in calculating CFU/ml of the original water specimen. Plates having fewer than 30 colonies or presenting "spreaders" or unusual bacterial colony types were dealt with as outlined in Standard Methods (ALPHA, 1985). Facultative tendencies in apparent psychrophiles or thermophiles were determined by subculturing each isolate onto fresh nutrient agar plates and incubating at 37 ± 1 C for 48 hours.

To ascertain if isolates were facultative with respect to oxygen, subcultures were also incubated at their optimum temperatures but under anaerobic conditions using the "GasPak" apparatus; in addition, this apparatus generates CO₂ to permit growth of capneic species.

Morphological Studies

Microscopic studies of each isolate were conducted and the organisms were characterized as to form of individual cells, characteristic cell arrangements, gram staining reaction, and presence or absence of endospores.

RESULTS

The temperatures for the sampling periods are given in Tables 1-5. It should be noted that the lake never attained the temperature (45 C) generally defined as the minimum for obligate thermophiles.

Samples from near the lake bottom presented higher counts for psychrophiles during the cold winter months while thermophiles were more numerous during the warmer months (Tables 1-5). In both winter and summer, the surface temperatures were slightly higher (by 0.1 - 1.0°C) than those at the deepest collection points; throughout the year, fewer psychrophiles were found in the samples collected near the surface.

All of the specimens that showed thermophilic tendencies in laboratory incubation were facultative in that respect; no obligate thermophiles were found. No significant differences due to sampling stations were noted. Obligate psychrophiles accounted for 37-49% of the total psychrophiles isolated while about 51-63% were facultative; 96-98% of the thermophiles were facultative (Table 6).

Lower water temperatures (4-8 C) coincided with higher concentrations of dissolved O₂ (Fig. 9; Tables 1-5); higher populations of psychrophiles were also found at the lower temperatures (Fig. 5). Thermophile populations were greatest when water temperatures were highest and dissolved O₂ lowest (Fig. 6, 8). Significant numbers of thermophiles were present only when water temperatures were 22° C or higher; thus, thermophiles showed a tendency to be facultative anaerobes (Fig. 8). Percentages of facultatively anaerobic species are given in Table 6.

In March, water temperatures were 4.2 - 4.6° C, dissolved O₂ reached a maximum of 11.8 - 12.9 ppm, and the population of psychrophiles was 6.7×10^4 CFU/ml (Tables 1-5, Fig. 5) while the population of thermophiles was at its lowest point (Fig. 6).

Percentages of obligate psychrophiles varied little between sampling stations or depths. There was no significant variation between the surface and bottom samples.

Populations of both psychrophiles and thermophiles fluctuated in relation to temperatures throughout the seasons (Fig. 3-6). Between January and March the temperatures changed from 4.3 to 6.5 C and the psychrophile population peaked accordingly (Tables 1-5).

Insignificant numbers of thermophiles were isolated between September and April, when the lake temperatures were 4.2 - 21 C (Table 1-5).

The gram-staining and spore-forming characteristics of the isolates are presented in Table 6; 50.6% - 61.6% of the psychrophiles were spore formers, an indication of the importance of sporulation to their survival.

DISCUSSION

Significant fluctuations in populations of bacteria occurred on a seasonal basis, with the psychrophiles reaching a maximum (ca. 6.75×10^4 CFU/ml) between January and March and a minimum (ca. 2.4×10^3 CFU/ml) between May and December. Thermophiles were at a maximum (6.5×10^3 CFU/ml) between June and August and a minimum (<30 CFU/ml) between September and May: the thermophiles isolated were all facultative (Table 6). Depth had no significant effect on population size of psychrophiles and there were no differences within either thermal group among the populations at the various sampling depths and stations (Table 1-5). The uniformity of distribution indicates that circulation sufficient to keep colloidal particles of bacterial size in homogeneous suspension occurs in the water. Wave action due to wind, along with thermal currents, are probable causes of the currents. More importantly, oxygen distribution was kept more or less uniform at the various depths sampled (Table 1-5). When the temperatures were lowest, the O_2 concentrations were highest (Fig. 9) and it was at these temperatures that the populations of psychrophiles were at maximum (Fig. 5). At higher temperatures and correspondingly lower O_2 concentrations, thermophile populations were maximum (Figs. 6, 8). No strictly anaerobic conditions occurred in any of the samples; the lowest O_2 concentration encountered was ca. 6 ppm (Table 1-5) and the thermophiles were not inhibited. When subcultured under strictly anaerobic conditions, 54-75% of the thermophilic isolates grew well and thus are facultative anaerobes (Table 6). The percentage of facultatively anaerobic psychrophiles was

52-68%; no statistical analysis was performed to see if this is a significant difference from the percentage found in the thermophiles. During the coldest times of the year, obligate psychrophiles accounted for approximately 37-50% of the total isolates, with facultative forms in the vicinity of 50-63% (Table 6).

Organic substances in water are decomposed chiefly by bacterial action and this process exerts a high demand on the dissolved oxygen in the lake. Thus in winter when decomposition is carried out primarily by psychrophiles, the oxygen concentration in the deeper water should tend to be low. According to a publication of the U. S. Department of the Interior (1967), diatoms generally will prevail in cold water and will be accompanied by a predominance of gram-positive heterotrophic bacilli. When the water temperature increases, as in summer, actinomycetes will become predominant and their presence may have an antibiotic effect resulting in a reduction of the gram-negative population. These conditions may favor the prevalence of gram-positive sporeformers (Table 6).

Strictly aerobic bacteria die off rapidly as dissolved oxygen concentrations decrease (Tables 1-5); this in effect will decrease the rate of decomposition, recycling and purification processes. As this happens, there will be a shift towards a predominance of facultatively anaerobic organisms (Fair et al. 1958).

Some inferences that may be drawn from these relationships are that the psychrophiles contribute most to decomposition and recycling in the winter; the mesophiles, facultative thermophiles and facultative psychrophiles are all active in the summer. However, the cold winter

temperatures, which have a retarding effect on growth and metabolic activities, along with the fact that psychrophiles generally grow more slowly than thermophiles probably means that the rate of biodegradation and thus the rate of recycling in winter is much slower than in the summer.

Future research along these lines could include simultaneous studies of decomposition rates and populations of the thermal groups to determine the nature and extent of any correlations that might exist. Interrelationships with factors such as pollen deposits in the springtime, algal blooms in the late summer and fall, and the shed foliage of deciduous trees in the autumn would be worthy of investigation.

Identification and biochemical characterizations of the organisms isolated in this study would provide many possibilities for future work and might reveal biochemical properties of significance in the over-all ecological picture of the lake.

Table 1. CFU/ml of bacteria isolated under psychrophilic (10°C), mesophilic (37°C), and thermophilic (55°C) incubation temps. with corresponding temps. (°C) and O₂ conc. (ppm) of the lake measured at time of sampling for station B (1.9 total depth and 0.3 m from the surface of water).

Date of Sampling	Sample Number	Temp of Lake	Oxygen Conc of Lake	CFU/ml Isolated at		
				10°C	37°C	55°C
9.28.86	1	25.7	8.5	1015	1015	9
10.12.86	2	20.1	7.8	1340	1805	30
10.26.86	3	15.4	7.8	1820	1220	4
11.9.86	4	17.2	9.6	2200	2020	5
11.23.86	5	10.1	8.8	2250	4400	3
12.7.86	6	9.2	10.0	4400	4000	5
12.21.86	7	7.4	11.2	----	3800	4
1.4.87	8	5.9	12.6	38000	20000	6
1.18.87	9	5.4	10.2	10200	5550	15
2.1.87	10	----	----	----	----	----
2.15.87	11	5.0	12.0	37500	17000	6
3.1.87	12	4.8	12.9	42000	18000	4
3.15.87	13	6.0	11.4	35000	----	6
3.29.87	14	10.2	10.6	15000	----	10
4.12.87	15	12.2	10.2	7650	5000	30
4.26.87	16	13.5	9.6	4000	3000	25
5.10.87	17	17.8	9.5	4600	----	28
5.24.87	18	20.2	9.0	3800	----	400
6.7.87	19	25.1	9.1	2000	4000	4500
6.21.87	20	----	----	----	----	----
7.5.87	21	----	----	----	----	----
7.19.87	22	----	----	----	----	----
8.2.87	23	----	----	----	----	----
8.16.87	24	----	----	----	----	----

Table 2. Temperature ($^{\circ}\text{C}$) and oxygen conc. (ppm) measured from sampling station B (1.9 m total depth and 0.3 m from bottom of lake) at time of sampling with corresponding CFU/ml of bacteria isolated at psychrophilic (10°C), mesophilic (37°C) and thermophilic (55°C) incubation temperatures.

Date of Sampling	Sample Number	Temp of Lake	Oxygen Conc of Lake	CFU/ml isolated at		
				10°C	37°C	55°C
9.28.86	1	25.9	8.5	1090	1110	19
10.12.86	2	20.1	8.0	1335	1535	43
10.26.86	3	16.3	7.5	1340	1700	4
11.9.86	4	17.2	9.6	2700	2725	7
11.23.86	5	9.3	8.1	4000	2700	14
12.7.86	6	8.2	9.3	6000	3200	5
12.21.86	7	6.8	9.4	----	3800	4
1.4.87	8	5.0	12.9	40000	18000	4
1.18.87	9	4.7	8.3	6200	5800	12
2.1.87	10	----	----	----	----	----
2.15.87	11	5.0	11.5	35000	16000	4
3.1.87	12	4.6	12.8	38500	18000	4
3.15.87	13	6.5	11.2	34000	----	8
3.29.87	14	10.0	10.8	18000	----	12
4.12.87	15	13.1	10.3	8000	5000	31
4.26.87	16	13.8	9.2	7600	4050	22
5.10.87	17	17.3	9.0	5100	----	24
5.24.87	18	20.8	8.5	3200	----	250
6.7.87	19	25.5	8.9	1940	3500	350
6.21.87	20	29.7	8.5	1800	5000	4500
7.5.87	21	31.3	7.6	2050	7500	6500
7.19.87	22	33.5	6.8	1400	8000	6450
8.2.87	23	34.0	6.0	1300	8500	6000
8.16.87	24	32.5	6.6	1350	7500	6050

Table 3. CFU/ml of bacteria isolated at psychrophilic (10°C), mesophilic (37°C), and thermophilic (55°C) incubation temperatures with corresponding temperatures (°C) and O₂ conc. (ppm) of the lake measured at time of sampling for station B (1.9 m total depth and 0.3 m from the bottom of lake).

Date of Sampling	Sample Number	Temp of Lake	Oxygen Conc of Lake	CFU/ml isolated at		
				10°C	37°C	55°C
9.28.86	1	24.5	6.4	1430	2620	14
10.12.86	2	20.0	6.9	2100	1550	18
10.26.86	3	15.5	5.4	7250	2010	23
11.9.86	4	15.7	8.3	2670	2820	14
11.23.86	5	8.7	6.1	12000	5050	17
12.7.86	6	7.5	7.6	18000	6000	7
12.21.86	7	6.0	8.0	----	5000	7
1.4.87	8	4.7	11.0	45500	14000	4
1.18.87	9	4.6	7.5	19700	6650	16
2.1.87	10	----	----	----	----	----
2.15.87	11	4.9	10.8	42500	18000	4
3.1.87	12	4.4	11.8	44500	17400	6
3.15.87	13	5.9	9.2	38000	----	8
3.29.87	14	9.5	11.3	25000	----	10
4.12.87	15	12.1	10.0	10200	8800	20
4.26.87	16	13.0	8.9	9450	7700	14
5.10.87	17	14.0	8.4	9450	7700	20
5.24.87	18	18.7	8.0	2500	----	120
6.7.87	19	23.9	9.8	2000	5500	300
6.21.87	20	28.4	8.7	1400	6500	3000
7.5.87	21	31.0	7.1	1550	7000	5500
7.19.87	22	32.0	6.7	1400	8500	5000
8.2.87	23	32.5	6.8	1300	8000	6500
8.16.87	24	31.4	7.0	1200	7050	5500

Table 4. Temperatures ($^{\circ}\text{C}$) and O_2 conc. (ppm) measured from sampling station C (3.7²m total depth and 0.3 m from the surface of water) at time of sampling with corresponding CFU/ml of bacteria isolated at psychrophilic (10°C), mesophilic (37°C), and thermophilic incubation temperatures.

Date of Sampling	Sample Number	Temp of Lake	Oxygen Conc of Lake	CFU/ml isolated at		
				10°C	37°C	55°C
9.28.86	1	26.0	8.3	1210	1360	17
10.12.86	2	20.4	7.6	1150	3335	35
10.26.86	3	16.0	6.7	1310	1630	4
11.9.86	4	16.0	9.9	2250	3020	8
11.23.86	5	8.9	8.2	4000	2650	5
12.7.86	6	7.6	7.7	6000	4000	6
12.21.86	7	6.6	9.2	----	4400	4
1.4.87	8	4.4	11.7	44000	14800	6
1.18.87	9	4.5	7.8	11900	7800	26
2.1.87	10	----	----	----	----	----
2.15.87	11	4.5	10.5	38000	14000	8
3.1.87	12	4.4	12.0	46000	13800	5
3.15.87	13	6.1	11.0	40000	----	8
3.29.87	14	10.9	10.2	20000	----	15
4.12.87	15	12.5	10.0	8200	6600	25
4.26.87	16	13.2	9.8	7400	5000	20
5.10.87	17	16.8	9.4	5450	----	23
5.24.87	18	20.6	8.8	3500	----	200
6.7.87	19	25.6	8.5	3000	4000	300
6.21.87	20	29.8	8.5	2000	6000	4000
7.5.87	21	31.6	6.8	2800	7500	5500
7.19.87	22	33.1	6.3	1600	8000	5000
8.2.87	23	33.7	6.1	1450	7500	6000
8.16.87	24	32.2	6.4	1400	7000	5500

Table 5. Temp ($^{\circ}\text{C}$) and O_2 conc. (ppm) measured from sampling station C (3.7 m total depth and 0.3 m from the bottom of lake) at time of sampling with corresponding CFU/ml of bacteria isolated at psychrophilic (10°C), mesophilic (37°C), and thermophilic (55°C) incubation temps.

Date of Sampling	Sample Number	Temp of Lake	Oxygen Conc of Lake	CFU/ml isolated at		
				10°C	37°C	55°C
9.28.86	1	22.2	5.7	6000	10300	240
10.12.86	2	19.4	5.2	4100	4000	65
10.26.86	3	13.0	5.5	1840	2400	35
11.9.86	4	14.7	7.9	27000	23000	30
11.23.86	5	8.4	5.8	26250	19900	16
12.7.86	6	6.9	6.4	26000	18000	9
12.21.86	7	5.6	8.0	----	15000	6
1.4.87	8	4.3	10.7	65000	16000	8
1.18.87	9	4.4	7.8	21300	13200	16
2.1.87	10	----	----	----	----	----
2.15.87	11	4.5	10.5	51000	15150	7
3.1.87	12	4.2	11.0	67500	15500	4
3.15.87	13	5.5	10.5	62000	----	6
3.29.87	14	9.2	11.8	22800	----	12
4.12.87	15	9.4	9.0	15000	10000	18
4.26.87	16	11.5	8.8	10200	8600	14
5.10.87	17	12.0	7.6	7050	----	16
5.24.87	18	16.4	7.4	2000	----	98
6.7.87	19	23.6	9.6	1800	5000	220
6.21.87	20	25.3	9.3	1600	5600	2500
7.5.87	21	30.0	7.8	1700	6500	3050
7.19.87	22	31.4	7.0	1200	7000	3000
8.2.87	23	32.0	6.7	1500	7000	3500
8.16.87	24	31.0	7.2	1100	7050	4050

Table 6. The percentages of facultative thermophiles and psychrophiles, facultative anaerobes, percent of spore formers and Gram stain characteristics by depths and incubation temperature.

Date of Collection	Temp of Incubation	Total of cfu/ml Isolated	Growth at 37°C		Growth at Anaerobic		Spore Stain		Gram Negative Rods		Gram Positive Rods		Gram Positive Cocci		Other	
			#	%	#	%	#	%	#	%	#	%	#	%	#	%
2ft(s)	10	73	37	50.7	41	56.2	45	61.6	45	61.6	15	20.6	6	8.2	7	9.6
	55	35	34	97.1	19	54.3	17	48.6	17	48.6	0	0	3	8.16	15	42.8
6ft(s)	10	85	52	61.2	57	67.1	49	57.7	49	57.7	16	18.8	10	11.8	10	11.8
	55	52	50	96.2	38	73.1	30	57.7	30	57.7	0	0	7	13.5	15	28.8
6ft(B)	10	88	53	60.2	46	52.3	46	52.3	46	52.3	20	22.7	8	9.1	14	15.9
	55	51	50	98.0	37	72.5	30	58.8	30	58.8	0	0	6	11.8	15	29.4
12ft(s)	10	87	55	63.2	51	58.6	44	50.6	44	50.6	20	23.0	10	11.5	13	14.9
	55	50	49	98.0	32	64.0	32	64.0	32	64.0	0	0	3	6.0	15	30.0
12ft(B)	10	92	56	60.9	63	68.5	50	54.4	50	54.4	15	16.3	8	8.7	19	20.7
	55	44	42	95.5	33	75.0	24	54.6	24	54.6	0	0	3	6.8	17	38.6

s = surface (about one foot from water surface)

b = bottom (about one foot from the bottom of the lake)

% = percent isolated = $\frac{\# \text{ isolated in each case}}{\text{Total \# of CFU/ml isolated}} \times 100$

Other = Other types of bacteria which did not fit into the gram stain characteristics (neither gram positive, cocci, gram negative bacilli, nor gram positive bacilli).

Fig. 1. Watershed of Westhampton Lake, Richmond, Virginia.
Shaded area denotes the campus of the University of
Richmond.
(From Bishop 1982)

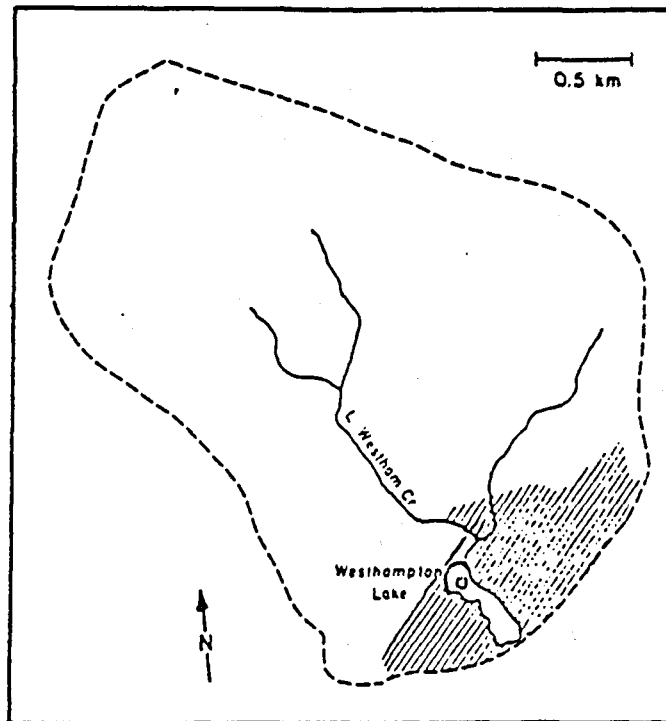


Fig. 2. Morphometric chart of Westhampton Lake, Richmond, Virginia. The chart was based on surveys taken after dredging between 1974-1976. The 7m depth was within the northwest section of the 6m contour. (Bishop 1982). The filled dots (. . .) indicate the sampling sites. Linear scale represents dimension of the lake.

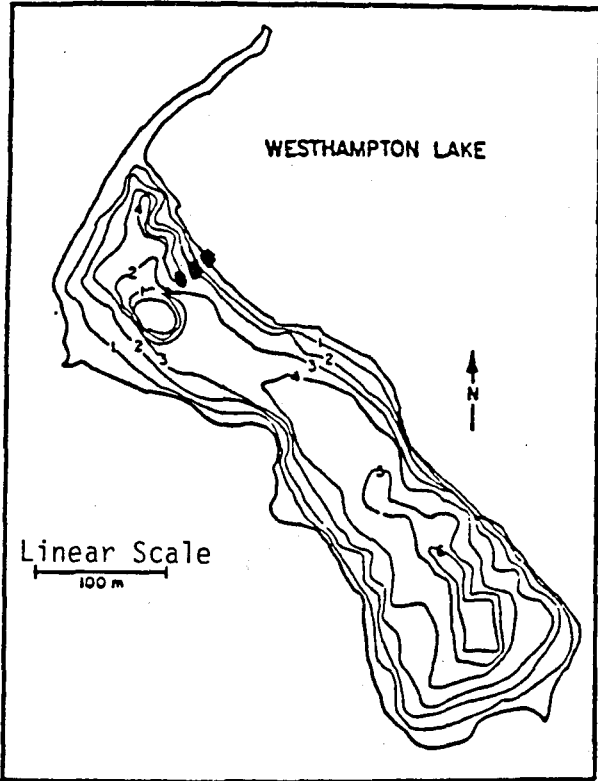


Fig. 3. Plot of colony forming units per ml of sample (CFU/ml) at 10°C against sample number.

Note: Sample numbers corresponds to the dates of collection. Sept. 1986 (sample #1) to August 1987 (sample #24)

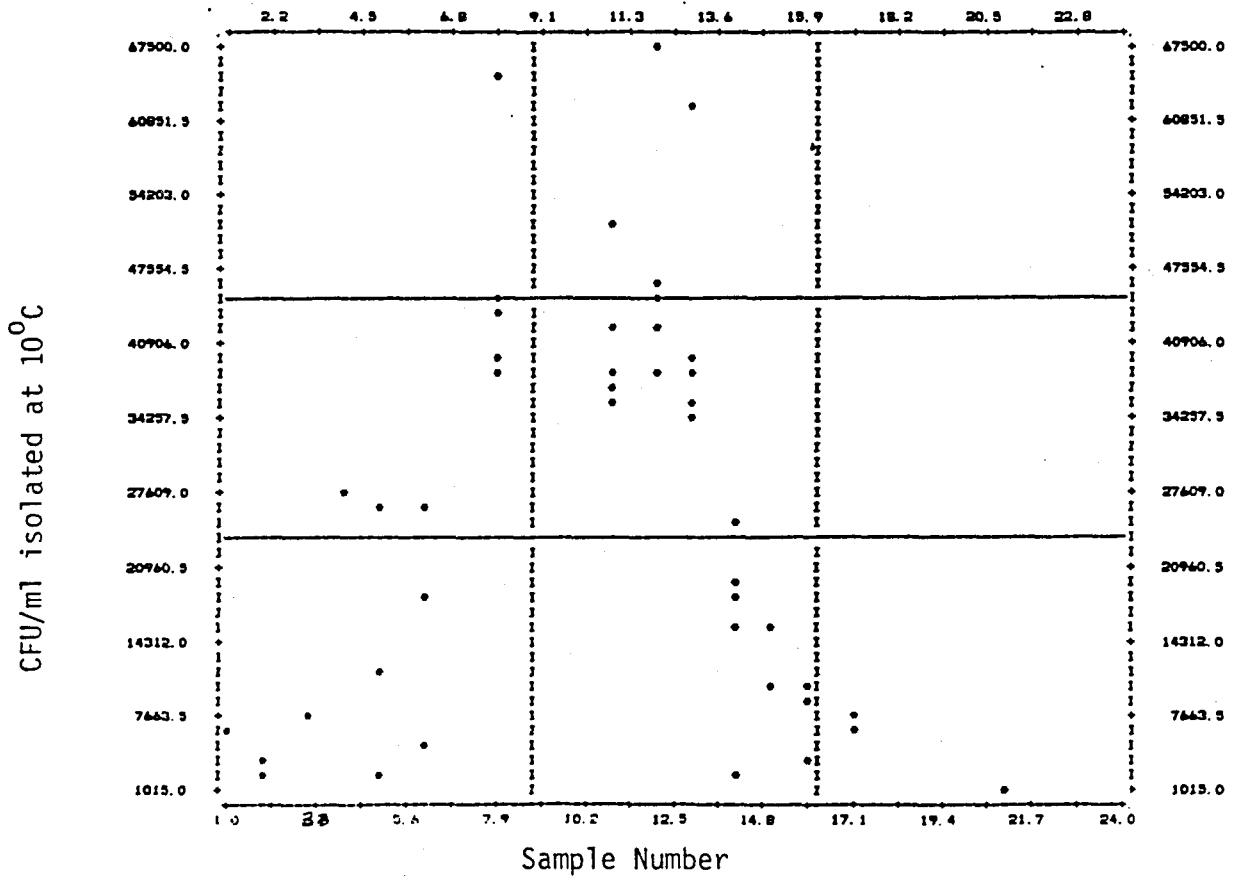


Fig. 4. Plot of CFU/ml isolated at 55°C against sample number. Absence of points between sample number 1 and sample number 17 indicate insignificance (less than 30 CFU/ml) CFU/ml isolated during this period.

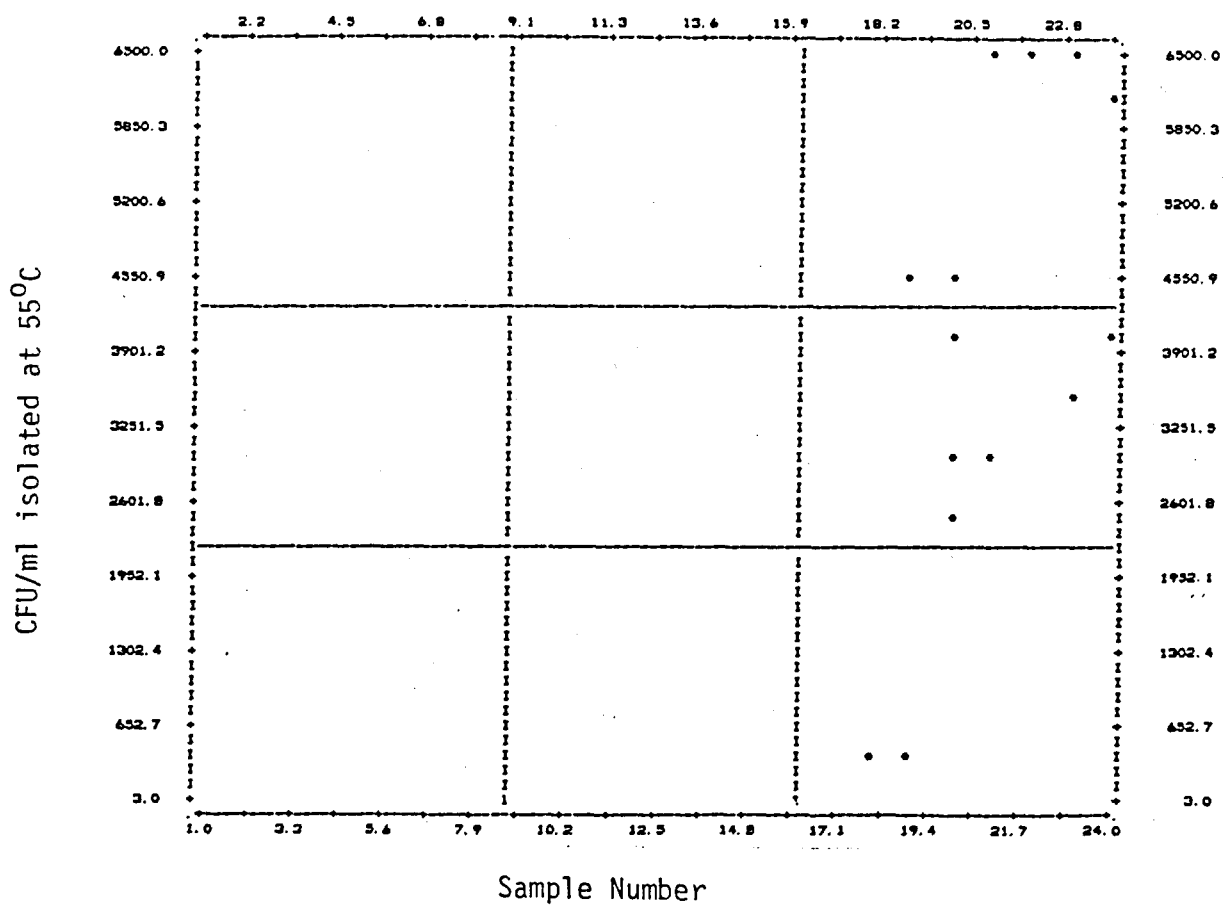


Fig. 5. Plot of CFU/ml isolated at 10⁰C against temperature of lake at time of sampling. CFU/ml isolated at 10⁰C incubation is inversely related to the temperature of lake at time of collection.

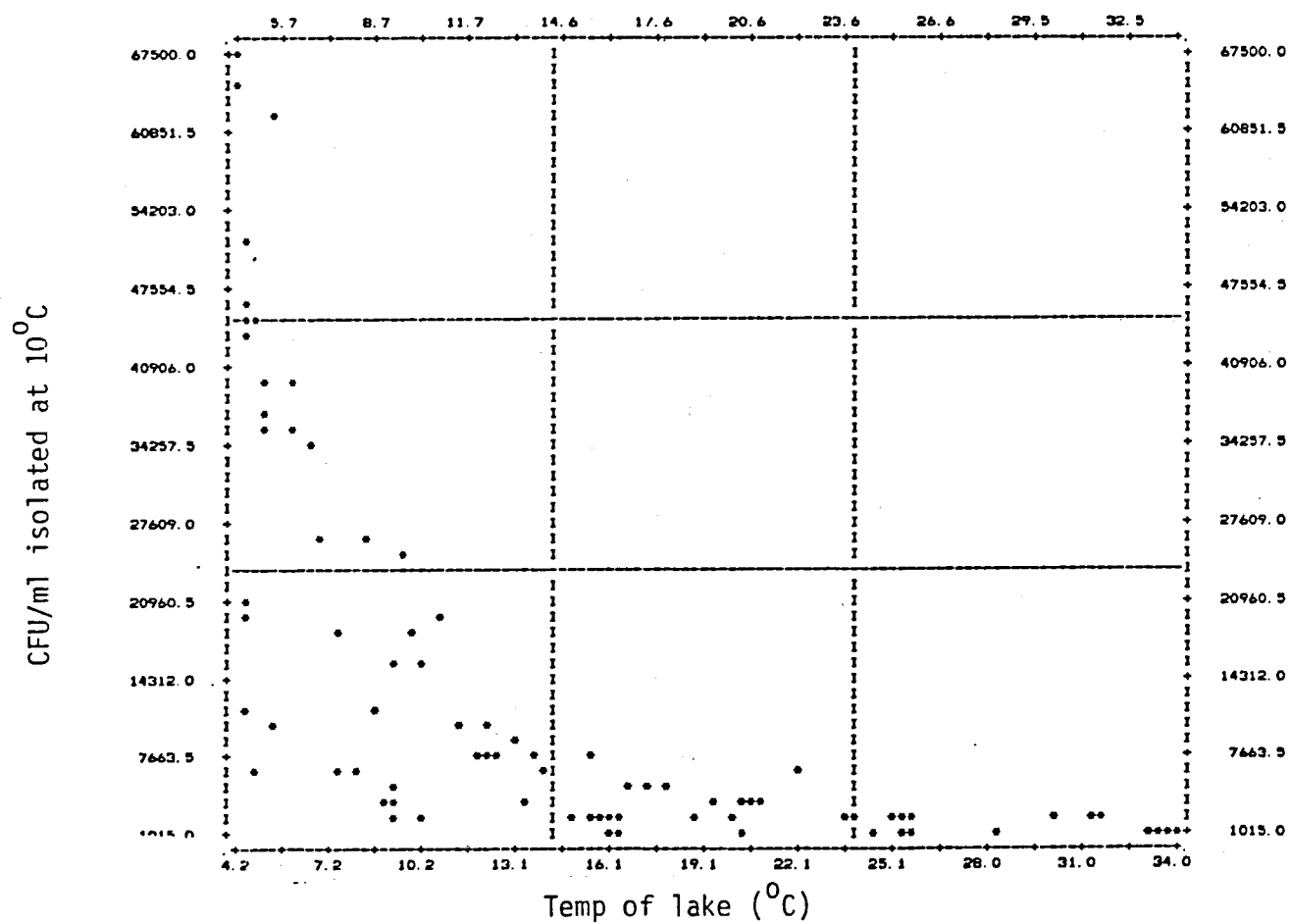


Fig. 6. Plot of CFU/ml isolated at 55°C (Thermophiles) against temperature of lake. The higher the temperature of the lake, the greater the number of CFU/ml isolated at 55°C.

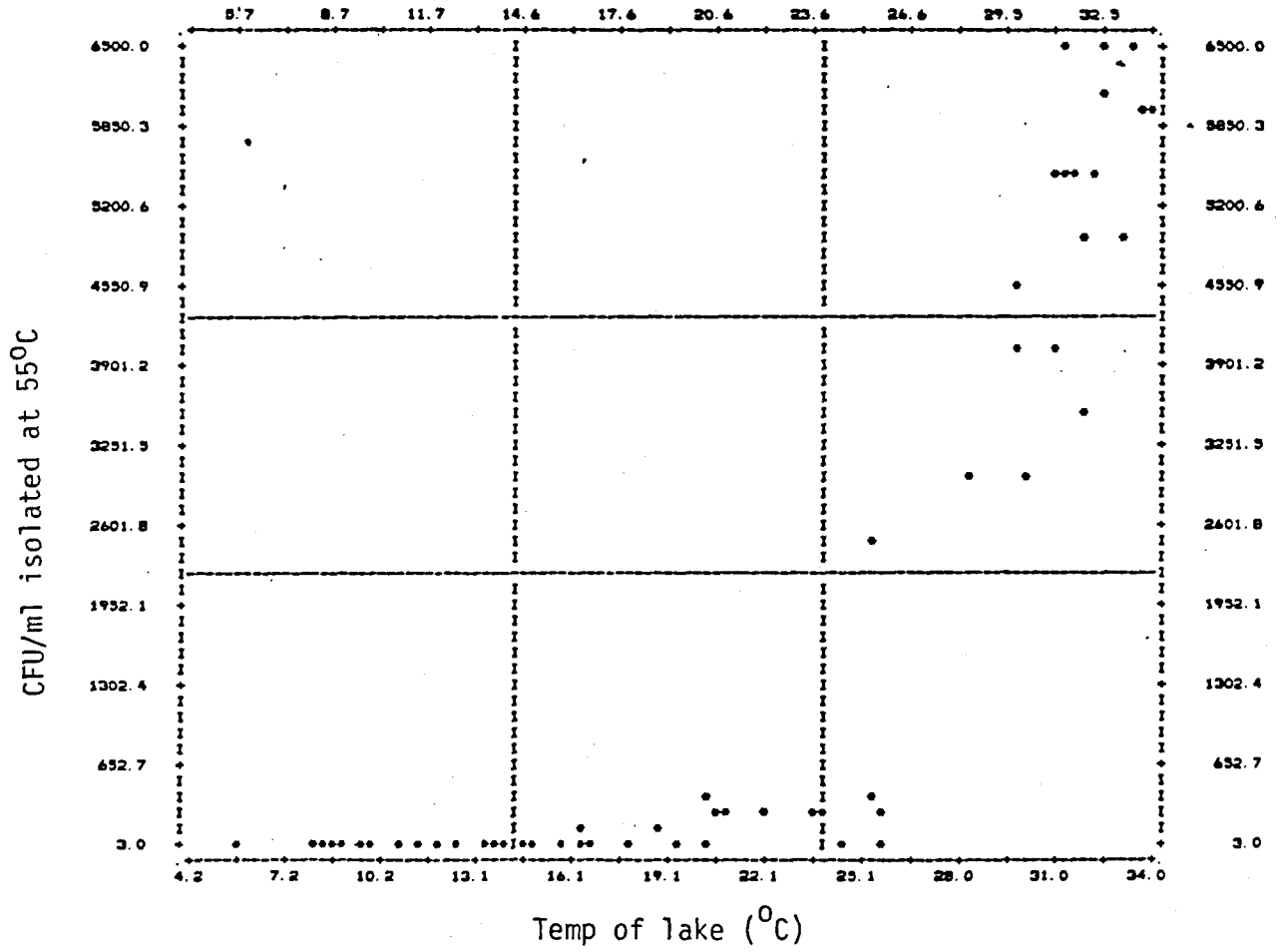


Fig. 7. The effects of oxygen on CFU/ml at 10°C (psychrophiles). Higher counts of CFU/ml at elevated oxygen concentration and a cluster of low counts of CFU/ml isolated at low O₂ conc of the lake.

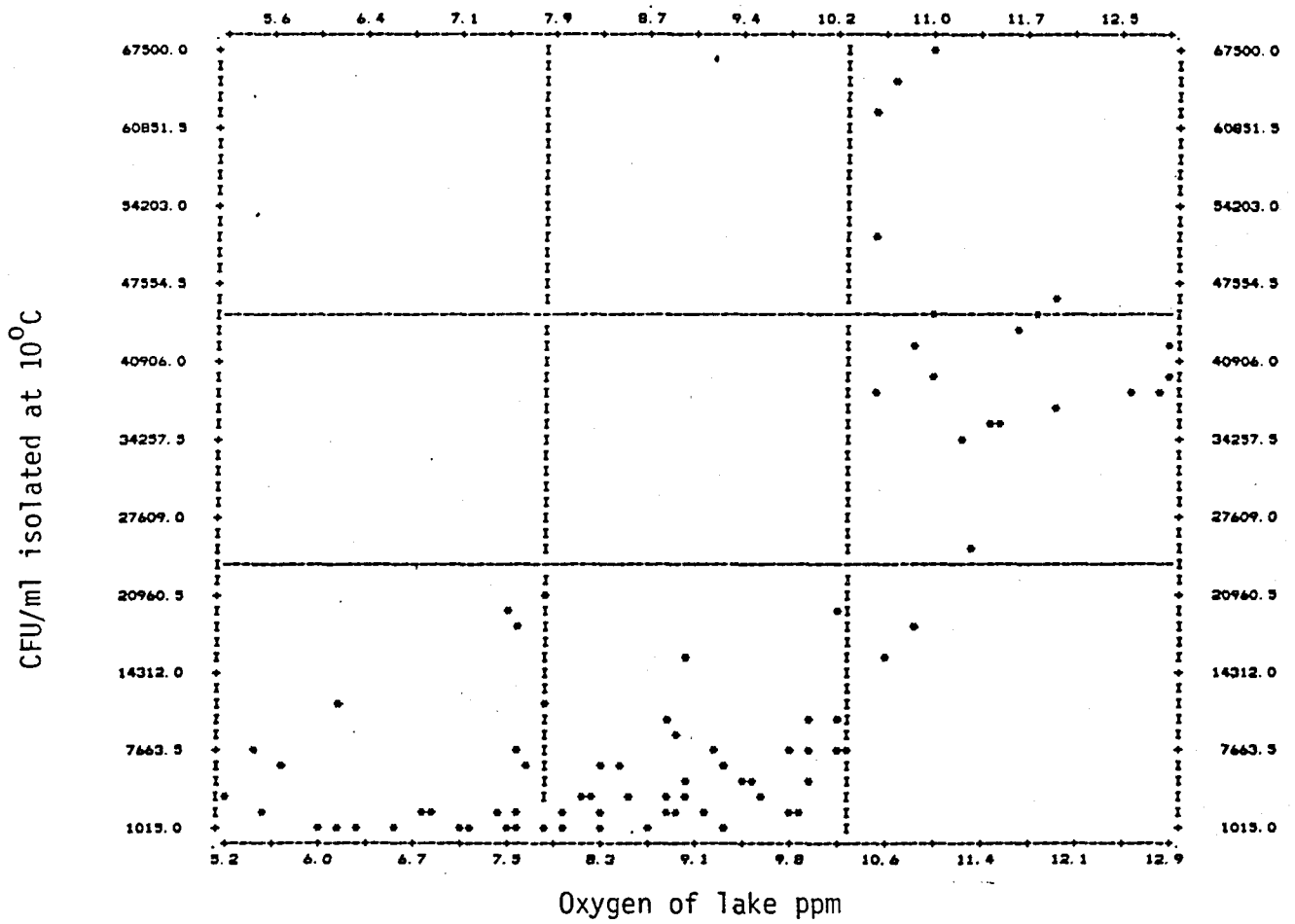


Fig. 8. O_2 effect on CFU/ml at $55^{\circ}C$. (Thermophiles) Greater number of CFU/ml was isolated at lower O_2 conc while insignificant number was isolated at higher O_2 conc.

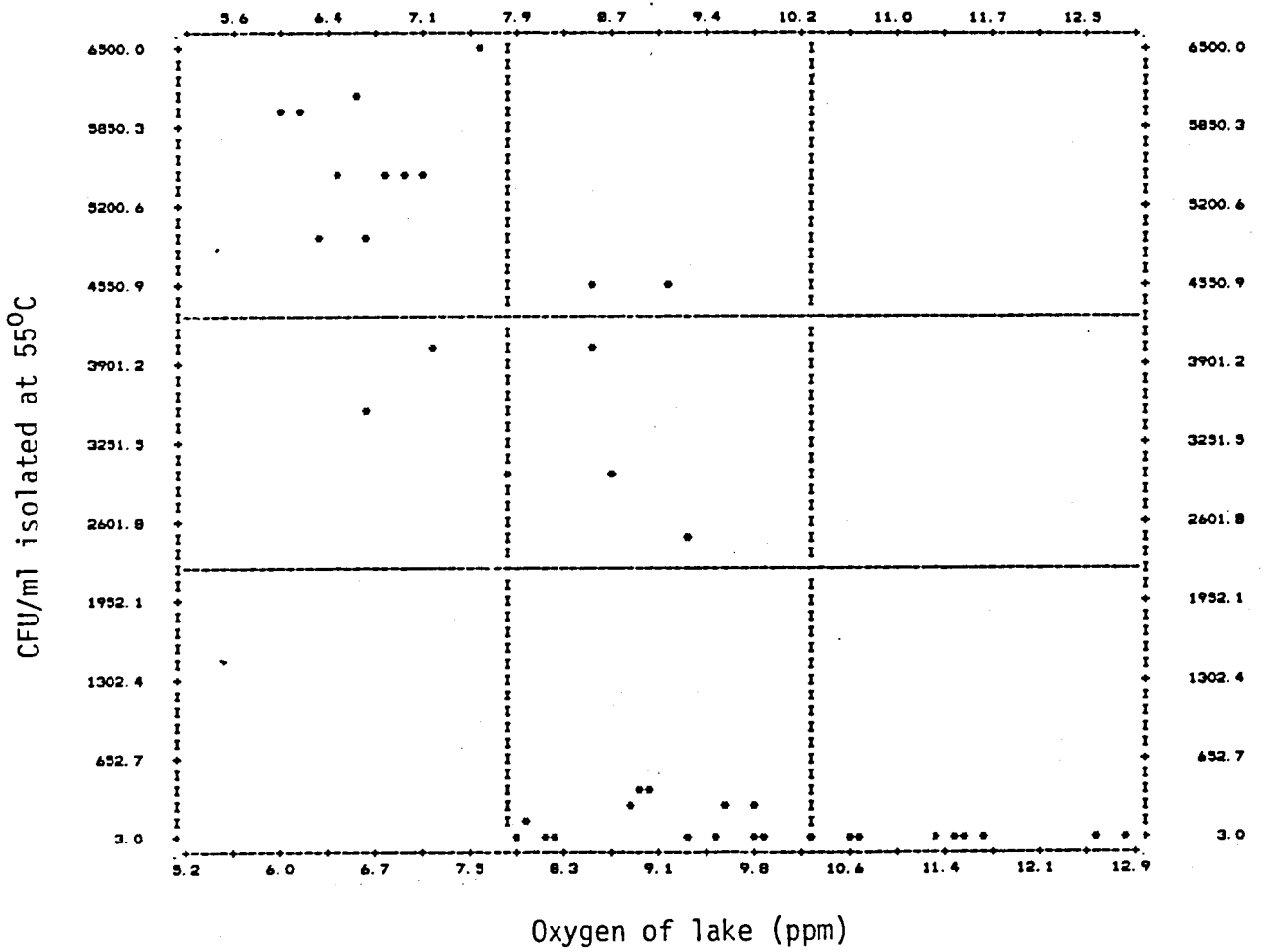
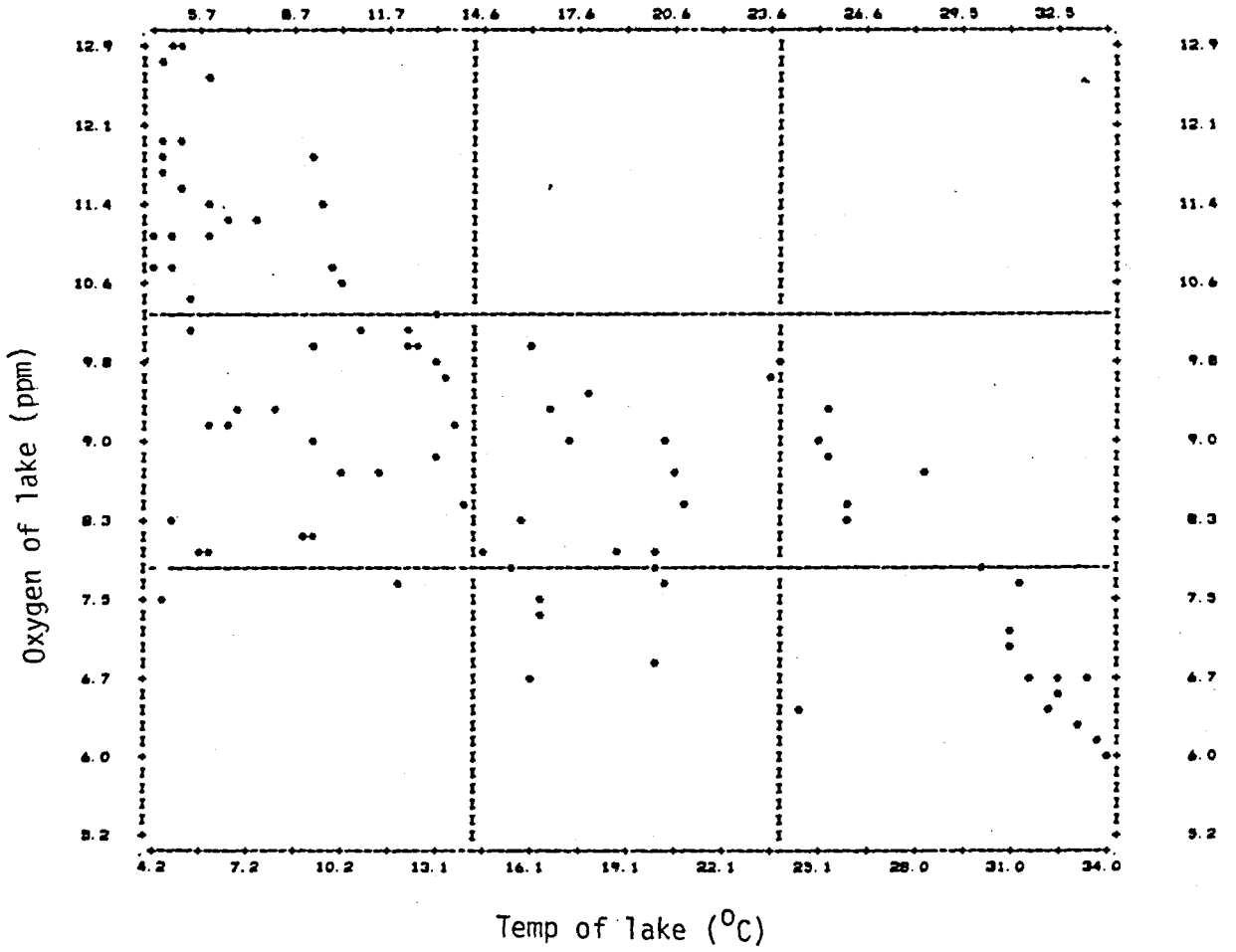


Fig. 9. Relationship of O_2 conc and temperature of lake. O_2 conc. is inversely related to temp.



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