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DEVELOPMENT OF HEALTH EFFECTS CRITERIA FOR FRESHWATER BATHING BEACHES BY USE OF MICROBIAL INDICATORS

The University of Oklahoma PH.D. 1980

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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

DEVELOPMENT OF HEALTH EFFECTS CRITERIA FOR FRESH WATER BATHING BEACHES BY USE OF MICROBIAL INDICATORS

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

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GARRY LEE MCKEE

Norman, Oklahoma

1980

DEVELOPMENT OF HEALTH EFFECTS CRITERIA FOR

FRESH WATER BATHING BEACHES BY USE

OF MICROBIAL INDICATORS

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ABSTRACT

An epidemiological-microbiological study was conducted at Lake Keystone, Oklahoma, near Tulsa, Oklahoma, to help develop recreational water quality criteria based upon health effects. Symptomatology rates among swimmers relative to non-swimmer controls were examined at a "barely acceptable" (BA) beach, Salt Creek North (I), and Keystone Ramp (II), and a "relatively unpolluted" (RU) beach at Washington Irving South (III).

This was accomplished by contacting family groups at the beaches on weekends and obtaining information on bathing activity by the use of interviewers. These beach-goers were questioned by telephone 8-10 days later concerning health related symptoms.

Measurements were made for a number of potential microbioal indicators of pollution during the time the interviews were being made. When the data from the BA and RU beaches was examined, the symptom rates categorized as gastrointestinal, respiratory and "other" were higher among swimmers than non-swimmers. Although the data was not statistically significant, definite trends could be shown in that direction. Good agreement was obtained between geometric means of Escherichia coli and enterococcus densities and the differential (swimmers minus non-swimmers) rate of gastrointestinal symptoms.

Therefore, the objective of relating illness as measured by symptomatology to an indicator of water quality has been addressed.

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DEVELOPMENT OF HEALTH EFFECTS CRITERIA FOR FRESH WATER BATHING BEACHES BY USE OF MICROBIAL INDICATORS

CHAPTER I

INTRODUCTION

Problem Statement

For many years individuals and committees have worked with the problem of what to do, if anything, about bathing water quality standards. The final product of these efforts has been the setting of widely varying limits for various bacterial groups, all of which are arbitrary, sometimes illogical, and are based upon a limited foundation in public health research.

The objective of control procedures against recreational related waterborne infectious diseases is the prevention of pathogen densities which could be elevated to a level that would result in a high risk of disease to swimmers. The major source of contamination of the water with etiologic agents is the feces of warm-blooded animals, especially man (1). The infective dose of certain waterborne pathogens is very low. At the same time, these

pathogens are difficult or virtually impossible to quantify at low levels. Therefore, recreational water quality indicators should ideally be microorganisms which will be present in high densities in the water and can be quantitatively correlated to potential health risk resulting from swimming.

Water quality standards are a plan established by governmental authority or a program for water pollution prevention and abatement. These standards are based upon current criteria. Water quality criteria are different from standards in that criteria are scientific requirements on which decisions or judgements may be based concerning the suitability of water quality to support a particular use. Bathing water quality standards are usually applied to primary contact recreation areas. These are defined as areas in which activities in the water cause a prolonged and intimate contact with the water involving considerable risk of ingesting water in quantities sufficient to pose a significant health hazard.

There is a need for recreational water quality criteria since swimming represents a major source of outdoor recreation. At the same time, the safe treatment and disposal of pathogen-laden sewage into lakes and streams is most difficult. Recreational water quality criteria are required so they can be used to help set source or effluent guidelines for designing and operating sewage treatment plants. Also, health officials need these criteria to help

set point source receiving water guidelines and standards designed to restrict recreation to those areas considered as "safe." Social, economic, and political factors may also influence these criteria.

Unfortunately, some of the most visible uses of criteria and standards for recreational waters have been beach closures and the posting of beaches as unsafe. Therefore, the restriction of recreational use of the water will not allow the public much needed areas in which to swim. In regard to public health hazards associated with the use of such recreational waters, many competent authorities have questioned the validity of these recreational closures, except in extreme cases where a beach is close to an untreated sanitary waste outfall. However, most of these same authorities would agree that standards and water quality criteria are essential in defining the type and extent of treatment required for sanitary and industrial wastes whose effluents are carried to recreational waters (2). Local regulatory agencies need information on the risk of disease, categorized by type, severity, and economic impact that may be associated with some indicator of water quality so that cost versus "acceptable risk" decisions required in the planning of treatment facilities can be more adequate. In many cases storm water runoff is frequently the cause of excessive bacterial levels in recreational waters. Control of these non-point sources and, in some cases, point sources is only now being developed.

Research Objectives

Recognizing the need as stated above, the U.S. Environmental Protection Agency has been conducting a program to develop health-effects recreational water criteria for marine and fresh waters. The overall program calls for studies to be done in several different geographic locations of the U.S. in order to correlate swimming-associated illness (measured by symptomatology), with densities of potential microbial indicators of water quality.

As part of the national program to develop health-effects criteria for recreational waters, the U.S. Environmental Protection Agency provided funds to conduct a prospective epidemiological-microbiological study at bathing beaches on Lake Keystone located near Tulsa, Oklahoma.

The four primary objectives of this project were:

1. To test the significance of differences in reported health symptoms classified by type and severity associated with swimming at a beach in the lower midwest region of the country under various conditions and levels of water pollution.
2. To test the association between various microbiological indicators of water pollution and reported symptoms classified by type.
3. To determine whether these associations differ significantly among sub-groups classified on the basis of age, sex, ethnicity, or socioeconomic level.

4. To correlate reported disease rates among swimmers by type and level of pollution at the time of the swimming event, using several microbiological indicators of pollution.

Research Scope

The project was similar to a study done by Haberman (3) at beaches around New York City in the summer of 1974. Comparisons were made between groups who swam on days when microbiological counts were high and those who swam on days with low counts. Therefore, it was possible to determine those points on a pollution gradient for which health effects as a result of swimming were most likely to occur.

Pre-test information collected during the summer of 1978 showed that a suitable site for the study existed on the Keystone Lake near Tulsa, Oklahoma. Three beaches were located, one being relatively non-polluted and the other two having much larger amounts of bacterial pollution. These areas also had sufficient numbers of visitors to allow a statistically valid epidemiological study of this type.

CHAPTER II

HISTORICAL BACKGROUND AND LITERATURE REVIEW

Causative Agents

Considerations of water quality criteria have been limited because of the lack of data from disease outbreaks or epidemiological studies which can clearly show that individual cases of disease were related to a particular level of pollution as measured by a particular indicator organism (4,5,6).

Although there have been a number of case reports where disease was attributed to swimming in recreational waters (7), there is a general lack of data in this area due to the fact that many of the infectious diseases which can be transmitted by this route are neither fatal nor "reportable" by most public health authorities (8). With the exception of "swimmers ear," an otitis externa usually caused by Pseudomonas aeruginosa (9), most of the diseases that are reported could be transmitted by routes other than recreational water use. Moore (10) has pointed out that reported outbreaks of waterborne disease have been very infrequent and in many cases the association with swimming was ill defined. Some researchers have expressed the belief

that in the absence of "reportable" or fatal disease, standards and even criteria are not required (11).

The primary object of the bacteriological examination of water to be used for recreation or domestic supplies is the detection of fecal pollution. Ill health caused by the consumption of water occurs only in rare instances from the presence of an excess of one of the inorganic salts which water contains or of metallic matter such as lead. The presence of excremental bacteria is a far greater danger to health, because the water that is contaminated may carry the causative organisms of disease (12).

The danger of swimming in polluted water comes principally from living organisms and not from dead organic matter. Most microorganisms in water are derived from air, soil, living and decaying plants or animals, and fecal excrement of humans and other warm-blooded animals. Many of these organisms come from unknown sources and have no sanitary significance because they are widely distributed in the natural environment and have no suspected association with human or animal wastes.

In addition to human influence on drinking water supplies and bathing beaches, Kunkle and Meiman (13) documented the impact of domestic livestock on the water quality of mountain watersheds. These researchers also observed higher coliform, fecal coliform, and fecal streptococcus concentrations on the grazed areas than on the ungrazed

areas. Another example of animal influence on water quality was reported by Stuart, et al, (14) who found four to six times the coliform counts on a Montana watershed closed to recreation than on an adjacent watershed open to recreation. The authors postulated that the large wild animal population was responsible for the increase in bacteria counts.

Human enteric pathogens have been detected in warm-blooded wildlife and domestic animal feces (15,16). Animals may become infected by human pathogens or act as natural carriers of human disease (17). Janssen and Meyers (18) suggested that freshwater fish may also act as natural carriers by acquiring pathogenic organisms through feeding in contaminated water and defecating these organisms in clean water.

Effects of swimming on water quality have been studied under controlled conditions by two research teams. Robinson and Wood (19) found that bathers, while swimming, contribute both fecal and eye-ear-throat-nose type bacteria to the water. Hanes and Fosea (20) demonstrated that swimmers can increase the amounts of chemical and bacterial indicators of bathing water quality.

Stevenson (21) conducted epidemiological studies on Lake Michigan at Chicago, Illinois, the Ohio River at Dayton, Kentucky, and Long Island Sound at New Rochelle, New York, to determine natural bathing water quality and the effect upon the health of swimmers. The author found some correlation between high coliform concentrations and

swimmers' illness. Epidemiological studies and laboratory studies concerning the health of water-based recreation users were the initial steps toward the development of recreational water quality standards.

Indicator Characteristics and Rationale for Their Use

The concept of microbiological examination of water to detect fecal contamination arose in the late nineteenth century after certain bacteria were described as characteristic of human feces (22). Pathogenic bacteria, few in number, and a wide variety of types, are not conveniently subject to routine analyses (23). Attention, therefore, has been focused on indicator strains that are abundant in feces and easily detected, even though they do not normally cause disease. The "ideal" bacterial indicator fecal pollution should have the following characteristics:

1. Be applicable in all types of water.
2. Always be present in water when pathogenic bacterial constituents of fecal contamination are present.
3. Be present in densities having direct relationship to the degree of fecal pollution.
4. Be non-pathogenic for laboratory safety.
5. Be rapidly and easily quantitated.
6. Be economical to monitor.

Coliform bacteria, consisting mainly of Escherichia coli,

are discharged by the billions in the feces of all warm-blooded animals, including humans. Many water quality studies have relied on the coliform test to indicate recent fecal contamination of surface waters (24,25). However, since some strains of coliform bacteria are common in unpolluted soils and plants, the sanitary significance of total coliform counts are questionable (26).

Each type of bacterial test has a specific purpose, limitation, and strength of inference. It is important to understand what types of organisms constitute the coliform group, which are of sanitary significance, and what the different tests actually measure. The coliform group includes the genera: Escherichia, Enterobacter, Klebsiella, and Citrobacter. The presence of coliform bacteria may indicate fecal contamination (either human or animal) of water. Humans may become infected by ingesting contaminated water. Intestinal diseases in man are commonly caused by Salmonella and Shigella which are transmitted primarily by fecal contamination of water (27).

Bacteriologists commonly rely on a general test which measures total coliforms present in a water sample. Included in this test is detection of fecal coliform organisms, such as Escherichia coli, as well as other types of coliforms, including some non-fecal forms. The assumption made is that the general test for total coliforms reflects fecal contamination. In order to prove that there is fecal

contamination, a more specific test for fecal coliforms must be made. The presence of fecal coliforms and other fecal bacteria, such as fecal streptococci, does not necessarily indicate pathogenic forms are present. A positive fecal coliform test only confirms fecal pollution is occurring and strongly suggests the possibility of the presence of pathogens. Routine analyses for the presence of pathogens are expensive and time consuming. For this reason, as well as other reasons, normally occurring bacteria in the intestines of warm-blooded animals have been used as indicators of fecal pollution, and indirectly as indicators of disease producing potential. The coliform group, fecal coliforms, and fecal streptococci are three indicator groups commonly used. Briefly stated, the routine bacteriological examination of water is concerned with the detection of normally harmless bacteria which are common inhabitants of the human and animal intestine.

Fecal bacteria are so abundantly present in the feces of man and animals that the pollution of water by exceedingly small traces of excrement can be demonstrated bacteriologically. Recent studies of the bacteria of the intestines by Upjohn Laboratories (28) indicated concentrations of bacteria in the large bowel in excess of 10^9 per gram of bowel contents, with most of these being anaerobic bacteria. Obligate anaerobic bacteria outnumber E. coli 1,000 (to 10,000) to one in the large intestine, with Bacteroides

fragilis being the anaerobic organism most frequently encountered. Facultatively anaerobic bacteria are in the minority here, but their quantity is no less than elsewhere in the intestinal tract. Enterococci and lactobacilli are each present in concentrations of about 10^6 to 10^8 organisms per gram of bowel contents. Escherichia coli is the most common species of bacteria, followed by species of enterococci (Streptococcus faecalis and Streptococcus faecium), Proteus, Pseudomonas, and Klebsiella. Staphylococcus aureus and Candida albicans, a yeast, are also present in the large bowel of normal individuals. Although seemingly large numbers of "potential" pathogens are present in the bowel, these organisms, when found in water, are greatly outnumbered by "normal" organisms, such as E. coli, which can survive longer in water than the majority of the pathogens. It follows, therefore, that water which is free from E. coli should, in natural circumstances, also be free from disease-producing organisms. On the other hand, if small quantities of the water show the presence of fecal bacteria, the possible presence of pathogens cannot be excluded and the water must be regarded as unsafe. Fecal bacteria may, then, be looked upon as indicators of pollution and as danger signs. If pathogenic bacteria are also present in the sewage or other source of contamination, the path is open for them to appear in the water.

A common misconception is that fecal coliforms only represent a potential danger when they come from human feces. This idea has led to proposals that concern should be limited only to fecal contamination from humans. However, these proposals are invalid because pathogens harmful to man can come from animals as well (16).

Constraints on Indicator Use

It is all too common that water quality indicator systems are used to obtain information far beyond their capabilities. In many cases, the data obtained is interpreted without taking into consideration the limitations of the indicator system (29). This seems to be the case when recreational waters are examined using a fecal indicator to assess the risk in relationship to the high number of indicator organisms to the pathogen ratio in feces, or sewage, that may be in the recreational waters.

Many microorganisms have been considered as recreational water quality indicators. Cabelli (30) has listed the possible water quality indicators, their significant sources and potential uses as shown in Table 2.1. All but the last four in the figure should be considered as potential indicators of contamination with feces of warm-blooded animals because they have been recovered from municipal sewage wastes. The last four along with Salmonella, Shigella and enteroviruses are used only on limited occasions. Total coliforms have been questioned as an

TABLE 2.1 Significance of Water Quality Indicators.

Indicator	Significant[a] Source	Potential[b] Use
Coliforms	F S I R A	S
Escherichia coli	F S	P F S A
Klebsiella sp.	S I R A	P S N
Enterobacter sp.	S I R A	S
Citrobacter sp.	S I R A	S
Fecal Coliforms	F S I R A	F[c] S
Enterococci	F S	F S A D
Clostridium perfringens	F S	F S A D
Candida albicans	F S	P F S
Bifidobacteria	F S	F S A D
Enteroviruses	F S	P
Salmonella sp.	F S	P
Shigella sp.	F[c] S	P
Coliphage	S	S
Pseudomonas aeruginosa	S I R A	P S N
Aeromonas hydrophila	S I R A	P S N
Vibrio parahemolyticus	A	P N

[a] Significant Source, F = feces of warm-blooded animals, S = sewage, I = industrial wastes, R = run-off from uncontaminated soils, A = fresh and marine waters.

[b] Potential use, P = pathogen, F = fecal indicators, S = sewage indicator, A = separation of human from lower animal sources, D = proximity to fecal source, N = indicator of nutrient pollution.

[c] Questionable.

indicator because of growth in the water and non-warm-blooded sources of the bacteria (31). Both of these characteristics make total coliforms of limited use for recreational water quality standards.

Hoadly (33) suggested that Pseudomonas aeruginosa be used as the indicator organism for recreational water because of its association with ear infections. Fecal streptococcus as an indicator was proposed by Mallman and Seligram (34) because of a relationship between streptococcus concentrations and bathing activity. In contrast, Foster, et al, (35), found that coliform, streptococcus, and Pseudomonas aeruginosa densities varied independently of swimming activity. Their study suggests that bacterial limits be considered only as a guide, requiring additional investigation based on epidemiological data for a particular area.

Other indicator systems which include pathogenic bacteria have been proposed (36). Geldreich (29) has suggested that the occurrence and density of pathogens in polluted water and in animal feces are highly variable. Thus, microbiological monitoring of water using waterborne pathogens would require a variety of complex, time consuming, and often insensitive procedures.

Fecal coliform bacteria, a subgroup of the total coliform population, have a direct correlation with fecal contamination from warm-blooded animals (23,36).

Geldreich (29) states "measurements of stream pollution must be based on the detection of fecal contamination from all warm-blooded animals, for this is the natural link to the occurrence of pathogenic microorganisms in polluted water." Geldreich suggests that fecal coliform bacteria should be used as a baseline indicator system for evaluating the suitability of recreational waters (29). Research of Smith and Twedt (38) supports the use of fecal coliforms as an indicator due to the isolation of Salmonella when fecal coliform levels were between 100 and 200 organisms per 100 ml. Reconfirming earlier work, Smith, Twedt, and Flanigan (39) found fecal coliforms beneficial as an indicator of recreation water quality.

If one accepts the hypothesis that coliform bacteria of fecal origin represent greater danger to health than bacteria native to other environments, separation of the fecal from non-fecal groups is necessary. Because the feces of numerous species of wild and domesticated animals may contain microorganisms capable of producing disease in man, it is necessary to consider all fecal coliforms as indicative of dangerous pollution. The fecal coliform content of a water source more nearly reflects its disease-producing potential than does the total coliform content determined simultaneously.

It has been shown that essentially all the coliforms in a freshly passed stool are enumerated by the fecal coliform test. In fresh sewage the fecal coliforms may constitute 30-40 percent of the total coliforms; in aged sewage and in polluted water, the fecal coliform fraction tends to decrease progressively with elapsed time. In heavily polluted surface waters, the fecal coliform component usually falls between 10-35 percent of the total coliform count.

Available information indicates non-fecal coliforms tend to survive longer than do fecal coliforms (40), and all coliforms survive longer in cold water than in warmer temperatures. The non-fecal group also tends to be somewhat more resistant to chlorination than fecal coliforms or the commonly occurring intestinal bacterial pathogens (41). Under certain conditions, some of the enteroviruses may survive longer in polluted waters and exhibit more resistance to chlorine than either fecal or non-fecal coliforms. In consideration of sanitary significance, the presence of fecal coliform organisms indicates recent and possibly dangerous fecal pollution. The presence of non-fecal coliforms suggests less recent pollution.

With respect to the coliform group in general, the fecal coliform component offers several distinct advantages as an indicator group. First, over 95% of the coliform bacteria from intestines of warm-blooded animals grow at elevated temperatures.

Second, survival of the fecal coliform group is shorter in environmental waters than for the coliform group as a whole. It follows then that high densities of fecal coliforms are indicative of relatively infrequent occurrence, except in association with fecal pollution. Fecal coliforms generally do not multiply outside the intestines of warm-blooded animals. One disadvantage that should also be mentioned is that little is known about the survival of fecal coliforms in polluted waters compared with that of enteric pathogenic bacteria.

Another important indicator group is the fecal streptococci consisting of streptococci commonly found in significant numbers in the feces of human or other warm-blooded animals. Other terms used for this group are "entercocci" or "Group D (Lancefield's) Streptocccci."

Enterococci are characterized by specific biochemistry. Serological procedures differentiate the Group D streptococci. Although they overlap, the three groups-fecal streptococcus, enterococcus, and Group D Streptococcus, are not synonymous (42). Because the principal emphasis is on indicators of unsanitary origin, fecal streptococcus is the more appropriate term.

The British Ministry of Health defines the fecal streptococci as "Gram-positive cocci, generally occurring in pairs or short chains, growing in the presence of bile

salt, usually capable of development at 45 degrees C., producing acid but not gas in mannitol and lactose, failing to attack raffinose, failing to reduce nitrate to nitrite, producing acid in litmus milk and precipitating the casein in milk in the form of a loose, but solid curd, and exhibiting a greater resistance to heat, to alkaline conditions and to high concentrations of salt than most vegetative bacteria" (43). Some workers consider growth at 45 degrees C and multiplication in 40% bile to be the most significant indications of fecal origin of streptococci.

Fecal streptococci have been considered indicators of fecal pollution for more than 50 years (44,45). Their poor acceptance in the past (especially in the United States) as a measure of pollution from human and warm-blooded animal excreta has been a result of several factors: low recovery rates, the multiplicity of detection procedures, poor agreement between the various enumeration methods, and lack of detailed and systematic studies of the sources, survival, and interpretation of fecal streptococci in various kinds of water. Furthermore, undue emphasis has been placed on the Streptococcus faecalis group (enterococci), with little or no regard for the numerous other streptococcal strains that may be present in varying numbers in the feces of mammals or birds. The predominating species in the excreta of various animals may vary markedly as to number and name. Interpretations are currently made by taking into

consideration both the numbers and species of streptococci present in the samples examined. Methods are now being developed to accurately select and quantitate for Group D Streptococci, with the idea that this group will possibly become a more reliable indicator to replace the current coliform method of indicating fecal pollution (46).

One advantage of the fecal streptococci is that they do not multiply in water. Fecal streptococci enter a water, survive for a period of time and then die without multiplying.

The data obtained by using health effects indicators to assess pathogen densities in recreational waters are used in three ways. These are: (1) to classify beaches in regard to existing standards and guidelines, (2) to evaluate pollution problems and assess possible long range problems, (3) to establish the source and condition of the water in waterborne outbreaks of infectious disease. It is therefore of utmost importance that limitations of these indicators be carefully assessed and that better methods for selection and quantification be developed.

Standards and Developmental Criteria

The need for standards governing sanitary quality of waters used for recreation has long been recognized by public health and environmental officials for many years. In response to this need, many states have adopted standards for the sanitary quality of recreational waters. The

standards that have been established were done so by use of the limited data available. Guidelines and standards vary from one country to another, and many countries have no standards at all. In the United States, they vary from state to state and, in some cases, from one municipality to another (1). Two microbiological guidelines appear most frequently: total coliforms and the fecal coliforms.

There is no readily defined minimum fecal coliform concentration that represents a health hazard. Unpolluted mountain streams typically contain fewer than 10 fecal coliforms per 100 ml (47). Raw sewage generally contains over 5 million fecal coliforms per 100 ml (48). Federal and state fecal coliform standards vary according to the degree of human contact likely with a particular type of water. Secondary sewage effluent must not exceed a 30 day geometric mean of 200 fecal coliforms per 100 ml (49). EPA guidelines for waters designated for primary or full body contact recreation require that the fecal coliform content must not exceed a geometric mean of 200 colonies per 100 ml, based on a minimum of five samples per month, nor shall more than 10% of samples exceed 400 colonies per 100 ml (50). Table 2.2 indicates bacteriological standards recommended by McKee and Wolf (1) and Geldreich (29).

Primary contact waters should include waters in which swimming occurs, as well as waters near campgrounds, picnic areas, fishing access points, streamside trails, and places

TABLE 2.2 Tentative Standards for Bacterial Levels.

	Acceptable Levels		
Test	Drinking Water	Recreational Body Contact	Inference
Total Coliform	1/100 milliliter average monthly count	50-3000/100 milliliters safe bathing areas (28) 1,000/100 milliliters mean monthly density (1)	Fecal contamination possible; pathogens may be present
Fecal Coliform	None	200/100 milliliters; not more than 10 percent of total samples in a 30-day period (1) exceed 400/100 milliliters	Warm-blooded animal fecal contamination pathogens may be present
Fecal Streptococcus	None	100/100 milliliters (28)	Fecal contamination absence suggests little or no warm-blooded animal contamination; pathogens could be present
Salmonella	None	None	Direct health hazard

where children are likely to play. Bacteriological standards for Oklahoma state that in areas designated as recreation (primary body contact) or public and private water supply, bacteria of the fecal coliform group shall not exceed a monthly geometric mean of 200 per 100 ml. The number of organisms are determined by multiple-tube fermentation or membrane filter procedures and are based on a minimum of not less than five samples for any 30-day period, with not more than ten percent of the total samples during any 30-day period allowed to exceed 400/100 ml. In this case primary body contact includes recreational uses where the human body may come in direct contact with the water to the point of complete body submergence. In areas designated as secondary body contact, bacteria of the fecal coliform group should not exceed a monthly geometric mean of 100 per 100 ml, as determined by multiple-tube fermentation or membrane filter procedures, nor shall more than ten percent of the total samples taken during any 30-day period exceed 2000 per 100 ml. Secondary body contact includes recreational uses, such as fishing, wading and boating, where ingestion of water is not probable (51). The fecal coliform standards adhere to research findings and field investigations (29,38, 39,52).

Compliance with these standards is by no means a guarantee that water is not hazardous. Pathogenic bacteria have been isolated with near 100% frequency from water

meeting partial body contact standards (52), and isolation of pathogens has been repeatedly accomplished from stream water with fewer than 24 fecal coliforms per 100 ml (53,54, 55,56).

Domestic wastewater is by no means the only potential source of stream pollution. Fecal material deposited on the soil by wild and domestic animals is commonly washed into streams by surface runoff (17,57).

Humans and animals are susceptible to many of the same pathogens (58). Consequently animal contaminators are as dangerous to humans as domestic sewage. It also follows that water pollution is a health hazard to animals as well as humans. Although the fecal streptococci have not been used extensively as an indicator of recreational water quality in the U.S., they have been widely used as indicators of recent fecal contamination in rivers and lakes (59). In freshly contaminated water that has not been chlorinated, the ratio of fecal coliforms to fecal streptococci can be helpful in differentiating between human and animal sources of pollution (60).

Developing a fecal coliform to fecal streptococci ratio is a valuable tool to determine the source of contamination. A ratio greater than 4 is characteristic of human contamination while a ratio less than 0.7 is indicative of animal sources.

Fecal Coliform-Fecal Streptococci Ratios (FC/FS)

Ratios of the various counts can be used to aid in interpretation of the source of bacterial contamination.

The following ratios have been suggested by Millipore Corporation (62):

$\frac{FC}{FS} \geq 4.0$ Contamination from human wastes

$\frac{FC}{FS} \leq 0.7$ Contamination from livestock or poultry

$2 < \frac{FC}{FS} < 4$ Human wastes predominate

$0.7 < \frac{FC}{FS} < 1.0$ Animal wastes predominate

$1 \leq \frac{FC}{FS} \leq 2$ Uncertain interpretation

Geldreich and Kenner (60) warn that the use of this relationship for natural waters would be valid only during the initial 24 hour travel downstream from the point of pollution discharge into the receiving waters. By utilizing individual strains and serotypes of fecal coliforms and fecal streptococci, progress is being made toward tracing pollution to more specific sources (64). But such specialized microbiological techniques are at present beyond the scope of most studies.

In addition to quantifying bacteria in surface water, fecal coliforms in bottom sediments should also be considered. Hendricks (65) and Van Dansel and Geldreich (52) found greater concentrations of fecal coliforms in bottom sediments than in the surface water. Hendricks (66)

observed higher recovery rates of Salmonella in stream bottom sediments than in surface water. Fecal coliforms in bottom sediments were 100 to 1000 times higher than in surface water; survival of fecal coliform in the bottom sediment closely paralleled survival of Salmonella (52).

Bottom sediment to water interface is not a static system. Consequently, the recirculation of older pollutants into bathing areas poses new problems in water quality, which must be considered potentially hazardous to swimmers (52). Hendricks (65) states that bacterial bottom sediment samples should be included in water quality monitoring programs.

The stream standards that are currently being used were adopted to protect the recreational use of water. These standards have had a great effect on sewage treatment cost and the techniques involved to control storm runoff water. The water treatment process is primarily done to protect the health of persons who use surface waters for recreation. Cabelli, et al, (68) states that if contact with water during recreation does not result in a major public health problem, it at least presents a major need for health effects research to define the criteria used in deciding how clean water must be to prevent illness.

Recent Criteria for the Development of New Standards

Information from past epidemiological studies to measure the health effects of swimming in recreational

waters near cities has been inadequate largely because of defects in the research design. Evaluation of such studies, particularly the work of Stevenson (21), has pointed to the need for clearer definitions and control over conditions prior to the period of observation. One of the weaknesses noted by Stevenson was in the definition of "swimmer." From the point of view of the study, a "swimmer" should be one exposed only to the water that was being tested for quality simultaneously during the experiment. Also "swimmers" should be those persons who had the upper body orifices in contact with the water. Additionally, there was the need to control for the possibility of disease prior to the swimming event which could influence the reporting of disease after the event. Also, due to another factor requiring control, was the considerable variability in levels of bacteria when the swimmer was exposed. It is only under these experimental conditions that such a correlation can be drawn between water exposure and a reported subsequent disease state for use as a basis for casual inference.

In 1973 and 1974, the methodology for an improved study was designed and implemented under the direction of Paul W. Haberman of the Center for Policy Research in New York and sponsored by the Office of Research and Development of the U.S. Environmental Protection Agency. The purpose of that study, "was to test the significance of differences in reported symptoms classified by type and severity associated

with swimming at beaches which are 'barely acceptable' in respect to local criteria for recreational waters, compared to 'relatively unpolluted' beaches" (67). The project was part of the Environmental Protection Agency Recreational Water Criteria Program to develop health effects criteria for marine recreational waters.

Trials were conducted at two beaches: Coney Island, which was designated as "barely acceptable" and Riis Park, labelled "relatively unpolluted." Trials consisted of initial beach interviews on Saturdays and Sundays and telephone follow-up eight to ten days later. The final sample consisted of 3,146 beach-goers at Coney Island and 4,923 at Riis Park, interviewed on eight weekend days in June, July, and August of 1974. Water samples were taken at the sites four times on each trial day and analyzed by a team of microbiologists from the West Kingston Rhode Island Field Station which is part of EPA's Health Effects Research Laboratory - Cincinnati.

Trial sites were selected not only on the basis of differential pollution levels but according to the population who use them. The criteria for this population was: (1) a large number of users on weekends, (2) utilization by groups (families) as opposed to couples or singles in order to provide a wide age range and the potentiality for "swimmers" and "beach-goers" (non-swimmers), and (3) a diversity of ethnic groups and socio-economic levels.

Follow-up interviews were conducted with 83% of the total sample. Such a high response rate is undoubtedly attributable to the interviewing and respondent contact procedures developed for the study. The procedures yielded a sample of such size and diversity that comparative analysis of health effects on swimmers could be done for groups by age, sex, ethnicity, and socio-economic status.

In general, symptoms and relative severity were reported more frequently by swimmers than by non-swimmers at both beaches. Gastrointestinal symptoms were reported significantly more often ($p=.05$) by swimmers than non-swimmers at Coney Island and there were significantly more respiratory symptoms for all respondents at Riis Park. The significant differences in gastrointestinal symptoms were due to higher reported rates for Latin American and younger (under age 20) swimmers than for their counterparts at Riis Park. There were, however, no significant differences in "other" symptoms and relative severity by beach or swimmer/non-swimmer status, nor in the symptom rates and severity by time spent in the water. Likewise, no consistent trends in "other" symptoms or severity were observed when controlling for demographic characteristics (67).

In the early 1950's, the Robert A. Taft Sanitary Engineering Center in Cincinnati, Ohio, conducted three studies at bathing beaches in regard to water quality and health effects. Stevenson (21) reviewed these studies and

concluded that there was sufficient evidence to indicate that the current bathing beach standards could be reduced without a significant detrimental health effect upon the bathers.

Moore (10) concluded after working with available morbidity statistics that unless there were large visible fecal aggregates in the water, there was little risk to the health of bathers.

A study done by Cabelli, et al, (68) found that there are measurable health effects associated with swimming in sewage-polluted waters. They found that Escherichia coli and fecal streptococci appeared to be the best indicators in association with indicator density symptomatology and not necessarily a cause and effect relationship to a specific disease entity. A recent attempt at predictive modelings of the risk of recreational waterborne disease was conducted by Dudley (69) using information from Michalas (32). They found a very low probability of salmonellosis while finding a much greater probability of recreational waterborne, enterovirus infection rate. This report is consistent with the literature on swimming associated with salmonellosis. However, the predictions for swimming associated virus infections are not consistent with case reports for virus diseases associated with swimming in feces polluted waters (70).

As part of a national program to develop health effects criteria for marine recreational waters, the U.S.

Environmental Protection Agency conducted a prospective epidemiological-microbiological study at bathing beaches in the vicinity of New York City, specifically at 20th Street on Coney Island and 67th Street and Riis Park at the Rockaways. The most consistent findings over the first two years of this study were that, for most of the water quality indicators examined, the mean densities at the Coney Island beach were appreciably and significantly higher than those at the Rockaways, and that the rate of gastrointestinal (GI) symptoms was significantly higher among swimmers relative to non-swimmers at the Coney Island beach but not at the Rockaways. When the data from two summers at both beaches (four points) were examined, good agreement was obtained between the mean Escherichia coli and enterococcus densities and the differential (swimmers minus non-swimmers) rate of GI symptoms. This preliminary finding addresses the objective of the study: relating illness as measured by symptomatology to some indicator of water quality.

Findings were described from the second year of an epidemiological-microbiological study conducted at New York beaches as part of the U.S Environmental Protection Agency program to develop health effects-recreational water quality criteria. Symptomatology rates among swimmers (defined as immersion of the head in the water) relative to non-swimming but beach-going controls at a "barely acceptable" (BA) beach and a "relatively unpolluted" (RU) beach were examined.

Data were collected by contacting family groups at the beach on weekends, obtaining information on bathing activity, and then questioning them by phone some 8-10 days later. In addition measurements were made for a number of potential water quality indicators.

It was observed that the symptom rates, categorized as gastrointestinal (GI), respiratory, "other," and "disabling" (stayed home, stayed in bed, consulted a physician), were higher among swimmers than non-swimmers. As in the pretest conducted the previous year, the rate of GI symptoms was significantly higher among swimmers relative to non-swimmers at the BA but not the RU beach. Children, Hispanic Americans, and the low-middle socio-economic groups were identified as the most susceptible portions of the population (8).

CHAPTER III

MATERIALS AND METHODS

Description of the Area

Keystone Lake got its name from Keystone Community where a post office existed from 1900-1962. The community was located at the point where the Cimarron and Arkansas Rivers met. This area was to be inundated by the proposed lake waters; hence, its name was passed on to the lake. Other townsites such as Mannford, Prue, Appalachia and part of Osage were abandoned because they were to be covered by the lake. Mannford was moved south and Prue was moved north as townsites.

Lake Keystone has beautiful blue water, wooded shorelines, sandy beaches, some rising bluffs, grasslands and even small, rolling hills to enjoy year round. The Lake snakes its way through small valleys creating many miles of enjoyable shoreline. There is a profusion of roads (county, state and Federal) around the lake which allow people to take advantage of sites overlooking the lake. Fishing, boating, skiing, picnicking, camping, swimming and hiking activities are easily accessible since Lake Keystone is only a twenty minute drive from metropolitan Tulsa.

Fourteen public use areas comprising 3,025 acres have been developed by the Corps of Engineers at Keystone Reservoir and the State of Oklahoma maintains three recreational sites covering 2,232 acres, including Keystone Park, Feiodi Bay Park, and Walnut Creek Peninsula Park. Two additional public recreation sites are East Levee Park, developed by the city of Cleveland, Oklahoma, and Cedar Creek Bay, a commercial concession area. The public use areas have camp sites, picnic areas, water systems, comfort stations and boat launching facilities. There are nine developed swimming beaches.

Keystone Dam is located at river mile 538.8 of the Arkansas River about 15 miles west of Tulsa, Oklahoma. It is about 2 miles downstream from the confluence of the Cimarron River with the Arkansas River.

The Keystone Dam Project was authorized by the Flood Control Act of 1950. It was designed by the Tulsa District, U.S. Army Corps of Engineers and built under supervision of the Corps.

Construction of the dam began in December, 1956, and was completed for flood control operation in September, 1964. Commercial operation of the Keystone powerplant in the production of electrical energy began in the spring of 1968. A regulating dam about 7 miles downstream from the dam was completed in 1968. Keystone Lake is a key unit in the main

control plan for flood control, water for navigation, generation of power, and other purposes in the Arkansas River Basin.

To accomplish its function, Keystone Lake has three kinds of storage that are separated by zones from the top to the bottom of the lake. These are flood control, conservation, and inactive storage.

The top or "flood control storage" portion of the lake has 1,218,500 acre-feet that is reserved to catch flood waters and will remain empty except during times of flood control operation. An acre-foot is enough water to cover one acre to a depth of one foot.

The middle or "conservation storage" provides 351,000 acre-feet of storage for water supply and power generation. The power generation provides water to support navigation on the McClellan-Kerr Arkansas River Navigation System.

The bottom or "inactive storage" provides minimum water pressure necessary for power generation and space to contain sediment.

Releases of water are generally made through the generation of power except in time of flood control operation and will vary from small flows to bank full flows of about 90,000 cubic feet per second. The releases depend on such factors as power requirements, navigation water requirements, the amount of water in storage, riverflows downstream and weather conditions.

Lake Keystone Dam

Watershed: Drainage area above the dam in square miles is 22,351.

Lake: Elevations, feet above mean sea level;

Top of flood control pool is 754 feet

Top of power pool is 723 feet

Top of inactive pool is 706 feet

Surface area of lake in acres;

At top of flood control pool is 55,300 acres

At top of power pool is 26,000 acres

Storage capacities in acre-feet;

Power pool is 330,500 acre-feet

Inactive pool is 287,500 acre-feet

Lake total is 1,836,500 acre-feet

Shoreline length miles:

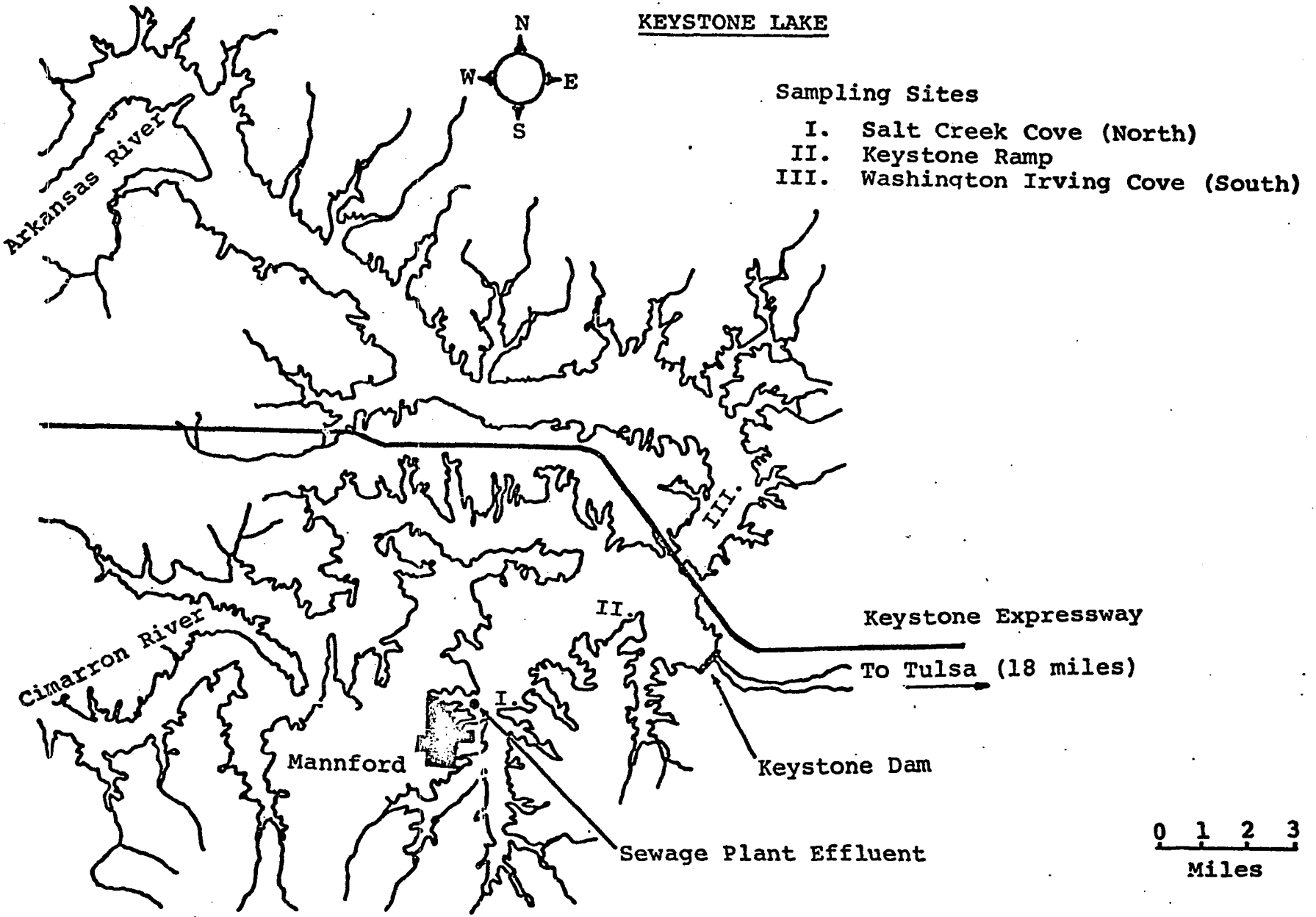
At top of power pool is 330 miles

Study Sites

The city of Mannford, Oklahoma, has a population of approximately 2,300 people. The sewage system for this community was two "full retention" lagoons. These lagoons were located near the Keystone reservoir in Creek county (see map). The legal description of the two lagoon facilities are as follows:

FACILITY	LEGAL LOCATION	SUB-SUB-BASIN
Mannford	NE/SE/NE 16-19N-9E	2-9-126 #55
Mannford Salt Creek Point	SE/NW/NW 23-19N-9E	2-9-126 #64

Figure 3.1 Keystone Lake Area



The lagoons were within one mile of the Salt Creek North bathing area and within 3 miles of the Keystone Ramp bathing beach area. These two beaches were used as the "barely acceptable" test beaches. The lagoons were too small to retain all of the sewage effluent that the city of Mannford discharged. This sewage effluent was 120,000 gallons per day on the average throughout the summer of 1979.

The test beaches had fecal coliform counts that usually exceeded 100 organisms per 100 ml of sample. Since Mannford was only 25 miles away from Tulsa, Oklahoma, a city with a population of approximately 380,000, a large potential source of bathers was provided for the beaches each weekend. The visitations for the weekend days in the summer of 1979 were:

Beach	Visitations/Day
Salt Creek North	4320
Washington South	2005

Pre-test bacterial sampling using E. coli and enterococci was done in the summer of 1978 (see Appendix A) and these organisms were consistently high at the test beaches. A "control" beach on the other side of the reservoir was selected. This beach was Washington Irving South, located on the Arkansas River arm of the Keystone Reservoir (see map). The fecal colifrom counts were relatively low at this beach and pre-testing of E. coli and enterococci showed it to be unpolluted (see Appendix A).

Field Procedures

Beach and Telephone Interviews

The methodology used in contacting and following the swimming and non-swimming populations was similar to that used in the study of Coney Island beach in New York City in 1974 (67). This included the interviewer approach to potential respondents at the beach, mail and telephone contact procedures following the initial interview, definition of swimming and socio-economic level, coding procedures, and categories of analysis. These procedures were pre-tested in Tulsa, Oklahoma, in 1978 and found to be effective in eliciting a high response rate. The use of similar procedures will make it possible to compare results from the two regions.

Collection of interview information and disposition of the sample data was obtained by means of personal interviews at Salt Creek Beach and Keystone Ramp area as the polluted areas and using the Washington Irving Cove South Beach as the unpolluted control area. Interviews were conducted on weekends with (family) group members contacted by a team of skilled interviewers. The interview schedule and procedures used in the 1974 New York City study were modified only as necessary to fit the Tulsa population. Interviewing was planned for every "good" weekend day, i.e., every Saturday and Sunday in June, July, and August of 1979, for which the probability of fair weather indicated a large number of beach-goers. Interviewers were instructed to

approach as many groups on the beaches as possible and to be attentive to groups who appeared to be near the point of leaving. The number of interviews possible on any one day, therefore, depended on the number of people at the beach, the size of groups, and the number of interviewers. Persons who swam between Monday and Friday of the previous week were not interviewed.

At the beginning of the following week, the addresses and telephone numbers given at the beach were posted. Post cards were sent to all addresses eliciting further cooperation for follow-up telephone calls to obtain information on health status nine to eleven days after the swimming event and also to validate some of the information given at the beach. Telephone follow-up was done by interviewers trained by project personnel. The interviewing schedule for this phase was also the same as that used in the New York study. Those persons reporting they swam between Monday and Friday after the initial beach interview were eliminated from the study at this point so as not to interpose the possibility of incubation of symptoms from a weekday swimming experience. Persons who swam on the weekend following the initial interview were retained in the study.

During the summer people were encountered more than once at the beach. Persons encountered on two successive weekends were not interviewed on the second weekend. However, those persons encountered a second time who had at least one intervening weekend but no midweek swimming

between interviews were included in the sample on both occasions.

In addition, persons who swam on both Saturday and Sunday of one weekend were included as swimming on the day with the highest microbial count.

The microbial counts on the day of swimming were linked to the interview data of each respondent retained in the sample.

Respondents were grouped into two categories according to their stated bathing activities; non-swimmers who either do not go in the water (non-bathers) or went in the water but did not get their head or face wet (waders) and "swimmers" who did swim or otherwise get the head or face wet. Respondents who reported that they were in the water for less than ten minutes were classified as non-swimmers irrespective of whether they got their head or face wet, in view of their short water exposure time. Non-swimmer controls were essential in order to obtain base rates on reported symptoms at the test beach and to adjust for other causes of symptoms at the beach, e.g., food.

The final sample then consisted of weekend beach-goers at Keystone Lake who, if they swam, swam only on weekends and only at that beach; who were willing to be interviewed and to give valid addresses and phone numbers; and who were able to be contacted nine to eleven days after the beach visit and who were able to supply the necessary health effects information.

Although there were several sources of interview mortality, (less of potential interviewees) none were viewed as biasing the sample in any known direction. Persons who refused to be interviewed or who gave invalid addresses or telephone numbers were probably motivated by a desire for privacy which was not related to their swimming habits or health status, and no health related selective factor was ascertained in failure to reach them by telephone. The sequence of events for the epidemiological-microbiological trials are shown in Figure 3.1.

Epidemiological Methods

The Sample. An attempt was made to obtain similar subsamples at the three test beaches based on sex, age, and ethnic groups.

The Approach. Groups of persons at the beaches who met the designated criteria; i.e., especially including persons under 20 who were getting ready to leave the beach but were still seated were selected.

The interviewer's introduction was exactly as it appeared on the questionnaire shown in Appendix B. They first asked, "Are you leaving the beach now?" If the answer was, "No," they terminated the interview with a "thank you."

If the answer to the question about leaving the beach was "Yes," they proceeded with the interview.

TABLE 3.1 Sequence of Events for the Epidemiological-Microbiological Trials

Day of Week	Day Number	Activity	Function
Saturday	1	(Beach interview, water sampling	(a) reject pretrial midweek swimmers (b) query on beach activity (c) obtain name, address, phone, etc. (d) assay of water samples
Sunday	2	As above	As above
Monday	3	Reminder letter	(a) reminder to note illness
Saturday	8	As for Day 1	As for Day 1
Sunday	9	As for Day 2	As for Day 2
Monday	10	As for Day 3 Telephone interviews	As for Day 3 (a) obtain illness information (b) obtain demographic information (c) reject post-trial midweek swimmers
Tuesday	11	As above for Day 10	As above

Setting Up the Interview. An appropriate person was selected to be the main respondent and questions were directed to him or her, taking care, however, to include the other persons present in the interview.

Who May Report for Whom. The most desirable circumstance was for knowledgeable persons to reply for themselves to questions they were able to answer since this was likely to provide the most accurate information. This applied even to children as yound as six who would know, for example, if they had gotten their heads or faces wet. A proxy, however, would need to provide answers to other questions such as, how long they were in the water, and approximately what time of day they swam, etc. It could also be possible that some of the persons in the group would not be present. The main respondent or another person in the group could reply for them. Whether they did or did not report for themselves was recorded on the questionnaire in the proper place. Item 9 of the questionnaire was provided to indicate whether persons did or did not answer questions 5, 6 and 7 for themselves.

Terminating the Interview. The interview was terminated in two other sets of circumstances: first, if respondents refused to give their first and last names; and second, if after asking question "1" to screen for persons who swam mid-week or got their heads or faces wet and it was found there were no eligible persons left to interview.

An explanation for elimination was read and the interview terminated gracefully with a "thank you." The screening sheet with no eligible respondents was attached to a non-interview form which then was completed.

Handling Refusals. If a respondent refused to start the interview or refused to give his name an attempt was made to convince him or her of the authenticity of the study by using credential cards. As with other social science research projects related to health, successful techniques to convert reluctants were: 1) communicating the conviction that the study was important and 2) emphasizing that that particular respondent's help was needed.

The interviewee was not unduly pressured, since a really reluctant respondent might complete the interview and not give an address and phone number and these cases would have limited value.

Non-Interview. Having decided that a case was either a refusal or ineligible, a Non-Interview Form was filled out. The sex, age, ethnicity, etc., of group members and the total number of persons in the group was recorded to the best of the interviewer's ability.

Telephone Follow-Up Interviews. Telephone follow-ups were made using the same criteria in regard to technique as the beach interviews. Appendix A gives detailed information in regard to beach and telephone interviews.

Quality control of the epidemology results was maintained by recalling 3% of the completed telephone interviews and verifying the information that had previously been given. Persons other than the original interviewers were used to do this. No discrepancies were found.

Collection and Analysis of Water Samples

Water samples were collected periodically during the time of maximum swimming activity at the beach on each interviewing day. One sample was collected at approximately 1:00 p.m., 3:00 p.m., and 5:00 p.m. The samples were taken at chest depth approximately four inches below the surface of the water. Upon collection the samples were iced and taken to the laboratory where they were assayed within six hours of collection. The laboratory facilities of the Tulsa City-County Health Department in Tulsa were used.

Bacteriological Procedures

The water samples arriving in the laboratory in coolers containing crushed ice were examined within six hours after collection. The bacteriological examinations included the following bacteria:

1. Total coliforms
2. Fecal coliforms
3. Fecal Streptococci

4. Thermotolerant E. coli.
5. Enterococci
6. Aeromonas hydrophila
7. Pseudomonas aeruginosa
8. Clostridium perfringens
9. Bifidobacterium
10. Acinetobacter

The indicators were enumerated by appropriate membrane filter procedures using Gelman filters (62,72). (Details are in Appendix C.) Total, fecal and streptococcus coliform densities were obtained using the technique described in Standard Methods for the Examination of Water and Wastewater (73) in conjunction with those of the Millipore Corporation described in "Biological Analysis of Water and Wastewater" Application Manual AM 302 (62) (Appendix C).

The M-Tec procedure of Dufour, et al, (74,75) was used to enumerate thermotolerant E. coli. Enterococci were quantified by the method described by Levin, et al, (46). Aeromonas hydrophila and Pseudomonas aeruginosa densities were determined by the M-A methods of Cabelli (76), Ewing, et al, (77), and Rippey, et al, (78), with M-PA methods of Levin, et al, (79), and Cabelli, et al, (80), respectively. The densities of anaerobic organisms were determined by using Clostridium perfringens (81,82), Bifidobacterium

(84) as anaerobic indicators. The density of Acinetobacter calcoaceticus was also examined to determine the nutrient level (85).

Enteroviruses were not examined due to the logistics involved. Salmonella sp. and Shigella sp. were not examined because of the relatively low numbers usually observed in swimming areas (84,86). Detailed procedures are included in Appendix C.

Bacteriological quality control was maintained by plating known quantities of selected bacteria for positive and negative controls. These were checked for the expected reactions.

Chemical-Physical Procedures

All chemical tests were performed according to standardized procedures (73). The following tests were performed by the Mannford Sewer Plant:

Final BOD
Final Suspended Solids
Final COD

Ammonia as N
COD High Level
Kjeldahl N
Organic N
Settleable Solids
Tot Org C
Water Temp.
BOD (5 Day)
D.O.
Nitrite-Nitrate
pH
Suspended Solids
Total Alkalinity
Total Phosphorous

Average Flow June
July
August

Chloride determinations were done on the BA border I (Salt Creek North) and II (Keystone Ramp) by the methodology in Appendix D. Temperatures of the water were obtained by using a standard thermometer and submerging it below the surface of the water. The air temperatures, wind velocities, evaporation readings, and lake elevations were performed by the Corps of Engineers at the Keystone dam.

All of the Chemical-Physical test results are included in Appendix F.

Statistical Procedures

The primary data summary effort focused on the estimation and comparison of symptom rates for swimmers and non-swimmers classified by beach and by various demographic and epidemiologic variables within the beach. For the purpose of hypothesis testing, a Chi Square statistic appropriate for a four fold table was used to associate a probability level with the difference between rates for swimmers and non-swimmers (89). As further data summaries the differences in rates (Δ = swimmer|non-swimmer) were calculated as were estimates, using sample estimates of probabilities, as:

$$R = \frac{P\ (\text{Symptom}|\text{swimmer})}{P\ (\text{Symptom}|\text{non-swimmer})}$$ where "R" is relative risk.

Rates were calculated for individual symptoms by dividing the total number of subjects responding "yes" to

having had the symptom by the total number of subjects responding, i.e., the sum of the "yes" and "no" responses (91). Unknown responses were excluded from both numerator and denominator. Also, some responses were excluded because the classification variable was missing. For example, a response may be included in calculating a rate for a beach, but excluded in the calculation for a sex specific rate because the value of the sex variable was missing.

In the calculation of rates for aggregates such as rates for respiratory symptoms, a subject was counted only once even if more than one respiratory symptom was reported. Thus, the numerator is the number of subjects with at least one symptom, not number of symptoms. Although a subject with multiple symptoms contributed only once to the numerator of the calculated rates for individual symptoms and for aggregates, the subject would contribute to more than one of the individual symptom rates and, perhaps, to more than one aggregate rate.

CHAPTER IV

RESULTS AND DISCUSSION

Data Presentation

Air and water temperature showed no extreme changes throughout the summer as shown in Appendix F (figures F-1 thru F-14). Evaporation and wind speed were considered to be average for the summer also. Precipitation occurred mostly on midweek days so that the weekends were not greatly affected. Lake elevations in Table F-2 also indicate no drastic rainfall in the surrounding watershed.

Chloride results are shown in Table F-3 and these were taken at the Salt Creek Beach area since the name of the beach implied that chloride levels might be elevated. The chloride levels ranging from 212 to 368 mg/l would not contribute to the general health hazard of the beach or bacterial survival in the beach waters. Table F-1 depicts the summer average for the Mannford sewage effluent that was within three miles of the BA beaches I and II. Bacteriological data from this sewage effluent shown in Table E-5 would have a high risk for containing large numbers of pathogens (5,7).

The epidemiological data presented here was obtained from the follow-up telephone questionnaires. Table 4.1 illustrates the response rate to the follow-up phone questionnaire. The success rate was approximately 84% of the individuals who gave a telephone number which could be completed by being called the next week. This response rate was considered satisfactory. Relevance was intended only to the population which had telephones.

The population studied was 6,469 and included those individuals for whom follow-up questionnaires were obtained and who did not swim during the week either before or after swimming on the interviewed weekend. Those individuals were divided into four sub-populations in groups of swimmers and non-swimmers at the BA (Barely Acceptable) and RU (Relatively Unpolluted) beaches. These are further classified demographically in Tables 4.2 through 4.6. The similarity in demographic variables among the beaches was such that adjustment on these was not deemed necessary.

Distribution of sex, age, ethnicity and socioeconomic status breakouts in Table 4.7 indicate similarity of groups in the population classified as swimmers.

The attack rates for the various symptoms among swimmers and non-swimmers at the two beaches are given in Table 4.8. One interesting point is that there is a higher percent illness for the gastrointestinal symptoms, vomiting,

TABLE 4.1 Follow-up Rates for Telephone Questionnaires.

Category	Salt Creek North Keystone Ramp I&II		Washington Irving South III
Total who gave a phone number at beach	4242		3457
No. follow-up phone interview	3610		2859
No answer, wrong phone disconnected phone	594		536
Uncooperative, unspecified	38		62
Total number of people with completed interviews on research project. (I, II, & III)		6463	
% Success	85.1		82.7
Total number of swimmers (I, II, & III)		5393	
Total number of Non-swimmers (I, II, & III)		970	

TABLE 4.2 Table of Percent of Respondents by Sex Category.

Demographic Group	Percent of Respondents by Category							
	BA						RU	
	I		II		I & II		III	
	Swim	Non-Swim	Swim	Non-Swim	Swim	Non-Swim	Swim	Non-Swim
Sex								
Male	49.1	38.1	50.8	40.5	50.0	38.9	52.0	38.2
Total(N)	1001	142	515	72	1516	214	1269	160
Female	50.9	62.0	49.2	59.6	50.4	61.2	48.0	61.8
Total(N)	1039	231	499	106	1538	337	1170	259
Total No. Male & Female	2040	373	1014	178	3054	551	2439	419
	I		II		I & II		III	
Total people Interviewed for each beach	2413		1192		3605		2858	

TABLE 4.3 Table of Population Density.

Persons/ room ratio *	Percent of Respondents by Category							
	BA						RU	
	I		II		I & II		III	
	Swim	Non-Swim	Swim	Non-Swim	Swim	Non-Swim	Swim	Non-Swim
<1	56.4	65.8	50.6	58.1	54.5	63.3	61.0	69.9
1.0-1.4	33.9	29.6	37.6	34.7	35.2	31.3	29.6	22.9
>1.4	9.7	4.6	11.8	7.2	10.4	5.5	9.4	7.2
Total (N)	1890	345	955	167	2845	512	2192	375
Number of Nonrespondents	155	28	59	11	214	39	248	44

* Number of persons in a household divided by the number of rooms in a household is used as an indicator of socioeconomic status (S.E.S.) Category < 1 persons/rooms indicates higher SES. Category 1.0-1.4 persons/rooms indicates middle SES. Category > 1.4 persons/rooms indicates lower SES.

TABLE 4.4 Table of Age by Bathers with Breakdown in Four Categories.

Age	Percent of Swimmers and Non-Swimmers							
	BA						RU	
	I		II		I & II		III	
	Swim	Non-Swim	Swim	Non-Swim	Swim	Non-Swim	Swim	Non-Swim
0-9 years	25.8	6.0	29.8	9.6	27.1	7.1	22.0	9.1
10-19	22.2	3.5	24.5	6.8	23.0	4.6	24.2	8.6
20-39	46.2	61.0	41.0	6.1	44.5	62.6	51.2	70.4
40	5.7	29.5	4.8	17.5	5.4	25.6	2.7	12.0
Total (N)	2005	369	998	177	3003	546	2395	409

For each beach, age and swim status is not independent ($P < .0001$) by Chi-Square.

TABLE 4.5 Table of Age by Bathers with Breakdown in Seven Categories.

Age	Percent of Swimmers and Non-Swimmers							
	BA						RU	
	I		II		I & II		III	
	Swim	Non-Swim	Swim	Non-Swim	Swim	Non-Swim	Swim	Non-Swim
0-5	11.9	3.8	11.6	6.2	11.8	4.6	9.9	7.1
5-9	13.9	2.2	18.1	3.4	15.3	2.6	12.0	2.0
10-14	11.1	.3	10.8	1.1	11.0	.6	9.7	1.5
15-19	11.2	3.3	13.6	5.7	12.0	4.0	14.5	7.1
20-39	46.2	61.0	41.0	66.1	44.5	62.6	51.2	70.4
40-59	5.1	23.3	4.7	16.4	5.0	21.1	2.6	10.0
60 or over	.7	6.2	.1	1.1	.5	4.6	.2	2.0
Total (N)	2005	369	998	177	3003	546	2395	409

For each beach, age and swim status is not independent ($P < .0001$) by Chi-Square.

TABLE 4.6 Table of Ethnicity.

	Percent of Respondents by Category							
	BA						RU	
	I		II		I & II		III	
	Swim	Non-Swim	Swim	Non-Swim	Swim	Non-Swim	Swim	Non-Swim
White	97.2	97.1	97.5	96.0	97.3	97.0	95.0	93.0
Other	2.8	3.0	2.5	4.0	2.7	3.3	5.5	6.9
Total (N)	1907	347	958	173	2865	520	2271	393

TABLE 4.7 Swimming Activity by Demography.

Demographic Group	Percentage Swimmers	
	BA I&II	RU III
Sex		
Male	49.6	52.0
Female	50.4	48.0
Age		
0-9 years	27.1	22.0
10-19	23.0	24.2
20-39	44.5	51.2
>40	5.4	2.7
Ethnicity		
White	97.3	95.0
Other	2.7	5.5
Persons/Rooms ratio*		
<1	54.5	61.0
1.0-1.4	35.2	29.6
>1.4	10.4	9.4

*Measure of socioeconomic status.

TABLE 4.8a Reported Symptom Rates Among Swimmers and Non-swimmers at BA and RU Beaches.

Symptom	Rate %						Relative Risk
	BA						
	I		II		I & II		
	Swim	Non-Swim	Swim	Non-Swim	Swim	Non-Swim	
Gastrointestinal							
Vomiting	2.2	2.1	1.7	1.1	2.0	1.8	1.1
Diarrhea	2.7	2.3	2.3	2.3	2.6	2.4	1.1
Stomach ache	3.8	2.4	3.3	3.4	3.6	2.7	1.3
Nausea	3.1	3.0	3.2	1.7	3.1	2.5	1.2
Respiratory							
Sore throat	4.1	4.3	5.3	2.3	4.5	3.6	1.2
Bad cough	2.6	1.9	2.8	1.7	2.7	1.8	1.5
Chest cold	2.1	1.6	1.8	1.1	2.0	1.5	1.4
Runny or stuffed nose	4.4	4.3	5.1	5.6	4.6	4.7	1.0
Earache or runny ears	2.4	1.9	3.1	1.1	2.6	1.6	1.6
Red, itchy or watery eyes (>1 day), stys	0.9	1.6	1.0	1.1	0.9	1.5	0.6
Other							
Fever (>100°F)	2.5	2.7	3.1	1.7	2.7	2.4	1.1
Headache (> few hrs)	2.2	3.5	3.3	1.7	2.6	2.9	0.9
Backache	0.6	1.1	0.9	0.0	0.7	0.7	1.0
Non specific							
Skin rash, itchy skin, welts	1.4	0.5	2.2	1.7	1.7	0.9	1.8
Sneezing, wheezing etc.	1.7	1.9	1.2	0.0	1.5	1.3	1.2

TABLE 4.8a Continued.

Symptom	Rate %						Relative Risk
	BA						
	I		II		I & II		
	Swim	Non-Swim	Swim	Non-Swim	Swim	Non-Swim	
Severe							
Home because of symptoms	0.4	0.0	1.2	2.2	0.7	0.7	0.6
Stayed in bed	2.7	4.0	1.5	1.1	2.4	3.1	2.0
Consulted medical help	2.3	1.6	3.1	0.0	2.5	1.1	1.0
Total number of persons	2045	373	1014	178	3059	551	

TABLE 4.8b Reported Symptom Rates Among Swimmers.

Symptom	Risk % RU III Swim	Risk % RU III Non-Swim	Relative Risk
Gastrointestinal			
Vomiting	1.6	1.2	1.3
Diarrhea	2.5	1.7	1.5
Stomach ache	3.5	3.1	1.2
Nausea	3.2	2.2	1.5
Respiratory			
Sore throat	3.4	3.3	1.0
Bad cough	1.5	2.4	0.6
Chest cold	1.1	1.4	0.8
Runny or stuffed nose	3.3	3.6	0.9
Earache or runny ears	1.4	2.2	0.6
Red, itchy or watery eyes (>1 day), stys	.7	.7	1.0
Other			
Fever (>100°F)	2.1	1.0	2.1
Headache (> few hrs.)	2.2	2.2	1.0
Backache	0.9	1.2	0.8
Non specific			
Skin rash	1.3	1.4	0.9
Sneezing	0.8	1.2	0.7
Severe			
Home because of symptoms	.2	1.2	1.0
Stayed in bed	2.0	2.1	1.5
Consulted medical help	2.2	1.4	1.1
Total number of people	2440	419	

diarrhea, and stomach ache at the BA beaches than at the RU beach.

Also the respiratory symptoms, other symptoms, and non-specific symptoms showed the same trend with more general sickness at the BA beaches in comparison to the RU beach. Individuals who responded affirmatively to queries in the non-specific category were further questioned concerning the onset of symptoms. This was used as a test for allergic reactions. It can be seen from Table 4.9 that affirmative answers to the onset questions were rare and were not more frequent among swimmers than non-swimmers.

The symptoms were categorized as gastrointestinal, respiratory, other, and severity as shown in Table 4.10. The symptom types along with the relative risk for swimmers was calculated showing a trend toward increased sickness for swimmers at the BA beaches.

The differential rates for the various symptom categories were examined by demographic grouping in order to identify the most susceptible portions of the populations. These are shown in Table 4.11. The differential rates which are in excess of 3.0% are underscored. It can be seen that most of the high differential rates occurred with respiratory and gastrointestinal symptoms at beaches I and II. The rates of gastrointestinal symptoms among children in the lower socioeconomic group ($>$ 1.4) who swam were higher than those who did not (Table 4.12). There were no significant

TABLE 4.9 Attack Rates for Allergic Symptoms.

Category	Attack Rate Percent for Possible "Allergy Related" Symptoms							
	BA						RU	
	I		II		I & II		III	
	Swim	Non-Swim	Swim	Non-Swim	Swim	Non-Swim	Swim	Non-Swim
Onset at beach or positive history*	8.3	9.1	11.1	8.4	9.2	8.9	7.2	7.2
No allergic symptoms	91.7	90.9	88.9	91.6	90.8	91.1	92.8	92.8
Total	2045	373	1014	178	3059	551	2440	419

*Headache, bad cough; chest cold; runny or stuffed nose; red, itchy or watery eyes, skin rask; itchy skin or welts; sneezing, wheezing or tightness in chest.

TABLE 4.10 Symptom Rate by Category

Symptom Type	Symptom Rate in %											
	BA								RU			
	I		II		I & II		Δ	Relative Risk	III		Δ	Relative Risk
	Swim	Non-Swim	Swim	Non-Swim	Swim	Non-Swim			Swim	Non-Swim		
G.I.	6.1	4.8	5.9	5.6	6.1	5.1	1.0	1.2	5.7	5.3	.4	1.1
Res.	8.6	8.0	11.1	8.4	9.4	8.2	1.2	1.2	7.0	8.6	-1.6	0.8
Other	6.6	6.4	8.1	5.1	7.1	6.0	1.1	1.2	5.5	4.3	1.2	1.3
No. of Partici-pants	2045	373	1014	178	3059	551			2440	419		

TABLE 4.11 Differential Rate of Gastrointestinal Symptoms of the Three Subpopulations of Demographic Grouping

Demographic Group	Symptom Rate (Swimmers minus Non-swimmers) Respiratory I&II	Respiratory III	Gastrointestinal I&II	Gastrointestinal III	"Other" I&II	"Other" III
Sex						
Male	2.2	-3.6	1.4	1.3	.5	.5
Female	.9	- .4	1.0	.1	1.7	2.1
Age						
0-9	9.7	-11.5	4.8	-.7	2.9	-3.4
10-19	-5.3	- 6.1	-11.0	-6.1	-13.9	1.5
≥20	.1	.1	.9	1.4	1.8	2.1
S.E.S. per room						
1	1.8	1.0	.9	.6	2.1	1.4
1.0-1.4	1.3	-4.7	1.9	1.4	1.0	1.9
>1.4	-1.1	3.9	-3.1	-.3	-.7	2.9

TABLE 4.12 Gastrointestinal Symptom Rate by Demographic Grouping.

Demographic Group	G.I. Symptom Rate in % at I&II		III	
	Swim	Non-Swim	Swim	Non-Swim
Age				
0-9	7.4	2.6	4.8	5.4
10-19	5.1	1.6	5.4	1.4
≥20	5.7	4.8	6.1	4.8
S.E.S. per room				
<1	5.8	4.9	5.9	5.3
1.0-1.4	7.5	5.6	7.2	5.8
>1.4	7.9	6.9	3.4	3.7

S.E.S. = persons/rooms ratio
G.I. = Gastrointestinal

differences between swimmers and non-swimmers using Chi-Square 2 X 2 tables. However, 12 out of the 18 reported symptom rates showed a greater attack rate among swimmers than non-swimmers. It therefore appears that swimmers are at a greater risk than non-swimmers in general. The relative risk reflects that this trend was also true. The difference between relative risk at the two beaches (Table 4.10) show that the barely acceptable beach I & II was higher than the control beach III. Except for the other category, this indicates a trend in favor of swimmers being at a greater relative risk in the categories of gastrointestinal and respiratory symptoms at the polluted beach. Reported symptoms were low in number and therefore this small sample size may not be large enough to detect the small differences between swimmers and non-swimmers or between the barely acceptable and the relatively unpolluted beach symptom rates.

The epidemiological methodology reflects trends observed in the rates of gastrointestinal symptoms among the four study populations (swimmers and non-swimmers at the BA and RU beaches) that has been reported in prior studies (86).

Criteria Development

The data can be analyzed to yield bathing beach criteria in two ways. It can be derived from data obtained within a given year (summer) by relating the symptom rates

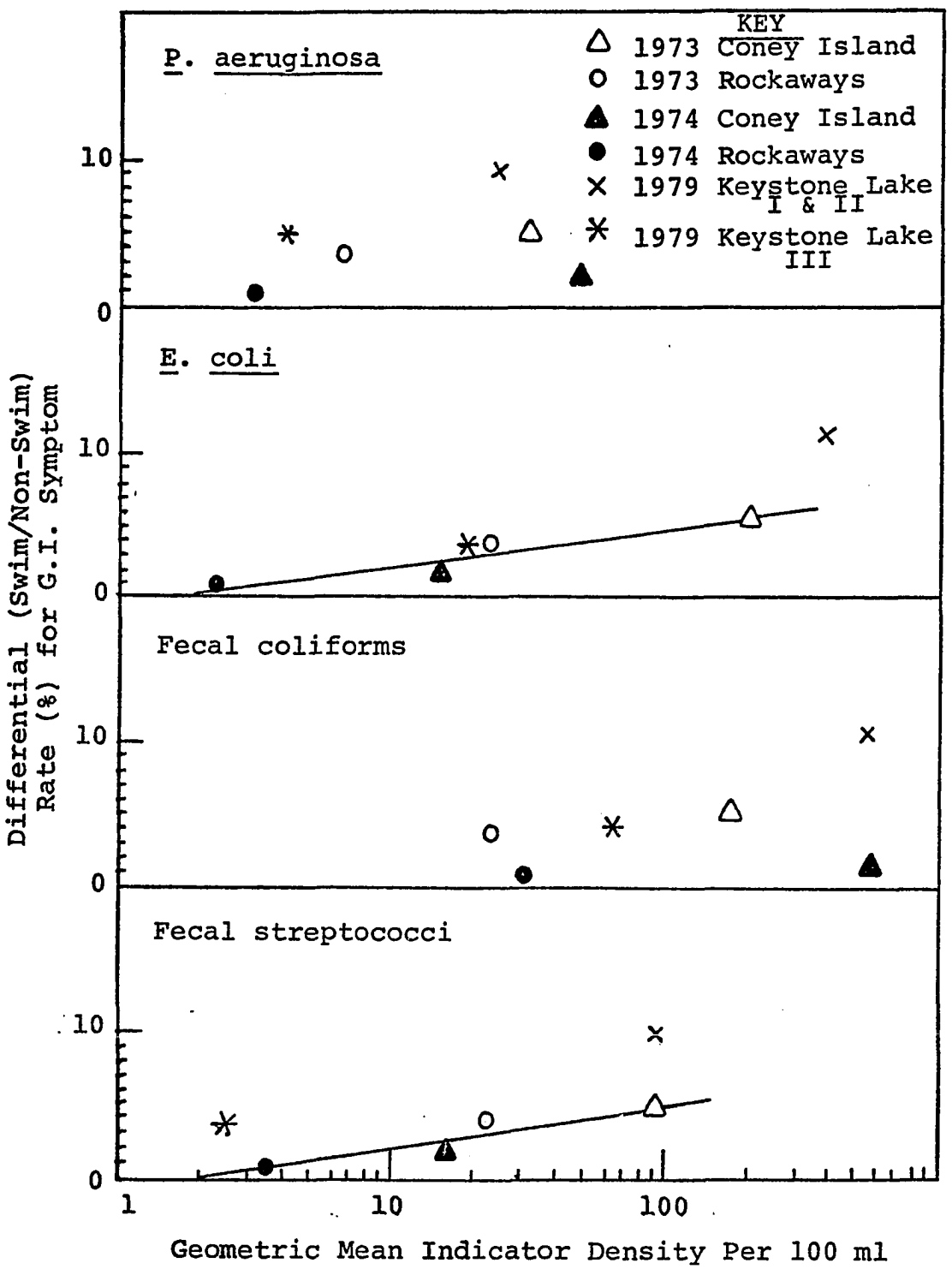


Figure 4.1. Relationship of Bacterial Indicators to G.I. Symptom Rates as Compared to Other Studies.

Lines of best fit for E. coli and Fecal streptococci were determined by Cabelli (8).

to the corresponding indicator densities for each trial (day) at each beach (68). The second approach is to analyze the data by years (summer). Thus, the overall symptom rates and associated mean densities for all the trials at each beach during a given summer are combined to yield a single data point. Results from Cabelli's study (68) are presented in Figure 4.1 along with data points from this study. Cabelli's work was done using bathing beaches in the vicinity of New York, specifically at Coney Island as the BA beach and Riss Park at the Rockways as the RU beach. Inspection of this data confirms the close relationships of gastro-intestinal symptomatology to E. coli and fecal streptococci densities. Enterococci densities are shown in Figure 4.2 and Table 4.13. If the lines were extended through E. coli densities of 10^6/100ml in order to determine what the symptom rates would be, the rate would be 12%. Approximately 10^5 to 10^6/100ml of E. coli could be expected in raw sewage. The data points obtained by using both methods of analysis (within summers and between summers) were quite similar. The lines indicated were obtained by Cabelli (67) using linear regression. The differential rate of gastrointestinal symptoms associated with a mean E. coli density of 200/100ml were 3.6 and 3.8 respectively (Figure 4.1). The data from this study support this relationship.

A basic component of the experimental design of this study was that no pre-determined judgments were made as to

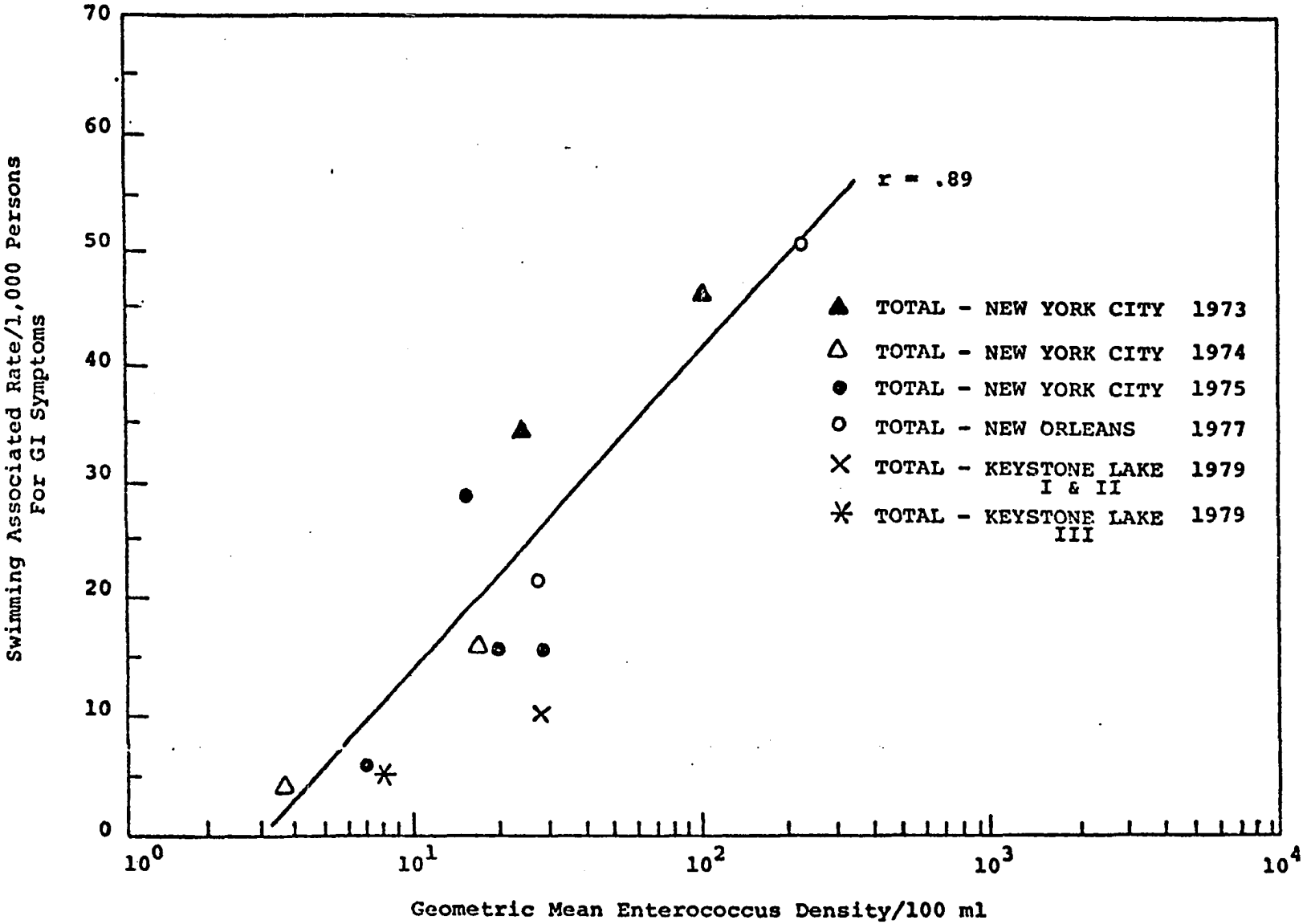


Figure 4.2. Relationship of a Bacterial Indicator to G. I. Symptom Rates as Compared to Other Studies.

Regression line by Cabelli (8).

TABLE 4.13 Epidemiological-Microbiological Trials at Keystone Beaches.

Beach	BA I & II	RU III
No. of participants	3,610	2,859
Gastrointestinal symptoms per 1000		
Swimmers (S)	61	57
Non-swimmers (NS)	51	53
Difference (S-NS)	10	4
Respiratory symptoms per 1000		
Swimmers (S)	94	70
Non-swimmers (NS)	82	86
Difference (S-NS)	12	-16
Other symptoms per 1000		
Swimmers	71	55
Non-swimmers (NS)	60	43
Difference (S-NS)	11	12
Severity symptoms per 1000		
Swimmers (S)	45	13
Non-swimmers (NS)	56	96
Difference (S-NS)	-11	-83
Geometric Mean Enterococcus Density per 100 ml	38.8	6.8

which microbe would be the best indicator. Therefore, measurements were made for a number of potential indicators. The geometric mean densities (hereafter referred to as mean densities) for the various bacterial indicators obtained at two locations over three time intervals at each of the beaches BA and RU are presented in Table 4.14. Also the ratio between beaches is shown. Summary graphs of the concentration of bacterial indicators are shown in Appendix E. E. coli appears to be the best indicator with a good range of about 100/100ml in the BA beach with 20/100ml in the RU beach. Fecal streptococci and enterococci were also good with adequate differences. The other indicators either showed too low or too high counts since the desired range for the millipore technique is 20-80 colonies per plate from each filtered sample.

E. coli and enterococci, as determined from the epidemiological study, are consistently associated with the source of pathogens, presumably human fecal wastes due to the sewage outfall being within three miles of beach I and II. Both of these organisms are present in the water in sufficient density to relate to the rather low rates of mild types of gastrointestinal symptoms and respiratory symptoms reported in this study. It also appears that both E. coli and enterococci will survive traveling from the source, the sewage effluent outfall, to the bathing beach well enough to provide a reasonably good correlation to the human

TABLE 4.14 Geometric Mean Densities of Potential Microbial Indicators.

	Indicator	Mean Recovery/100 ml BA I & II	Mean Recovery/100 ml RU III	Ratio Between Beaches
1.	Total coliform	19,000	6,700	2.8
2.	Fecal coliform	436	51.0	8.5
3.	Fecal streptococci	96.6	19.0	5.1
4.	*Escherichia coli*	138	19.1	7.2
5.	Enterococci	38.8	6.8	4.2
6.	*Aeromonas hydrophila*	27,000	7,024	3.8
7.	*Pseudomonas aeruginosa*	18.7	4.2	4.5
8.	*Clostridium perfringens*	5.0	3.9	1.3
9.	Bifidobacteria	17.3	1	17.3
10.	Acinetobacter	662	718	.9

health effects. This symptomatology that was seen at the beaches was presumably caused by pathogenic microorganisms from the sewage.

A microbiological criterion used as an indicator of possible health effects has its limitations because it represents the "average situation" (68). At one end of the spectrum there are no pathogenic organisms being shed into the sewage and thus presenting no hazard to swimmers and, on the other hand, during epidemic situations, the indicator guideline may not be strict enough, even though a safety factor is built into it. The first situation is unlikely, but the last one remains the one that health effects recreational water quality guidelines and standards must be used in conjunction with, but not to the exclusion of good public health surveillance practices.

An attack rate for gastrointestinal symptoms of 3-4% associated with swimming in waters containing about 200 E. coli per 100ml would appear to be somewhat of a concern, especially when projected to the large numbers of individuals who swim at this level of polluted beach during the entire summer. It should be pointed out that there were no "severe" symptoms that required hospitalization. However, few, if any of these cases of illness would have been reported to public health authorities except in an "outbreak" situation. In declaring that a beach is a possible hazard to the swimmers

by posting it or even closing it must be weighed against the social, economic and health consequences of denying the use of the beach to the public, especially in large urban areas.

CHAPTER V

CONCLUSIONS

As part of the national program to develop health-effects criteria for recreational waters, the U.S. Environmental Protection Agency provided funds to conduct a prospective epidemiological-microbiological study at bathing beaches on Lake Keystone located near Tulsa, Oklahoma. Symptomatology rates among swimmers relative to non-swimmer controls were examined at a "barely acceptable" (BA) beach, Salt Creek North (I), and Keystone Ramp (II), and a "relatively unpolluted" (RU) beach at Washington Irving South (III).

This was accomplished by contacting family groups at beaches on weekends and obtaining information on bathing activity by the use of interviewers. These beach-goers were questioned by telephone 8-10 days later concerning health related symptoms.

The four stated objectives revealed the following:

1. The differences in reported health symptoms classified by type and severity associated with swimming in this lower midwest region of the country under various conditions and levels of water pollution showed that the symptom rates categorized as gastrointestinal, respiratory and "other" were higher among swimmers than non-swimmers. Although the data was not statistically significant, definite trends could be shown in that direction.

2. The association between various microbiological indicators of water pollution and reported symptoms classified by type was calculated. Good agreement was obtained between geometric means of Echerichia coli and enterococcus densities with the differential (swimmers minus non-swimmers) rate of gastrointestinal symptoms.

3. These associations did not differ significantly among sub-groups classified on the basis of age, sex, ethnicity, or socioeconomic level. However, symptom rates were higher for the age group between 0 and 9. A higher symptom rate was also observed for the lower socioeconomic group that swam. There was no noticeable difference in symptom rates with regard to sex or ethnicity.

4. A correlation of reported symptom rates of disease among swimmers by type and level of pollution at the time of the swimming event, using several microbiological indicators of pollution was addressed. This was done by comparing the

results of this study with Cabelli's work (8) with regard to lines of best fit and linear regression.

The data presented suggests that there are measurable health effects associated with sewage polluted waters.

Although the data obtained in this study is encouraging, the overall program to develop health effects recreational water quality criteria is far from complete. We have shown trends in the same direction as studies done in this field by E.P.A. More work needs to be done in this area to help define the disease-indicator associations, especially a study that could be done using the same population and swimming sites but with no pollution at beach (I) and (II) to see if the illness rates at these beaches would decrease.

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APPENDIX A

Instructions for Beach Interviewers

INSTRUCTIONS FOR BEACH INTERVIEWING: A TRAINING GUIDE FOR INTERVIEWERS

Overview of Questionnaire

I. Qualifications for Inclusion in the Study

A. Weekend (Saturday or Sunday) beach-goers only - that is, persons who have not been swimming at any beach, pool, or lake anywhere during the preceding Monday through Friday.

B. Persons who may have been near or in the water at this or some other location during the previous week but who did not get their heads or faces wet.

Rationale: Persons who were swimming during the previous week and who got their heads or faces wet were exposed to water conditions on which we have no measures. If these persons were included in the study, and if they reported symptoms, it would not be known which swimming event might have precipitated the symptoms.

Question 1 A. and 1 B. is for the purpose of eliminating mid-week swimmers.

II. Information Required on Each Weekend Beach-goer

A. First and last name.

Rationale: Since we have to get in touch with these people later by telephone, to find out if they had any symptoms of illness, we have to have their full names in order to identify them and link the information obtained from them at the beach to that obtained by telephone.

Recorded: Top of page 2 of the questionnaire.

B. Sex, ethnicity or race (E/R on the questionnaire), and age.

Rationale: We want to look at the results to see if there are differences in symptom rates by sex, by ethnicity and by age. These characteristics must be obtained at the beach interviews from observations by the interviewers.

Definitions: Ethnicity/Race is White (WI), Black (B2), Spanish-Speaking (L3 for Latin), and Other (O4). Age will be estimated by the observer in terms of the broad categories used but can be asked for those under 20.

Recorded: In indicated spaces top of page 2 and Q. 4.

C. Swimming experience the day of the interview

1. Swimmers

Definition: Persons in the water and who got their heads or faces wet.

Rationale: A person is exposed to water conditions only if he/she gets his/her head or face wet.

Q. 5 and Q. 5 A are for the purpose of distinguishing the swimmers from the non-swimmers.

a. Place person swims

Rationale: Although most people will be swimming in the roped-off area, they may also walk along the levee and swim from there.

Q. 6 is a check to see where they swim.

b. Time person swims

Rationale: Water conditions will be measured at noon, mid- and late afternoon. It is important to find out when each person was swimming in order to link his record to the water conditions.

Q. 7 is for this purpose.

c. Amount of time in the water

Rationale: Only persons who are in the water for at least 10 minutes can be adequately exposed to water conditions. Therefore, it is necessary to distinguish swimmers from "dippers."

Q. 8 is for this purpose.

2. Non-swimming beach-goers

Rationale: People who do not swim may also report illness symptoms the following week. Comparison of symptom rates of non-swimmers and swimmers is important for determining the association between water conditions and swimming.

Q. 5 distinguishes the non-swimmers.
Q. 5 B provides information on whether the person is not swimming because of illness symptoms or some other reason.

D. Illness symptoms during past week

Rationale: It is important to know whether people who report symptoms after swimming had some symptoms prior to swimming.

Q. 9 is to get this information on each person.

E. Appearance at time of interview (by observation)

Rationale: Noting appearance gives the interviewer some idea of whether or not the person went into the water and recording it gives the office the interviewer's impression.

Q.10 is for this purpose.

F. Source of information

Rationale: Sometimes one member of a group will report the information on other people. It is important to know whether the person was reporting his/her own swimming experience or whether it was reported by another.

Q. 11 is for this purpose.

III. Information Needed for Follow-Up

Rationale: Since we will be writing the respondents a letter to thank them for their cooperation and since we will be telephoning many of them to inquire about any symptoms following their day at the beach, it is imperative that accurate addresses and telephone information be obtained at the time of the beach interview.

Q. 12 on page 4 is for recording this information.

IV. Cooperative Rating

Rationale: The beach interviewer's impression will provide a clue to the degree of difficulty of telephone follow-up. Also it provides documentation of feasibility of this type of interview situation in the planning of further studies.

Q. 13 is the location for recording this impression after the interview is terminated.

V. Language Used for the Interview

Rationale: This is important to know in assigning telephone interviewers.

Q. 14 is for reporting language.

DETAILED INSTRUCTIONS

1. Time to Interview: Since we want to know about swimming experiences that day, we need to talk to people who are about ready to leave the beach. It is anticipated that morning swimmers may be leaving around noon. Some interviewers should be there to contact that group. Picnickers probably stay longer. The heaviest exodus should be between 4 P.M. and 7 P.M. A larger number of interviewers should be on the beach between those hours.

2. Check List of Things to Have With You:
   1. Interviewer's badge
   2. Letter of identification
   3. Referral cards
   4. 4 - 6 sharpened pencils or pen and 2 pencils
   5. Legal-sized clipboard
   6. Interviewer's Manual
   7. Enough questionnaires to cover the time you expect to work. Allow 5 questionnaires per hour

3. Who to Approach: Look for family groups or groups which seem to contain a wide age range of persons. One or two persons in such a group can probably give you information on others in the group. Also several members of such a group probably live at the same address and have the same phone. This simplifies the information you will have to obtain on page 4.

   Single persons or couples in groups constitute a large percentage of beach-goers. Do not avoid them, but concentrate on your family groups and try to balance the number of groups of singles you encounter.

   In the event that there are more than 6 persons in a group, you will have extra copies of pages 2 and 3 to attach to the questionnaires. Write the last name of the family on these pages to identify.

4. What Constitutes an Interview:
   a. An interview is the information obtained from a group of persons and will contain information on as few as two and as many as six or more persons. Our goal is 2000 interviews. This should provide information on 6000 beach-goers since it has been estimated that three is the average number of persons per interview.

b. A few groups you encounter may have no week-end swimmers. In other words, their response to the first question would indicate that they had all been swimming mid-week and had gotten their heads or faces wet. You would have no more questions to ask of these persons but it would constitute an interview.

c. A completed interview should take about fifteen minutes.

5. General Format of the Questionnaires:
   a. Items typed in upper case (CAPS) are instructions, not to be read to the respondents.

   b. Questions to be asked are typed in lower case.

   c. The numbers with a /, e.g. 15/, or numbers circled in the "p" columns, e.g. 15 are column locations for key punching into IBM cards.

6. Introduction:
   a. Your introduction statement is written at the top of page 1. Memorize this so it becomes free and easy.

   b. If people respond that they are not ready to leave the beach, TERMINATE with a statement to the effect that you would like to come back to talk to them later.

7. Beginning an Interview: (Persons about ready to leave and willing to talk to you.)
   Record the DATE and approximate TIME in the upper right hand corner of page 1. (The Group # and the D. Code will be filled in in the office.)

8. The Questions:

   Question 1: If Yes to this question, print the first names in the spaces provided on page 1. Then ask Q. 1 B about each. If 1 B is Yes, circle the 1, next to the person's name. If No, circle 2 and print the first name at the top of page 2 in the space indicated. P_1 would be the first person you record, P_2 the second person, etc. At that point you might ask the person's last name to record it on page 2.
   When you have finished the list of persons who have been swimming mid-week, go to Q. 2 with the explanatory statement leading into the question. Be sure to circle the name of the person on page 2 who gives you the information.

Question 2: Ask as given and print the names under one of the "P" columns on page 2. If there is no one else, check the box indicated. (Note: the item "Total eligible" will be filled in in the office.)

If NO ONE IS LISTED ON PAGE 2, TERMINATE the interview with a statement of appreciation for their help.

Responses to the items on pages 2 and 3 are to be recorded for each person whose name is listed. Take each person one at a time and then go back to the next person, and record your observations and the answer for that person. The answer for each person is recorded by circling the appropriate code number in the column for the person you are talking about. The meaning of these codes is given in the second column on pages 2 and 3.

RELATIONSHIP TO RESPONDENT IF VOLUNTEERED OR OBVIOUS - The name of the respondent will be circled on page 2. If the relationship of the person being talked about is apparent or mentioned by the respondent, record it. It is not necessary to ask and you might not obtain it for each person. It only helps the interviewer and hence the office to have an idea of the composition of the group.

SEX - By observation. Circle 1 if male and 2 if female in the column under the person being talked about.

E/R (Ethnicity/Race) - By observation. Circle 1 if White, 2 if Black (but not Spanish-speaking or Spanish accent), 3 if Spanish-speaking or Spanish accent (here the names may give you a clue), 4 if Other (you may encounter a number of Oriental families).

Question 3: To be asked only for interviews conducted on a Sunday. If the answer is Yes, indicating the person swam on Saturday, check the box for Yes in the column for that person, and ask the A part of the question about getting head or face wet. If the answer is Yes, check the box for Yes in the column for that person and ask the B part of the question to find out where the person swam. Circle 1 for Shell Beach, 2 for other place in Lake Keystone or 3 for any other location including swimming pool. If the person swam more than one place, you may circle all that apply.

If the person did not swim on Saturday, check the No box for that question in the column for that person and go to Q. 4 (Age).

If the person went in the water on Saturday but did not get his head or face wet, check the No box for that question and go to Q. 4 (Age).

Question 4, (Age): Ask age only if you are not sure or if the person is a child. If not sure about an adult, you may ask: "Are you over 20 and under 40?" As you will

see, exact age is not required. Circle 0 for children under 5, 1 for those 5-9, 2 for those 10-14, 3 for 15-19, 4 for 20-39, 5 for 40-59, and 6 for 60 or over.

Question 5: If the answer is Yes, the person did go into the water on the interview day; check the Yes box in the column for that person and ask the A part of the question. If the answer to the A part is Yes, circle 1 for Yes in the column for that person and move on to Question 6. If the answer is No circle 2 in that column and move on to Question 6.

If the answer to Question 5 is No, the person did not go into the water; check the No box for the question in the column for the person and ask the B part of the question. If the answer indicates that the reason has nothing to do with health, circle 6 in the column for that person and go to Question 9. If health is the reason, diplomatically ask what's wrong and circle the appropriate category, such as 1 for sunburn, 2 for respiratory (e.g., cold, cough, runny nose), 3 for skin problems (other than sunburn), 4 for gastro-intestinal (e.g., upset stomach, nausea, diarrhea), 5 for unspecified/other illness (e.g., "just don't feel well," slight fever, allergy). Then go to Q. 9 for that person. You may circle more than one code for this question.

Question 6 (For person who went into the water and got their head or face wet.): Under the column for the person, circle the code for the place they went into the water. Circle 1 if they swam within the roped-off area, 2 if they swam outside the area, east towards the Bayou St. John outlet, and 3 if they swam west of the area. Circle as many as mentioned.

Question 7: Time of day in the water. Circle 1 if they were in the water around noon or earlier, 2 if they were in the water around 3 P.M. (or between 1 P.M. and 4 P.M.), 3 if in the water around 5 P.M. or after (anytime after 4 P.M.). Circle as many times as apply. (e.g., for a person who had been in and out of the water from the time they arrived around noon or before until their departure around 6 P.M., all three numbers would be circled.)

Question 8: Try to get an estimate of the total accumulated time in the water up to an hour or more. Exact time is not required. You might help the respondent by asking the categories we are using. Circle 1 for less than 10 minutes, 2 for 10-29 minutes, 3 for at least a half hour but less than an hour, and 4 for an hour or more.

Question 9: FOR EVERYONE. When asking this question, read the symptoms off as a check list the way a nurse or technician might do in a doctor's office, in a matter-of-fact sort of way. Circle 1 for Yes and 2 for No for each symptom read in the column for that person.

Question 10: For those persons present at the interview, note their appearance in terms of wetness. Circle 1 for head and suit wet, 2 for head wet, 3 for suit wet, 4 for neither wet or not in a suit, and 5 if the person is not there to be observed, in the column for each person.

Question 11: Circle 1 if the information about experiences in the water (Q.'s 5-8) and Q. 9 (symptoms) were given by the person him-/herself and 2 if the information was given by another person.

When Pages 2 and 3 have been completed for all persons named across the top of Page 2, go to Page 4 and contact information.

Question 12: Memorize this statement, (or your version thereof), explaining why we would like addresses and phone numbers.

EXPLAIN THAT THIS INFORMATION WILL BE KEPT CONFIDENTIAL AND IS NEEDED ONLY TO BE ABLE TO FIND OUT WHETHER PEOPLE DO DEVELOP ANY SYMPTOMS AFTER THEY HAVE BEEN TO THE BEACH.

PRINT the full name of the person you have talked to at the beach whom we can contact. PRINT the complete address and phone number. If no phone, find out if there is another phone number where they can be reached.

Get the first names of all persons in the group at the beach on the interview day who live at this address. (Their last names will be on page 2.)

Get the names, addresses, and phone numbers of all persons in the group on whom you have obtained information who do not live at the first address.

9. Ending an Interview: Express appreciation and thank the respondents for their help.

Leave the group to go on to another interview. At a distance fill in:

Question 13: Degree of cooperation. Check the category that best describes your impression, very _____, somewhat _____, little _____. If there were unusual circumstances (e.g., difficult, suspicious, etc.), check other _____ and write a word of explanation.

Question 14: Check the language used as indicated.

Write your name at the bottom in the indicated place. Phone/address code will be assigned in the office.

10. Termination of an Interview Before Completion: In the event that a respondent begins the interview and does not want to go on with it, encourage them to go on, assuring them of the importance and the confidentiality of the information. Do not pressure them because their participation is voluntary. If they doubt your credibility, you can show them your letter of introduction and provide them with a card indicating where they can get more information about the study. Write the reason for non-completion at the bottom of Page 3.

11. Checking Interviews: Before turning in your interviews, check the forms for completeness and accuracy.

12. Where to Return Interviews: Procedures for returning the completed interview forms will be attached.

Dear Beach Visitor,

A few days ago one of our interviewers working for the University of Oklahoma spoke to you at a beach on Lake Keystone. We are checking on the relationship between swimming and health. This is a reminder that we will be calling you on Monday or Tuesday.

Thanks for your assistance,

James M. Robertson

James M. Robertson
University of Oklahoma
Research Team

The above is a copy of a post card that was used to remind the people interviewed that we would be calling them to complete the interview.

APPENDIX B

SAMPLE INTERVIEW FORM

(Beach and Telephone)

OKLAHOMA BEACH STUDY

GROUP # _____/1-4

SITE/ID _____/5

SAT...1 SUN...2 MON...3 TUES...4 WED...5 THURS...6 FRI...7 /6

DATE: _____/7-8

TIME: _____

Hello, I'm _____. Oklahoma University is working in a study to help improve swimming conditions at public beaches throughout the country. We want to find out about conditions here at _____ Beach and we need the help of people who come to the beach. We would like to ask you about the experiences that you and your group have had at the beach today.

Are you about ready to leave the beach now? IF NO, TERMINATE.

1. During the past week, between Monday and Friday, did you or anyone at the beach with you <u>now</u> go in the water anywhere -- here or at some other beach or pool?

YES (ASK A & B).....[ ]
NO (GO TO Q.2).....[ ]

A. who was that? RECORD FIRST NAME OF EACH ON A SEPARATE LINE	B. Did (PERSON) actually swim, or get (his/her) head or face wet? YES	NO	
	1	2	(RECORD FULL NAME ON PAGE 2)
	1	2	(RECORD FULL NAME ON PAGE 2)
	1	2	(RECORD FULL NAME ON PAGE 2)
	1	2	(RECORD FULL NAME ON PAGE 2)
	1	2	(RECORD FULL NAME ON PAGE 2)
	1	2	(RECORD FULL NAME ON PAGE 2)
	1	2	(RECORD FULL NAME ON PAGE 2)

GROUP # _____

We are only interested now in those who did not actually swim or get (their) heads or faces wet during the past week between Monday and Friday.

2. What is your name and the names of the (other) people who are with you now (who you haven't told me about)? RECORD FULL NAMES BELOW.

No one else.....0

Total Number of eligible respondent in (family) group _____

IF NO ONE LISTED BELOW, TERMINATE

		P_1	P_2	P_3	P_4	P_5	P_6
COMPLETE Q.'s 3-11 FOR PERSON 1 BEFORE COMPLETING 3-11 FOR PERSON 2, ETC. FOR SUNDAY INTERVIEWS, ASK OTHERWISE BEGIN WITH Q.4.	FIRST NAME						
	LAST NAME /9-14						
	RELATIONSHIP TO RESPONDENT IF VOLUNTEERED OR OBVIOUS						
	SEX: Male...1 Female..2 /15	1 2	1 2	1 2	1 2	1 2	1 2
	E/R: W1 B2 L3 O4 /16	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4
SUNDAY ONLY 3. Did (PERSON) go swimming or in the water anywhere yesterday? /17	YES (ASK A).....[1] NO (GO TO Q.4).[2]	(ASK A) [1] (GO TO Q.4) [2]	(ASK A) [1] (GO TO Q.4) [2]	(ASK A) [1] (GO TO Q.4) [2]	(ASK A) [1] (GO TO Q.4) [2]	(ASK A) [1] (GO TO Q.4) [2]	(ASK A) (GO TO Q.4)
IF YES: A. Did (PERSON) actually swim or get his/her head or face wet then? WHERE? /18	YES (ASK A).... [1] NO (GO TO Q.4) [2]	(ASK B) [1] (GO TO Q.4) [2]	(ASK B) [1] (GO TO Q.4) [2]	(ASK B) [1] (GO TO Q.4) [2]	(ASK B) [1] (GO TO Q.4) [2]	(ASK B) [1] (GO TO Q.4) [2]	(ASK B) (GO TO Q.4)
/19	SALT CREEK BEACH.1 KEYSTONE BEACH...2 WASH. I.....3 OTHER.....4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4
4. CODE AGE WITHOUT ASKING. IF CHILD OR NOT SURE, ASK: What is (PERSON'S) age? /20	Under 5....0 20-39..4 5-9.....1 40-59..5 10-14.....2 60 or 15-19.....3 over..6	0 4 1 5 2 6 3	0 4 1 5 2 6 3	0 4 1 5 2 6 3	0 4 1 5 2 6 3	0 4 1 5 2 6 3	0 4 1 5 2 6 3

GROUP # _____

		P_1	P_2	P_3	P_4	P_5	P_6
5. Did (PERSON) go into water at all today?	/21 YES (ASK A).....[1] NO (ASK B).....[2]	(ASK A).....[1] (ASK B).....[2]	(ASK A).....[1] (ASK B).....[2]	(ASK A).....[1] (ASK B).....[2]	(ASK A).....[1] (ASK B).....[2]	(ASK A).....[1] (ASK B).....[2]	(ASK A).....[1] (ASK B).....[2]
A. IF YES TO Q.5: Did (PERSON) actually swim or get his/her head or face wet?	/22 YES (GO TO Q.6)...1 NO (GO TO Q.8)...2	(GO TO Q.6) 1 (GO TO Q.8) 2	(GO TO Q.6) 1 (GO TO Q.8) 2	(GO TO Q.6) 1 (GO TO Q.8) 1	(GO TO Q.6) 1 (GO TO Q.8) 2	(GO TO Q.6) 1 (GO TO Q.8) 1	(GO TO Q.6) 1 (GO TO Q.8) 2
B. IF NO TO Q.5: Why not? IF ILL: Is anything wrong? (Symptoms.)	/23 Sunburn.....1 Respiratory.....2 Skin rash.....3 Gastro-intestinal.4 unspecified/other illness.....5 Not because of illness.....6 (GO TO Q.9)	1 2 3 4 5 6 (GO TO Q.9)	1 2 3 4 5 6 (GO TO Q.9)	1 2 3 4 5 6 (GO TO Q.9)	1 2 3 4 5 6 (GO TO Q.9)	1 2 3 4 5 6 (GO TO Q.9)	1 2 3 4 5 6 (GO TO Q.9)
C. If a child, PROBE: Does (he/she) usually go into the water?	/24 YES.....1 NO.....2	1 2	1 2	1 2	1 2	1 2	1 2

IF INITIATED INTERVIEW IS TERMINATED, GIVE REASON:

Group # ___ ___ ___ ___ ___		P_1	P_2	P_3	P_4	P_5	P_6
6. Could you please tell me if (PERSON) was in the water around 1 or before: around 3; around5 or after?	CODE AS MANY AS APPLY: (25) Around 1 or before... .... 1 Around 3 ..... 2 Around 5 or after ..... 4 ..... –	1 2 4 ___	1 2 4 ___	1 2 4 ___	1 2 4 ___	1 2 4 ___	1 2 4 ___
7. How long altogether was (PERSON) in the water? We are only interested in the time actually in the water, not the total time at the beach.	Minutes (26) Less than 10 .. 1 10 – 29 ..... 2 30 – 59 ..... 3 60 or more 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4
8. FOR EVERYONE During the past week did (PERSON) have any of the following symptoms: ASK EACH SYMPTOM GROUP	Sunburn	Y N	Y N	Y N	Y N	Y N	Y N
	Skin rash	Y N	Y N	Y N	Y N	Y N	Y N
	Backache, headache, fever (27)	Y N	Y N	Y N	Y N	Y N	Y N
	Sore throat, Cough, runny nose (28)	Y N	Y N	Y N	Y N	Y N	Y N
	Vomiting, nausea diarrhea (29)	Y N	Y N	Y N	Y N	Y N	Y N
	Wheezing, or asthma-like attack	Y N	Y N	Y N	Y N	Y N	Y N
	Any other symptoms?	Y N	Y N	Y N	Y N	Y N	Y N
9. FOR EVERYONE APPEARANCE AT TIME OF INTERVIEW (BY OBSERVATION)	Head and suit wet ..1 Suit wet....3 Head wet....2 Neither wet.4 Not present.5	1 3 2 4 5	1 3 2 4 5	1 3 2 4 5	1 3 2 4 5	1 3 2 4 5	1 3 2 4 5
10. ANSWERED Q.'S 5, 6, 7, 8, FOR SELF	YES ...Y NO ..N	Y N	Y N	Y N	Y N	Y N	Y N

IF INITIATED INTERVIEW IS TERMINATED, GIVE REASON:

Group # ____ ____ ____ ____ ____

11. We are interested in whether or not anyone who is at the beach with you today gets any eye, ear, nose, throat, skin, or stomach trouble in the near future. Could you please give me your complete name, address and telephone number so we can get in touch with you next week? IF NO PHONE: Is there any phone number where you can be reached - at a neighbor's or friend's phone, or at work? IF RELUCTANT TO GIVE PHONE: Could we reach you at work or at some other phone?

PRINT FULL NAME AND ADDRESS

Name_____ Address (Street and No.) _____

City, State, Zip _____

Home Phone_____ Other Phone_____ What is the best time to call?_____

Other Persons' Names at this address _____, _____, _____, _____.

FOR EACH PERSON ON PAGE 2 NOT LISTED ABOVE, ASK:

11A. What is (PERSON'S) address and phone number?

IF (PERSON) IS UNDER 16, AND NO RELATED ADULT IS LISTED, GET NAME, ADDRESS AND PHONE NUMBER OF A RESPONSIBLE ADULT AND SPECIFY RELATIONSHIP NEXT TO NAME.

NAME	ADDRESS	PHONE NUMBER	BEST TIME TO CALL?

We appreciate your help very much. Thank you again.

---

12. HOW COOPERATIVE WAS THIS FAMILY/GROUP? VERY___(1) SOMEWHAT___(2) LITTLE___(3) OTHER___(4)

OTHER - EXPLAIN:_____

_____
Interviewer's Name

GROUP # _____

SITE/ID _____

Telephone Follow-up

PERSONS #'s ___,___,___,___; PHONE_____

PERSONS #'s ___,___,___,___; PHONE _____

Hello, is this (CONTACT PERSON)? (May I speak to (CONTACT PERSON))? This is_____.
I'm working with the Oklahoma University Beach Study. About a week ago one of our interviewers spoke to you at _____Beach. Do you recall?

GROUP # _____

		P1	P2	P3	P4	P5	P6
NAME:							
First, have you, _____, _____, _____, or _____ gone swimming or in the water anywhere -- at _____ Beach or any other beach, pool or lake - since the weekend we talked to you at the Beach?							
	YES (GO TO SECTION A) /30 NO (GO TO SECTION B, P.3)	YES 1 No 2	YES 1 NO 2	YES 1 NO 2	YES 1 NO 2	YES 1 NO 2	YES 1 NO 2
SECTION A							
IF YES							
Who was that? CIRCLE EACH NAME GIVEN. Probe: Anyone else? CIRCLE NAMES							
FOR EACH NAME CIRCLED ASK:	/31						
1. DID (PERSON) acutally swim or get his/her face wet?	YES...GO TO Q.2 NO....GO TO Q.1 FOR NEXT NAME CIRCLED	1 2	1 2	1 2	1 2	1 2	1 2
IF YES TO Q.1 ABOVE:	/32						
2. What days did they do that?	1. Sun. 2. Mon.-Fri. 3. Sat. or later	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3
3. Where did (PERSON) go into the water?	/33 CIRCLE ANY NAMED:						
	1. =Salt Creek North 2. =Keystone Ramp 3. =Washington Irving So. 4. =Other	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4

Group # ___ ___ ___ ___ ___

SECTION B.

Now I would like to ask some questions about the people who were with you at the beach on the day of the interview who didn't swim at another beach during the following week.

That is, _____, _____, and _____. READ THE NAMES YOU HAVE NOT CROSSED OFF BELOW.

1. Let's start with (PERSON). HAS (PERSON) had any of these symptoms since you were at the beach on _____ (day), _____ (date)? ASK EACH SYMPTOM

IF "YES" TO ANY OF THE FOLLOWING SYMPTOMS ASK:

What day did that start?

PROBE: FIX EXACT DATE. WRITE DATE IN BOX UNDER THE PERSON AND BY THE SYMPTOM

		NAME:	P1	Onset	P2	Onset	P3	Onset	P4	Onset	P5	Onset	P6	Onset
A.	GASTROINTESTINAL 1. Stomach ache	/34/35	Y N		Y N		Y N		Y N		Y N		Y N	
	2. Diarrhea or loose bowels	/36/37	Y N		Y N		Y N		Y N		Y N		Y N	
	3. Nausea or feeling nauseous	/38/39	Y N		Y N		Y N		Y N		Y N		Y N	
	4. Throwing up or vomiting	/40/41	Y N		Y N		Y N		Y N		Y N		Y N	
B.	RESPIRATORY 1. Sore throat	/42/43	Y N		Y N		Y N		Y N		Y N		Y N	
	2. Bad cough	/44/45	Y N		Y N		Y N		Y N		Y N		Y N	
	3. Chest cold	/46/47	Y N		Y N		Y N		Y N		Y N		Y N	
C	"OTHER" 1. Fever (temperature) over 100 degrees	/48/49	Y N		Y N		Y N		Y N		Y N		Y N	
	2. Headache lasting more than a few hours	/50	Y N		Y N		Y N		Y N		Y N		Y N	
	3. Backache	/51	Y N		Y N		Y N		Y N		Y N		Y N	

Group # _____

		P_1	Onset	P_2	Onset	P_3	Onset	P_4	Onset	P_5	Onset	P_6	Onset
	NAME:												
D.	EYE, EAR, NOSE	/52											
	1. Runny or stuffed nose	Y N		Y N		Y N		Y N		Y N		Y N	
	2. Earache or runny ears	/53 Y N		Y N		Y N		Y N		Y N		Y N	
	3. Red, itchy, or watery eyes for more than one day, or sties	/54 Y N		Y N		Y N		Y N		Y N		Y N	
E.	ALLERGENIC	/55											
	1. Skin rash, itching skin, or welts	Y N		Y N		Y N		Y N		Y N		Y N	
	2. Sneezing, wheezing, tightness in the chest breathlessness for more than a few minutes	/56 Y N		Y N		Y N		Y N		Y N		Y N	
F.	SUNBURN which bothered (you/him/her)	Y N		Y N		Y N		Y N		Y N		Y N	
2.	TALLY TOTAL NUMBER OF SYMPTOMS (IF NO SYMPTOMS, GO TO SECTION B, Q 1A (PAGE 3) FOR NEXT PERSON)												
	IF ANY DATE IS GIVEN, ASK Q.'S 3, 4, and 5:	/57											
3.	Did (PERSON) stay home because of (SYMPTOMS)?	Y(1) N		Y (1) N		Y(1)N		Y(1)N		Y(1) N		Y(1)N	
4.	Did (PERSON) stay in bed?	Y(2) N		Y (2) N		Y(2)N		Y(2)N		Y(2) N		Y(2)N	
5.	Did (PERSON) consult anyone for medical help ?	Y(3) N		Y (3) N		Y(3)N		Y(3)N		Y(3) N		Y(3)N	

GO BACK TO Q. 1A (PAGE 3) FOR EACH ELIGIBLE PERSON BEFORE CONTINUING TO PAGE 5.

GROUP #_____

		P_1	P_2	P_3	P_4	P_5	P_6
WHEN SYMPTOMS HAVE BEEN ASKED FOR ALL PERSONS, ASK ONE ADULT Q.6, AND Q.7:	NAME:						
1. Could you please tell me how many people including yourself, live in your home (house or apartment)? IF NECESSARY - Your answer will be completely confidential.	/58-59						
2. And how many rooms do you have at home, not including the kitchen or bathroom(s)?	/60-61						
3. WHO WAS THE RESPONDENT? S=SELF; O=OTHER PERSON		S O	S O	S O	S O	S O	S O
4. HOW COOPERATIVE WAS RESPONDENT? 1=VERY, 2=MODERATELY, 3=LITTLE		1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3

We appreciate your help very much. The information you gave will help us in our study to improve swimming conditions at public beaches.

DATE OF COMPLETION: _____

TIME OF COMPLETION: _____

GROUP # _____

REASON FOR NONCOMPLETION:

	P_1	P_2	P_3	P_4	P_5	P_6
NO ANSWER	0	0	0	0	0	0
NOT HOME	1	1	1	1	1	1
WRONG NO. PROVIDED	2	2	2	2	2	2
REFUSED WHEN CALLED	3	3	3	3	3	3
DISCONNECTED/OOS.	4	4	4	4	4	4
NO TELEPHONE	5	5	5	5	5	5
MOVED	6	6	6	6	6	6
UNLISTED NO.	7	7	7	7	7	7
OUT OF STATE	8	8	8	8	8	8
REFUSED INFO. AT BEACH	9	9	9	9	9	9

INTERVIEWER: _____

APPENDIX C

PROCEDURES FOR LABORATORY TESTING

GENERAL PROCEDURES FOR LABORATORY TESTING USING MEMBRANE FILTERS (62)

For all tests during which an indicator organism produces a known colony type on a known medium, count only those typical colonies. In the majority of situations, workers may feel sure that indicator organism counts per 100 ml based on counts of typical colonies are accurate provided all other test requirements are fulfilled.

Be sure to take into account the presence of dissolved solids and other foreign substances in the sample. Typical colonies may be produced by the indicator bacteria, but distortions due to physical or chemical interference may make their recognition somewhat more difficult.

For all indicator tests, it is assumed that one organism trapped on the surface of the membrane filter (MF) produces one colony. Although the colonial behavior of bacteria can vary from group to group and environment to environment, it is assumed that at least one and (for counting purposes) only one organism generates one colony on the MF. If the assumption were otherwise, a workable count would be very difficult to obtain. When dealing with the particular groups of bacteria used in sanitary water testing - i.e., gram negative rods and gram positive cocci which are harmless commensals of the gut or skin - their behavior is such that "one organism makes one colony" is a reasonable assumption. This provides a built-in safety margin such that minimal data will yield maximum information about the condition of the water being tested.

The Basic Equation:

The above assumption allows the formulation of the basic equation for indicator organism counts per 100 ml of source water, as follows:

The no. of indicator organisms per 100 ml =

$$100 \times \frac{\text{no. of indicator colonies counted}}{\text{no. ml of sample filtered}}$$

a simple rearrangement of this equation gives the equation for determining the proper Sample Filtration Volume (SFV) quantity. Within the basic equation, the number of indicator organism colonies counted by the worker is divided by the number of milliliters of sample in the SFV quantity. The result is the number of colonies per one milliliter of sample. Since it is assumed that one organism produces one colony on the MF, this result might also be interpreted as the number of indicator organisms per one milliliter of sample. According to current practice developed from nearly 100 years of research, results are best expressed as the number of indicator organisms per one hundred milliliters. The result obtained from the above division (i.e., the number of organisms per 1 ml) is multiplied by 100 to produce the required expression for the indicator organism concentration in the source water.

When incubation is complete, follow these steps:

1. Remove the fully incubated cultures from the incubator, keep them in the inverted position and take them to the microscope area on the bench.

2. Open all cultures in the inverted position with the butt-end of the forceps.

3. Place the petri dish half containing the MF culture on the stage of the stereomicroscope.

4. Adjust magnification to 10X.

5. Position the fluorescent illuminator so that the light falls as nearly vertically on the culture as is possible.

6. Examine the entire surface of the MF for the presence of indicator colonies, the shapes and sizes of indicator colonies, the estimated number of indicator colonies, and the over-all number of indicator colonies.

7. If a series of cultures from the same sample are examined, determine after examination of the entire series which culture or cultures shall be used to furnish a colony count for the sample.

Counting Colonies on Acceptable Cultures

After the preliminary examination is completed satisfactorily, (this amounts to a "screening" procedure) a colony count is taken as follows:

1. Stage the culture under fluorescent illumination as above. The petri dish half may retain the culture; or the entire MF and pad combination may be transferred very carefully into the bowl of a petri dish or onto the surface of a 2" x 3" slide.

2. Proceed with indicator organism colony counting by adhering to the description of a typical indicator colony and any atypical growth descriptions given.

3. Proceed from top to bottom and left to right, counting the organisms in each square.

4. Use the grid system on the MF surface to locate colonies along the counting path.

5. Record on the data sheet the finished count for each acceptable MF culture.

The Indicator Organism Count

To obtain the indicator organism count per 100 ml of sample, two values are needed. These are the MF culture colony count, and the value of the corresponding SFV quantity. This data should be collected as follows. When a particular MF culture is chosen as acceptable for colony counting, the SFV quantity should be immediately available from the incubation label on the petri dish. If the SFV quantity is not listed on the label (for lack of space or out of laboratory preference), it should be listed according to a petri dish label on the test data sheet. Once an MF culture is chosen for colony counting, however, the corresponding SFV quantity should appear on the test data sheet as identification for the MF culture. The colony count is then taken, and that quantity is recorded with the SFV quantity.

The two values are plugged into the basic equation, which as given previously is:

$$\text{the no. of indicator organisms per 100 ml} = 100 \times \frac{\text{MF colony count}}{\text{SFV quantity}}$$

Since the values needed as "known values" are easily obtainable (as indicated above) and the basic equation is a matter of simple division and multiplication, the determination of the indicator organism count per 100 ml of sample is a straight-forward procedure.

Typical Steps

1. Choose an MF culture suitable for colony counting.
2. Record the SFV quantity associated with MF culture.
3. Take the indicator organism colony count as instructed.
4. Use the two values obtained in steps (2) and (3) and construct the basic equation as discussed above.
5. Perform the necessary division and multiplication to obtain the indicator organism count per 100 ml of sample.

Example - Optimum Conditions

Suppose that the SFV quantity associated with a particular MF culture is 10 ml. Suppose that after colony counting, this MF culture yielded 50 colonies. According to the above discussion, construct the basic equation as follows:

$$\text{indicator count per 100 ml} = 100 \times \frac{50 \text{ colonies}}{10 \text{ ml (SFV)}}$$

The division of the number of colonies (50) by the SFV quantity (10) produces:

$$\text{indicator count per 100 ml} = 100 \times 5$$

Since the basic assumption states that one colony equals one organism (see discussion), the number 5 above might be read, "5 organisms per 1 ml (SFV)." Multiply this quantity times 100 to obtain the indicator count per 100 ml.

$$\text{indicator count per 100 ml} = 500 \text{ organisms}$$

Good for All Acceptable MF Cultures

Use the above equation and the values discussed as a typical procedure for any MF culture yielding a countable number of colonies on the surface of the membrane. Most of a worker's MF culture examinations will be of indicator organism colonies. Thus, while new MF procedures continue to emerge from research, the steps involving MF colony counting and indicator organism calculations per 100 ml will remain essentially the same for all MF tests.

Reporting Counts Under Unacceptable Conditions

Occasionally, a worker will be stuck without any MF culture whose colonies are within the acceptable boundaries for the organism cultured. Standard Methods recommends that a worker should test a new SFV quantity or a new series of quantities to obtain a countable culture. In the meantime, the report on the unacceptable MF cultures should read as follows:

> When colonies are too numerous to the point of being uncountable, the report should read: TNTC = Too Numerous To Count.
>
> When the colony count is obtainable but above the recommended upper limit perform the count and basic calculation, and record: Estimated Count "XX" organisms per 100 ml from a non-ideal colony count.
>
> When colonies are too scarce to the point of being absent under the stereomicroscope, assume that a larger SFV might have yielded a count. Perform the basic calculation and record: "Less than 1 organism per 100 ml."
>
> When the colony count is obtainable but below the lower limit, perform the count and basic calculation for the MF showing the highest count and record: Estimated count "XX" organisms per 100 ml from a non-ideal colony count.

Special Situations

Certain situations will demand special consideration from the workers. The following examples include general cases whose only major relation to each other lies in their being out of the ordinary.

Standard Counts

Usually when a worker reports a Standard Count, the count is to be recorded no matter what occurs on the MF. If colonies are absent, the count is "less than 1 organism per 100 ml." If colonies are present but the colony count is less than 20, the organism count is recorded as for an ideal number of colonies. The idea in Standard Counts is not to exceed the standard in any one sample. Thus, anything passes for a colony count and is reported as such.

Composite Counts

When a series of SFV quantities are tested, the results may vary as drastically as "TNTC" all the way down to no visible indicator colonies at all in extreme cases. If careful choosing of SFV quantities produces results which are ideal and fairly close together, however, it is often helpful to combine values to produce one total for the colony count and one total for the SFV quantity before calculating with the basic equation.

This is called compositing the values, and it is especially useful when two or more cultures are considered whose colony count values fall within the acceptable limits set for the various indicator organism tests. When reporting results, perform the basic calculation as follows:

$$\text{indicator count per 100 ml} = 100 \times \frac{\text{total no. of colonies counted}}{\text{total of the SFV quantities}}$$

Add up the number of colonies for the cultures being composited. Add the corresponding SFV quantities to obtain the total amount of sample filtered. Substitute these totals in the basic equation as given above.

I. M-Endo MF Broth

1. Using a spatula or scoop, weigh out 4.8 grams of dehydrated medium into a weighing dish placed on the laboratory balance

2. Pour out 100 ml (0.1 liter) of distilled water into a clean 100 ml graduated cylinder.

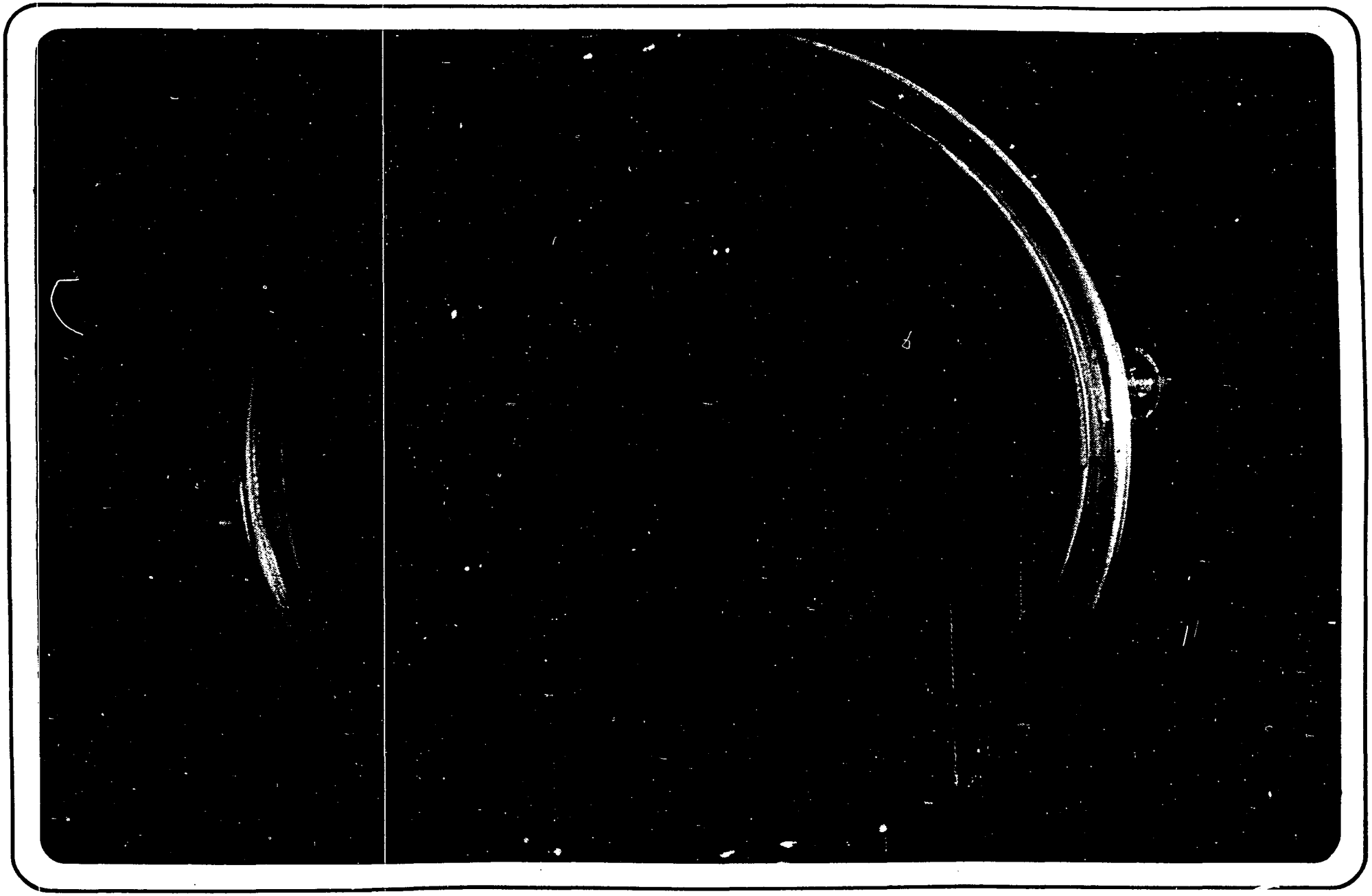
3. Add 2 ml of 95% ethyl alchohol to the distilled water in the graduated cylinder. (Do not pipette directly from the reagent bottle. Pour a portion in a beaker first.)

4. Pour out approximately 20 ml of solution from a graduated cylinder into a clean 250 ml screw-cap Erlenmeyer flask without spilling.

5. Empty the contents of the weighing dish carefully into the prepared 250 ml Erlenmeyer flask and swirl to disperse the dehydrated medium.

6. Pour the remaining contents of the graduated cylinder into the 250 ml Erlenmeyer flask without spilling.

Figure C-1. Total Coliform on Membrane Filter (4X).



7. Place the flask loosely covered, in a boiling water bath (or in the make-shift beaker and hot-plate water bath).

8. Parboil the medium for 3-5 minutes.

9. Remove and cool to 45° C. Adjust the pH to between 7.1 and 7.3.

10. Left-over medium may be refrigerated at 2-10° C for 96 hours maximum and then discarded. It is best, however, to use up all fresh media each day.

II.

Procedure: Follow the basic MF procedure using sample dilutions that will yield approximately 50 coliforms but no more than 200 colonies of all types. Size of the sample is dependent on expected bacterial density and may vary in potable waters from 100 to 500 ml or more.

Incubation: Incubate inverted broth pad or agar cultures 22-24 hours at 35°C ± 0.5°C with approximately 90% relative humidity.

Counting: Count typical pink to dark red colonies with a golden green metallic sheen on a filter which has a colony range of 20-80 coliforms, with a total colony count of no more than 200.

Interpretation: Coliforms in water are indicators of possible fecal contamination and may indicate the presence of pathogenic enteric bacteria, enteric viruses, and protozoa.

III. M-FC Broth

1. Using a spatula or scoop, weigh out 3.7 grams of dehydrated medium into a weighing dish on the laboratory balance.

2. Pour out 100 ml (0.1 liter) of distilled water into a clean 100 ml graduated cylinder.

3. Pour out approximately 20 ml of the distilled water from the graduated cylinder into a clean 250 ml screw-cap Erlenmeyer flask without spilling.

4. Empty the contents of the weighing dish carefully into the prepared 250 ml Erlenmeyer flask and swirl to disperse the dehydrated medium.

5. Pour the remaining contents of the graduated cylinder into the 250 ml Erlenmeyer flask without spilling.

6. Obtain dehydrated rosolic acid from the reagent shelf.

7. Weigh out 1 gram of dehydrated rosolic acid on the laboratory balance according to the weighing procedure above.

8. Measure out 100 ml of 0.2 N sodium hydroxide solution into a clean 100 ml graduated cylinder.

9. Pour out approximately 20 ml of sodium hydroxide from the graduated cylinder into a second clean 250 ml screw-cap Erlenmeyer flask without spilling.

10. Carefully empty the contents of the weighing dish (dehydrated rosolic acid) into the second prepared 250 ml Erlenmeyer flask and swirl to disperse the dehydrated medium.

11. Pour the remaining contents of the graduated cylinder into the second 250 ml Erlenmeyer flask without spilling. This produces a 1% rosolic acid solution.

12. Pipette out 1 ml of 1% rosolic acid solution.

13. Dispense 1 ml into the flask containing the dissolved M-FC broth.

14. Place the flask, loosely covered, in a boiling water bath.

15. Heat the medium to the boiling point, then remove and cool.

16. Dispense at room temperature. pH should be 7.4.

17. Store unused portion at 2-10°C and discard after 96 hours.

IV.

Procedure: Follow the basic MF procedure using sample dilutions that will yield a colony range of 20-60 fecal coliforms per membrane filter.

Incubation: Place the prepared cultures in waterproof plastic bags. With cultures inverted and submerged, incubate for 22 ± 2 hours in a circulating water bath at 44.5°C ± 0.2°C. (Start incubation within 30 minutes after filtration to discourage growth of non-fecal coliforms.)

Counting: Count blue colonies on a filter with a colony range of 20-60 fecal coliforms. Non-fecal coliforms are grey to cream in color.

V. KF Streptococcus Agar

1. Using a spatula or scoop, weigh out 7.64 grams of dehydrated medium into a weighing dish placed upon the laboratory balance.

2. Pour out 100 ml (0.1) liter of distilled water into a clean 100 ml graduated cylinder.

3. Pour out approximately 20 ml or distilled water from the graduated cylinder into a clean 250 ml screw-cap Erlenmeyer flask without spilling.

4. Empty the contents of the weighing dish carefully into the prepared 250 ml Erlenmeyer flask and swirl to disperse the dehydrated medium.

5. Pour the remaining contents of the graduated cylinder into the 250 ml Erlenmeyer flask without spilling.

6. Place the flask, loosely covered, in a boiling water bath.

7. Heat until the medium appears completely dissolved; then heat 5 minutes more. Do not boil this medium.

8. Remove and cool to 50-60°C.

9. Add 1 ml of pure aqueous 1% solution of 2, 3, 5-triphenyltetrazolium chloride (TTC).

10. Adjust the pH to 7.2 with 10% sodium carbonate (Na_2CO_3) if necessary.

11. Liquid agar may be held in a water bath up to 4 hours at 45-50°C before pouring plates.

12. Poured plates may be stored in the dark at 2-10°C for up to 2 weeks.

VI.

Procedure:	Follow the basic MF procedure using sample dilutions yielding a colony range of 20-100 colonies on the membrane filter surface. Sample size may vary from 100 to 10, 1, 0.1, or 0.01 ml, depending on the bacterial density of the sample.
Incubation:	Invert culture plates and incubate at 35°C ± 0.5°C for 48 hours.
Counting:	Count dark red to pink colonies on a filter which has a colony range of 20-100 colonies.

VII. Preparation of Sterile Phosphate Buffer Water

1. Dissolve 34.0 grams of potassium dihydrogen phosphate, KH_2PO_4, in a clean 1000 ml beaker filled with 500 ml of distilled water.

2. Adjust the pH to 7.2 with 1N NaOH (available commercially).

3. Dilute to 1000 ml (1 liter) with distilled water to produce stock buffer solution.

4. Pour the contents of the beaker into a clean Fenwall bottle and label it Stock Buffer Solution.

5. Place the stopper on the Fenwall bottle and autoclave it for 15 minutes at 121°C and 15 psi so that the level of contamination in the stock buffer solution will remain at a minimum

6. Allow the stock buffer solution to cool before dispensing it.

7. Pour out 1 liter portions of distilled water into clean Fenwall bottles, as many as needed.

8. 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.

9. Replace the stoppers on the Fenwall bottles and autoclave them for 15 minutes at 121°C and 15 psi.

Properly autoclaved bottles produce a "pop" when they are opened for use.

10. Label each bottle PHOSPHATE BUFFER WATER and store on the shelf until needed.

11. Store the Stock Buffer Solution at 2-10°C or on a cool, dark shelf. Check the pH before each use to make sure it is 7.2.

Preparation of Sterile Dilution Blanks

1. Obtain either clean standard milk dilution bottles or clean screw-cap 15 x 150 mm culture tubes (see page 16).

2. 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.

3. Autoclave the dilution water containers, loosely capped, at 121°C for 15 minutes at 15 psi.

4. After autoclaving, the amounts of water present in each bottle should be 99 ml ± 2.0 ml, and the amount of water in each tube should be 9 ml ± 0.2 ml at room temperature. Workers may experiment with various preautoclaved 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.

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

METHOD: Membrane Filter Technique for Thermotolerant *E. coli* (m-TEC) (75).

PROCEDURE:

The medium formulated for the enumeration of thermotolerant *E. coli* (m-TEC) contains ingredients common to a number of coliform media. It has the following composition: Proteose peptone #3, 5.0 g; yeast extract, 3.0 g; lactose, 10.0 g; NaCl, 7.5 g; K_2HPO_4, 3.3 g; KH_2PO_4, 1 g; sodium lauryl sulfate, 0.2 g; sodium desoxycholate, 0.1 g; brom cresol purple, 80 mg; phenol red, 80 mg; agar, 15 g; distilled water to one liter. The ingredients are dissolved by stirring sterilized by autoclaving at 121°C for 15 min., and poured in 10 x 47 mm plates (4 ml per plate).

a) Filter appropriate volumes of the water sample through a sterile membrane so that 20-80 colonies will result.

b) Place the membranes on the agar surface taking care to avoid trapping air bubbles on the underside.

c) Place plates in whirlpack bag (single layer). Invert and place in stainless steel rack. Incubate for 2 hrs. at 35°C, then transfer rack/plates to a 44.5°C water bath for 20-22 hours.

d) Mark all yellow colonies that are $\geq$ 1 mm in diameter.

e) Flood a sterile filter pad with urease reagent (see Note B). Carefully remove filter and place on the saturated pad. After 20 minutes count all marked colonies that remain yellow (thermotolerant *E. coli*).

NOTE: a) Store m-TEC plates at 4°C in the dark.

b) If the urease reagent turns red or orange readjust the pH by pouring it into a beaker with a stirring bar, and, using an applicator stick dipped into concentrated HCl, watch for the reagent to turn yellow again. The reagent should turn instantly. Store reagent in the brown bottle provided at 4°C.

c) Test for the production of urease:

Peptone	1 gm
Sodium chloride	5 gm
Glucose	1 gm
Monobasic potassium phosphate	2 gm
Phenol red	0.012 gm (6ml of 1:500 solution)
Urea	20 gm
Distilled water	100 ml

Adjust to pH 6.8 to 6.9. Filter sterilize.

$$O = C(NH_2)_2 + 2H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2 + H_2O$$

Urea - pH 6.8 Ammonia - pH 8.1

FILTRATION

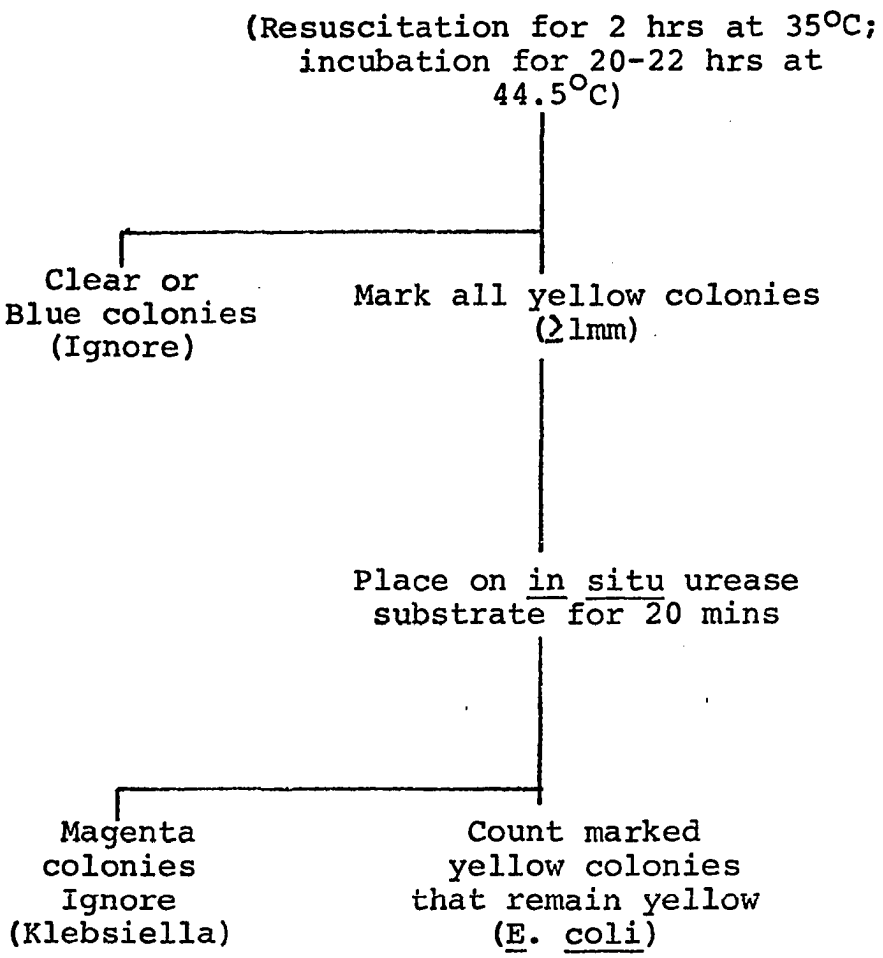
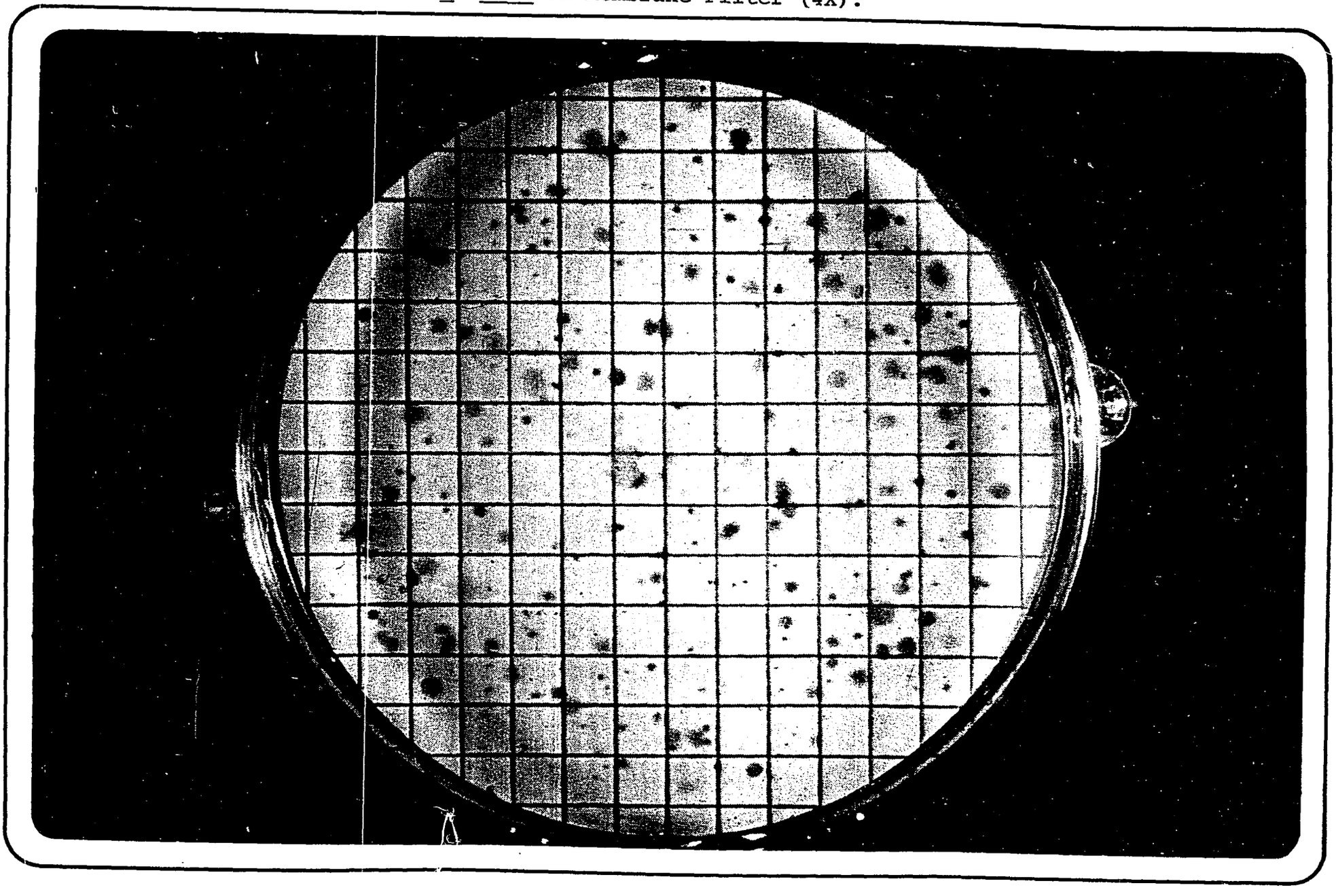

(Resuscitation for 2 hrs at 35°C; incubation for 20-22 hrs at 44.5°C)
Clear or Blue colonies (Ignore)
Mark all yellow colonies (≥1mm)
Place on in situ urease substrate for 20 mins
Magenta colonies Ignore (Klebsiella)
Count marked yellow colonies that remain yellow (E. coli)

Figure C-2. Thermotolerant E. coli on Membrane Filter (4X).



METHOD: Membrane Filter Technique for Group D Streptococci (m-E) (46).

INTRODUCTION: This is not a "standard method" in the sense that is has not been evaluated by several laboratories. However, the accuracy, precision, selectivity, and sensitivity of the method have been evaluated and found satisfactory with marine waters collected in the northeast. It is being used routinely with samples collected from New York City recreational waters. This procedure selects for and quantitates Group D Streptococci in marine waters. It can be used with highly polluted samples with a high degree of accuracy. It was developed to overcome the inaccuracy and lack of sensitivity observed when existing techniques were applied to marine samples.

SAMPLES: Collect and hold as described in Section 405 of Standard Methods for the Examination of Water and Wastewater.

PREPARATION: The preparation of sampling bottles, samples, and equipment is described in "Standard Methods" (Sections 405 and 408).

PROCEDURE:

a) Filter appropriate volumes of the water sample through a sterile membrane so that 20-80 colonies will result.

b) Place the membranes on the agar surfaces of the m-E plates, taking care to avoid trapping air bubbles on the underside.

c) Incubate for 48 hours at 41°C.

d) The filters are then transferred to EIA agar plates (which have been allowed to reach room temperature) and allowed to stand for 20-30 minutes. Red or pink colonies which form a black or reddish-brown precipitate in the EIA medium are counted as Group D Streptococci.

CALCULATIONS: Results are presented as organisms per 100 ml of water.

MATERIALS:

m-E Medium

Ingredients	g/l
Agar	15.0
Peptone	20.0
NaCl	15.0
Esculin	1.0
Yeast extract	30.0
Actidione	.050
Sodium azide	.150
Distilled water	1000 ml

Autoclave 15 lbs/15 minutes. Add, after autoclaving Nalidixic Acid, .240 gm; Triphenyl tetrazolium chloride; .15 gm and adjust pH to 7.1 ± 0.1.

EIA Medium

Ingredients	g/l
Agar	15.0
Esculin	1.0
Ferric citrate	0.5
Distilled water	1000 ml

pH 7.1 ± .1 before autoclaving.

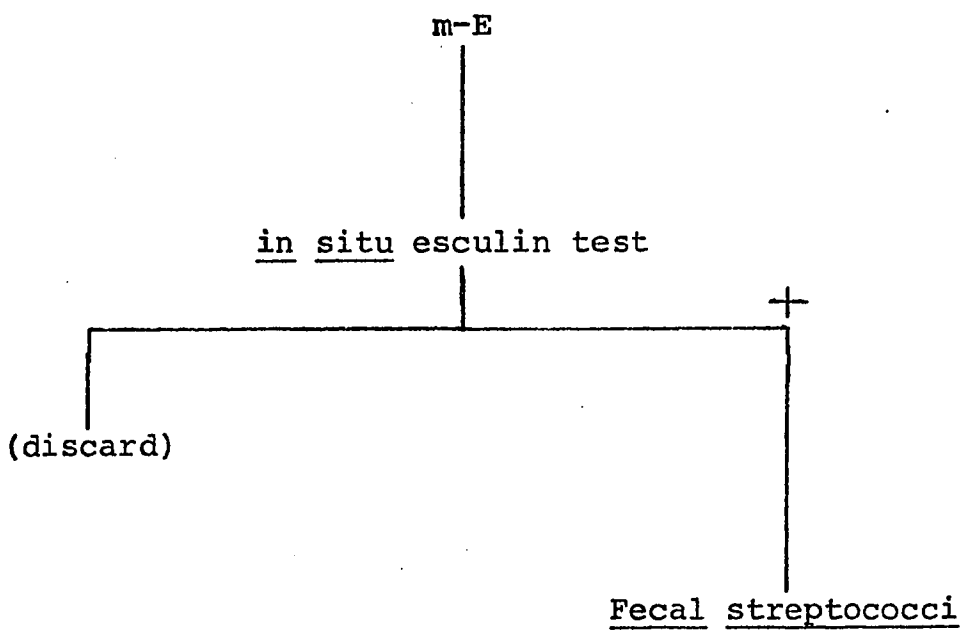
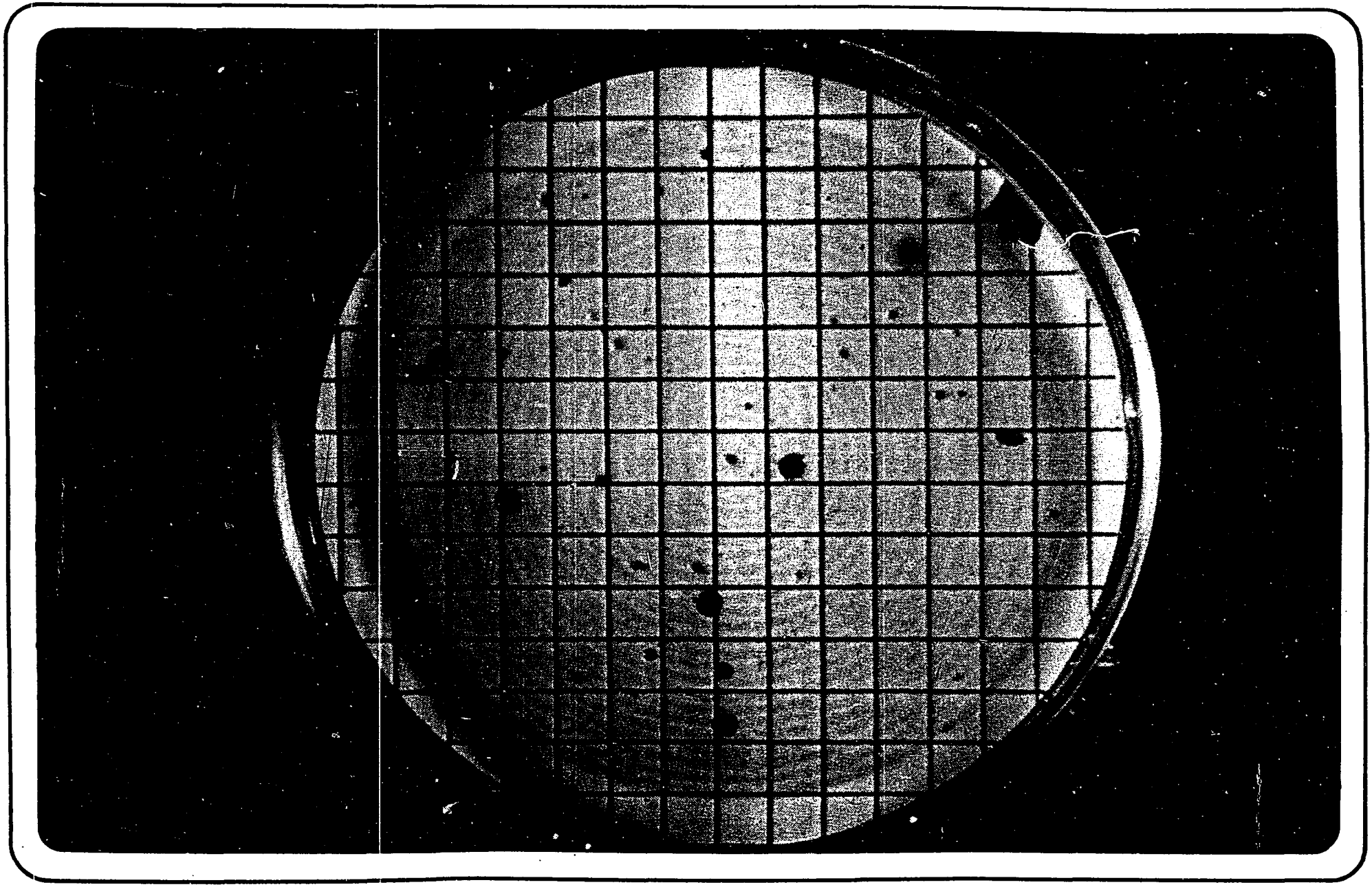

m-E
in situ esculin test
+
(discard)
Fecal streptococci

Figure C-3. Group D Streptococci on Membrane Filter (4X).



METHOD: Membrane Filter Procedure for Aeromonas hydrophila (m-A) (73,77,78).

INTRODUCTION: This is not a "standard method" in the sense that it has not been evaluated by several laboratories. However, the performance characteristics of the method have been determined and found satisfactory with fresh waters collected throughout the United States. A. hydrophila is a human, as well as a fish and reptile, pathogen. It is considered a resident, aquatic bacterium in fresh waters and presumably multiplies therein under appropriate conditions. A. hydrophila is seasonably distributed with maximal densities occurring in the water column from summer through early fall. The densities of the organism found in fresh waters are a reflection of their trophic states and are indicative of nutrient pollution.

SAMPLES: Collect and hold as described in Section 405 of Standard Methods for the Examination of Water and Wastewater.

PREPARATION: Sample container, membrane filter apparatus and other equipment are prepared as described in Sections 405 and 408 of "Standard Methods."

a) Filter appropriate volumes of the water sample through a sterile membrane so that 20-80 colonies will result.

b) Place the membranes on the agar surfaces of the m-A plates, taking care to avoid trapping air bubbles on the underside.

c) Follow the flow scheme in figure 1 to complete identification.

INTERPRETATIONS: A. hydrophila ferments trehalose and mannitol and hence produces a yellow colony on m-A medium due to the color change of the brom thymol blue indicator. Neither A. salmonicida nor A. shigelloides will grow on m-A. The inhibitors notwithstanding, some colofirms will "break through," as do pseudomonads. The former are oxidase negative; and the latter generally are small colonies, which on oxidase testing, are colored purple throughout the colony. For further confirmatory procedures, see Ewing, et al.

MATERIALS: See Section 408 A of "Standard Methods."

Aeromonas hydrophila Medium (m-A)

Ingredients	gm/100 ml
Tryptose	0.5
Trehalose	0.5
Yeast Extract	0.2
NaCl	0.3
KCl	0.2
$MgSO_4 \cdot 7H_2O$	0.02
$FeCl_3 \cdot 6H_2O$	0.01
Brom thymol blue	0.004
Distilled water	100 ml

Dissolve at room temperature, adjust the pH to 8.0 with 1N NaOH, add 1.5 g agar and autoclave at 121°C for 15 minutes. Immediately after autoclaving, add 1.0 ml ethanol; cool the mixture to 50°C; add 2 mg Ampicillin and 10 mg sodium desoxycholate; dispense the medium into sterile petri plates at 5 ml/plate.

It is recommended that the plates be stored in the dark at 4°C and used within 6 weeks.

In situ Mannitol Medium

Ingredients	gm/100 ml
Tryptose	0.5
Mannitol	0.5
Yeast Extract	0.2
NaCl	0.3
KCl	0.2
$MgSO_4 \cdot 7H_2O$	0.02
$FeCl_3 \cdot 6H_2O$	0.01
Brom thymol blue	0.008
Distilled water	100 ml

The ingredients are dissolved at room temperature, the pH is adjusted to 8.0 with 1N NaOH, 1.5 g agar is added; the medium is autoclaved at 121°C for 15 minutes. After cooling to 50°C, 100 mg of sodium desoxycholate are added and the medium is dispensed into petri dishes (50x12 mm) at 5 ml/plate.

The plates of medium can be stored for 2 months at 4°C.

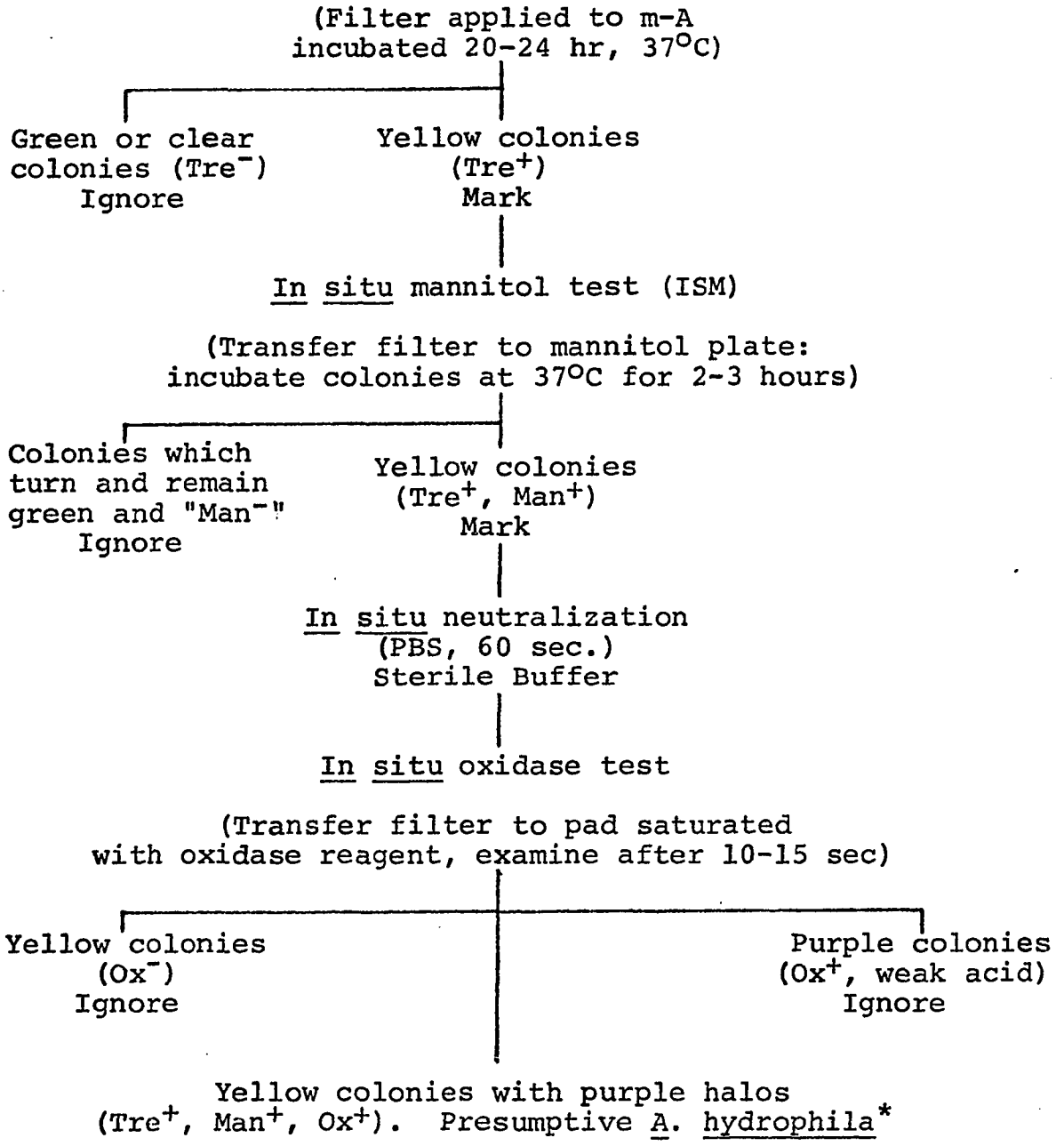
In situ Oxidase Medium

Ingredients

N-N-N'-N' - tetramethyl-para-phenylenediamine dihydrochloride	100 mg
Deionized water	10 ml

Prepare fresh in small quantities when needed.

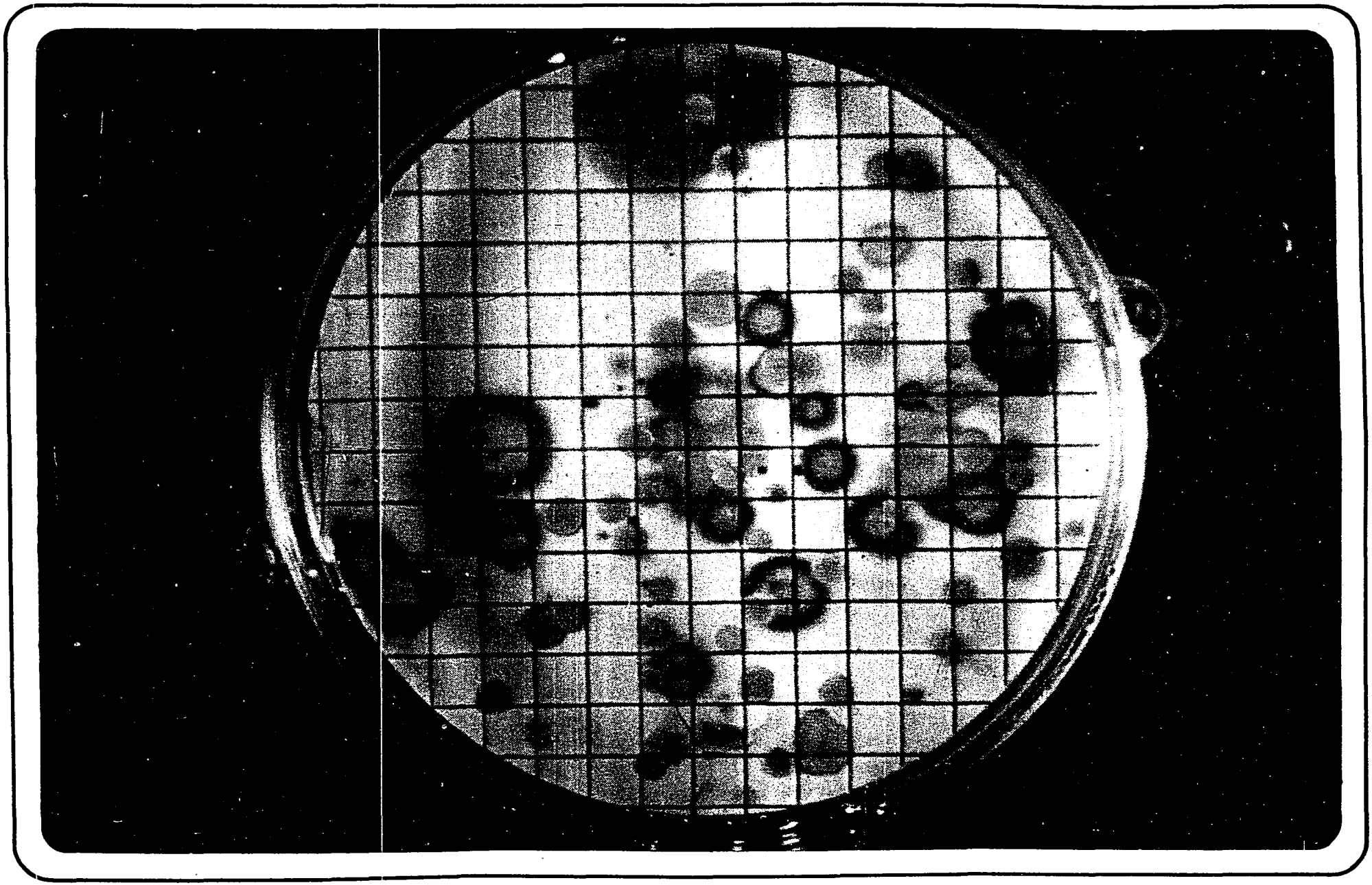
FILTRATION



Tre-trehalose; Man-mannitol; Ox-oxidase

*A. shigelloides is rarely isolated on m-A. If its presence is suspected, colonies can be picked to inositol broth.
A. shigelloides is inositol positive.

Figure C-4. Aeromonas hydrophila on Membrane Filter (4X).



METHOD: Membrane Filter Technique for Pseudomonas aeruginosa (m-PA) (73,79).

INTRODUCTION: This procedure has been shown to recover P. aeruginosa accurately and quantitatively from both fresh and salt water samples.

SAMPLES: Collect and hold as described in Section 405 of Standard Methods for the Examination of Water and Wastewater.

PREPARATION: Sample containers, membrane filter apparatus and other equipment are prepared as described in Sections 405 and 408 of "Standard Methods."

PROCEDURE:

a) Filter appropriate volumes of the water sample through a sterile membrane so that the 20-80 colonies will result.

b) Place the membranes on the agar surfaces of the m-PA plates, taking care to avoid trapping air bubbles on the underside.

c) Filters are incubated for 48 hours at 41°C.

d) Positive colonies are flat, approximately $\geq$ 0.8 mm in diameter with a dark-brown or greenish-black center and a pale outer edge, not yellow.

e) These plates can be refrigerated with no effect on the color of colonies.

CALCULATIONS: Results should be reported as organisms per 100 ml of sample.

MATERIALS:

Pseudomonas Medium (m-PA)

Ingredients	gm/100 ml	Antibiotics	mg/100 ml
L-lysine	0.5		
NaCl	0.5	Sulfapyridine	17.6
Yeast extract	0.2		
Xylose	0.25	Kanamycin sulfate	0.85
Sodium thiosulfate	0.68	Nalidixic acid	3.7
Sucrose	0.125	Actidione	15.0
Lactose	0.125	Sulfapyridine	
Agar	1.5		
Phenol red	0.0008		
Ferric ammonium citrate	0.08		
Distilled Water			

Autoclave 15 minutes at 121°C. Add antibiotics as dry powders to medium after cooling to 55°C. Adjust pH to 7.1$\pm$ 0.1

Confirmation Media - Milk Media

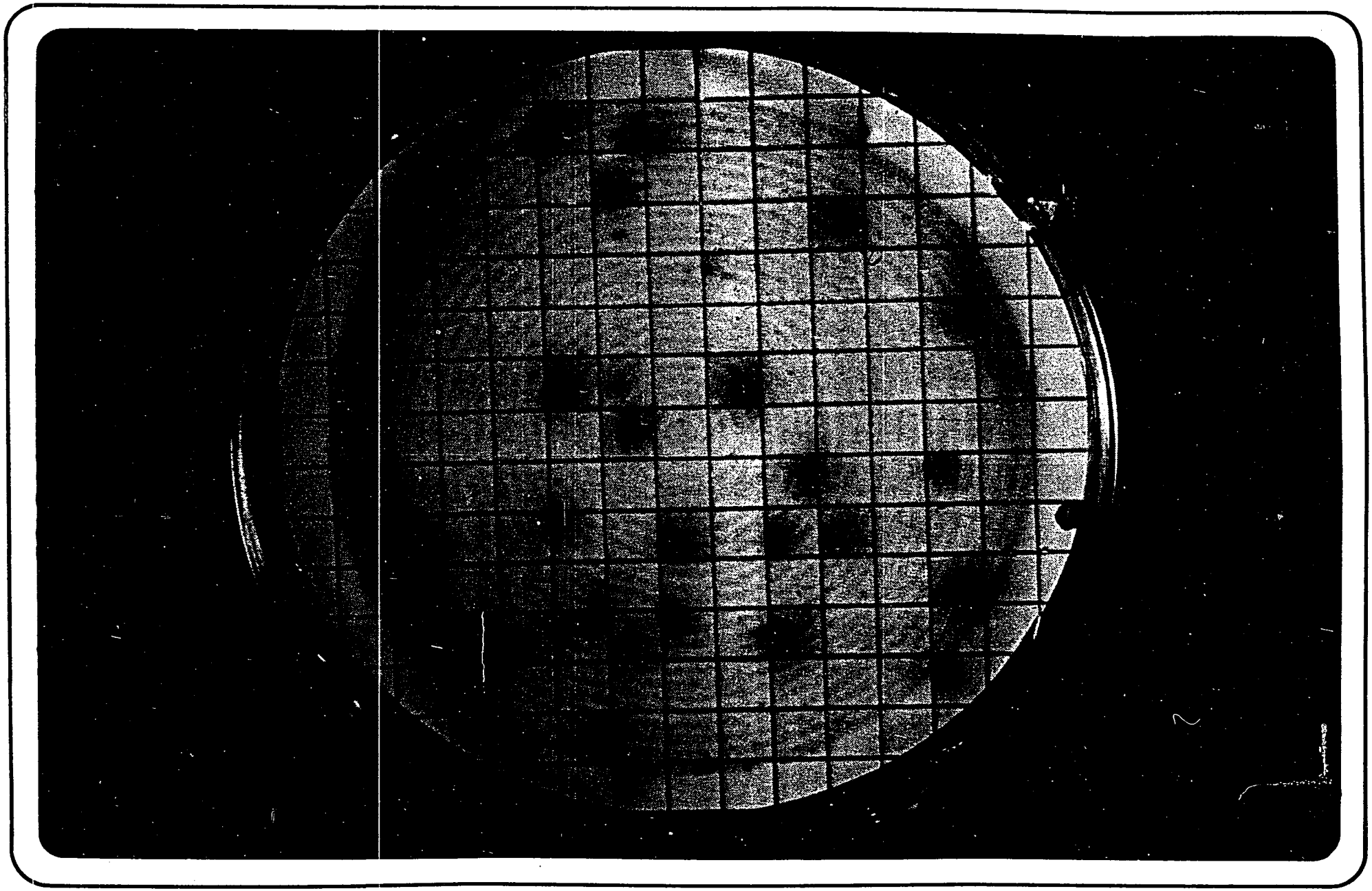
Solution 1	Amounts
Pseudocel agar Nutrient Broth	According to directions
Agar	2.0%
Distilled water	1/2 quantity

Solution 2	Amounts
Carnation Dry Milk	20%
Distilled water	1/2 quantity

Autoclave and cool milk immediately. Add the two solutions; mix well and pour in large petri dishes. Inoculate no more than 8/plate. Incubate 24 hours at 35°C.

Positive: Clearing around streak.
Negative: Streak usually remains white with no clearing.

Figure C-5. Pseudomonas aeruginosa on Membrane Filter (4X).



METHOD: Membrane Filter Technique for Clostridium perfringens (m-CP) (80).

SAMPLES: Collect and hold as described in Section 405 of Standard Methods for the Examination of Water and Wastewater.

PROCEDURE: The recovery medium developed by E.P.A. (m-CP) was prepared by adding the following ingredients (in grams per 90 ml) to distilled water: tryptose, 3.0; yeast extract, 2.0; sucrose, 0.5; L-cysteine hydrochloride, 0.1; $MgSO_4 \cdot 7H_2O$, 0.01; bromocresol purple, 0.004; and agar, 1.5. The ingredients were dissolved, and the pH was adjusted to 7.6. After autoclaving at 121°C for 15 minutes, the medium was allowed to cool to 50°C and the following ingredients were added: 40 mg of D-cycloserine (Sigma Chemical Co.) and 2.5 mg of polymyxin-B sulfate (Sigma) as the dry ingredients; indoxyl β-D-glucoside (IBDG; Reliable Chemical Co.), 60 mg dissolved in 8.0 ml of sterile distilled water; 2.0 ml of a filter-sterilized, 0.5% solution of phenolphthalein diphosphate (Sigma); and 0.2 ml of a filter-sterilized 4.5% solution of $FeCl_3 \cdot 6H_2O$. Once it had cooled to 50°C, the medium was dispensed in 5 ml quantities into sterile petri dishes (50 by 12 mm). The poured plates were stored in an anaerobic jar (Baltimore Biological Laboratory (BBL) GasPak anaerobic unit) until use.

a) Filter appropriate volumes of the water samples through a sterile membrane so that 20-80 colonies will result.

b) Place the membranes on the agar surfaces of the m-CP plates, taking care to avoid trapping air bubbles on the underside.

c) Heat a pair of forceps or tweezers and carefully burn 6 holes in the lid of the plate. Incubate anaerobically (Anaerobic jar with GasPak and anaerobic indicator strip) for 24 hours at 45°C.

NOTE: a) Hydrogen sulfide is often generated by C. perfringens and this will inactivate the catalyst. Frequently remove the catalyst beads from the holder on the jar lid into a beaker. Heat in a 300°F oven for 3 hours to reactivate.

b) Store m-CP in an anaerobic jar with a GasPak at room temperature.

FILTRATION

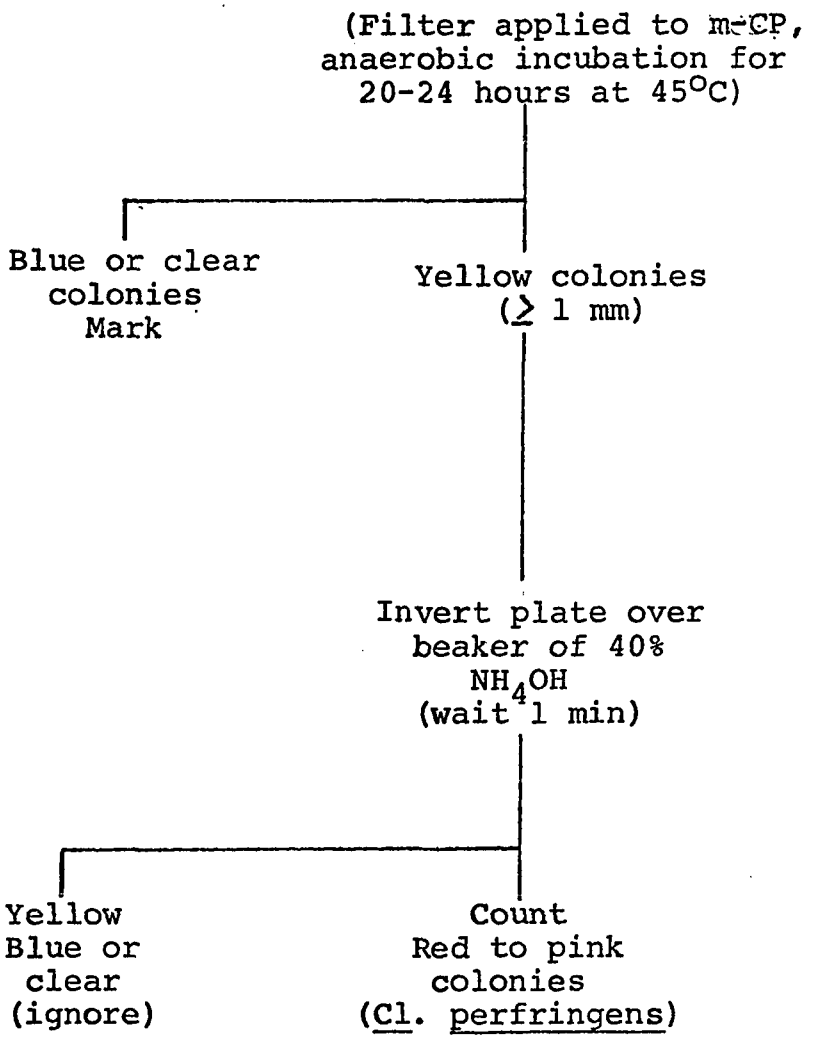
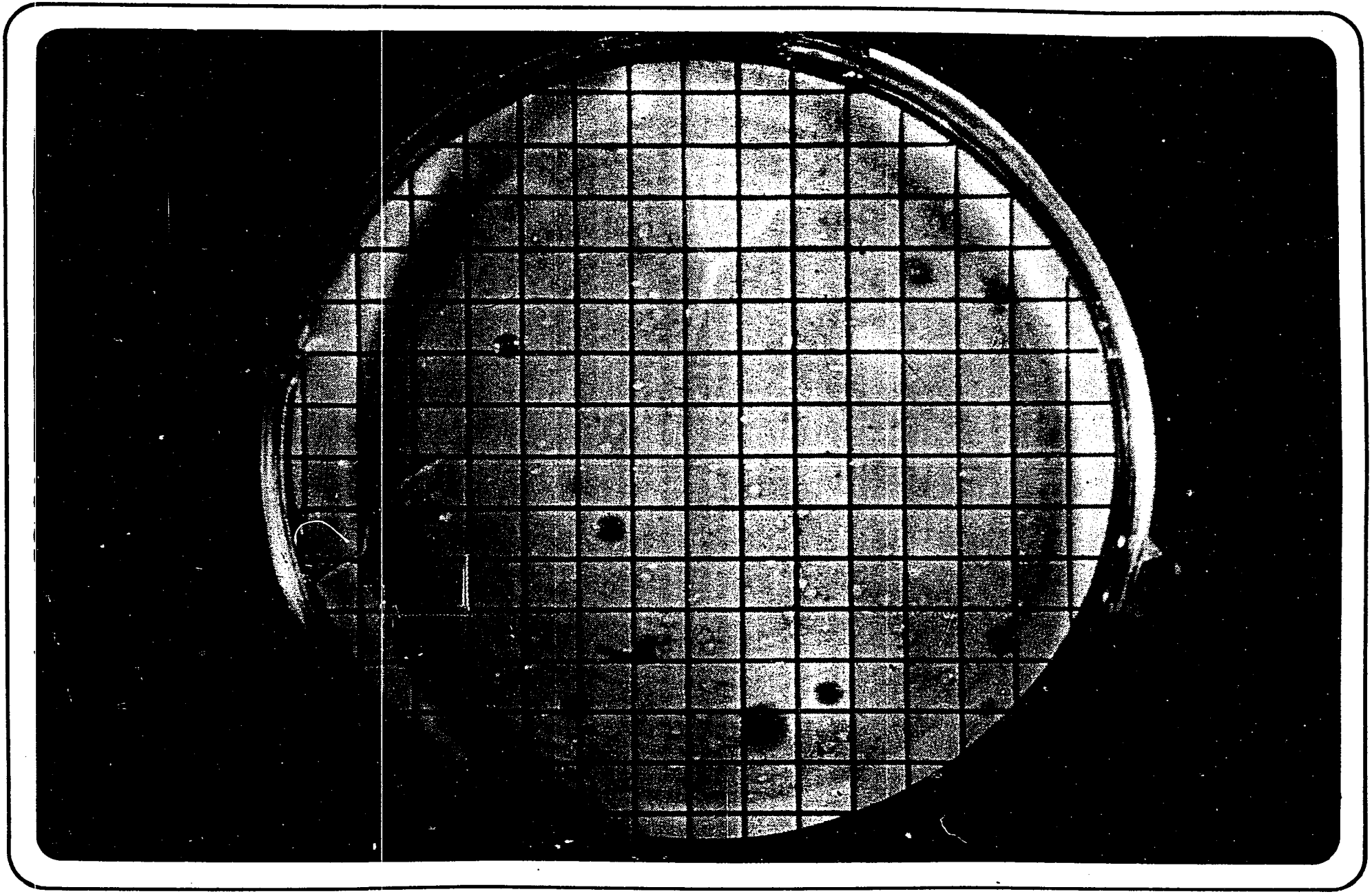

(Filter applied to m-CP, anaerobic incubation for 20-24 hours at 45°C)
Blue or clear colonies Mark
Yellow colonies (≥ 1 mm)
Invert plate over beaker of 40% NH_4OH (wait 1 min)
Yellow Blue or clear (ignore)
Count Red to pink colonies (Cl. perfringens)

Figure C-6. Clostridium perfringens on Membrane Filter (4X).



METHOD: Membrane Filter Technique for Acinetobacter (85,88).

INTRODUCTION: This procedure is used to select for and enumerate Acinetobacter calcoaceticus in fresh waters. It is not a standard method and is still in the developing stages of its evaluation. It is being developed because there is presently no membrane filter technique for Acinetobacter isolation.

SAMPLES: Collect and hold as described in Section 906 of Standard Methods for the Examination of Water and Wastewater.

PROCEDURE:

a) Filter appropriate volumes of the water sample through a sterile membrane so that 20-80 colonies will result.

b) Place membrane on agar surface of prepared Acinetobacter plates, taking care to avoid trapping bubbles on the underside.

c) Invert and incubate at 31°C for 45 hours.

d) Transfer filter to carbohydrate differential medium (SR). Incubate 2 hours at 31°C.

e) Acinetobacter colonies will be relatively large (≥ 1 mm), green-blue in color. Score on the filter by punching a hole in the membrane with a needle next to target colonies.

f) Transfer the filter to a pad saturated with oxidase reagent (N, N, N', N' - Tetramethyl-P-phenylenediamine dihydrochloride, 0.1 g/10 ml) for 5-10 seconds and place back on the differential carbohydrate medium.

g) Oxidase positive colonies will develop a purple halo or turn dark purple. Green-blue, oxidase negative colonies, 1 mm in diameter or greater, are counted as Acinetobacter.

Preparation of Acinetobacter Medium (mAc)

	g/l
Na Acetate·$3H_2O$	2.0 g
KNO_3	2.0 g
Purified Agar (Oxoid)	10.0 g
Deionized H_2O	960.0 ml

Autoclave 15 minutes at 121°C, cool to 50°C, then add the following while stirring:

	per liter
$MgSO_4 \cdot 7H_2O$ (10T sterile solution)	1.0 ml
Concentrated base (sterile solution)	20.0 ml
2.0 M KH_2PO_4 (sterile solution)	5.7 ml
2.0 M Na_2HPO_4 (sterile solution)	14.3 ml
Formic acid	2.0 ml

Adjust the pH to 7.2 by adding 4.3 ml of 1N NaOH. Dispense 6 ml amounts into standard MF petri dishes.

Preparation of Carbohydrate Differential Media (SR)

	g/l
Tryptose	2.5
Mannitol	5.0
Sucrose	5.0
Lactose	5.0
Yeast Extract	2.0
NaCl	3.0
KCl	2.0
$MgSO_4$	0.2
$FeCl_3$	0.1
Bromthymol blue	0.08
Deionized H_2O	1000.0 ml

Adjust the pH to 8.5; add 15 g/l agar and autoclave 15 min. at 121°C; cool to 50°C, then add 1.0 g/l sodium desoxycholate; mix thoroughly and dispense 6 ml amounts into standard MF petri plates.

Preparation of Stock Solutions

Concentrated base (add and dissolve in the order given)

Deionized water	250.0 ml
Nitrilotriacetic acid*	10.0 g
$MgSO_4$	14.45 g
$CaCl_2 \cdot 2H_2O$**	3.335 g
$(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$	9.25 mg
$FeSO_4 \cdot 7H_2O$	99.0 mg
Nicotinic acid	50.0 mg
Thiamine·HCl	25.0 mg
Biotin	0.5 mg
Metals "44"	50.0 ml

*Prepare concentrated base by dissolving nitrilotriacetic acid and neutralize with KOH (about 7.3 g) after which the rest of the ingredients are added.

**Adjust the pH to 6.0 - 6.6 before adding 3.335 g/l $CaCl_2 \cdot 2H_2O$. Add the rest of the ingredients, adjust the pH to 6.6 - 6.8, then bring to volume (1000 ml) with H_2O and autoclave.

In autoclaving the concentrated base a precipitate forms but redissolves if allowed to cool with mixing.

Metals "44"	mg/100 ml
Ethylenediaminetetraacetic acid	250.0
$ZnSO_4 \cdot 7H_2O$	1095.0
$FeSO_4 \cdot 7H_2O$	500.0
$MnSO_4 \cdot H_2O$	154.0
$CuSO_4 \cdot 5H_2O$	39.2
$Co(NO_3)_2 \cdot 6H_2O$	24.8
$Na_2B_4O_7 \cdot 10H_2O$	17.7

A few drops of sulfuric acid are added to retard precipitation.

METHOD: Membrane Filter Procedure for Bifidobacterium (YN6) (82,83,89,90).

PROCEDURE:

a) Filter appropriate volumes of sample through a sterile filter so that 20-80 colonies will result.

b) Place the membrane on the agar surface so as to avoid trapping air bubbles on the underside.

c) Heat a pair of forceps or tweezers and carefully melt 6 holes in the lid.

d) Incubate anaerobically (jar with GasPak and anaerobic indicator strip) for 48 hrs. at 35°C.

e) Gram stain smears with green, glistening, smooth entire colonies with sunken centers.

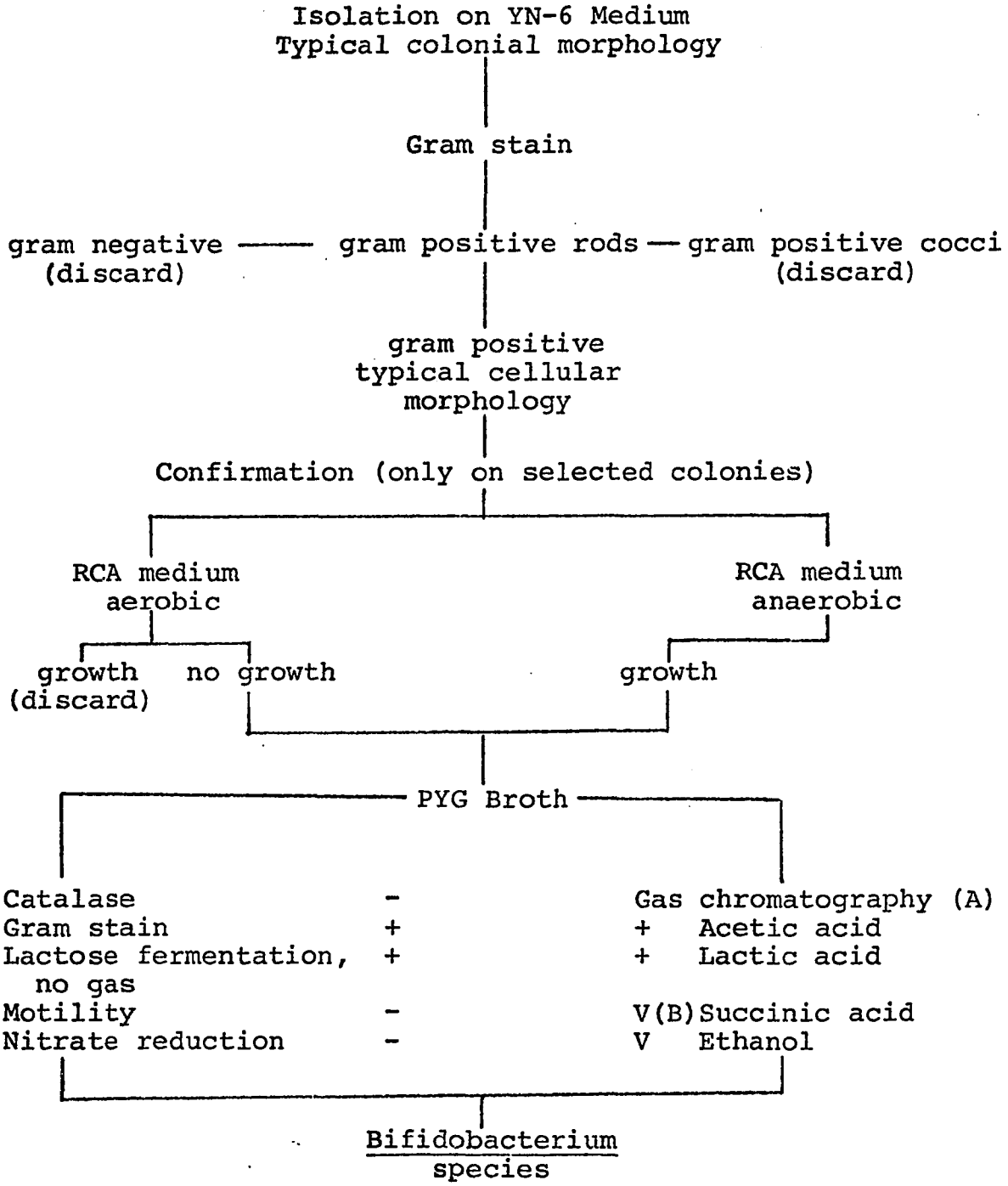
f) Store plates at 4°C in the dark.

Preparation of YN-6 Medium

Ingredients[1]	Quantity
Yeast extract	20 g
Peptone	10
Lactose	10
Casamino acids	8
Sodium chloride	3.2
Brom cresol green	0.3
Deionized water	1 L

[1]Add ingredients to water and boil for 10 minutes. Cool to ambient temperature, add cysteine hydrochloride (0.4g) and nalidixic acid (80 mg). Adjust to pH 6.9 with 1 N sodium hydroxide. Add agar (15.0 g) and autoclave for 15 min. at 121°C. Cool to 60°C before adding 1 ml of a stock solution containing 2.5 mg of neomycin sulfate per ml of deionized water. Dispense 4 ml volumes to 50 mm petri dishes with tight lids and store at 4°C in the dark.

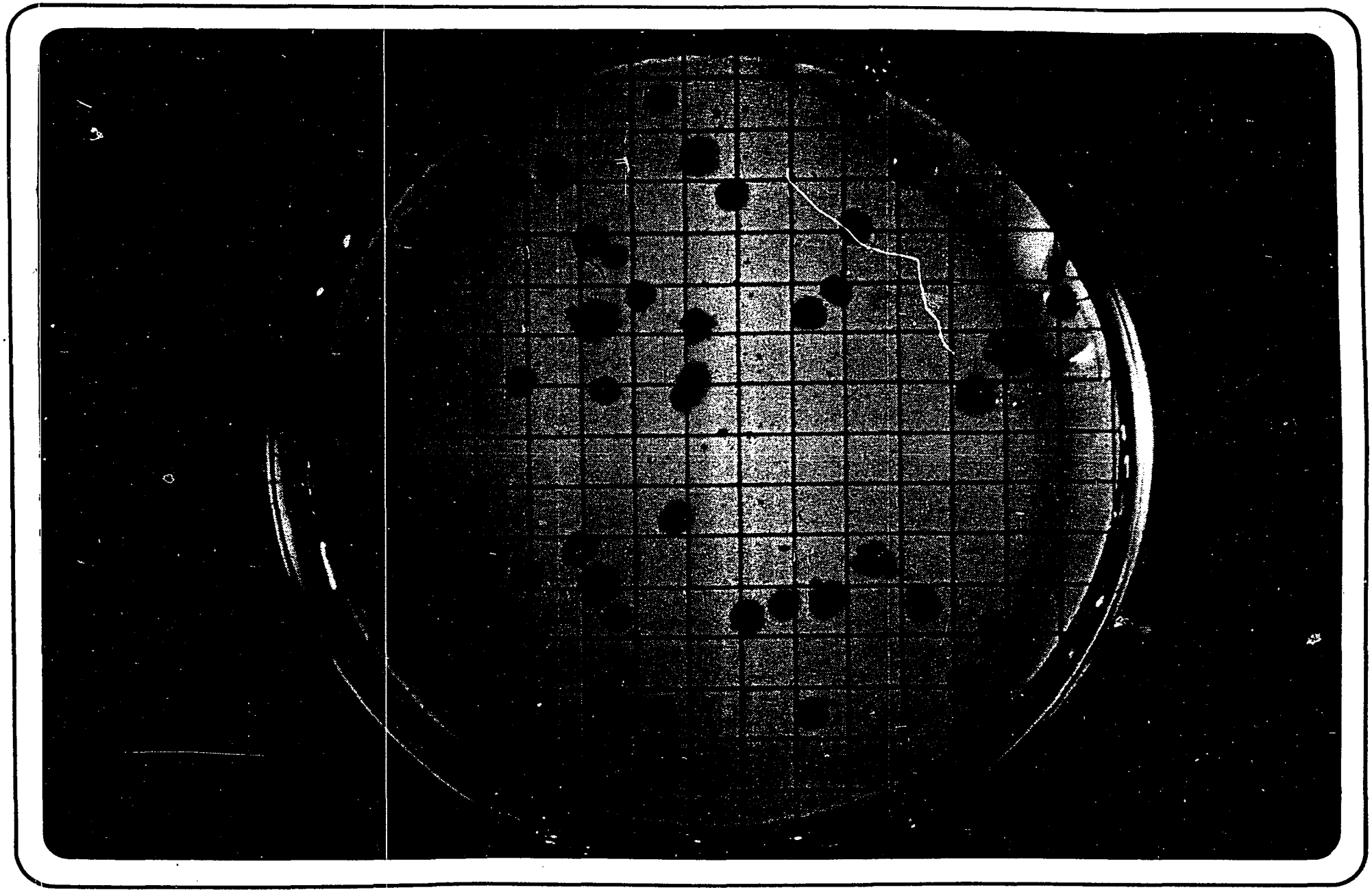
Identification of genus Bifidobacterium



(A) Ratio of acetic to lactic acids must exceed 1:1.

(B) Variable; may or may not be present.

Figure C-7. Bifidobacterium on Membrane Filter (4X).



Dilution Methodology

	Media	Beach	Dilution
1.	m-ENDO	I & II	1/1000,1/10
		III	1/1000, 1/10
2.	m-FC	I & II	1/10, 1/1
		III	1/10, 1/1
3.	m-KF	I & II	1/10, 1/1
		III	1/10, 1/1
4.	m-Tec	I & II	1/100, 1/10, 1/1
		III	1/10, 1/1
5.	m-E	I & II	1/100, 1/10, 1/1
		III	1/10, 1/1
6.	m-A	I & II	1/1000, 1/100
		III	1/1000, 1/100
7.	m-PA	I & II	1/10, 1/1
		III	1/1
8.	m-CP	I & II	1/10, 1/1
		III	1/10, 1/1
9.	m-BIFID	I & II	1/10, 1/1
		III	1/10, 1/1
10.	m-Ac	I & II	1/1000, 1/100, 1/10
		III	1/1000, 1/100, 1/10

Chlorides

Source:

Chlorides are found in practically all natural waters. They may be of natural mineral origin or derived (a) from salts spread on fields for agricultural purposes (b) from human or animal sewage or (c) from industrial effluents, such as those from paper works, galvanizing plants, water softening plants, oil wells, and petroleum refineries.

Significance:

The U.S.P.H.S. recommends that chlorides do not exceed 250 mg/l (250 ppm) in drinking water supplies.

Chloride concentrations in excess of 4000 mg/l (4000 ppm) have been reported to cause injury to livestock. Chloridity is closely related to the total salinity and its effects on osmosis; hence it is evident that fresh-water fish cannot tolerate excessive changes in or levels of salinity. The following concentrations of chloride will not be normally deleterious to the specified beneficial uses: (a) irrigation 100 mg/l (100 ppm) and (b) stock and wildlife 1500 mg/l (1500 ppm).

Equipment:

Units	Description
1	Automatic buret assembly, 10 or 25 ml
4	Casserole dishes with handles
2	Stirring rods
2	Bottles, dropping about 60 ml
2	Medicine dropper
2	100 milliliter volumetric flask
8	Volumetric pipets
2	1 milliliter
2	5 milliliter
2	10 milliliter
2	50 milliliter

Reagents:

Volume	Conc.	Description
2 liter		Standard mercuric nitrate
4 oz		Chloride indicator
100 ml	0.1N	Nitric acid

Procedure:

1. Measure out 50 milliliters of the water sample and pour into a white porcelain dish.

2. Add about 1 ml of chloride indicator.

3. Add 0.1N Nitric acid dropwise while stirring until the sample will not turn more yellow with addition of more 0.1N Nitric acid.

4. Add mercuric nitrate titrant and stir until a definite purple endpoint is reached. Record amount of titrant used and calculate mg/l Cl (ppm).

5. If the endpoint is not reached before 20 mls of the titrant is used then dilute according to one of the following, depending on the estimated concentration of the sample.
   a) Add 1 ml of the original sample with a volumetric flask and dilute with distilled water to the mark on the volumetric flask. This will make a 1 to 100 dilution.
   b) Add 5 ml of the original sample with a volumetric pipet to a 100 ml volumetric flask and dilute with distilled water to the mark on the volumetric flask. This will make a 1 to 20 dilution.
   c) Add 10 ml of the original sample with a volumetric pipet to a 100 ml volumetric flask and dilute with distilled water to the mark on the volumetric flask. This will make a 1 to 10 dilution.

   Then using a, b, or c, titrate according to steps 1, 2, and 3, calculate according to one of the following.

Calculations:

A x factor = mg/l (ppm) Chloride	when no dilution is made
A x factor x 100 = mg/l (ppm) Chloride	when 1 to 100 dilution is used
A x factor x 20 = mg/l (ppm) Chloride	when 1 to 20 dilution is used
A x factor x 10 = mg/l (ppm) Chloride	when 1 to 10 dilution is used

Factor = About 20, should be on titrant bottle.

Determining Cl Factor:

1. 25 mls of 1000 ppm technicon standard
2. Add indicator + .1N HNO_3 = yellow
3. Titrate to purple with mercuric nitrate titrant
4. Factor = 500/mls of titrant

Appendix D

Bacteriological Pre-test Results

Saturday - June 24, 1978

	Count per 100 ml		
	E. coli	Enterococci	C. perfringens
Salt Creek Cove North (East)			
11:00am	70	50	1
3:00pm	150	40	4
Salt Creek Cove North (West)			
11:00am	160	150	0
3:00pm	11	11	0
Keystone Ramp			
12:00Noon	90	20	2
3:30pm	93	28	0
Washington Irving South			
1:00pm	49	38	0
3:30pm	20	24	0
Washington Irving North			
4:00pm	17	52	4

Sunday - June 25, 1978

	Count per 100 ml		
	E. coli	Enterococci	C. perfringens
Salt Creek Cove North (East)			
10:45am	50	180	10
Salt Creek Cove North (West)			
10:30am	5	20	0
1:00pm	4	210	20
Keystone Ramp			
11:00am	150	120	110
2:15pm	110	150	20
Washington Irving South			
10:00am	40	140	-
3:15pm	120	310	20

Monday - June 26, 1978

	Count per 100 ml		
	E. coli	Enterococci	C. perfringens
Salt Creek Cove North (East)			
12:00Noon	140	20	9
3:00pm	46	4	7
Salt Creek Cove North (West)			
12:00Noon	26	6	5
3:00pm	26	7	2
Keystone Ramp (West)			
12:00Noon	50	16	6
3:00pm	30	8	5
Keystone (East)			
12:00Noon	70	15	5
3:00pm	60	29	6

Tuesday - June 27, 1978

	Count per 100 ml		
	E. coli	Enterococci	C. perfringens
Salt Creek Cove North (East)			
12:00Noon	120	15	8
4:00pm	44	2	6
Salt Creek Cove North (West)			
12:00Noon	22	7	6
4:00pm	21	6	1
Keystone Ramp (West)			
11:00am	40	12	5
4:00pm	35	7	4
Keystone Ramp (East)			
11:00am	40	9	2
4:00pm	31	15	3

Saturday - September 2, 1978

		M TEC *E. coli* per 100 ml
8:00 AM	Salt Creek Cove Beach	14
	Keystone Ramp Beach	11
	Washington Irving Cove South	7
10:00 AM	Salt Creek Cove Beach	18
	Keystone Ramp Beach	17
	Washington Irving Cove South	6
12:00 PM	Salt Creek Cove Beach	23
	Keystone Ramp Beach	19
	Washington Irving Cove South	9
2:00 PM	Salt Creek Cove Beach	21
	Keystone Ramp Beach	24
	Washington Irving Cove South	12
4:00 PM	Salt Creek Cove Beach	62
	Keystone Ramp Beach	48
	Washington Irving Cove South	16
6:00 PM	Salt Creek Cove Beach	89
	Keystone Ramp Beach	67
	Washington Irving Cove South	19

Sunday - September 3, 1978

		M TEC *E. coli* per 100 ml
8:00 AM	Salt Creek Cove Beach	12
	Keystone Ramp Beach	9
	Washington Irving Cove South	3
10:00 AM	Salt Creek Cove Beach	16
	Keystone Ramp Beach	12
	Washington Irving Cove South	14
12:00 PM	Salt Creek Cove Beach	27
	Keystone Ramp Beach	14
	Washington Irving Cove South	11
2:00 PM	Salt Creek Cove Beach	94
	Keystone Ramp Beach	32
	Washington Irving Cove South	12
4:00 PM	Salt Creek Cove Beach	110
	Keystone Ramp Beach	74
	Washington Irving Cove South	21
6:00 PM	Salt Creek Cove Beach	147
	Keystone Ramp Beach	122
	Washington Irving Cove South	63

Monday - September 7, 1978

		M TEC *E. coli* per 100 ml
8:00 AM	Salt Creek Cove Beach	89
	Keystone Ramp Beach	64
	Washington Irving Cove South	28
10:00 AM	Salt Creek Co	113
	Keystone R	73
	Washingt	27
12:00 PM	Salt	
	Key	
	Wa	
2:00 PM		
4:00 PM		
6:00 PM		



Monday - September 7, 1978

		M TEC *E. coli* per 100 ml
8:00 AM	Salt Creek Cove Beach	89
	Keystone Ramp Beach	64
	Washington Irving Cove South	28
10:00 AM	Salt Creek Cove Beach	113
	Keystone Ramp Beach	73
	Washington Irving Cove South	27
12:00 PM	Salt Creek Cove Beach	220
	Keystone Ramp Beach	114
	Washington Irving Cove South	19
2:00 PM	Salt Creek Cove Beach	214
	Keystone Ramp Beach	100
	Washington Irving Cove South	26
4:00 PM	Salt Creek Cove Beach	167
	Keystone Ramp Beach	128
	Washington Irving Cove South	38
6:00 PM	Salt Creek Cove Beach	190
	Keystone Ramp Beach	212
	Washington Irving Cove South	54

Saturday - September 16, 1978

		M TEC *E. coli* per 100 ml
8:00 AM	Salt Creek Cove Beach	28
	Keystone Ramp Beach	32
	Washington Irving Cove South	19
10:00 AM	Salt Creek Cove Beach	12
	Keystone Ramp Beach	38
	Washington Irving Cove South	14
12:00 PM	Salt Creek Cove Beach	27
	Keystone Ramp Beach	63
	Washington Irving Cove South	19
2:00 PM	Salt Creek Cove Beach	98
	Keystone Ramp Beach	133
	Washington Irving Cove South	27
4:00PM	Salt Creek Cove Beach	210
	Keystone Ramp Beach	301
	Washington Irving Cove South	51
6:00 PM	Salt Creek Cove Beach	314
	Keystone Ramp Beach	281
	Washington Irving Cove South	79

Sunday - September 17, 1978

		M TEC *E. coli* per 100 ml
8:00 AM	Salt Creek Cove Beach	10
	Keystone Ramp Beach	8
	Washington Irving Cove South	7
10:00 AM	Salt Creek Cove Beach	17
	Keystone Ramp Beach	9
	Washington Irving Cove South	6
12:00 PM	Salt Creek Cove Beach	34
	Keystone Ramp Beach	19
	Washington Irving Cove South	9
2:00 PM	Salt Creek Cove Beach	128
	Keystone Ramp Beach	39
	Washington Irving Cove South	14
4:00 PM	Salt Creek Cove Beach	151
	Keystone Ramp Beach	101
	Washington Irving Cove South	21
6:00 PM	Salt Creek Cove Beach	136
	Keystone Ramp Beach	143
	Washington Irving Cove South	47

Results of samples taken from the Mannford sewage effluent along a line to the Salt Creek Beach. These samples were taken in a straight line from the sewage effluent to the beach.

Saturday 6:00 AM 9-16-78 M-TEC

Sewage effluent samples		Distance from effluent (miles)	E. coli per 100 ml.
	1.	0.25	28,000
	2.	0.5	17,000
	3.	0.75	14,000
	4.	1.0	13,000
	5.	1.25	7,000
	6.	1.5	5,500
	7.	1.75	600
	8.	2.0	510
	9.	2.25	300
Salt Creek Cove Beach	10.	2.5	314

APPENDIX E

BACTERIOLOGICAL DATA

TABLE E-1a Bacteriological Data From Beaches I and II (BA)
(Counts are per 100ml)

Date	Time P.M.	Total Coliform	Weekend Geometric Mean	Fecal Coliform	Weekend Geometric Mean	Fecal Strepto-cocci	Weekend Geometric Mean	Thermo-tolerant E. coli	Weekend Geometric Mean	Entero-cocci	Weekend Geometric Mean
6-16	1:00	5,800						90		180	
Sat.	3:00	8,300						530		640	
	5:00	1,700	6,700					120	100	120	100
6-17	1:00	7,100						67		33	
Sun.	3:00	24,000						50		110	
	5:00	6,300						51		22	
6-30	1:00	18,000						39		38	
Sat.	3:00	26,000						80		14	
	5:00	21,000	23,000					150	26	16	26
7-1	1:00	22,000						19		43	
Sun.	3:00	28,000						35		31	
	5:00	24,000						30		26	
7-7	1:00	26,000						140		24	
Sat.	3:00	22,000						110		28	
	5:00	21,000	23,000					87	26	34	26
7-8	1:00	26,000						61		21	
Sun.	3:00	29,000						100		22	
	5:00	28,000						70		30	
7-14	1:00	11,000						60		17	
Sat.	3:00	13,000						95		12	
	5:00	13,000	11,000					110	140	29	19
7-15	1:00	8,700						140		11	
Sun.	3:00	9,400						310		31	
	5:00	9,100						300		23	
7-21	1:00	14,000						120		18	
Sat.	3:00	19,000						130		19	
	5:00	21,000	16,000					180	130	22	21
7-22	1:00	16,000						130		19	
Sun.	3:00	12,000						98		21	
	5:00	14,000						150		26	

TABLE E-1b Bacteriological Data From Beaches I and II (BA)
(Counts are per 100ml)

Date	Time P.M.	Total Coliform	Weekend Geometric Mean	Fecal Coliform	Weekend Geometric Mean	Fecal Strepto-cocci	Weekend Geometric Mean	Thermo-tolerant E. coli	Weekend Geometric Mean	Entero-cocci	Weekend Geometric Mean
7-28	1:00	12,000						82		16	
Sat.	3:00	14,000						110		27	
	5:00	24,000	19,000					120	180	33	21
7-29	1:00	19,000						170		12	
Sun.	3:00	31,000						890		28	
	5:00	23,000						180		19	
8-4	1:00	7,100						81		25	
Sat.	3:00	8,600						100		32	
	5:00	9,400	14,000					130	140	27	29
8-5	1:00	21,000			"			160		31	
Sun.	3:00	24,000						210		33	
	5:00	22,000						190		26	
8-11	1:00	11,000		330		28		150		22	
Sat.	3:00	19,000		420		32		160		29	
	5:00	16,000	16,000	380	410	41	56	160	230	31	32
8-12	1:00	13,000		380		86		220		33	
Sun.	3:00	19,000		510		92		560		41	
	5:00	20,000		440		110		350		40	
8-13	1:00	18,000		410		30		96		18	
Sat.	3:00	22,000		460		31		110		22	
	5:00	21,000	26,000	520	530	42	80	120	140	24	25
8-19	1:00	29,000		550		230		140		31	
Sun.	3:00	35,000		630		190		190		29	
	5:00	34,000		690		150		180		26	
8-25	1:00	27,000		1,200		330		910		25	
Sat.	3:00	23,000		970		220		640		28	
	5:00	22,000	27,000	870	540	180	200	560	350	26	21
8-26	1:00	34,000		240		180		200		12	
Sun.	3:00	32,000		340		160		190		25	
	5:00	29,000		320		150		140		18	
9-1	1:00	41,000		220		38		120		19	
Sat.	3:00	47,000		200		49		130		26	
	5:00	39,000	54,000	190	310	53	99	120	42	24	42
9-2	1:00	63,000		460		190		200		52	
Sun.	3:00	74,000		510		230		220		100	
	5:00	67,000		430		220		240		91	

TABLE E-2a Bacteriological Data From Beaches I and II (BA)
(Counts are per 100ml)

Date	Time P.M.	Aeromonas hydrophila	Weekend Geometric Mean	Pseudomonas aeruginosa	Weekend Geometric Mean	Clostridium perfringens	Weekend Geometric Mean	Bifido-bacteria	Weekend Geometric Mean	Acineto-pacter	Weekend Geometric Mean
6-16	1:00	19,000		12		26		4		30	
Sat.	3:00	20,000		480		21		10		230	
	5:00	47,000	38,000	280	29	23	15	10	12	180	170
6-17	1:00	37,000		4		8		30		170	
Sun	3:00	55,000		29		13		23		290	
	5:00	80,000		13		10		10		360	
6-30	1:00	4,600		9		16		20		10	
Sat.	3:00	14,000		25		8		100		3,000	
	5:00	18,000	22,000	13	21	11	8	20	25	10	480
7-1	1:00	21,000		18		4		11		200	
Sun.	3:00	51,000		32		5		20			
	5:00	100,000		46		12		30		180	
7-7	1:00	37,000		32		5				1,000	
Sat.	3:00	55,000		44		4				1,300	1,100
	5:00	30,000	32,000	37	20	1	6			1,100	
7-8	1:00	34,000		5		3					
Sun.	3:00	21,000		13		11					
	5:00	26,000		17		11					
7-14	1:00	26,000		13		8				900	
Sat.	3:00	41,000	32,000	26		5				470	
	5:00	28,000		17		5				530	1,400
7-15	1:00			11	12	4	6			7,000	
Sun.	3:00			7		6				2,700	
	5:00			8		8				1,700	
7-21	1:00			19		3				2,000	
Sat.	3:00			17		6				3,300	
	5:00			18		8				3,000	3,100
7-22	1:00			9	16	8	6			2,400	
Sun.	3:00			22		6				3,900	
	5:00			15		5				4,600	

TABLE E-2b Bacteriological Data From Beaches I and II (BA)
(Counts are per 100ml)

Date	Time P.M.	Aeromonas hydrophila	Weekend Geometric Mean	Pseudomonas aeruginosa	Weekend Geometric Mean	Clostridium perfringens	Weekend Geometric Mean	Bifido-bacteria	Weekend Geometric Mean	Acineto-bacter	Weekend Geometric Mean
7-28	1:00	4,200		13		3				300	
Sat.	3:00	9,400		12	11	7				2,000	
	5:00	12,000	8,700	9		4	5			6,400	1,800
7-29	1:00	7,100				4				520	
Sun.	3:00	11,000				5				4,800	
	5:00	12,000				7				3,600	
8-4	1:00	20,000				4				380	
Sat.	3:00	18,000				7				420	
	5:00	18,000	16,000			5	4			400	870
8-5	1:00	14,000				2				2,200	
Sun.	3:00	12,000				5				800	
	5:00	14,000				3				3,800	
8-11	1:00	41,000				6				380	
Sat.	3:00	29,000				10				700	530
	5:00	36,000	42,000			7	8			550	
8-12	1:00	51,000				8					
Sun.	3:00	58,000				8					
	5:00	45,000				11					
8-18	1:00	20,000				5					
Sat.	3:00	22,000				7					
	5:00	24,000	27,000			6	6				
8-19	1:00	33,000				4					
Sun.	3:00	28,000				7					
	5:00	38,000				5					
8-25	1:00	36,000				2					
Sat.	3:00	31,000				2					
	5:00	27,000	29,000			1	1				
8-26	1:00	25,000				1					
Sun.	3:00	28,000				2					
	5:00	29,000				1					
9-1	1:00	39,000				3					
Sat.	3:00	49,000				2					
	5:00	46,000	47,000			2	2				
9-2	1:00	43,000				2					
Sun.	3:00	56,000				3					
	5:00	51,000				3					

TABLE E-3a Bacteriological Data From Beach III (RU)
(Counts are per 100ml)

Date	Time P.M.	Total Coliform	Weekend Geometric Mean	Fecal Coliform	Weekend Geometric Mean	Fecal Strepto-cocci	Weekend Geometric Mean	Thermo-tolerant E. coli	Weekend Geometric Mean	Entero-cocci	Weekend Geometric Mean
6-16	1:00	2,500						33		52	
Sat.	3:00	1,700						29		100	
	5:00	1,900	3,300					20	27	98	32
6-17	1:00	5,100						65		23	
Sun.	3:00	6,000						22		9	
	5:00	5,600						15		10	
6-30	1:00	4,300						4		6	
Sat.	3:00	7,800						11		9	
	5:00	2,500	3,700					16	4	6	4
7-1	1:00	2,800						1		1	
Sun.	3:00	5,700						10		2	
	5:00	1,900						1		4	
7-7	1:00	3,100						15		8	
Sat.	3:00	4,700						27		7	
	5:00	8,500	9,200					33	19	18	12
7-8	1:00	1,800						12		11	
Sun.	3:00	5,200						17		14	
	5:00	10,000						15		24	
7-14	1:00	3,000						5		3	
Sat.	3:00	6,200						20		8	
	5:00	6,700	3,900					17	26	9	5
7-15	1:00	2,300						57		4	
Sun.	3:00	3,100						65		2	
	5:00	3,900						53		6	
7-21	1:00	8,000						10		3	
Sat.	3:00	9,400						14		6	
	5:00	12,000	9,200					19	26	9	6
7-22	1:00	9,400						41		4	
Sun.	3:00	8,900						44		7	
	5:00	8,100						68		7	

TABLE E-3b Bacteriological Data From Beach III (RU)
(Counts are per 100ml)

Date	Time P.M.	Total Coliform	Weekend Geometric Mean	Fecal Coliform	Weekend Geometric Mean	Fecal Strepto-cocci	Weekend Geometric Mean	Thermo tolerant E. coli	Weekend Geometric Mean	Entero-cocci	Weekend Geometric Mean
7-28	1:00	6,700						12		3	
Sat.	3:00	7,800						40		5	
	5:00	9,700	8,300					36	23	9	6
7-29	1:00	12,000						19		4	
Sun.	3:00	7,700						30		9	
	5:00	6,900						16		10	
8-4	1:00	2,800						24		8	
Sat.	3:00	3,400						31		10	
	5:00	4,200	7,300					29	38	11	9
8-5	1:00	14,000						20		7	
Sun.	3:00	16,000						83		11	
	5:00	17,000						81		9	
8-11	1:00	3,200		100		13		20		7	
Sat.	3:00	3,900		140		14		30		6	
	5:00	4,400	5,500	130	150	12	19	25	27	7	6
8-12	1:00	7,700		150		26		24		6	
Sun.	3:00	8,400		190		31		33		7	
	5:00	8,000		200		28		30		5	
8-18	1:00	4,100		34		9		12		7	
Sat.	3:00	7,100		51		12		13		8	
	5:00	8,100	8,800	66	80	13	7	14	15	8	4
8-19	1:00	11,000		130		2		12		1	
Sun.	3:00	13,000		120		4		20		3	
	5:00	14,000		140		8		21		2	
8-25	1:00	9,000		20		90		10		4	
Sat.	3:00	11,000		20		120		16		5	
	5:00	12,000	11,000	20	21	80	72	16	18	3	4
8-26	1:00	12,000		20		50		19		5	
Sun.	3:00	11,000		30		80		25		6	
	5:00	10,000		20		40		26		4	
9-1	1:00	19,000		19		4		8		3	
Sat.	3:00	24,000		17		5		12		9	
	5:00	20,000	19,000	18	27	3	14	12	13	5	6
9-2	1:00	13,000		37		40		11		6	
Sun.	3:00	19,000		44		70		18		7	
	5:00	20,000		41		50		20		8	

TABLE E-4a Bacteriological Data From Beach III (RU)
(Counts are per 100ml)

Date	Time P.M.	Aeromonas hydrophila	Weekend Geometric Mean	Pseudomonas aeruginosa	Weekend Geometric Mean	Clostridium perfringens	Weekend Geometric Mean	Bifido-bacteria	Weekend Geometric Mean	Acineto-bacter	Weekend Geometric Mean
6-16	1:00	2,600		10		19		1		300	
Sat.	3:00	3,000		13		24		1		260	
	5:00	2,700	3,500	24	12	26	15	1	1	1,400	610
6-17	1:00	1,400		24		19		2		12,000	
Sun.	3:00	11,000		4		6		1		180	
	5:00	6,000		1		9		1		210	
6-30	1:00	800		1		2		1		60	
Sat.	3:00	3,300		3		3		1		150	
	5:00	3,800	1,600	2	2	4	3	1	1	50	46
7-1	1:00	1,100		4		4		1		10	
Sun.	3:00	1,200		1		3		1		200	
	5:00	1,900		1		4		1		10	
7-7	1:00	6,100		12		3				1,400	
Sat.	3:00	5,400		7		4				1,600	1,600
	5:00	5,700	3,800	19	10	5	5			1,700	
7-8	1:00	2,100		6		7					
Sun.	3:00	2,600		8		8					
	5:00	1,100		13		3					
7-14	1:00	6,300		3		10				1,200	
Sat.	3:00	9,700	8,100	6		7				1,600	
	5:00	8,700		4	4	6	5			2,100	2,300
7-15	1:00			5		3				4,600	
Sun.	3:00			1		4				4,700	
	5:00			2		4				1,800	
7-21	1:00			2		1				2,400	
Sat.	3:00			2		2				2,900	
	5:00			9	4	4	3			8,100	3,900
7-22	1:00			9		6				6,800	
Sun.	3:00			3		7				3,200	
	5:00			5		4				2,900	

TABLE E-4b Bacteriological Data From Beach III (RU)
(Counts are per 100ml)

Date	Time P.M.	*Aeromonas hydrophila*	Weekend Geometric Mean	*Pseudomonas aeruginosa*	Weekend Geometric Mean	*Clostridium perfringens*	Weekend Geometric Mean	*Bifido-bacteria*	Weekend Geometric Mean	*Acineto-bacter*	Weekend Geometric Mean
7-28	1:00	3,400		3		4				480	
Sat.	3:00	5,600		2	3	7				650	
	5:00	6,300	4,200	3		5	5			8,100	940
7-29	1:00	3,100				4				1,400	
Sun.	3:00	4,300				6				1,100	
	5:00	3,600				5				180	
8-4	1:00	15,000				9				470	
Sat.	3:00	10,000				8				1,700	
	5:00	11,000	8,400			6	9			280	850
8-5	1:00	6,700				9				300	
Sun.	3:00	5,100				13				2,500	
	5:00	6,100				11				2,200	
8-11	1:00	9,000				4					
Sat.	3:00	12,000				7					
	5:00	9,000	14,000			5	4				
8-12	1:00	18,000				2					
Sun.	3:00	20,000				3					
	5:00	21,000				3					
8-18	1:00	8,000				3					
Sat.	3:00	13,000				2					
	5:00	9,000	11,000			2	1.5				
8-19	1:00	12,000				1					
Sun.	3:00	14,000				2					
	5:00	10,000				1					
8-25	1:00	13,000				1					
Sat.	3:00	16,000				1					
	5:00	15,000	13,000			1	1				
8-26	1:00	11,000				1					
Sun.	3:00	12,000				1					
	5:00	10,000				1					
9-1	1:00	24,000				2					
Sat.	3:00	26,000				3					
	5:00	28,000	22,000			3	3				
9-2	1:00	16,000				4					
Sun.	3:00	21,000				6					
	5:00	18,000				3					

TABLE E-5 Bacteriological Data From Mannford Sewage Effluent (Counts are per 100ml)

	Thermotolerant *E. coli*	Fecal Coliform	Fecal Streptococci
7-28-79	28,000		
8-5-79	38,000		
8-11-79	210,000	180,000	40,000
8-18-79	110,000	140,000	33,000
8-25-79	52,000	161,000	29,000
9-1-79	98,000	130,000	21,000

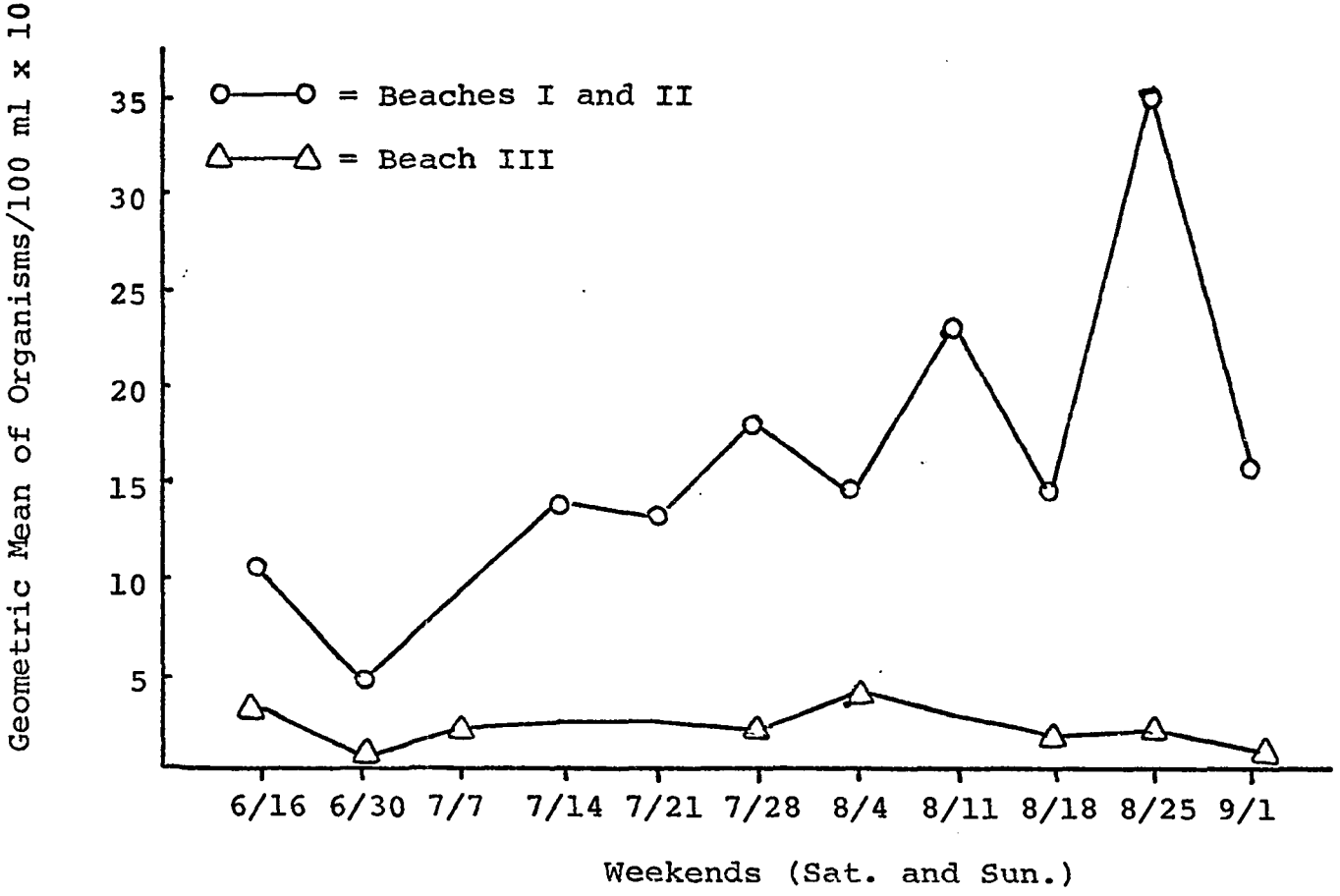


Figure E-1. Weekend Concentrations of Thermotolerant E. coli for Test Beaches I and II and Control Beach III.

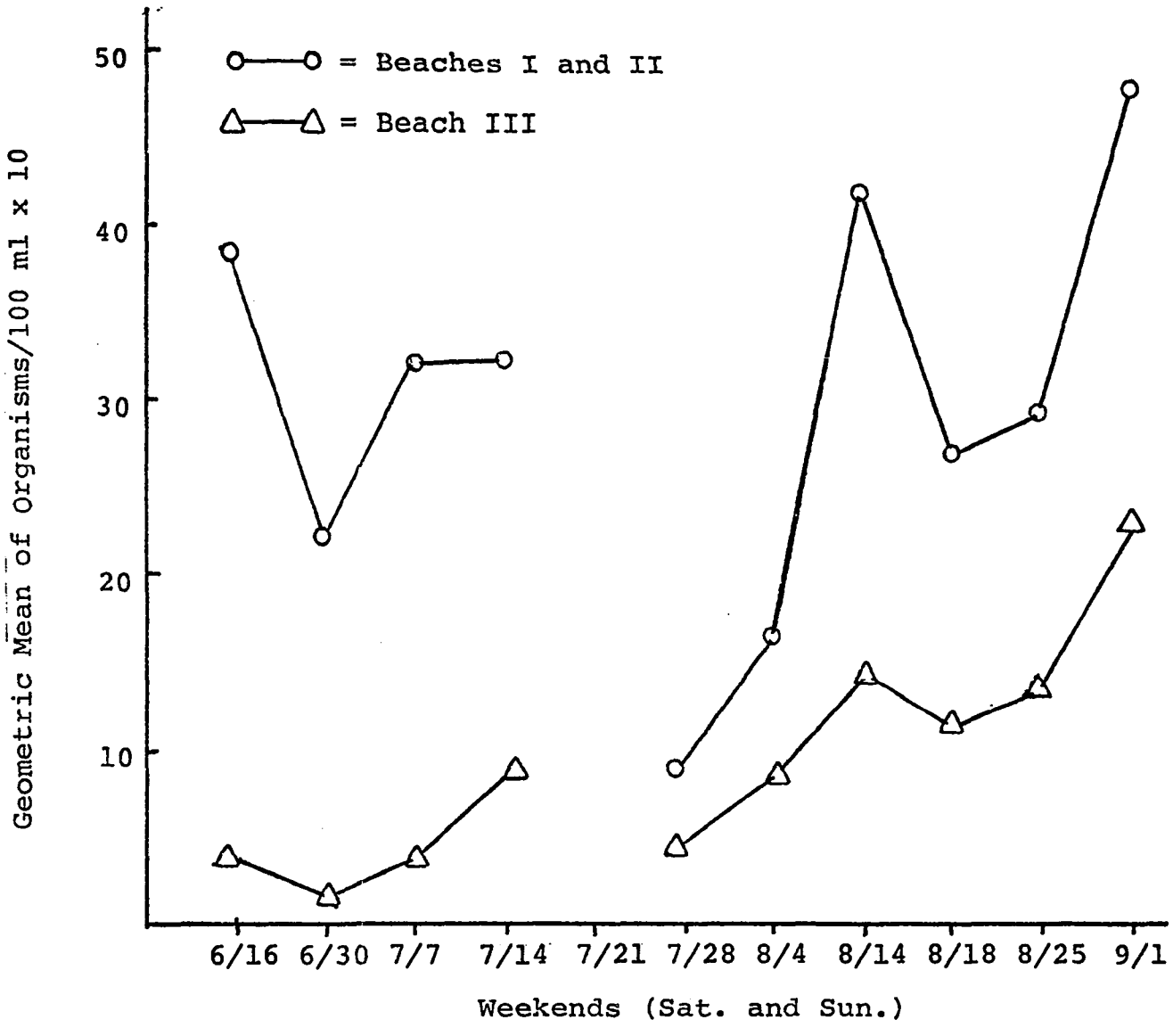


Figure E-2. Weekend Concentrations of Aeromonas hydrophila for Test Beaches I and II and Control Beach III.

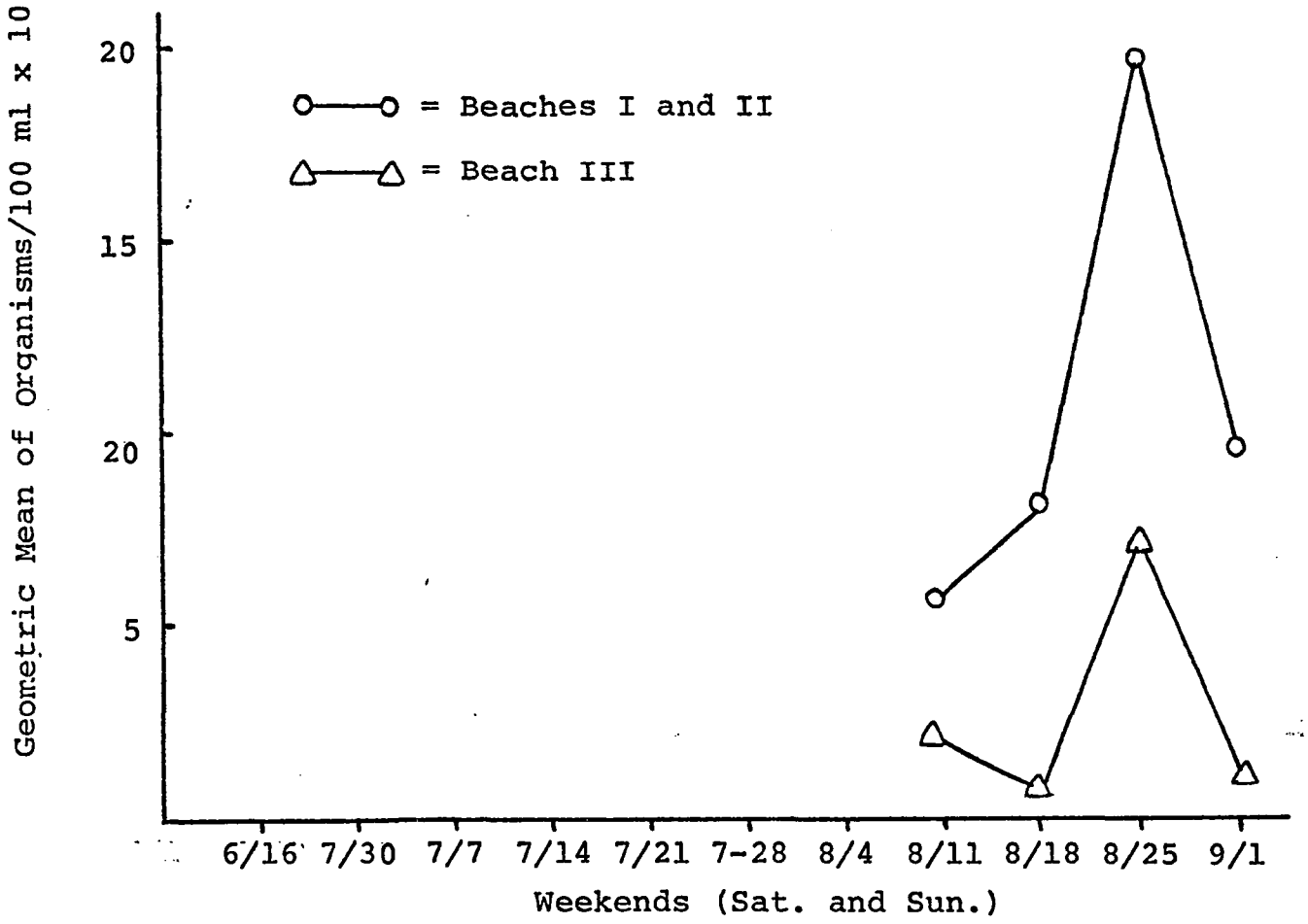


Figure E-3. Weekend Concentrations of Fecal Streptococci for Test Beaches I and II and Control Beach III.

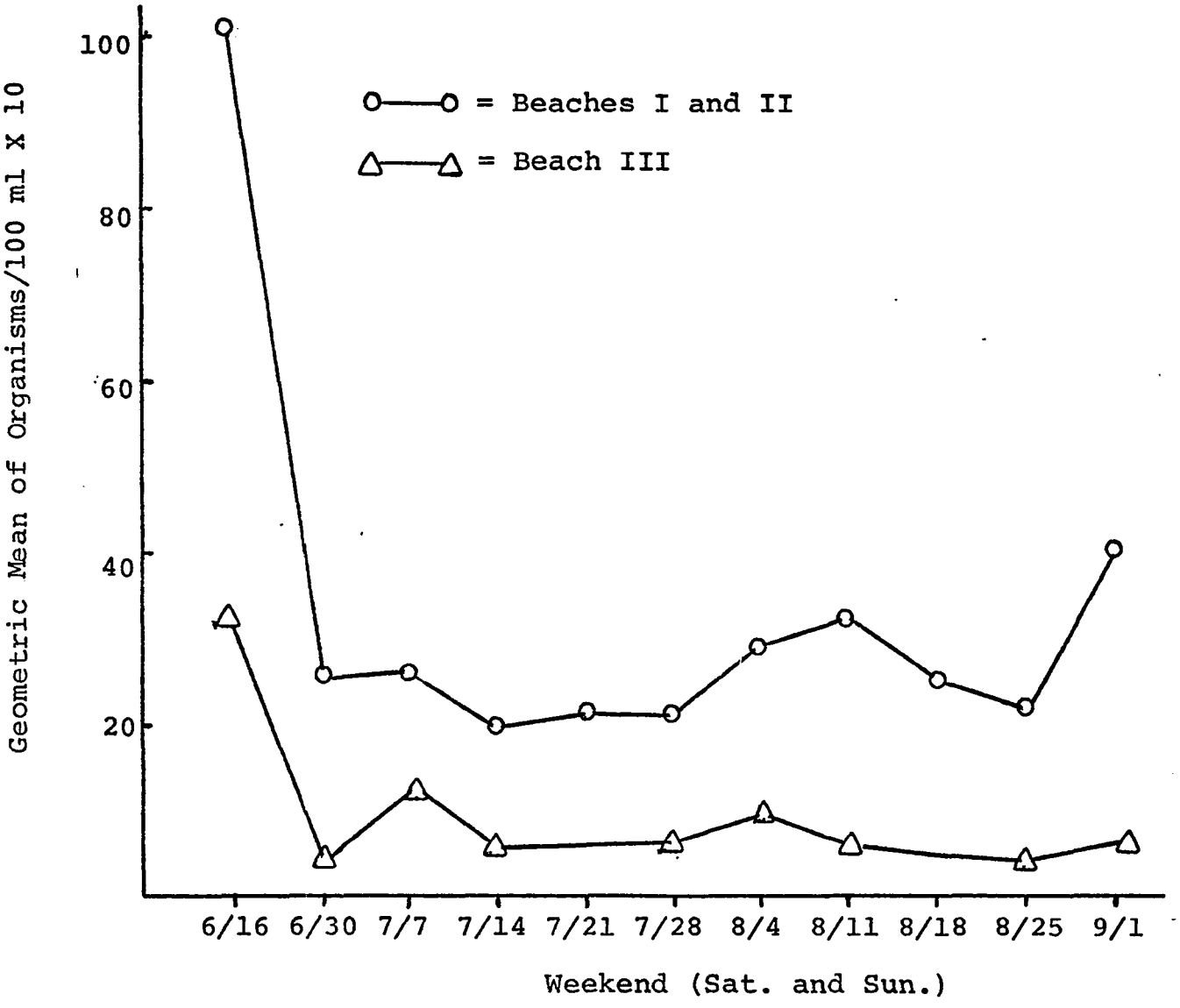


Figure E-4. Weekend Concentrations of Enterococci for Test Beaches I and II and Control Beach III.

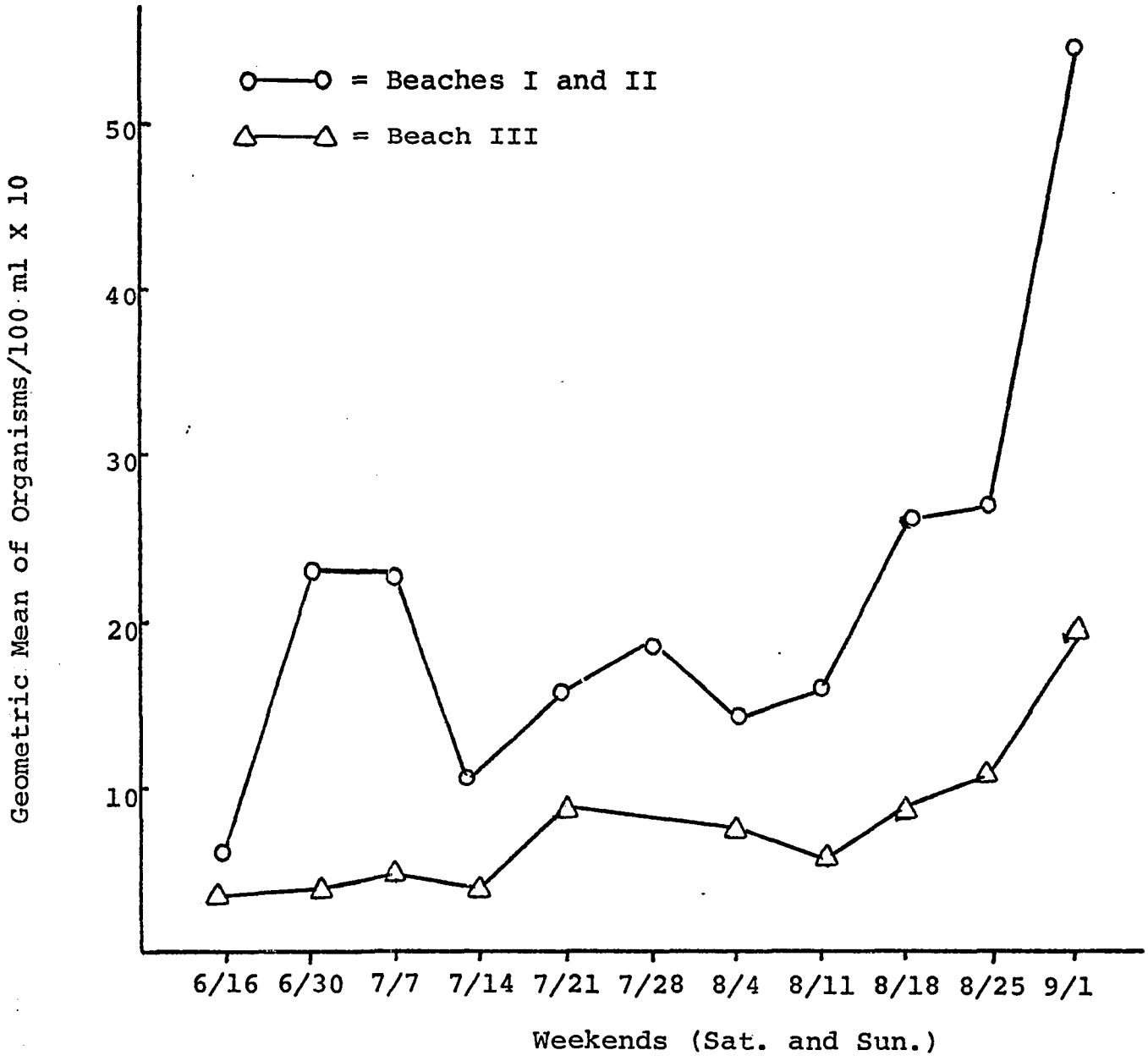


Figure E-5. Weekend Concentrations of Total Coliform for Test Beaches I and II and Control Beach III.

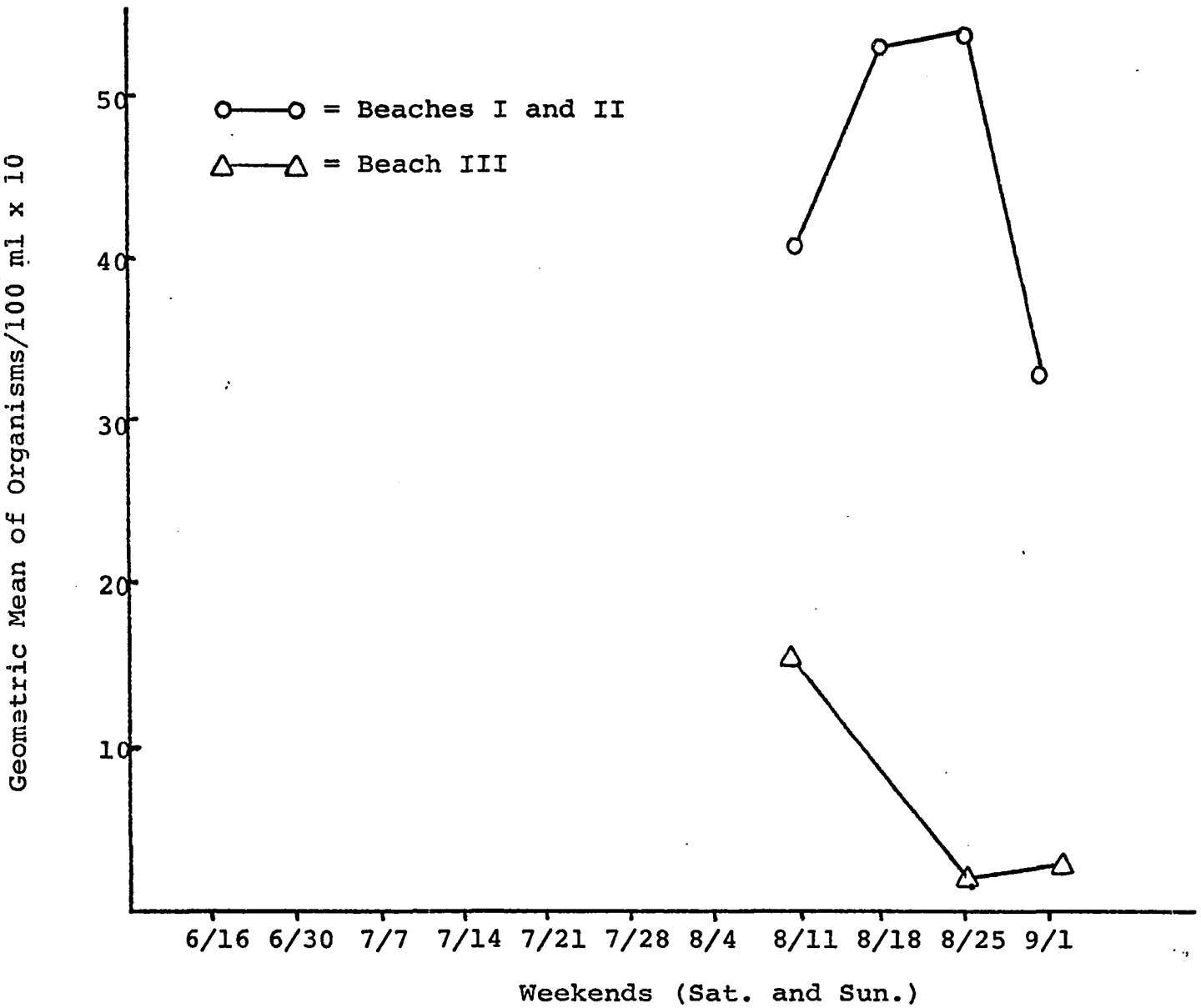


Figure E-6. Weekend Concentrations of Fecal Coliforms for Test Beaches I and II and Control Beach III.

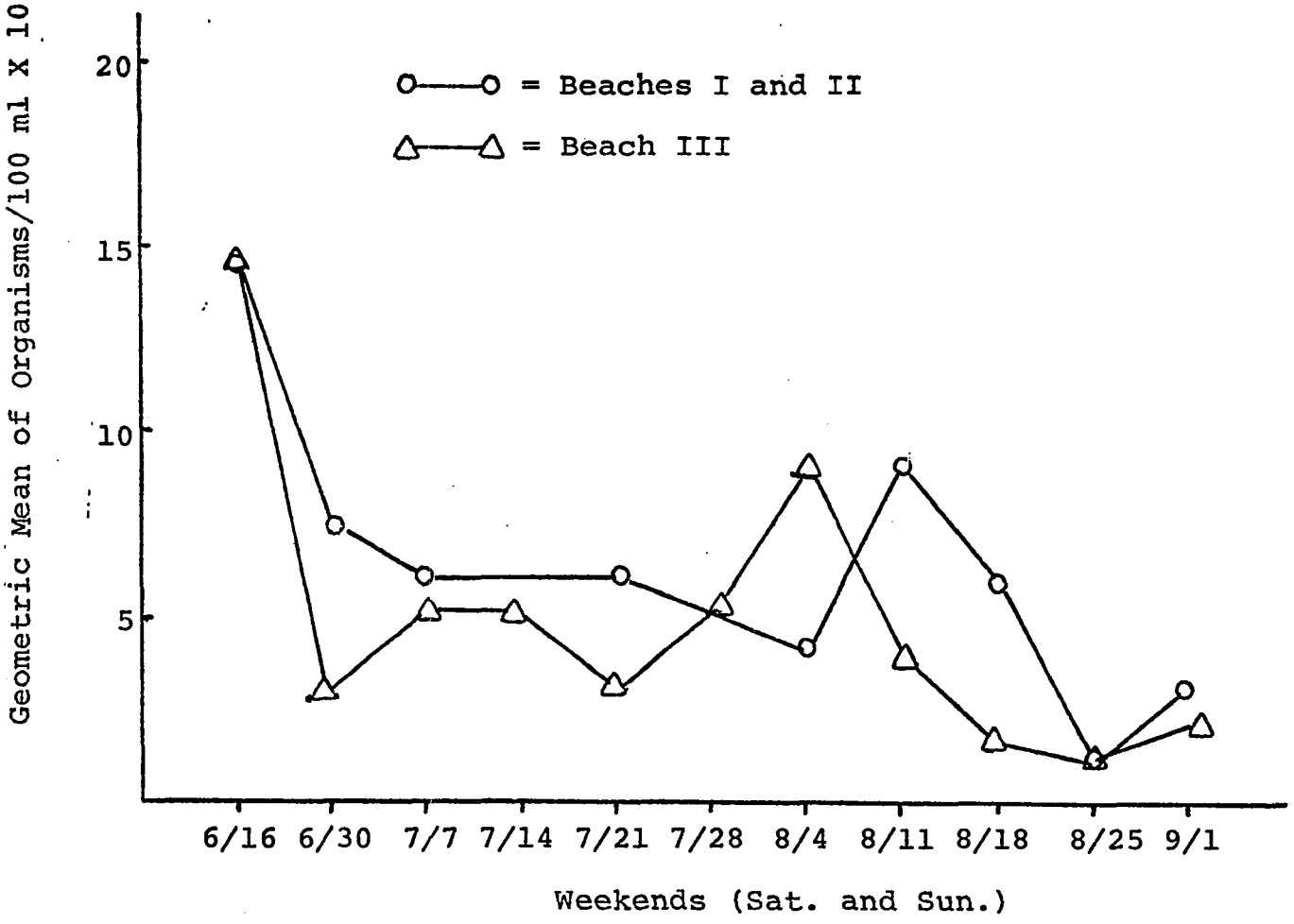


Figure E-7. Weekend Concentrations of Clostridium perfringens for Test Beaches I and II and Control Beach III.

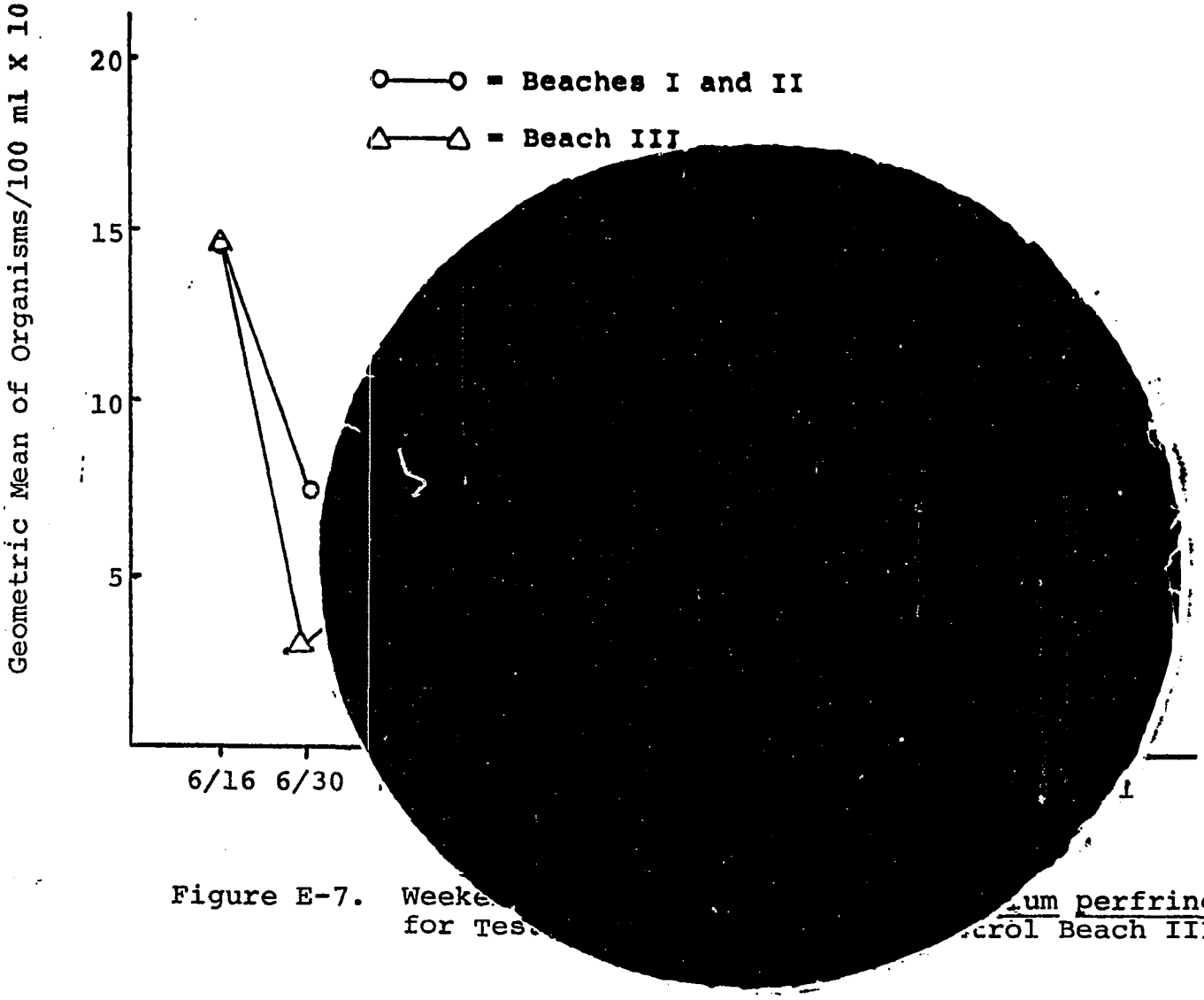


Figure E-7. Weeke… …um perfringens
for Tes… …trol Beach III.

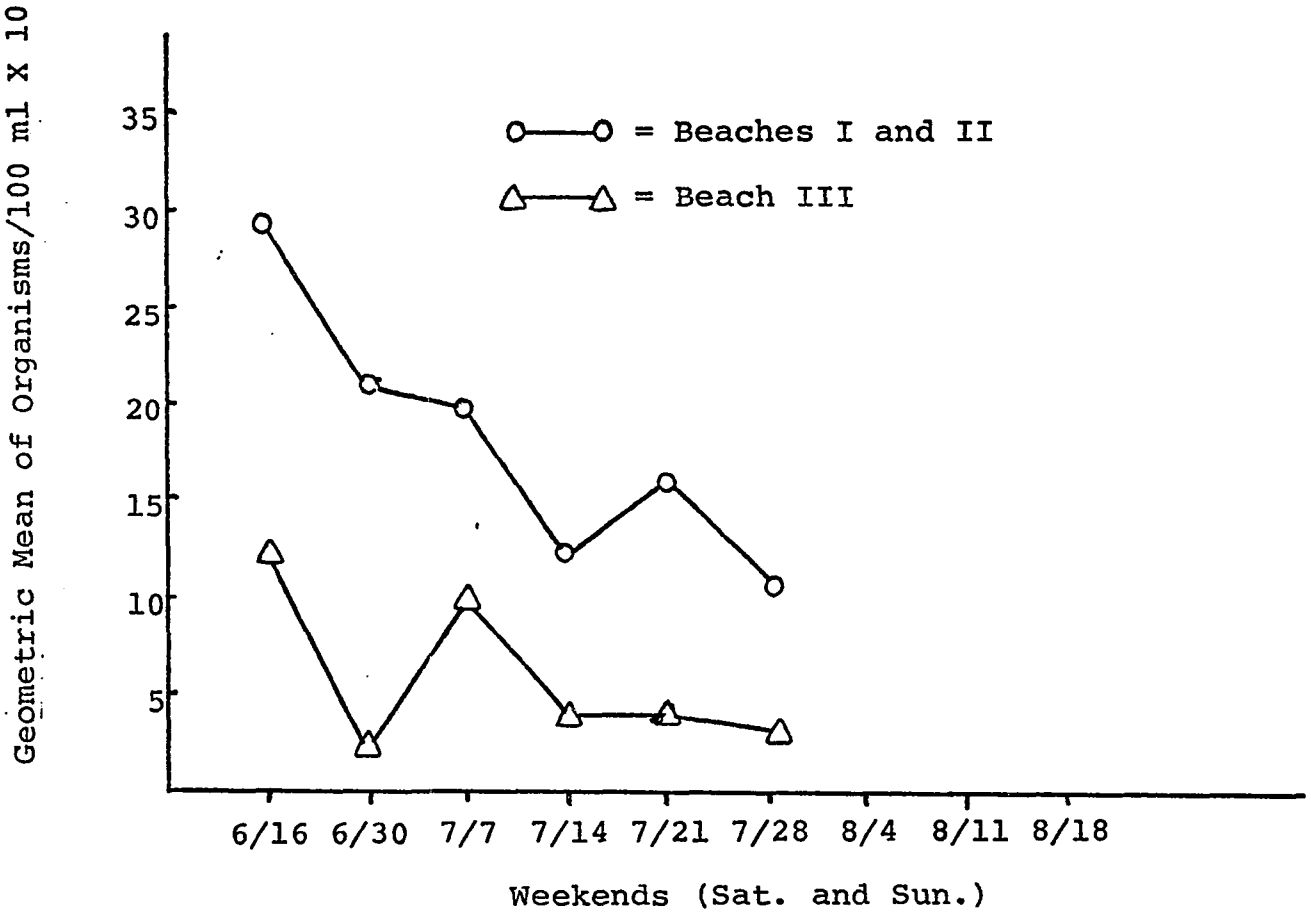


Figure E-8. Weekend Concentrations of Pseudomonas aeruginosa for Test Beaches I and II and Control Beach III.

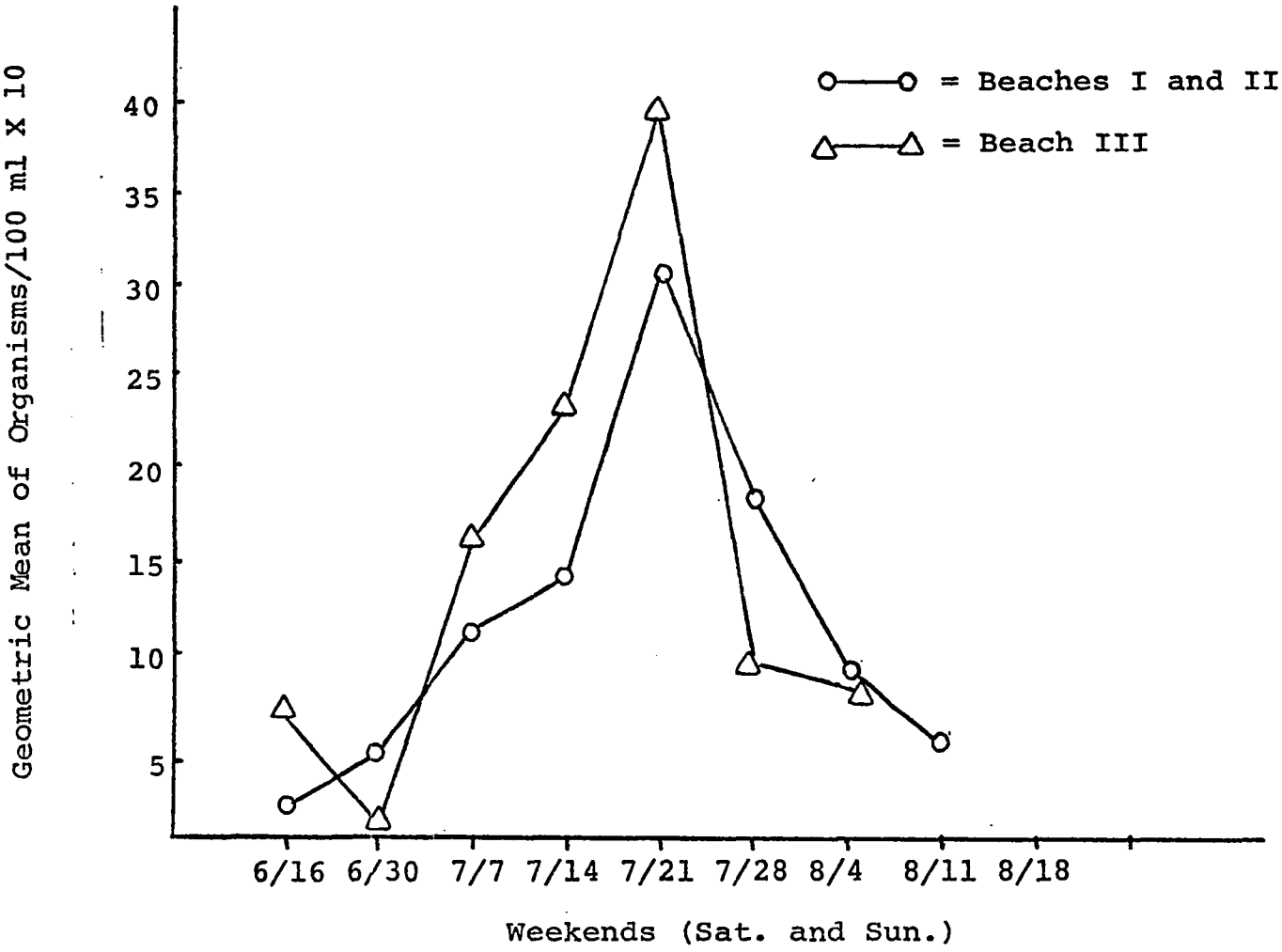


Figure E-9. Weekend Concentrations of Acinetobacter for Test Beaches I and II and Control Beach III.

APPENDIX F

CHEMICAL - PHYSICAL DATA

TABLE F-1 Average Test Results from the Mannford Sewer Plant for the Summer

Final BOD	153.0	Mg/L
Final Suspended Solids	115	Mg/L
Final COD	255.7	Mg/L
Ammonia as N	5.03	Mg/L
COD High Level	255.7	"
Kjeldahl N	22.68	"
Organic N	17.65	"
Settleable Solids	0.3	"
Tot N (Calc)	22.78	"
Tot OrG C	--Sample Rejected--	
Water Temp.	26.5 degrees C.	
BOD (5 Day)	153.0	
D.O.	5.5	
Nitrite-Nitrate	0.1	
pH	7.0	
Suspended Solids	115.0	
Total Alkalinity	149.0	
Total Phosphorous	14.5	
Average Flow June	268,000 GPD	
July	244,000 GPD	
August	250,000 GPD	

TABLE F-2 Average Daily Pool Elevation in Feet

Date	June	July	Aug.	Sept.
1	723.93	726.27	725.57	724.49
2	723.85	726.18	725.61	724.66
3	723.90	726.06	725.74	
4	724.08	726.00	725.85	
5	724.01	725.99	725.95	
6	723.92	726.44	725.83	
7	723.84	726.61	725.64	
8	723.78	726.63	725.46	
9	724.28	726.73	725.13	
10	725.11	726.78	724.88	
11	725.03	726.69	725.04	
12	724.50	726.57	725.23	
13	724.32	726.39	725.30	
14	724.89	726.22	725.12	
15	725.62	725.96	724.85	
16	725.97	725.52	724.63	
17	726.27	725.02	724.39	
18	726.39	724.63	724.22	
19	726.47	724.46	724.25	
20	726.54	724.51	724.21	
21	726.44	724.85	723.95	
22	726.34	725.18	723.79	
23	726.27	725.44	723.65	
24	726.21	725.54	723.78	
25	726.11	725.51	723.96	
26	726.08	725.37	724.12	
27	726.14	725.26	724.23	
28	726.24	725.15	724.52	
29	726.36	725.25	724.78	
30	726.36	725.47	724.65	
31		725.54	724.53	

TABLE F-3 Chloride Results

Composite Weekend	Beach I and II mg/l	Sewage Effluent mg/l
7-7 & 7-8	309	231
8-4 & 8-5	306	133
8-11 & 8-12	212	-
8-25 & 8-26	368	112

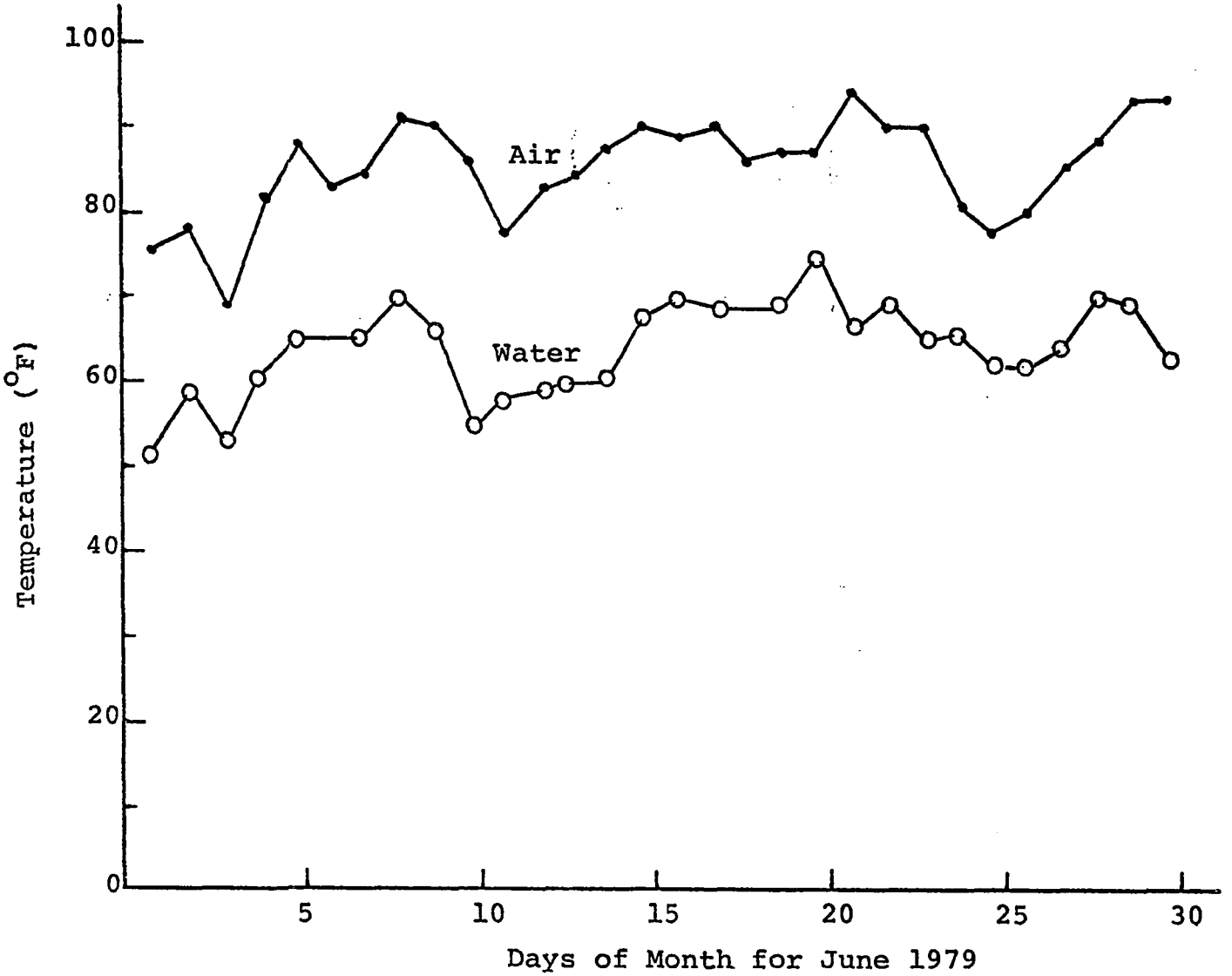


Figure F-1. Air and Water Temperatures for Lake Keystone

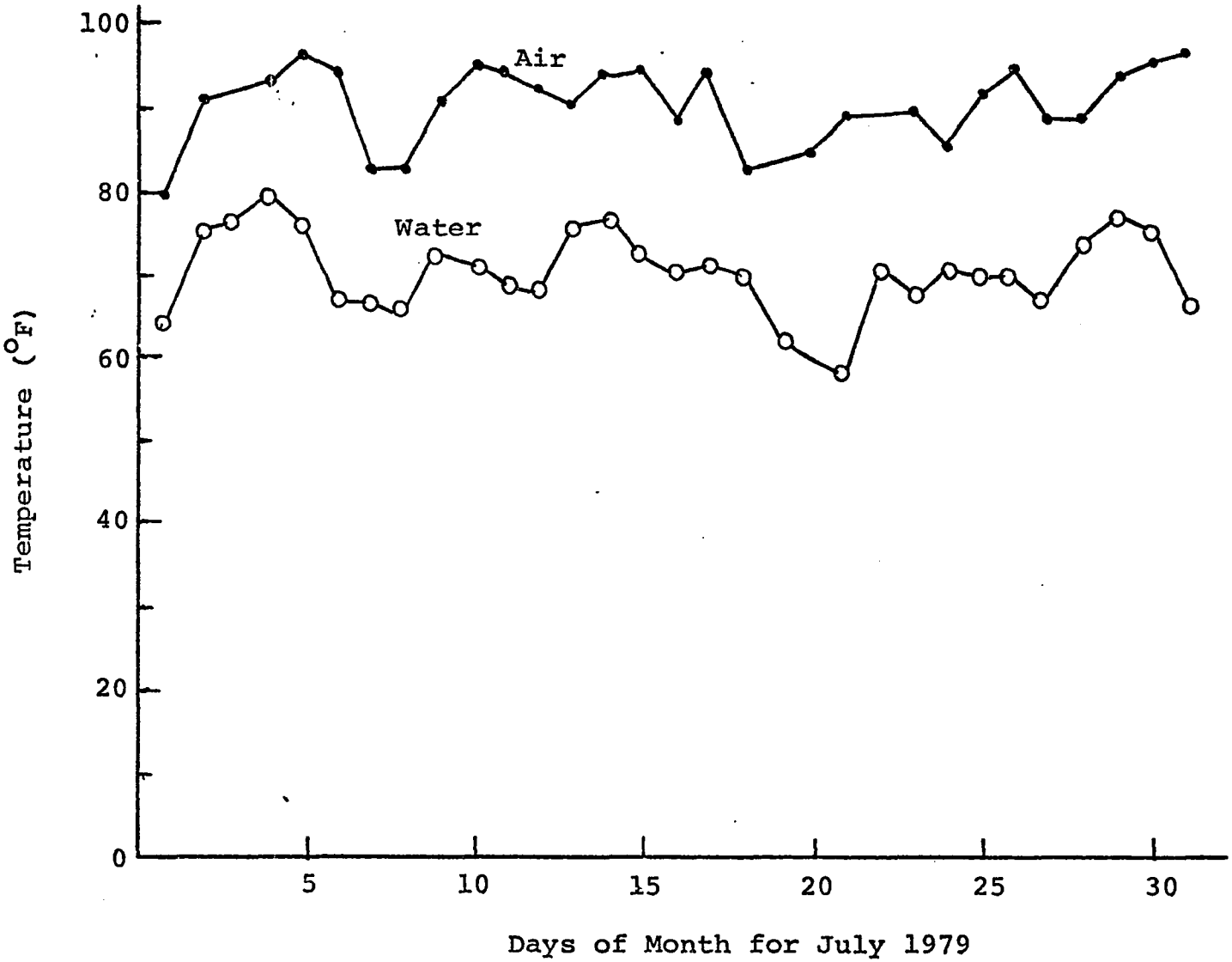


Figure F-2. Air and Water Temperatures for Lake Keystone.

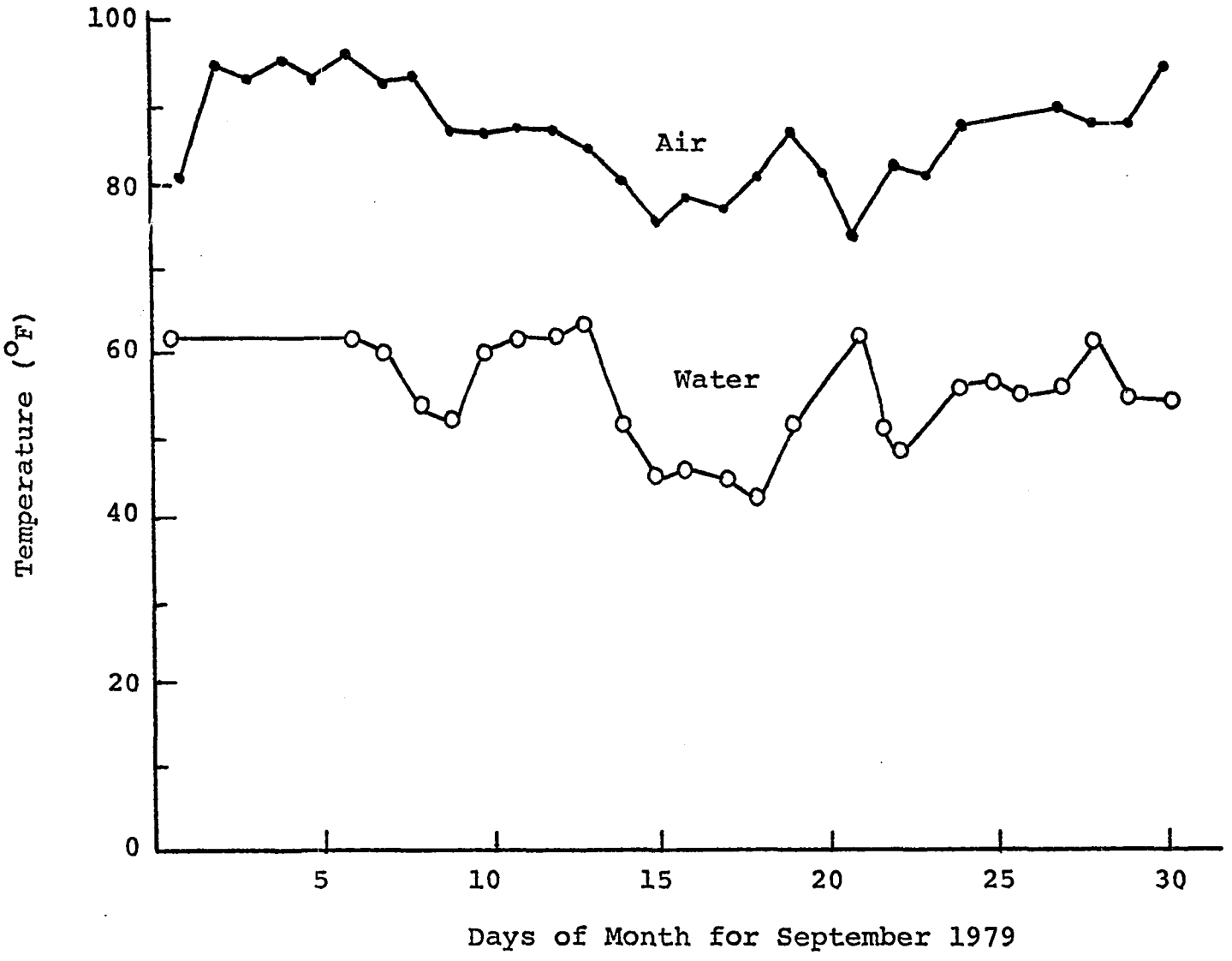


Figure F-3. Air and Water Temperature for Lake Keystone.

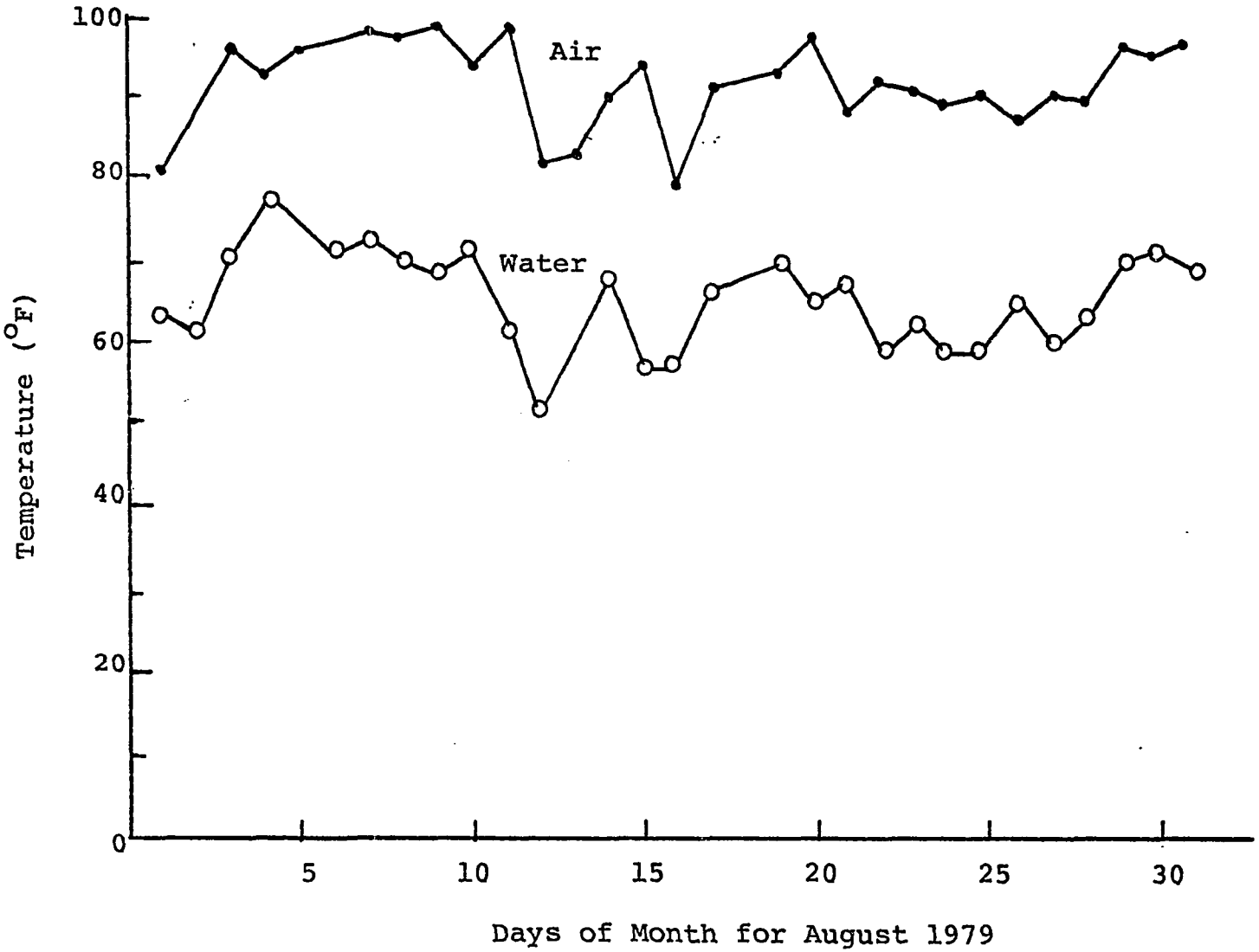


Figure F-4. Air and Water Temperatures for Lake Keystone.

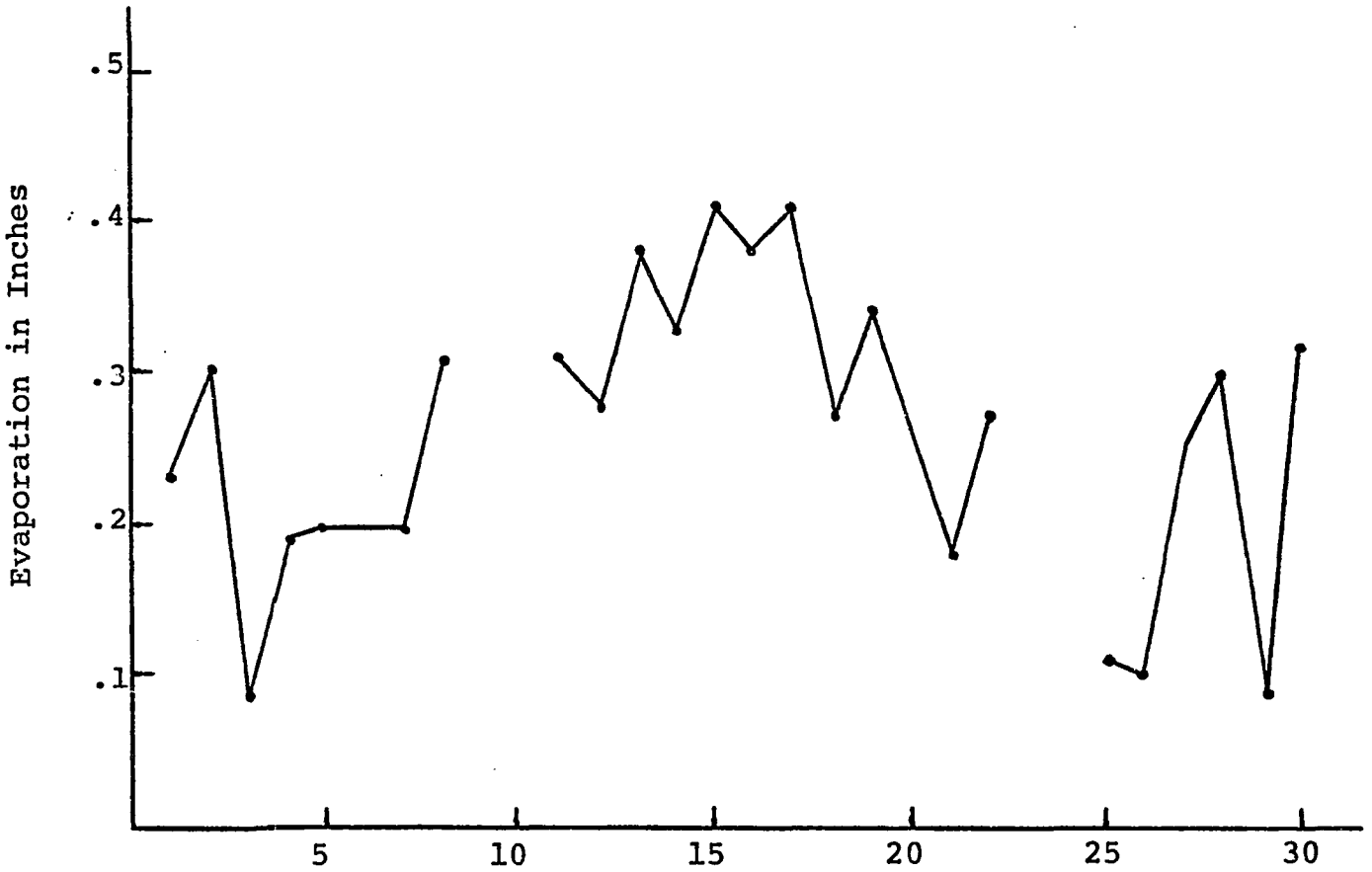
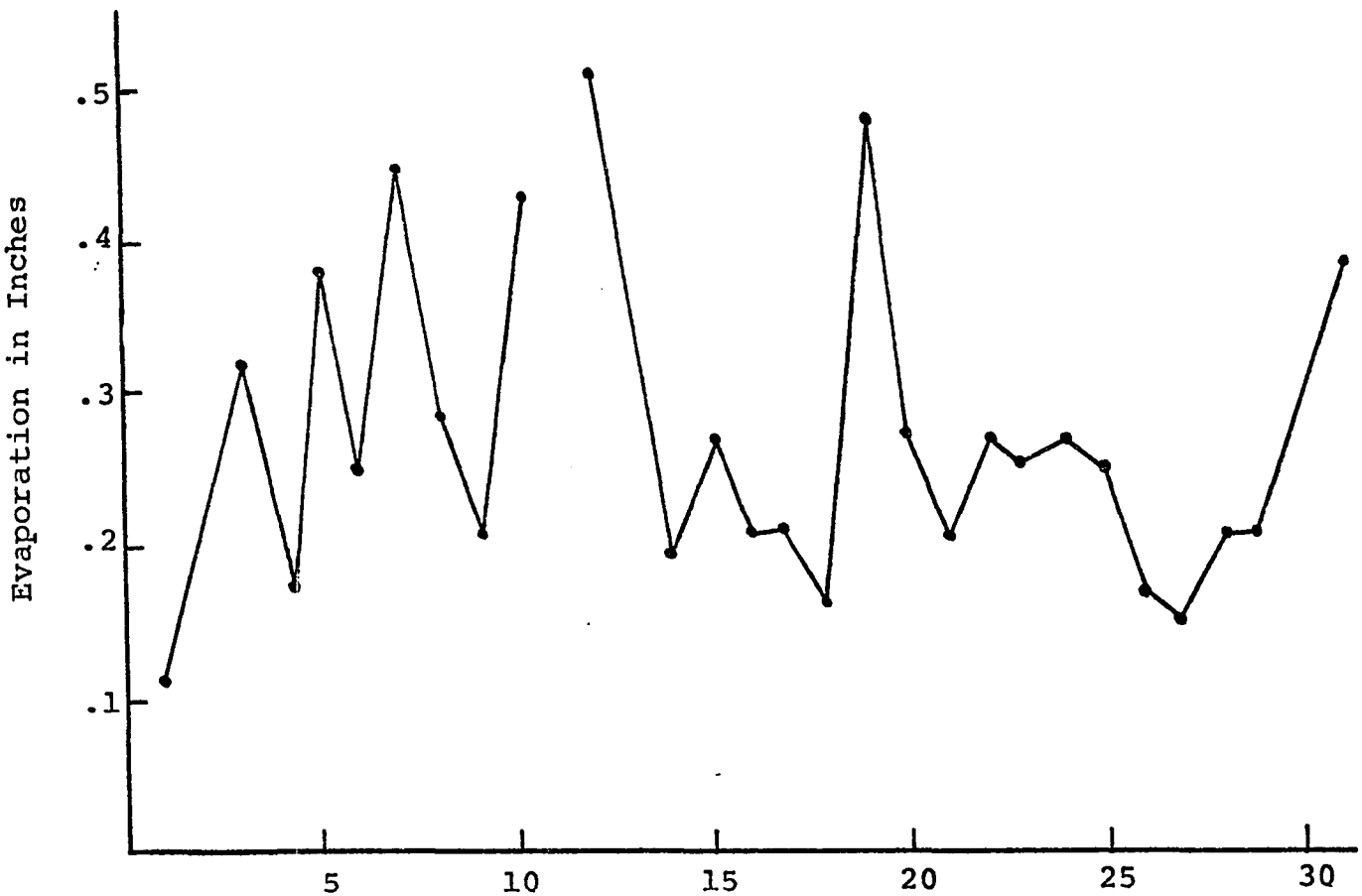


Figure F-5. Rate of Evaporation for Lake Keystone.



Daily Evaporation Recorded During August 1979

Figure F-6. Rate of Evaporation for Lake Keystone.

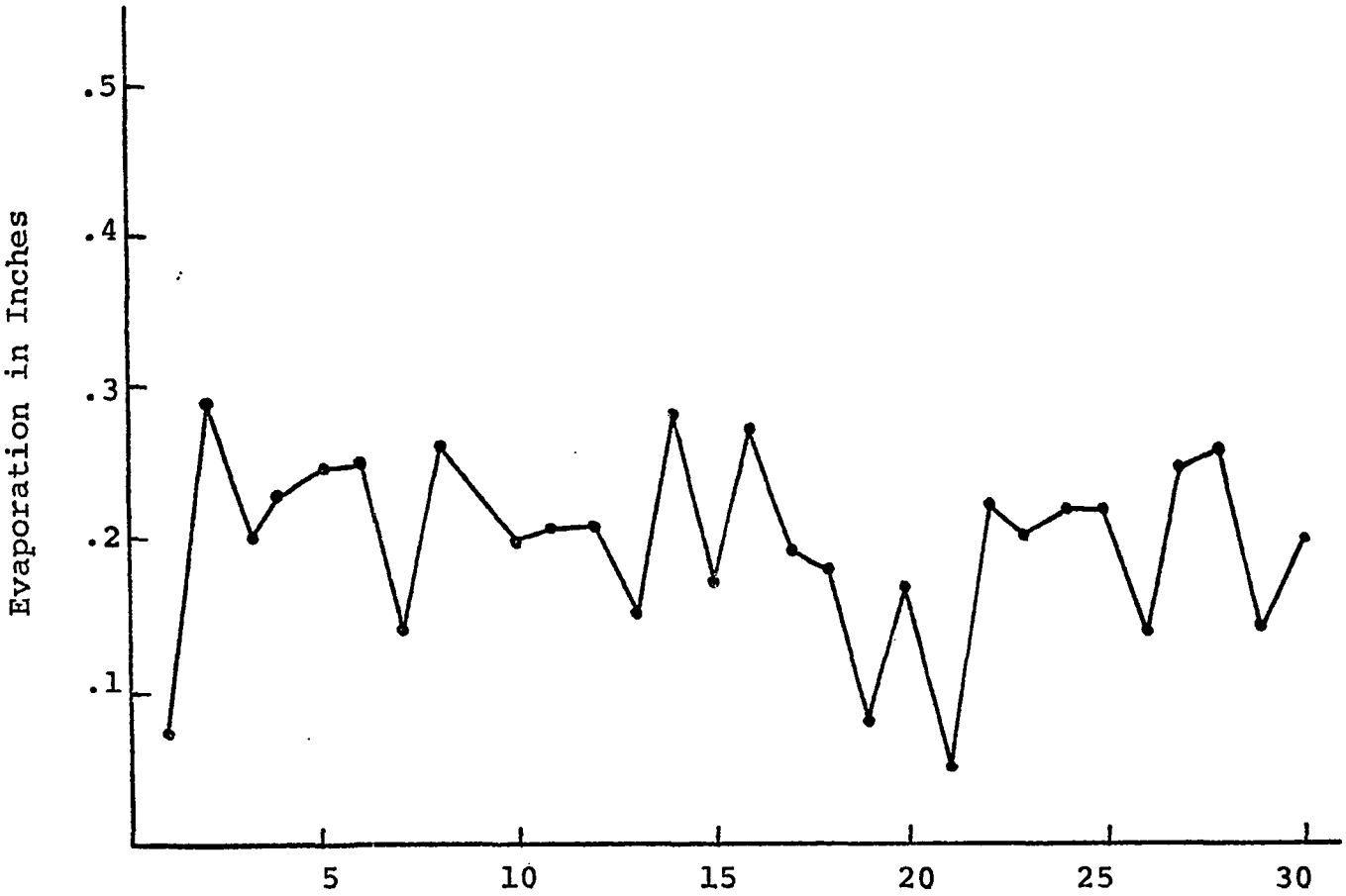


Figure F-7. Rate of Evaporation for Lake Keystone.

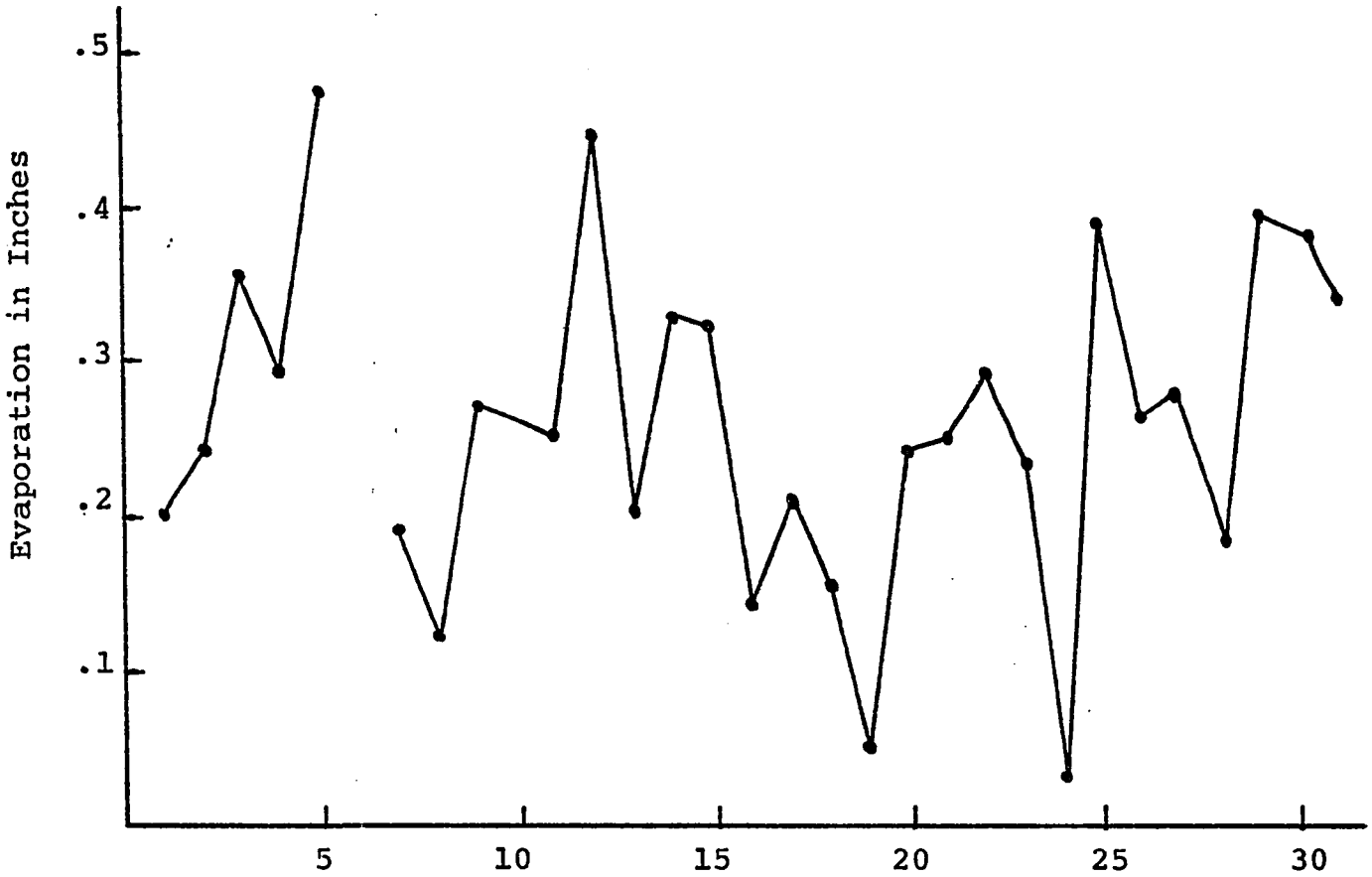


Figure F-8. Rate of Evaporation for Lake Keystone.

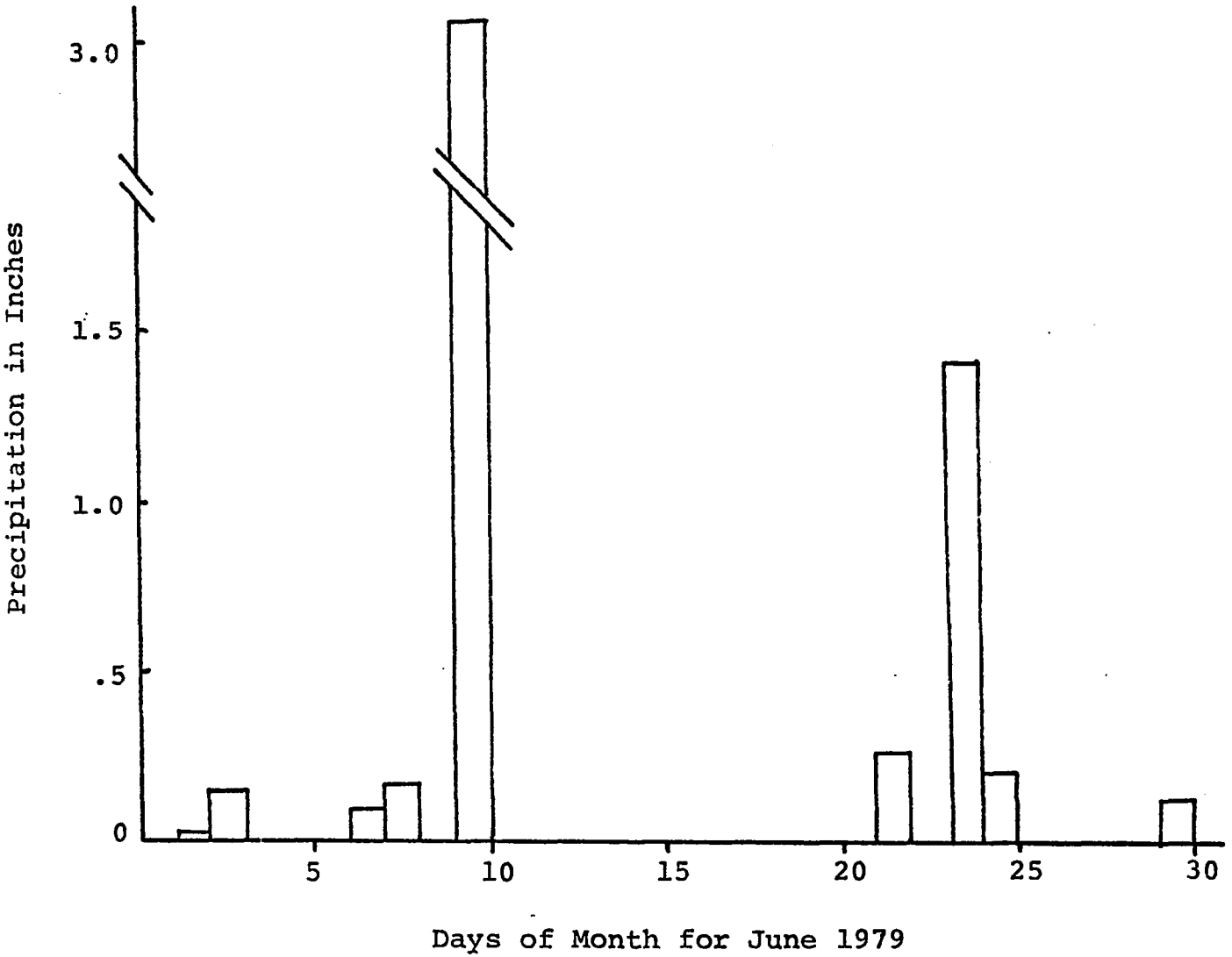


Figure F-9. Precipitation for Lake Keystone.

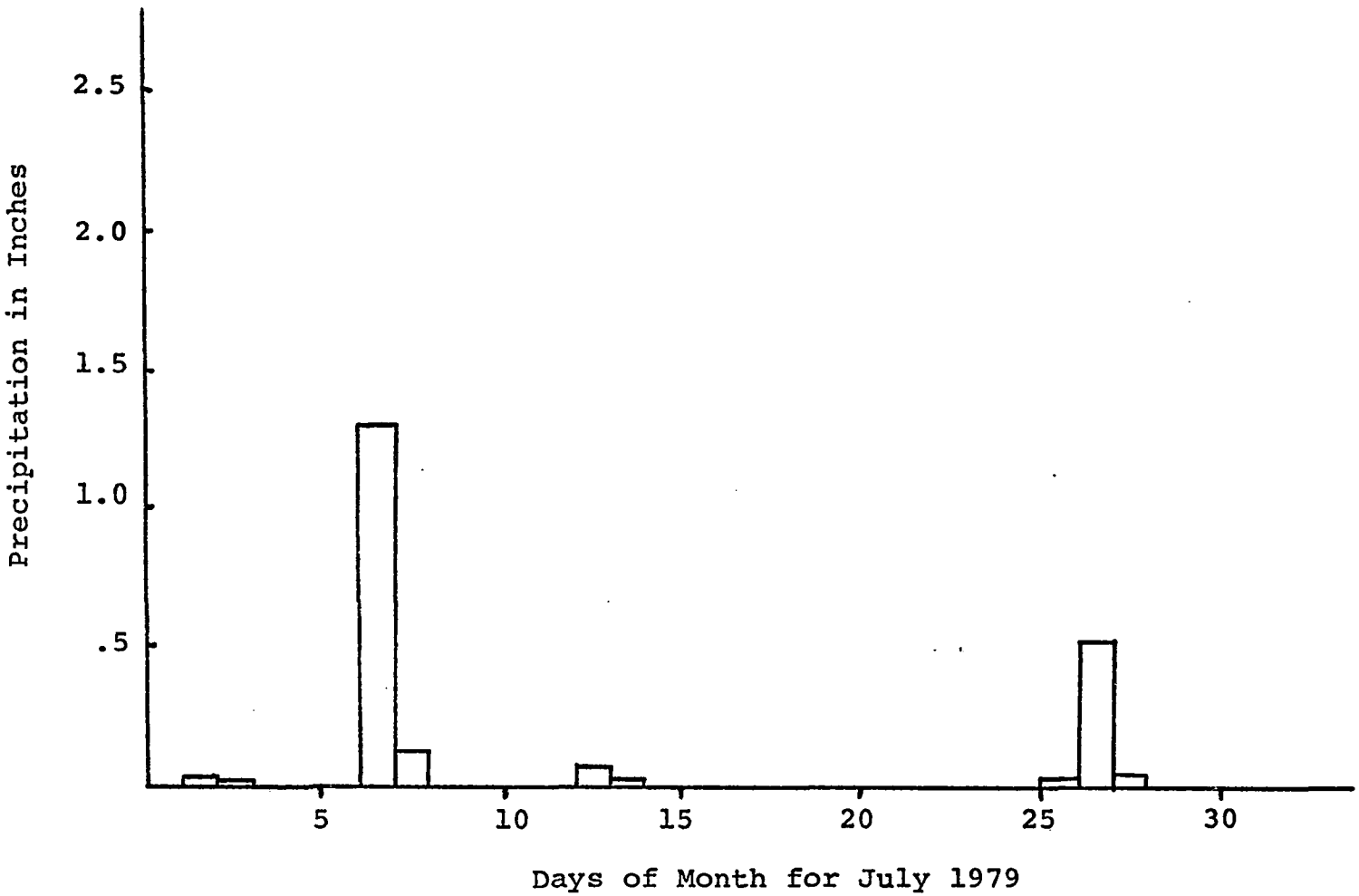


Figure F-10. Precipitation for Lake Keystone.

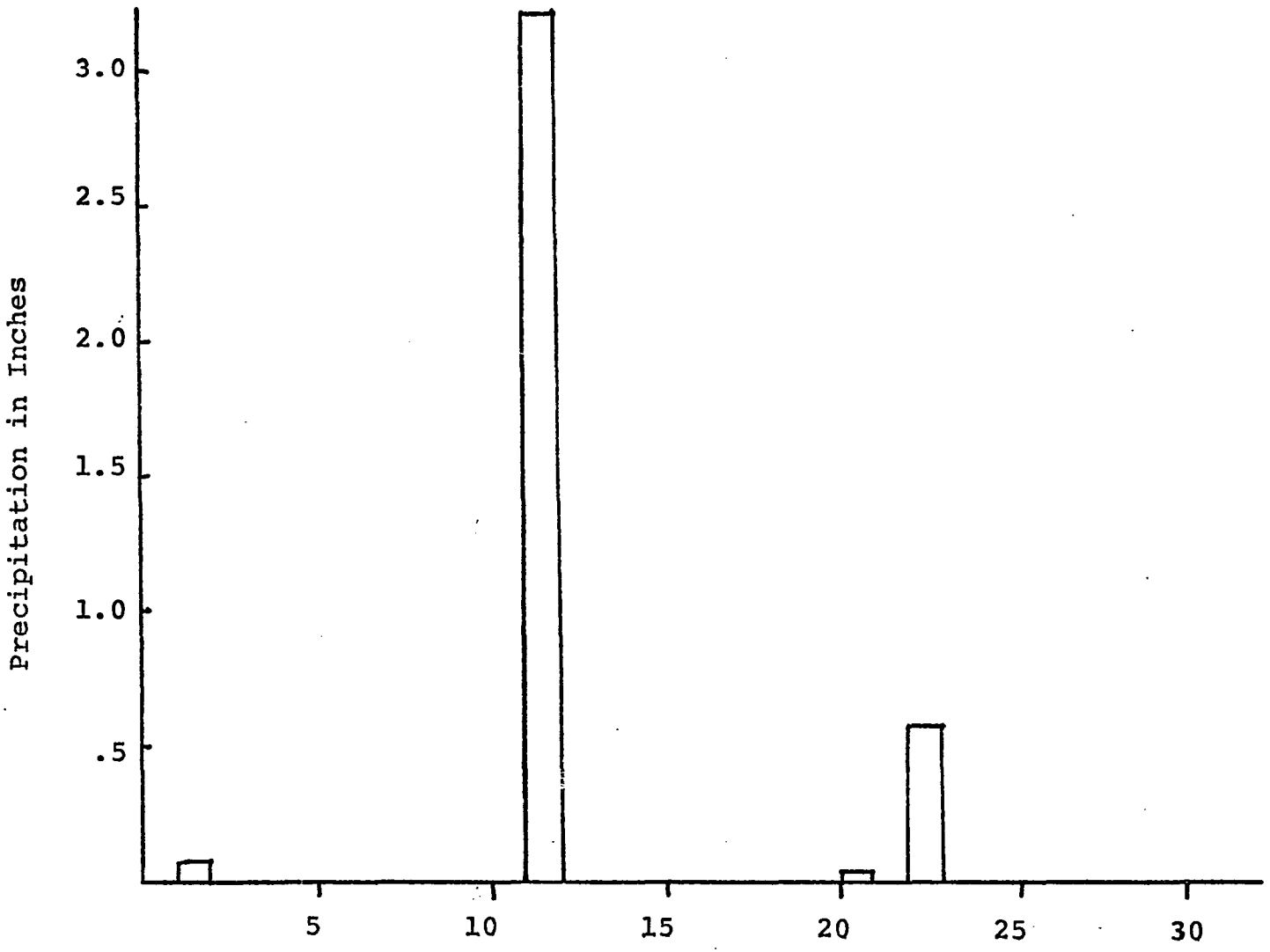


Figure F-11. Precipitation for Lake Keystone.

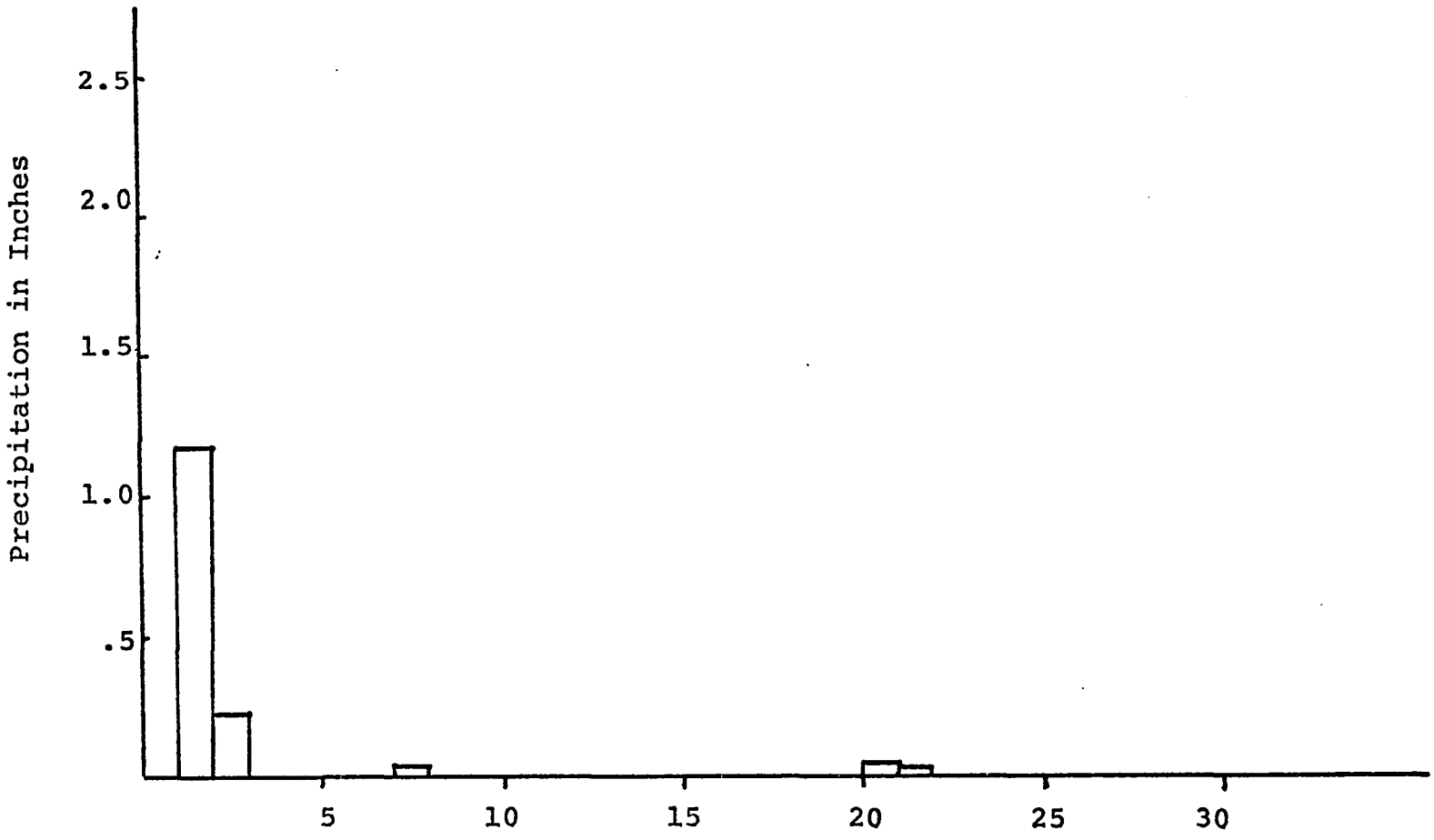


Figure F-12. Precipitation for Lake Keystone.

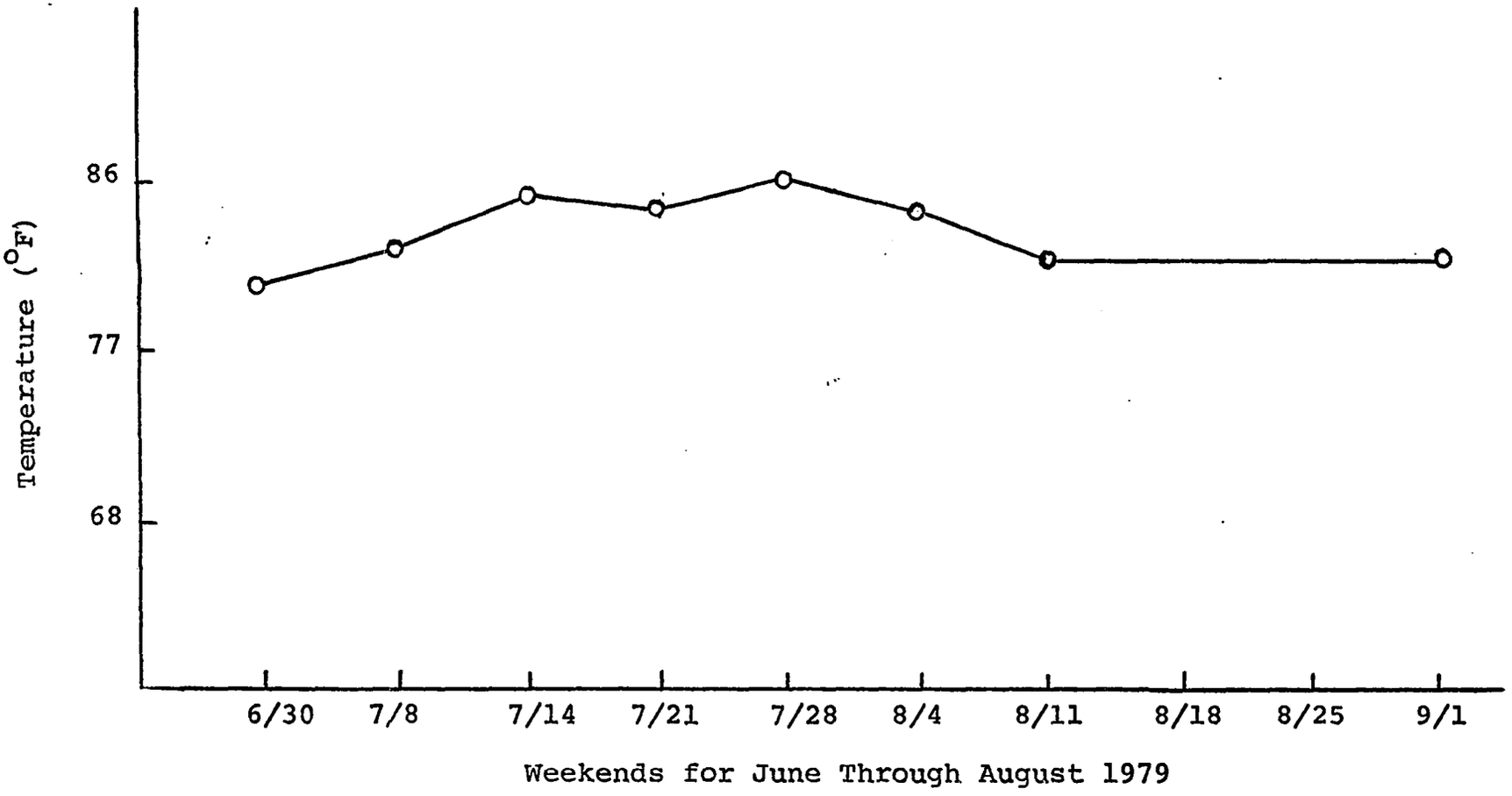


Figure F-13. Weekend Water Temperatures for Lake Keystone.

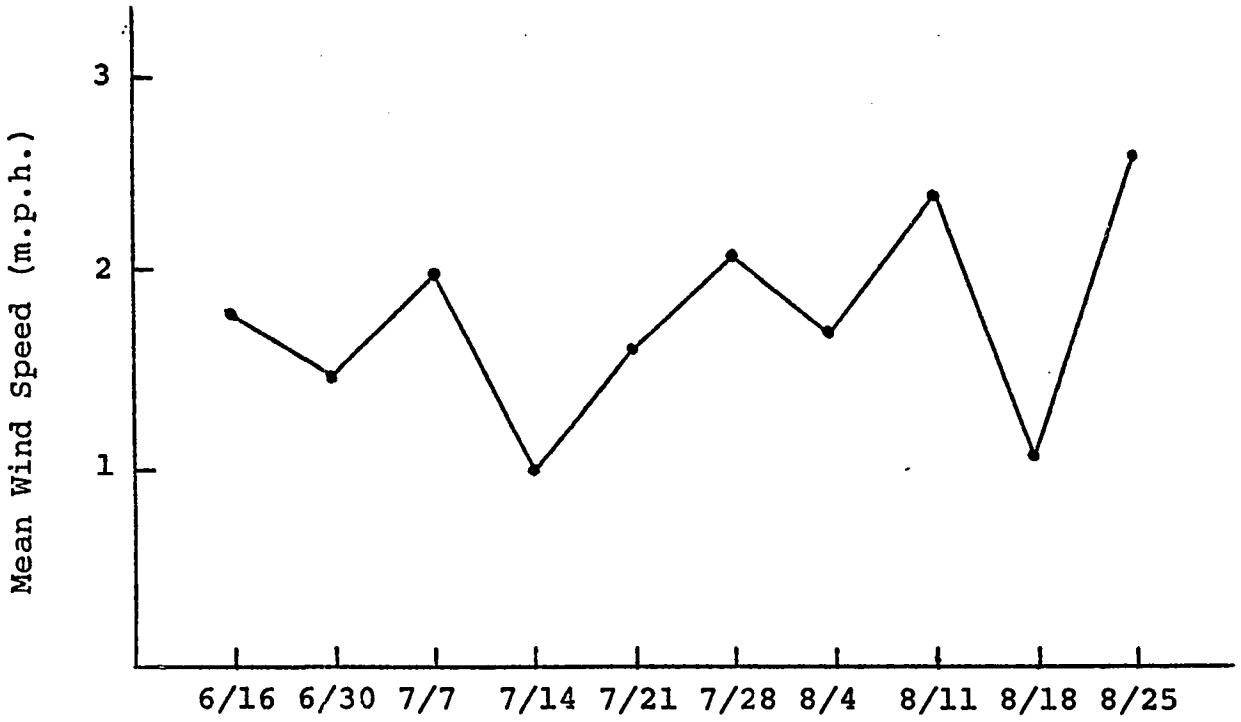


Figure F-14. Weekend Wind Speed for Lake Keystone.

APPENDIX G

HUMAN EXPERIMENTATION CONTROL COMMITTEE REPORTS

The University of Oklahoma 1000 Asp Avenue, Room 314 Norman, Oklahoma 73069 73019

Office of Research Administration
(405) 325-4757

July 20, 1978

Re: HECC Review of Swimming Beach Standards Proposal

Dr. Leale E. Streebin
School of Civil Engineering and
Environmental Science
University of Oklahoma

Dear Dr. Streebin:

The University of Oklahoma Human Experimentation Control Committee has approved your proposed swimming beach standards project, as presented orally by Mr. Garry McKee, contingent upon your making the following written assurances to the Committee:

1. The anonymity of all subjects will be preserved in project reports and other public releases by data coding and/or other appropriate means.

2. The subjects will be provided with the name and telephone number of a designated project member who will answer all questions about the project openly and fully.

3. The subjects will be assured during the initial contact and again during the follow-up telephone call that their participation in the project, including contribution of any information, is completely voluntary at all times.

These assurances are in addition to the assurances and methodology descriptions that Mr. McKee gave the Committee orally during its July 19 meeting. Please send your reply to me for distribution to Committee members.

Sincerely yours,

Mark Elder

Mark Elder
Administrative Officer
Human Experimentation Control Committee

ME:bjg

The University of Oklahoma 202 West Boyd Street, Room 334 Norman, Oklahoma 73019

School of Civil Engineering
and Environmental Science

July 28, 1978

Re: HECC Review of Swimming Beach Standards Proposal

Mr. Mark Elder
Administrative Officer
Human Experimentation Control Committee
Office of Research Administration
1000 Asp Avenue, Room 314
Norman, Oklahoma 73019

Dear Mr. Elder:

All interviewers will be instructed to inform the participants that the anonymity of all subjects will be preserved in project reports and other project releases by data coding and/or other appropriate means. They will be provided with the name and telephone number of a designated project member who will answer all questions about the project openly and fully. They will also be assured during the initial contact and again during the follow-up telephone call that their participation in the project, including contribution of any information, is completely voluntary at all times.

Sincerely,

Leale E. Streebin
Professor of Civil Engineering and Environmental Science

carbon copy to Garry McKee