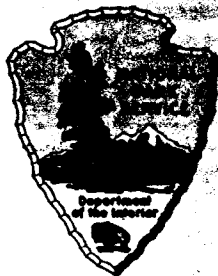


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NATIONAL PARK SERVICE COOPERATIVE RESEARCH UNIT



INSTITUTE FOR MAN
AND ENVIRONMENT
University of Massachusetts at Amherst

BACTERIOLOGICAL ASSESSMENT OF
FRESHWATER PONDS AT CAPE COD NATIONAL SEASHORE
FINAL REPORT

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by

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RECOMMENDATIONS: MANAGEMENT OF THE PONDS IN THE CAPE COD NATIONAL SEASHORE

In 1974 an increase in upper respiratory infections (URI) in the towns near the Cape Cod National Seashore (CCNS) precipitated a bacteriological investigation of the ponds in CCNS during 1975 and 1976. Because of a 1974 study, the ponds were suspected to have harbored and transferred β hemolytic Streptococcus and thus contributed to the rise in URI. The findings of our investigation, which are presented in this report, have elicited the following advice for management of the ponds.

Pollution factors. Bathing-water quality of the ponds in CCNS will vary depending, in part, on the fixed sources of pollution, such as houses on the ponds' perimeters with various sewage disposal systems. The incidence of disease within the nearby towns will also greatly affect the opportunities for disease-producing organisms to be transmitted through the pond waters, depending on the swimmer load; this load could be an important factor not only because it indicates the population at risk but also because it may be a significant source of infectious agents. This causal relationship, however, could not be established. Still, although a relationship between bacterial pollution in the ponds and swimmer load could not be demonstrated, gross observations indicate a potential for future problems.

The physical properties of the ponds, though we have little control over them, are also important since the wind, weather, temperature, and composition of the pond water all relate to the "die-off," settling, dispersion, dilution, natural filtration, and predation of various pathogenic microorganisms that may be present in the ponds.

Risks. The ponds offer three basic areas of risk to the health of the individual bather:

1. infections caused by pathogenic organisms;
2. allergic or debilitating responses to chemical or physical factors; and
3. injuries due to poor pond accessibility or poor visibility in turbid pond water.

Specific recommendations. In recommending recreational policy concerning the use of the ponds, the first priority must be the control of pathogenic microorganisms within the ponds. The most obvious method of control is through the proper handling and disposal of sewage at the beach sites.

The proper disposal of sewage at the ponds, particularly Great Pond (Wellfleet) and Long Pond, is necessary for the protection of the public and the environment. This will ensure that there is no contamination of the surface or ground waters, as well as offer protection to the visitors and wildlife at these sites from the various health hazards associated with sewage contamination. In this respect, all ponds should have some type of sanitary facilities. Presently, only Gull Pond has sanitary facilities.

Along with sewage control are the other necessary sanitary facilities for drinking water and solid waste disposal. If the ponds are not adequately equipped with these facilities, the visitors will be forced to fend for themselves, causing aesthetically displeasing sights of trash and other waste products scattered about, and will allow the possibility of the bathers drinking unsafe water from the ponds. If possible, an extension of a water line to state-approved sources with adequate quality and quantity would be advisable. The proper handling and storage of the solid waste products would alleviate unsightly conditions as well as reduce the incidence of certain diseases.

In addition, adequate toilet and handwashing facilities should be established in proportion to heavy swimmer-load. Furthermore, limiting the number of swimmers per pond would minimize gross environmental impact and simultaneously reduce the probability of health hazards.

An assessment of sewage disposal facilities of all houses and structures around the ponds should also be undertaken. Inspections of all facilities by the local health sanitarian should be made in order to insure a policy of noncontamination of the water table. Also, a program of routine pump-out of septic tanks will minimize problems.

The major cause of death at recreational areas is due to accidents and drownings. Accidents occur most frequently when people are traveling to and from recreational sites, so a careful observation should be made of the accessibility of each pond. Great Pond (W), in particular, has an extremely hazardous access route that also causes an increased rate of erosion into the pond, which contributes to an increase in cultural eutrophication. The installation of a proper access route at Great Pond (W) would not only be a safety and ecological feature but would aid in the more even dispersal of swimmers at the pond.

The distribution of swimmers should be proportional to the beach frontage available. Gull Pond is sometimes greatly overused while
 ✓ Great Pond (W) is underused. A system should be employed to distribute the swimmers more evenly. One method might be to decrease parking space at Gull Pond, specifically at the upper parking areas where the soil is eroding into the pond, in conjunction with an equal increase of parking space at Great Pond (W). Each area should have the facilities and access designed so as to evenly distribute the bathers along the beach frontage.

Animals of all kinds should be eliminated from the ponds wherever possible. It has been shown in the literature that wildlife can carry human pathogens (25) and can distribute them in the environment. This problem could be partially eliminated with the mandatory exclusion of all domestic animals from the bathing areas as well as careful disposal of animal wastes, specifically of dogs and horses, in an area away from the ponds. Accumulated manure is a breeding ground for flies as well as a source of pond pollution in rainwater runoff. The proper control and disposal of animal wastes is also important in the control of certain communicable diseases, such as salmonellosis and shigellosis.

Horse stalls should be properly situated so that drainage into the ponds cannot occur. The horses kept near Long Pond, to cite a particular case, may be a factor in the high bacterial counts obtained from that pond.

The natural physical characteristics of the ponds were within the limits of toleration to the bathers' health. The water temperatures were fairly constant between 23°C and 28°C throughout the summer. The major concern of high water temperatures would be the loss of heat-dissipating abilities, with subsequent significant changes in the aquatic biota. Furthermore, high temperatures with the proper nutrients available would encourage bacterial multiplication. The pH of the water was between 5.0 and 9.0. Any waters varying significantly from these values may cause severe eye irritation. The eye has great buffering abilities, but if the lacrimal fluid deviates 1.1 pH from the normal pH of 7.0, irritation and pain can result.

Water clarity is a physical safety factor; a low Secchi disk reading (less than four feet) indicates a hazard to the bathers walking or diving in areas around the docks. Clarity is not only important from safety and aesthetic points of view, but it is also important in that it reduces light penetration. The decrease of ultraviolet rays would cause an increase in bacterial growth if the turbidity were due to suspended particles. We should, therefore, closely monitor the ponds for signs of cultural eutrophication, for it brings with it a decrease in clarity and the possibility of an increase in algal blooms and bacterial growth.

It is essential that external chemical pollution be kept out of the ponds. It is advised that along with sanitary facilities, showers be installed; and it should be made mandatory that any person coming from swimming in the ocean take a cleansing shower before entering the ponds. The reason for such showers should be posted. The use of soap and shampoos at the ponds should be prohibited and this regulation strictly enforced. The reason for this is that the above compounds contain high quantities of phosphates, which add nutrients aiding in the growth of microorganisms.

The environmental health factors discussed here are a necessary consideration for the complete enjoyment and satisfaction of the town residents and vacationers who use the ponds. The installation of sanitary facilities, though a major cost factor, is justified by the fact that they lead to optimal development of the ponds for recreation. If these basic environmental health needs are neglected, the health of the swimmers will be endangered and the ponds as a recreational source will not be developed to their full potential.

Furthermore, visitors might be much more respectful of the ponds and take care of them if information plaques are posted, giving the history and ecological nature of the ponds plus specifying means of reducing pollution. Naturally, it should be stated that any restric-

tions placed on the use of the ponds are done for the safety of the bathers and preservation of the ponds.

Finally, implementation of a periodic program of assessment of both microbiological and limnological parameters would monitor the ponds for the presence of public health and environmental hazards.

The availability of such data to those officials concerned will aid in the rational management of a valuable resource.

STATEMENT OF PROBLEM

In the summer of 1974, a student from Tuft's University, doing bacteriological analysis of water from the various freshwater ponds on Cape Cod's National Seashore, plated water on blood agar plates and obtained hemolytic colonies, which he called β hemolytic Streptococcus (18). His findings were corroborated by local physicians (16) who had suspected that a high incidence of upper respiratory infection (URI) in the population during the summer months was due to swimming in the ponds.

The National Park Service was made aware of the problem, and in the winter of 1974-1975, a meeting was held at the Cape Cod National Seashore Headquarters in Wellfleet, Massachusetts in which one of the authors (45) was present. At that meeting, it was decided that a full investigation be conducted in order to determine, mainly, if β hemolytic Streptococcus was actually present in the ponds, and also to determine to what extent swimming in the ponds contributed to URI in the community.

This report incorporates the findings of bacteriological work conducted during the summers of 1975 and 1976. The data, however, are limited to those ponds that were found to be of suspicious character in the summer of 1975. Statistical analysis comparing data from 1975 to data from 1976 was done only for Long, Great (Wellfleet), and Gull Ponds, using Duck Pond as a control (see page 19).

Bacteriological classification of isolates incorporates both isolates from 1975 and 1976. Bacteriophage typing of Staphylococcus aureus, on the other hand, is based only on isolates from 1975 clinical and pond samples.

LITERATURE REVIEW

The role water plays in the transmission of diseases has been well established. Even in ancient times, water and air were considered two important vehicles of disease dissemination. The words "mal-aire" and "miasma" were coined to describe illnesses thought to be related to air and water respectively. Most of the major waterborne disease epidemics have been due to contamination of drinking water with human or animal feces or urine (28,50). The first incident cited in the literature is the control of an epidemic of cholera in England through the relocation of a water pump above the sewerage effluent outfall (53). Recent studies have centered on more specific disease-producing etiologies, among them cholera (24,30), typhoid fever (salmonellosis) (23, 42, 61, 62), shigellosis (23, 51), leptospirosis (17), enteropathogenic E. coli (14, 50, 51), hepatitis (3, 37), amebic encephalitis (7), and possibly polio and other viral diseases (10, 47).

Attention has been given to the role swimming plays in the transmission of diseases in recreational waters. Although many instances of swimmer infections have been cited, few have been clearly proven through microbiological and epidemiological studies. In many instances, the association with a measured indicator of water quality has been vague. Some confirmed cases of the role that water used for swimming plays in the transmission of disease are:

1. "swimmer's itch," due to schistosomes of nonhuman origin (13);
2. swimming pool conjunctivitis (5);
3. swimming pool granuloma (58);
4. "swimmer's ear," an otitis externa whose major etiological agent is Pseudomonas aeruginosa (33); and
5. skin rash due to Pseudomonas aeruginosa (31, 39, 41, 43, 44).

An apparent epidemic of typhoid fever in New Haven, Connecticut, was attributed to bathing in contaminated waters (61), but a definitive conclusion could not be reached, because of a lack of epidemiological data and confirming bacteriological studies.

One major limitation of recreational water studies is the fact that most infections transmitted through swimming are not reportable and can also be transmitted by other means. This problem is evident in all studies done to date. However, one well-controlled investigation supported by the USPHS found that 50% of all swimming-related ailments were eye, ear, nose, and throat infections, while gastrointestinal complaints accounted for 20% and skin irritations and other ailments accounted for 30% (57). This study of bathing practices concluded that illnesses were higher among swimmers than nonswimmers regardless of the water quality. The swimmers showed no significant difference in the prevalence of illness when comparing high level to low level of pollution. There

was, however, a definite suggestion of a relationship, with gastrointestinal disorders being slightly higher among swimmers at beaches that were considered barely acceptable. This study is in contrast to other epidemiological studies clearly showing that individual cases of disease or the rate of illnesses associated with the use of recreational water are correlated with a given level of pollution as measured by any of several indicators of water quality (8).

The presence or absence of actual pathogenic organisms is the logical indicator of water quality. Because of their low numbers and the difficulty involved in isolating pathogens, however, this is impracticable for routine use. For this reason, the indicator of choice has been the coliform group, based on earlier studies by Escherich (21), who equated the presence of coliforms with the close association of feces of warm-blooded animals. This choice was based on the following criteria:

1. coliforms are present in higher numbers than other pathogens;
2. they are not able to proliferate to a greater extent than other pathogens when in the environment;
3. they are more resistant to environmental conditions, thereby surviving longer than other pathogens;
4. they are absent when other pathogens are absent; and,
5. they are readily and easily identified with simple laboratory procedures.

The use of the coliform group as an indicator of the sanitary quality of the water has long been practiced by microbiologists in the health field. Using coliforms as an indicator of the relative chance of intestinal infections has been significant in the past reduction of enteric infections. Recently, however, the use of total coliforms as an indicator of water quality has been questioned. The relationship of total coliforms isolated from water to the occurrence of fecal contamination is variable, at best. The use of coliforms as an indicator has been investigated and some major deficiencies have been found. It has been demonstrated that coliforms can grow and multiply in a new environment (19) with a 10- to 40-fold increase within 30 hours of discharge (20).

On the other hand, environmental stress has been found to have an effect on the recovery of coliform organisms from the environment (2). When pure cultures of E. coli and Streptococcus fecalis were exposed to aquatic environment, a significant proportion of the cells lost their ability to produce colonies on selective medium, yet retained this capability on a nutritionally rich nonselective medium. It seems, therefore, that these organisms survive much better along with other bacteria in the environment than when they are in pure form.

While total coliform as an indicator of fecal pollution has been losing acceptance, fecal coliform has been gaining in popularity. Investigations have shown that fecal coliforms show a positive correlation with warm-blooded animal fecal contamination. Many scientists have suggested that a test be developed whereby all fecal pollution from nonhuman sources could be disregarded. This would be in definite conflict, however, with basic public health goals since human pathogens have been isolated from most warm-blooded animals (25).

In addition to fecal coliforms, the use of fecal Streptococcus has become widely employed as a pollution indicator. One of the major drawbacks concerning fecal Streptococcus is the fact that S. fecalis var. liquifaciens may frequently be found in substantial numbers even when there has been no recent contamination. The inability to differentiate among fecal Streptococci without lengthy biochemical analyses thus limits its sanitary use (25, 35, 48).

The absence of coliforms from natural recreational water has been taken to indicate the absence of potential intestinal pathogens (38). However, recent studies indicate that pathogens may be present in water even when there is an absence of coliforms (19, 53). An outbreak of salmonellosis in Riverside, California was confirmed when Salmonella was isolated from drinking water containing less than 2.2 coliforms per 100 ml (19). In Galilee, Israel, Salmonella was isolated from the drinking water containing ratios of Salmonella to coliforms of 5:0, 5:1, 1:4, 5:4, and 5:2 (52). These cases illustrate that coliforms are not the ideal indicators of water quality they were once thought to be. As a matter of fact, Salmonella has been proposed as an index of pollution of surface waters, by Cherry and Hanks (11). They used both cultures and fluorescent antibody (FA) techniques to test for the presence of Salmonella from waters in the natural environment. The FA test proved to be a highly sensitive tool for the detection of Salmonella. Their significance as pathogens may make them superior to coliforms as indices of water quality from the public health point of view.

Another pathogen that has continuously been isolated from waters is Klebsiella pneumonia. The presence of Klebsiella pneumonia in surface waters (36, 40) was found to be ubiquitous since it was isolated concomitant to E. coli.

Even the use of fecal coliform and fecal Streptococci as indicators has been questioned because of their omnipresent relationship to the environment (1, 19, 26). In their place, the use of Pseudomonas and Staphylococcus has been suggested as indicators of recreational water quality (8, 22).

Intestinal diseases are only a minor part of the recreational water quality criteria as the organisms of concern are usually Salmonella and/or Shigella. The danger of disease transmission in swimming waters is greater when the pathogens are derived from the skin and the upper respiratory tract.

Pseudomonas aeruginosa has been confirmed as the primary etiological agent of "swimmer's ear" (33) and has been incriminated as the casual agent of skin rash in swimmers (31, 34, 39, 43, 44). In this occurrence, Pseudomonas of the same phage type was isolated from both the swimming pools and the infected skin of persons swimming in them. The outbreak was attributed to both heavy bather load and low disinfectant levels.

More recently, Jacobson et al. (31) showed that Pseudomonas serotype 11 was continuously isolated from both swimming pools and natural waters and from patients having acquired skin infections through swimming. The presence of Pseudomonas in waters used for full body immersion, therefore, indicates that the water is of questionable quality.

Staphylococcus, a known pathogen, has also been proposed as an indicator of recreational water quality. Staphylococcus counts in swimming pools show a definite correlation to the bather load on the pool, as they are derived from the nose, throat, and skin of swimmers (22). Although Staphylococcus has been isolated from swimming pools, no relationship has been established between their presence and the health of the bather despite several studies (1, 12, 22, 57).

On the other hand, the use of β hemolytic Streptococcus as an indicator of human pollution has never been proposed. A review of the literature revealed little clinical or epidemiological evidence incriminating β hemolytic Streptococcal infections associated with swimming waters. One instance reported is the work of Johnson in the winter of 1957-1958 (32), when, using a modified membrane filter overlay technique, he isolated groups A, B, and G β hemolytic Streptococcus from swimming pool waters. Again, however, it was not established whether or not they were also isolated from persons swimming in the pool.

METHODS AND MATERIALS *

1. Survey Area. This survey was limited to those freshwater ponds used for swimming within the boundaries of Cape Cod National Seashore near and around the towns of Wellfleet and Truro, Massachusetts. This area is bounded on the south by the town of Eastham, on the north by the town of Truro, on the east by the Atlantic Ocean, and on the west by Cape Cod Bay and Wellfleet Harbor. There are twenty-one freshwater ponds within the area. Only fifteen ponds used for swimming were sampled, but in 1976 only three ponds that are heavily used for swimming and a control pond were surveyed. These will be listed below.

2. Sample Collection and Processing. Samples were obtained in such a manner and time as to insure maximum recovery of microorganisms. Standard procedures were followed (56) for sample collection, to minimize contamination of the test sample. The frequency of sampling the ponds was set up to correspond with swimmer use and pond condition. Eight ponds were chosen to be sampled three times per week because of their heavy swimmer use, and the remaining seven were sampled once a week.

Water samples were collected three times a week from the following ponds: Great Pond (Wellfleet), Dyer Pond, Long Pond, Spectacle Pond, Gull Pond, Higgins Pond, Great Pond (Truro), and Snow Pond.

The following ponds were sampled once a week: Duck Pond, Horseleech Pond, Slough Pond, Round Pond (T), Herring Pond, Ryder Pond, and Round Pond (W).

In the summer of 1976, the survey was limited to Gull Pond, Long Pond, Great Pond (W), and, as a control, Duck Pond. All four ponds were tested twice daily for four days a week, on Monday, Tuesday, Friday, and Saturday.

Grab samples were taken 10 feet from shore at a depth of 6 to 12 inches in the bathing areas between the hours of 10:00 A.M. and 1:00 P.M., the hours of peak use.

In 1976, the first sample was collected at 8:30 A.M. in the morning before swimming started. The second sample was collected at 3:00 P.M. Water temperature and number of bathers were noted, as well as the time of the sample collection. All samples were returned to the laboratory within one hour after collection and kept cool while being processed.

* Survey area, sample collection and processing, bacteriological methods, and materials used in the summer 1975 survey were also used in summer 1976, except where otherwise indicated.

All samples were aseptically processed within three hours of collection time. Processing was done by the membrane filtration technique according to Standard Methods, 14th edition (56).

The samples were filtered through the membranes, grid side up, using two sample volumes of 10 ml and 100 ml. The membrane filters were rinsed twice with 100 ml each of peptone buffer. The membrane was then removed from the holder and placed grid side up on the surface of the appropriate bacteriological medium. Furthermore, samples collected in 1976 were also tested using the MPN method in order to maximize recovery and estimate the number of organisms. All samples were done in duplicate.

From August 15 to 25, 1975, Gull Pond, was closed down for road repairs, during which time we interrupted our standard sampling route in order to do an hourly survey of this pond before and after closing. Great Pond (W) was also studied at this time because the town switched its swimming programs to that pond from Gull Pond. This hourly survey was done to see what effect the closure and change of swimming patterns would have on the bacterial flora of the ponds in question. The standard sampling route was resumed after the hourly survey was completed.

All water samples for bacteriological analysis were collected using sterile one-liter widemouthed polypropylene (PP) sample bottles. Petri dishes and membrane filters (0.45 microns) were purchased from Millipore Corporation presterilized and ready for use. All Pyrex glass filter holders (used for sample filtrations in conjunction with a six-place stainless steel and three-place polyvinyl chloride manifold vacuum system) and sample-collecting bottles were steam-sterilized in a pressure cooker-canner at 15 psi for fifteen minutes prior to use.

3. Bacteriological Methods and Processing of Organisms.* The bacteriological media employed were prepared according to manufacturers' instructions. Prepared media were stored at 4°C for periods no longer than recommended in the 13th edition of Standard Methods (56).

a. Total Coliform.

Membrane filters were placed on M-Endo L.E.S. agar (BBL) and incubated for 24 hours at 36°C.

All colonies displaying a metallic green sheen were counted as coliforms.

b. Fecal Coliform.

Membrane filters were placed on M.F.C. broth (BBL) with 1.5% agar (Difco) added. Plates were placed in Whirlpac bags

* See Appendix A for details of the "Bacteriological Media, Formulas, and Preparation."

and incubated in a water bath at 44.5°C for 24 hours. All colonies showing blue to blue-green pigment were counted as fecal coliforms.

c. Fecal Streptococcus.

Enumeration was accomplished by placing the membrane filter on KF agar plates and incubating at 35°C for 48 hours. Fecal Streptococci colonies were identified by their characteristic small, pinpoint, red to red-pink colonies.

d. Pseudomonas aeruginosa.

Recovery of Pseudomonas from the ponds in 1975 was accomplished using Pseudosel agar (BBL) plates and incubating at 35°C for 48 hours. Typical colonies have green-diffusing pigment. Because it has not yet been established that all Pseudomonas will yield pigmented colonies, only a qualitative analysis was possible.

Because of difficulties encountered in 1975 in differentiating Pseudomonas from other organisms that may have become greenish from diffusible pigments produced by Pseudomonas, in 1976 these organisms were tested by the MPN method in Asparagine broth and with the aid of a Woods lamp in order to determine fluorescence of the organism. Presumptive positive cultures were further confirmed in Acetamide media for pigment production (see also pp. 20, 21, 23, 26, & 27-29).

e. Staphylococcus.

Initially two media, Chapman-Stone (BBL) and Vogel-Johnson (BBL) were employed. Chapman-Stone agar proved to be unsatisfactory due to high background counts. Vogel-Johnson medium, therefore, was selected for use because of its high inhibitory quality for background colonies at 100 ml volumes of sample. Filtered samples placed on Vogel-Johnson agar plates were incubated for 24 hours at 35°C. Typical Staphylococcus aureus colonies were identified by their characteristic deep black color and confirmed by the coagulase test (see Appendix A).

4. Clinical Specimens. In an attempt to correlate epidemiologically, bacterial isolates from the ponds with their effect upon the health of the bathers, five local physicians were contacted and agreed to participate in collecting swimming-related clinical specimens.* The clinicians involved with the study interviewed patients with symptoms indicative of swimming-related infections -- skin lesions and ear, eye,

* In 1976 only AIM and Wellfleet Medical Associates cooperated in the study.

nose, and throat complaints. The patients were also questioned regarding the ponds they used.

The physicians were supplied with blood agar plates and sterile cotton-tipped swabs. They cultured material from ear, throat, and skin lesions and placed the material directly on the blood agar plates. These plates were picked up daily.

Upon receipt, all clinical specimens were streaked and placed into candle jars and incubated at 37°C for 18 to 24 hours. After incubation, plates were examined for the presence of characteristic hemolysis. Typical colonies were isolated and confirmed as follows:

Staphylococcus aureus was tested for presence of coagulase using BBL rabbit plasma. β hemolytic Streptococcus was confirmed by using BBL bacitracin discs. Pseudomonas was checked for pigment production on TSA and pellicle production on TSB.

All positive cultures were then transferred to nutrient agar slants for further studies.

5. Most Probable Number (MPN) Method. In 1976 the afternoon sample was also tested by the MPN method on dates indicated for total coliform and fecal coliform to attain an MPN index and range. Results of MPN were computed from standard tables (56) in order to obtain a range, which was in turn compared to results obtained by the membrane filter technique.

6. Bacteriological Classification of Isolates. In order to make a complete assessment of bacteria found in the ponds, all plates containing typical colonies of total coliforms, fecal coliforms, and fecal Streptococcus were saved for further classification.

a. Identification of isolates from M-Endo and M-FC agar plates were based on the reactions obtained from the following biochemical tests:

1. Indole
2. Methyl red
3. Voges-Proskauer
4. Citrate
5. H₂S (triple sugar iron)
6. Urease
7. Motility
8. Lysine decarboxylase
9. Arginine dihydrolase
10. Ornithine decarboxylase
11. Lactose
12. Phenylalanine malonate

The results from these tests were compared to tables of biomedical reactions for Enterobacteriaceae, Aeromonas, and Vibrio (15). These results gave a profile of the various species of bacteria isolated

from water samples incubated on M-FC and M-Endo agar plates.

b. Identification of isolates from KF agar plates were based on the reactions obtained from the following biochemical tests:

1. Catalase test
2. Growth of BHI
 - a. 45°C
 - b. 10°C
 - c. in 6.5% NaCl
 - d. in 9, 6 pH
3. Reduction of
 - a. K_2TeO_2
 - b. TTC
 - c. 0.1% Methyl blue milk
4. Hydrolysis of
 - a. Gelatin
 - b. Starch
5. Fermentation of
 - a. D-Sorbitol
 - b. Glycerol
 - c. L-Arabinose
 - d. Lactose
6. Hemolysis of 10% horse blood

Biochemical reactions were compared to the table of reactions for identification of fecal Streptococcus (48). These results gave a profile of the fecal Streptococci species from water samples incubated on KF agar plates.

7. Additional Data Collected

- a. Weather Conditions
 1. Data on weather were obtained by personal observations on whether or not it was sunny (S), cloudy (C), humid (H), partly cloudy (PC), or rainy (R), and these were confirmed by weather stations set up within the survey area.
 2. Water temperature was recorded by immersing a thermometer (calibrated in degrees centigrade) in the water and allowing it to stand for two minutes before reading.
 - b. Number of People at the Ponds
 1. A visual count was taken of the number of persons at the ponds. These included persons both in and out of the water (see Tables 1-8).
8. β Hemolytic Streptococcus (see special section on page 17).
9. Staphylococcus phage typing (see special section on page 23).
10. Statistical analyses were done by chi-square, linear regression, and analysis of variance, using the Wilcoxon rank of sum and Mann-Whitney test (see Appendix C, page 48).

RESULTS AND DISCUSSION

A. β HEMOLYTIC STREPTOCOCCUS (STREPTOCOCCUS PYOGENOUS)

The Streptococci are among the more fastidious of bacteria with respect to nutritive requirements. They will not grow in ordinary meat extract mediums, and growth is ordinarily poor even in infusion mediums but may be somewhat improved by the inclusion of phosphate buffer and a small amount of glucose. For the most part, infusion mediums enriched by the addition of 5% to 10% defibrinated blood, ascitic fluid, and/or similar substances are used, and a medium such as blood agar is satisfactory for routine culture of the pathogenic forms.

In addition, the Streptococci grow over a wide temperature range, from 10°C to 42°C. Those of the pyogenic group (β hemolytic), which is made up of human and animal parasites, have an optimum growth at 37°C and a relatively restrictive temperature range (6).

Pathologically, the Streptococci are responsible for specific diseases in man and domestic animals. Streptococcus equi is the cause of "strangles" in horses, a suppurative infection of the upper respiratory tract that is characterized by abscess formation in the throat and submaxillary region. This species is apparently not pathogenic for man (6).

Some strains of group C Streptococci infect horses, causing upper respiratory infections and suppurative lesions in various parts of the body. A small proportion of group C Streptococci also cause infections in man that are indistinguishable from those of group A β hemolytic Streptococcus.

The most important group so far as man is concerned is group A β hemolytic Streptococcus, not only because its pathogenicity in streptococcal pharyngitis or pyoderma, but because of its sequela of rheumatic fever and acute glomerulonephritis (6).

Group A Streptococci are also the cause of erysipelas and scarlet fever in man and bovine mastitis in cows.

The enterococcus, which includes Streptococcus faecalis (fecal strep), is occasionally the cause of food poisoning, especially at picnics in the hot summer months.

Although the spectre of infection from Streptococci is high, they cause infections only in large numbers or in environmental conditions where they can easily multiply. However, because of the relatively complex nutritive requirements of these organisms, it is questionable that they will multiply in the open environment. It is also doubtful that they will survive for any length of time (except for fecal Streptococci)

outside their natural habitat.

The Spectre of β Hemolytic Streptococcal Infection. When Doolittle (18) reported in 1974 that β hemolytic Streptococcus was present in Cape Cod ponds, his report was based on the presence of β hemolysis on blood agar plates. No attempt was made to further identify the organism(s) by colony morphology, gram staining, or further subculturing and/or antibiotic inhibition. Doolittle was not aware at the time (personal communication) that organisms other than β hemolytic Streptococci are capable of producing β hemolysis on blood agar.

Not being able to find enough substantiative data in the literature, we approached the problem on a systematic trial/error fashion. First, the following questions were asked: Are there β hemolytic Streptococci in the ponds? If so, in what proportions? Would it be possible to pick them up in just a small amount of water (1 ml), or would we have to concentrate, through filtration, large volumes of water (100 ml or more) in order to recover the organism?

Other questions that needed answers were: If we find β hemolytic Streptococcus, how did it get there? If it were introduced by the swimmer(s), would it survive long enough for us to recover it, and will it multiply in a nutrient-deficient environment?

In an attempt to isolate β hemolytic Streptococcus, we applied the following methods:

1. 1 ml of water was spread over the surface of blood agar plates.
2. 10 ml and 100 ml of water were filtered through a membrane and the membrane placed over a blood agar plate, with 0.04% of sodium azide added to the medium.
3. A modified membrane filter procedure (water volumes same as above) that used Todd-Hewitt agar containing 0.01% triphenyl-tetrazolium chloride (TTC) and 0.04% sodium azide. In this method, the membrane containing the filtered sample was incubated on the blood agar plate for two hours and then overlaid with 2 to 3 ml of blood agar.

All plates were incubated in candle jars for 24 hours and then examined for hemolysis. Hemolytic colonies presented in any of the various plates used above were removed and streaked on blood agar. A bacitracin disc was placed over the heavily streaked portion, and the plates incubated for 24 hours, then checked for the presence of inhibition by bacitracin, against β hemolytic Streptococci.

Findings. Although many β hemolytic colonies appeared on the various plated techniques used above, not one single colony was confirmed, either by morphology, staining characteristics, or bacitracin inhibition, as β

hemolytic Streptococci. Some of the colonies were confirmed as Staphylococcus aureus, Pseudomonas aeruginosa, fecal Streptococcus, yeasts, and bacilli.

It is therefore our conclusion that Doolittle was dealing with β hemolytic colonies pertaining to one or more of the above-mentioned organisms and not to β hemolytic Streptococcus. In other words, we were not able to isolate β hemolytic Streptococcus from the various ponds in spite of all our efforts. We, therefore, conclude that β hemolytic Streptococci are not present in the Cape Cod ponds tested (see also pp. 27-29).

B. COMPARISON OF 1975 DATA WITH 1976 DATA

Bacteriological data presented in Tables 1 to 4 represent mean values per day, including duplicate samples collected in the mornings and afternoons for 1976 and duplicate samples per day for 1975. The 1976 data used for computing mean values extend only to 7/27/76, when we obtained both morning and afternoon samples.

Table 1 (Long Pond) shows a statistical significance between the means for total coliform recovered in 1975 and 1976, and also between the means for fecal Streptococci recovered in two years. Total coliforms were significantly higher in 1975 and fecal Streptococci much higher in 1976. Although the number of fecal coliforms and Staphylococcus aureus recovered in 1975 were higher than for those recovered in 1976, these were not statistically significant.

Table 2 (Gull Pond) compares bacteriological data recovered for 1975 and 1976. The recovery of total coliform, fecal coliform, and fecal Streptococcus was not statistically significant between 1975 and 1976. There was, however, a significant statistical difference between the recovery of Staphylococcus aureus in 1975 and its recovery in 1976 (Table 2).

Table 3 presents data for Great Pond for 1975 and 1976. The data indicate that there was a significant difference in the means for total coliform, for fecal coliform, and for fecal Streptococcus between 1975 and 1976. It is interesting to note that in 1976 we were able to isolate more fecal Streptococci than in 1975. Also, although the mean for fecal coliform isolated in 1975 seems much higher than that of 1976, this difference can be attributed to an abnormally high count in a single day (7/28/75); therefore, the emphasis here should be based on the daily recovery of fecal coliform in 1976, not on an average that includes this isolated high count.

Duck Pond (Table 4) was selected as the control pond in computing statistical analysis for the other three ponds tested in 1976 (Tables

1-3) because of the very low number of bacteria found in Duck Pond in 1975; also because of poor access, few people swam in Duck Pond, enhancing its value as a control pond for our study. However, we were unable to do statistical analysis on this table because the data for 1976 were based on weekly counts. (See "Results: Summer 1976" for original counts, and Appendix B for 1975 original counts).

Table 5 summarizes the statistical analysis for Tables 1, 2, and 3. The data were analyzed by the Wilcoxon rank of sum and by the Mann-Whitney test.

The data presented for bacterial indicators tested between 1975 and 1976 highly suggest that there are both seasonal as well as yearly variations working together in Cape Cod ponds. These variations, however, cannot be predicted with the limited data (two seasons) obtained. If such data were to be collected for several consecutive seasons, a better prediction could be made.

Pseudomonas. We were unable to do statistical analysis for Pseudomonas because of two different methods employed. In 1975 we used the membrane filter technique, which proved to be unsuitable because of pigments diffusing throughout the medium. In 1976 we employed the MPN method using Asparagine broth. This latter technique has been hailed as superior by experts in the field (9). Using this technique, we obtained unusually low results in 1976.

C. CONFIRMATION AND IDENTIFICATION OF ISOLATES, 1975 AND 1976

If any relationship between swimming in the ponds and health problems in the community is to be established, we must be able to confirm and identify isolates obtained from the ponds and from the patients suspected of having swimming-related illnesses. Identification of isolates from the patients created no problem since the number of patients was low.

Confirmation and identification of isolates from the ponds is presented in Tables 6-12. Confirmation of the typical green sheen isolates from M-Endo LES agar (BBL) plates gave satisfactory results as shown by Table 6. Seventy-seven percent of the typical green sheen colonies were confirmed as the coliform group as defined by the 14th edition of Standard Methods for the Examination of Water and Wastewater (56); however, 39% of the atypical (background) colonies were also confirmed as coliforms.

Typical blue-pigmented isolates from M-FC (BBL) agar yielded a high percentage of confirmation, as shown in Table 8. Ninety-one percent of the atypical blue colonies were confirmed to be fecal coliforms, using the E.C. test; however, 19% of the atypical (background) colonies were confirmed as fecal coliforms. The high percentage of atypical colonies being confirmed as coliform, fecal coliform, and fecal Streptococci, clearly indicates that the background bacterial flora of these ponds may be of significant sanitary value. This conclusion is further sub-

stantiated when one looks at the classification of isolates for atypical (background) colonies (Tables 7, 9). Klebsiella, Serratia, Aerobacter, and Shigella were isolated and classified by biochemical tests, with Klebsiella being the only one isolated in significant quantity. Klebsiella accounted for approximately 22% of the fecal coliforms and 15 to 22% of the total coliforms isolated on their respective agars. The high concentration of Klebsiella may be due to their natural presence in the environment and may be a cause for concern as a potentially pathogenic microorganism (36).

Confirmation of typical fecal Streptococci colonies from KF media gave 69% positive and 31% negative results (Table 10). Atypical colonies (although few were tested) gave 45% false negative results. One must therefore question the validity of the fecal Streptococcus test using KF media since it is slightly over 50% accurate.

Furthermore, the relative significance of fecal Streptococcus as an indicator of water quality is questionable when the classification of isolates from the KF media are categorized (Table 11). Almost 50% of the isolates classified as fecal Streptococcus were of the subspecies S. faecalis var. liquifaciens, which have been associated with nonfecal sources (e.g., plants and insects). The sanitary significance of fecal Streptococcus as an indicator of fecal pollution may be limited due to the presence of this subspecies in such high quantities.

The significance of the enteric indicators has been questioned (19, 26) and our results confirm previous researchers' suspicions. In place of the enteric indicators, both Staphylococcus and Pseudomonas are being proposed as alternatives due to their skin and upper respiratory association (8, 22). In trying to confirm our results, we discovered that S. aureus was a far superior test, giving a low false positive of 11% and no false negative (Table 12). Even if our proposed standard for S. aureus of 50 colonies per 100 ml of water is accepted (see pp. 24, 25 & 28), we find that the ponds rarely exceed this level. Based on these results, we feel the ponds are quite safe to use for total body contact.

The second nonenteric indicator proposed is Pseudomonas aeruginosa. Our efforts to confirm this organism on isolates obtained in 1975 met with failure. We were able to confirm it only in low numbers. Confirmation of Pseudomonas obtained in 1976 was done, but with difficulty, due to the extremely low counts ascertained for this organism. The confirming test is difficult and requires experienced hands. We feel, however, that since this organism was isolated in such low numbers, it is not a health problem at the present time.

D. CLINICAL SAMPLES: 1975 AND 1976

One of the major objectives of this study was to attempt to correlate, epidemiologically, swimming (bathing) to upper respiratory infections in

the community.* The local physicians cooperated in this part of the study by collecting clinical and demographic data from patients presenting complaints of upper respiratory infection.

The results of these clinical trials are presented in Tables 13 to 16. Ninety-seven patients in 1975 and 67 patients in 1976 (a total of 164) presented symptoms of throat, ear, ear and throat, and skin infections (Table 13). There were 86 males and 78 females (Table 14); 26 cases were positive for one or more organism. Of the 26 positive cases, 14 were males and 12 females. There was no statistical difference between the number of males and females. The data thus indicate that these infections are not sex related since they occur with equal frequency between males and females.

The sources of clinical samples are presented in Table 13. Seventy-four percent of the infections were for throat complaints, 11% for ear complaints, and 9% for both ear and throat infections. Statistically, there was no significant difference between infections of one source and infections of another source.

The types of organisms isolated from positive cases are shown in Table 15. Fifty percent of positive cultures were for Staphylococcus aureus, 37.5% for Streptococcus pyogenus, and 12.5% for Pseudomonas aeruginosa. Although there was no difference between the isolation of one organism and another organism, there was a statistical difference between the isolation of Pseudomonas and the source of infection. Pseudomonas aeruginosa was isolated from the ear only ($p=.015$).

Ponds incriminated by patients with clinical symptoms are shown in Table 16. Thirty-one percent of the patients incriminated Gull Pond, 29% Long Pond, and 25% Great (W) Pond. There was no statistical difference between the incrimination of one pond and another. The data thus show that these infections (if obtained by bathing in the ponds) are equally distributed among the various ponds.

We also noticed that 84% of these infections were aseptic infections (no bacterial pathogens found) and were probably due to other causes, possibly viruses. The scope of this study was such that it did not allow for the testing of viruses in the water nor in patients.

Further analysis of the clinical data revealed that the incubation period of these infections ranged from one day to two weeks, with an average of two days. The data also show that all positive cultures for S. aureus occurred when the Staphylococcus aureus counts in the ponds exceeded 50 organism/100 ml of water.

*Community: persons having bathed in the ponds and subsequently having contracted upper respiratory infections as defined by physicians in the area.

In summary, the small number of clinical samples (164) with only 26 positive (16%) show that these infections occurred as often in males as in females and that there was no difference relating to which pond they had used. The isolation of Pseudomonas aeruginosa from the ear proved to be significant since it was the only place where it was isolated. The average incubation period for these infections was two days, and S. aureus was isolated from patients when counts of this organism in the ponds exceeded 50/100 ml of water.

E. STAPHYLOCOCCAL PHAGE TYPING: 1975 ISOLATES ONLY -- DESCRIPTION AND SIGNIFICANCE OF RESULTS

Bacteriophages (phages) are viruses that attack bacteria. In the process of infecting a bacterium, the virus (phage) multiplies inside the cell, and when sufficient numbers are present the bacterium ruptures (lyses), thus releasing the viruses. Phages are host-specific, attaching only to bacteria that are compatible with their attachment sites.

The host specificity of these bacterial viruses provides additional means of characterization of bacteria extending to strain (type) differences. This method allows characterization within a single serotype and has been extremely useful for epidemiological studies, especially since most bacteria have their own specific phage type.

The application of bacteriophage typing, or phage typing, of Staphylococcus (60) has been invaluable in providing a means of identification where the conventional biochemical and serological methods have failed.

In brief, phage typing is based upon the collection of a group of phages of varied specificity but which in the aggregate will lyse (rupture) the majority of strains of Staphylococcus encountered. These phages are numbered arbitrarily. Although the maintenance of such standard phages requires technical precision, and although test conditions must be defined, the typing procedure is simple but time-consuming. Phage typing is based upon a kind of spectrum or pattern of activity that is defined for the standard phage against standard test of bacteria. The number of such patterns is very large. For this reason it is recommended that a set of basic phages should be used and grouped as follows:

Group I: 29, 52, 52A, 79, 80
Group II: 3A, 3B, 3C, 55, 71
Group III: 6, 7, 42E, 47, 53, 54, 75, 77
Group IV: 42D
Miscellaneous: 81, 187

Thus, it is possible to characterize a strain of Staphylococcus as belonging to one or another of these groups and phage types. Inevitably, some strains cannot be typed with the present available spectrum of phages.

Now, what does this mean to the layperson? It means that certain bacteria are capable of being infected with one or more types of phages but not with others. Therefore, if you isolate a species of bacteria from two different sources and they happen to be infected by the same type of phage, then you could certainly say that such bacteria have a common source.

In our case, we were able to isolate various strains of S. aureus from different patients and similarly from Gull Pond. Three strains isolated from three different patients had the same phage types as three strains isolated from Gull Pond (Table 17). Therefore, a definite relationship can be established between pathogenic bacteria found in the ponds and those isolated from some patients. We were able to isolate other phage-sensitive Staphylococci, both from patients and from the ponds. However, because of the high cost of the technique, we were limited in the number of strains we could type. Should more strains have been typed, we would have been able to match more of them.

The problem that we are faced with is extremely difficult to solve. We cannot tell whether the patients obtained their S. aureus infections from the pond or whether they themselves were the source of contamination to the pond. From our present knowledge of staphylococcal carriers, we are inclined to say that the patients were the source of contamination to the pond.

Is there, then, a danger of contracting a staphylococcal infection from swimming in Gull Pond? As mentioned in last year's report, there are no national standards for S. aureus for waters used for total body immersion (swimming). The magnitude of Staphylococci isolated from the various ponds was so low that it was hard to make a judgment. We have taken the liberty of making an arbitrary decision as to what level of Staphylococci concentration is safe (45, 46). This level has been set up at 50 isolated colonies per 100 ml of water. Any level above 50 would be considered dangerous. The reader must keep in mind that this is an arbitrary decision and it lacks both epidemiological and bacteriological confirmation.

When we compared staphylococcal isolates (for Gull Pond) obtained in the summer of 1976 with results reported in the summer of 1975, we found a great difference. In 1975 we isolated Staphylococci with much greater frequency and a much higher level than in 1976. Twelve times in 1975 S. aureus isolates exceeded the proposed 50/100 ml safety level. In 1976 there were only two occasions in which the safety margin was exceeded. Similarly, in 1975 we isolated Staphylococci from ear, throat and skin infections in twelve different patients, while in 1976 no patients presented themselves with Staphylococci as the predominant organism isolated. We are not altogether certain why there is such a great discrepancy but we will attempt to clarify it in the general discussion.

F. RESULTS: SUMMER 1976

Results of total coliform, fecal coliform, fecal Streptococcus, and Staphylococcus aureus are presented in Tables 18-21A. Morning and afternoon samples are presented for Gull, Great (W), Long, and Duck ponds.

1. Total Coliform. Total coliform counts on the morning sample differed considerably from counts obtained in the afternoon for all ponds. The lower morning count may indicate that these organisms do not survive in the ponds overnight or that they may be unable to grow on selective media after being exposed to the environment overnight. Although the afternoon counts were high, they never exceeded the national standards of 1,200 total coliforms per 100 ml of water. On only one occasion, 7/13/76, did the total coliform count for Gull Pond approach the national standard. Total coliform, however, is not a very good indicator because there is always a high background count that tends to interfere with results obtained.

2. Fecal Coliform. Fecal coliform counts follow the same trend as total coliforms. Counts were low in the morning and somewhat higher in the afternoon. Gull Pond exceeded the national standard of 200 fecal coliforms per 100 ml of water on only two occasions. None of the other ponds exceeded this limit nor closely approximated it. In 1975 also, Gull Pond exceeded the national limit two times. These two times occurred on nearly the same days in both years: 7/7 and 7/16 in 1975; 7/9 and 7/13 in 1976. Long Pond exceeded the national standard two times in 1975 but not at all in 1976. These two times occurred on 7/15 and 7/16 while in 1976 the counts for these two dates were extremely low.

3. Fecal Streptococcus. Fecal Streptococci counts obtained in 1976 followed the same sporadic trend seen in 1975. The indication here is that fecal Streptococci survive longer in the environment than do the coliforms. This observation has also been made by other researchers in the field (251). We found out that all the ponds tested in 1976 exceeded the proposed national standard of 100 fecal Streptococci per 100 ml of water on more than one occasion. These high counts were obtained early in the season (June) for Great (W), Long, and Duck ponds and once late in the season (August) for Gull and Duck ponds.

4. Staphylococcus aureus. Since there are no national standards for S. aureus on waters used for total body immersion, we have proposed, after a thorough study of both clinical samples and S. aureus isolates from Gull Pond, that 50 S. aureus per 100 ml of water could be adopted as a safety level (45, 46). Basically, there is the usual trend of low counts in the morning sample, followed by higher counts in the afternoon sample. Using the above safety-level standard, we found that it was exceeded twice in 1976 in Gull, Great (W), and Duck ponds and once in Long Pond. In 1975 this level was exceeded more than 12 times in Gull Pond. As presented in the phage-typing section, this unusually high count of S. aureus obtained from Gull Pond in 1975 did show a definite relationship with bather pollution.

5. Pseudomonas. Because of difficulty in enumeration and quantification by membrane filtration, Pseudomonas was tested by the MPN method using asparagine broth as a presumptive test and acetamide broth as a confirmed test. Results obtained are presented in Tables 22-25. All ponds tested showed an unusually low count for Pseudomonas. On one occasion (7/31), Long Pond had a count that was above the mean count obtained for the other ponds. The high Pseudomonas counts obtained in 1975 may be explained by the fact that there may be other organisms that produce green pigment in Pseudosel agar (the medium used in 1975) but not in asparagine or acetamide broth. In other words, what we called Pseudomonas in 1975 might not have been Pseudomonas at all. This is possible especially since we were not able to confirm our 1975 isolates as Pseudomonas.

6. MPN versus Membrane Technique. Results obtained by the MPN method and by membrane filter techniques are presented in Tables 22-25. Results obtained by the MPN method correlated closely with those obtained by the membrane filtration method. Since the methods correlated well, and since the membrane filtration method is a simpler and faster method, we recommend that future bacteriological studies on the ponds be done by the membrane method except, however, for Pseudomonas. The membrane technique for isolation of Pseudomonas requires further refinement. Until such refined techniques are available, we recommend the use of MPN for the isolation of this organism.

7. Statistical Analyses. Statistical analyses for Tables 18-21A are presented in Appendix C. No relationship could be established between the number of swimmers and fecal coliforms or fecal Streptococci.

CONCLUSION

This report incorporates studies done in the summers of 1975 and 1976 on the sanitary quality of the various freshwater ponds on the Cape Cod National Seashore bordering the towns of Wellfleet and Truro, Massachusetts, and on the epidemiology of relating swimming in the ponds to upper respiratory infections (URI) in the community. High incidence of URI in the community in the summer of 1974 had led the local physicians to suspect that these infections were related to swimming in the ponds. We, therefore, undertook these studies to determine the sanitary quality of these ponds and to find out whether or not these freshwater ponds were hazardous to the health of the persons swimming in them. We will discuss, then, the data as two separate entities: (1) upper respiratory infections and swimming in the ponds and (2) sanitary quality of these ponds.

A. UPPER RESPIRATORY INFECTIONS AND SWIMMING IN PONDS

The basic organism suspected by the physicians as the cause of the upper respiratory infection was Streptococcus pyogenus (β hemolytic Streptococcus), because in the summer of 1974 Doolittle, a student, doing bacteriological analysis in the ponds isolated β hemolytic colonies that he called β hemolytic Streptococcus (18). We undertook a systematic analysis of these pond waters in an attempt to isolate Streptococcus pyogenus. We tried several standard and modified methods, but we were not able to isolate this organism even when water samples were concentrated 100 times. We did, however, isolate β hemolytic colonies from the ponds, but none proved to be Streptococcus pyogenus. It is our belief, then, that Doolittle (18) was dealing with β hemolytic colonies belonging to organisms other than Streptococcus pyogenus (see pp. 17-19). We, therefore, conclude that Streptococcus pyogenus is not capable of surviving in the waters of these ponds. However, this conclusion did not answer the question raised by the physicians (i.e., why the increase in the incidence of upper respiratory infections?).

Therefore, in conjunction with the physicians in the area, we undertook an epidemiological study to discover the relationship, if any, between upper respiratory infections in the community and swimming in the ponds. In 1975 we received 97 clinical samples from throat, ear, eye, and skin infections, while in 1976 we only received 67 samples for a total of 164 samples for the two years. Over 86% of the patients incriminated Long, Gull, and Great (W) ponds. However, only 16% of the clinical samples were positive for either Streptococcus pyogenus, Staphylococcus aureus, or Pseudomonas aeruginosa. As already mentioned, we were not able to isolate S. pyogenus from the ponds, so therefore no correlation could be made between this organism and swimming in the ponds.

Both Pseudomonas and Staphylococcus have been incriminated as the cause of skin infections derived from swimming in contained waters (i.e.,

swimming pools) (31, 39, 43, 44, 45). And Pseudomonas has further been implicated in pneumonia cases (4). However, standards for concentration of these organisms based on epidemiological data have not been established. We were not able to correlate the isolation of Pseudomonas from patients to the isolation of this organism from the ponds. In essence we were not able to confirm the identity of Pseudomonas-like colonies isolated from the ponds in 1975, and in 1976 they were isolated in very low numbers and not at all from patients. Staphylococcus aureus was the most frequent isolate from patients (50% of positive cultures).

Furthermore, on the analysis of clinical data, all positive S. aureus cultures isolated from patients occurred when this organism was isolated from the ponds in counts above 50/100 ml H₂O. Based on these limited data, a standard of 50 S. aureus per 100 ml could be a safety margin allowed in waters used for total body immersion (i.e., swimming).

We further tried phage typing of staphylococcal isolates from both patients and the ponds and thus were able to establish a link between swimming in the ponds and URI in these patients.

We must, however, be extremely cautious in establishing this relationship. It is estimated that over 200,000 persons swim in the ponds every year, so that 164 clinical cases out of 400,000 persons in 2 years would occur naturally by chance and do not show any statistical significance. Therefore, based on the clinical cases reported to us, it can reasonably be said that there is no statistical correlation between swimming in the ponds and infections in the community. Any correlation would have to be based on the laboratory analysis of the positive clinical and pond samples and not statistically made.

It is also safe to say that any increase in URI observed by the physicians during the summer months may be directly because of an increase in the population during the summer and not to swimming in the ponds. If the ponds were to be incriminated at all, this would be more as a precipitating factor rather than a direct cause of URI. In other words, a person may be incubating a low-grade infection and, by swimming in the ponds, they may develop a full-blown infection.

The question may be raised, "What about the staphylococcal infections?" There is a great possibility that a direct relationship occurs between the isolation of Staphylococcus aureus from the ponds and those from patients. The phage typing of these isolates indicates that this is so. However, we cannot prove whether the patients were the source of contamination to the ponds or whether the ponds were the source of infection to the patients.

In summary then, we were able to isolate S. aureus, S. pyogenus, and P. aeruginosa from patients with clinical symptoms and S. aureus and P. aeruginosa from the ponds. The only link between infection in the

community and swimming in the ponds was based on phage typing of S. aureus. The isolation of P. aeruginosa from the ponds was in very low numbers in 1976 and could not be confirmed by further biochemical tests.

Because of the low number of patients presenting clinical symptoms, we must therefore conclude that the ponds are not the source of infections but may be a precipitating factor to URI.

B. SANITARY QUALITY OF CAPE COD NATIONAL SEASHORE PONDS

The bacteriological study conducted by us in 1975 was the first systematic study ever done on these freshwater ponds. Not having any previous data to compare our results with, we raised several questions as to the sanitary quality of the ponds. Nonetheless, in 1975 we observed that the sanitary quality of these ponds, with a few excepted days, complied well within national set standards (Appendix D). Still, several pathogens were isolated: Klebsiella pneumonia, Staphylococcus aureus, and Pseudomonas aeruginosa. However, such pathogens we are sure are present in almost any waters with a limited amount of pollution. The reason that they are not mentioned in published reports is that most people are concerned with the levels of pollution and not with the classifications of isolates.

More intensive and systematic studies conducted in 1976 showed these pathogens to be in very low numbers and probably not hazardous at these levels.

The difference in the counts for indicator organisms obtained between 1975 and 1976 may be explained in the fact that the 1975 counts were based on double testing of one sample per day compared to two samples per day in 1976. The 1976 data may, therefore, be a more accurate indication of the level of pollution encountered in the ponds.

Using the national set standards for waters used for total body immersion (27, 49, 59), we find the ponds to be quite clean. There were days when the national standards were exceeded, but they were not in consecutive sampling, except once. Furthermore, in any natural body of water one would expect certain amounts of pollution from rainwater draining into the ponds and bringing with it fecal coliform and fecal Streptococci from feces deposited nearby by wild animals. We have no control over naturally occurring situations like this.

The national standards are:

Total coliform -- 1200 colonies/100 ml H_2O
 Fecal coliform -- 200 colonies/100 ml H_2O
 Fecal Streptococci -- 100 colonies/100 ml H_2O

When applying national standards, we find that the quality of water in Gull Pond would be questionable with respect to fecal coliforms and fecal Streptococci. Gull Pond is heavily used because of its easy access and also because of several houses on its border. There always is the possibility that these houses may be contributing to the level of pollution of this pond.

Other uncontrollable factors that might account for the low level of pollution might be the publicity generated in 1975. This publicity instructed regular pond users how to reduce pollution in the ponds. However, it is very difficult to assess this factor without actually doing sociological studies of pond users.

Another point that deserves attention is the nature of our sampling. From the public health point of view, sampling must be done at the scene of the action. That is, one must take samples where people are swimming in order to pinpoint the infection. Therefore, for the sake of duplication and standardization, we always took samples at the same place each time.

There may be occasions when a "pool" of pollution may occur outside this sampling area, which may have a direct effect on the swimmer(s). Again, however, we have no control over such an occurrence.

A further point to consider is external chemical pollution. Many persons go swimming in the ocean and afterward cleanse themselves of salts and minerals in the ponds. This type of pollution, being investigated by Soukup et al. (54), adds nutrients to the ponds that help the multiplication of bacteria and algae. The curtailing of such contamination would enhance the safety of the ponds immensely.

All the literature we have dealt with has been on pollution parameters based on research done for "closed systems" (i.e., swimming pools). We must be careful, however, not to make irrational judgments based on such research and not to compare "closed systems" to open, natural bodies of water like the ponds we are studying. For instance, it is easy to do controlled experiments on a closed system such as a swimming pool because the number of persons allowed in is restricted, the amount of water dilution can be controlled, and one can treat (chlorinate) the system very easily. The ponds, on the other hand, present a different kind of system. Basically there is little control that can be exerted, except possibly prohibiting people from using them. The number of persons can be limited but not completely controlled since there is no way to keep people out at odd times (i.e., evenings or early mornings). Secondly, if chlorinated, the whole pond is affected. Thirdly, there is a dilution factor that is quite different from that of a swimming pool. The dilution factor is significant because one contamination source (i.e., a fecal sample) in a swimming pool would be extremely dangerous, but the same sample in one of the ponds may be completely insignificant. Furthermore, since the pond's bottom is composed of sand it has an excellent bacterial

filter system that pools do not have. The indications based on our 1976 morning samples are that these ponds tend to cleanse themselves several hours after swimming has ceased because of this natural filtration system.

Statistical analysis of our data gave us extremely disappointing results. In 1975 we found a partial correlation between the isolation of Staphylococcus and fecal Streptococcus from Gull Pond. In 1976 we were not able to establish such a relationship. In spite of the fact that we kept more accurate data on the number of swimmers, the number of indicator organisms isolated was so low that we could not establish a direct relationship between level of pollution and bather load.

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APPENDIX A

BACTERIOLOGICAL MEDIA, FORMULAS, AND PREPARATION

I. Total Coliform Analysis

A. M-Endo Agar LES (BBL 11203)

B. Formulation and Preparation

1. Formula in grams per liter distilled water:

Yeast extract	1.20
Trypticase peptone	3.70
Thiotone peptone	3.70
Biosate peptone	7.50
Lactose	9.40
Dipotassium phosphate	3.30
Monopotassium phosphate	1.00
Sodium chloride	3.70
Sodium desoxycholate	0.10
Sodium lauryl sulfate	0.05
Sodium sulfite	1.60
Basic fuchsin	0.80
Agar (dried)	14.00
pH 7.2	

2. This medium contains desoxycholate as an inhibitor and basic fuchsin as the dye. When the basic fuchsin-sodium sulfate is broken down due to a lowering of the pH produced by the growing organism, the water-soluble basic fuchsin precipitates, thus giving the typical metallic green sheen to coliform colonies.

3. This medium is prepared according to manufacturer's instructions, and plates were stored at 4°C in cardboard boxes for a maximum of two weeks.

II. Fecal Coliform Analysis

A. M-FC Broth (BBL 11365) with 1.5% Bacto Agar (Difco)

B. Formulation and preparation

1. Formula in grams per liter of distilled water:

Biosate peptone	10.0
Polypeptone peptone	5.0
Yeast extract	3.0

Sodium chloride	5.0
Lactose	12.5
Bile salts	1.5
Aniline blue	0.1
Agar (dried)	15.0
pH 7.4	

2. This medium is prepared with the addition of rosolic acid salt solution as an inhibitor with aniline blue as an indicator of pH change, thus obtaining the typical blue coliform colonies.

3. This medium is prepared according to the manufacturer's instructions, and plates were stored at 4°C in cardboard boxes for a maximum of four days.

III. Fecal Streptococcus Analysis

A. KF Streptococcal Agar (BBL 11313)

B. Formulation and preparation

1. Formula in grams per liter distilled water:

Polypeptone peptone	10.0
Yeast extract	10.0
Sodium chloride	5.0
Sodium glycerophosphate	10.0
Maltose	20.0
Lactose	1.0
Sodium azide	0.4
Agar (dried)	20.0
pH 7.2	

2. This medium contains sodium azide as the inhibitory agent and the addition of TTC solution as the indicator gives the fecal Streptococci colonies their typical red-pink color due to their ability to reduce the TTC.

3. This medium is prepared according to manufacturer's instructions, and plates were stored at 4°C in cardboard boxes for a maximum of four weeks.

IV. Staphylococcus aureus Analysis

A. Vogel and Johnson Agar (tellurite glycine red agar base) (BBL 11812)

B. Formulation and preparation

1. Formula in grams per liter distilled water:

Trypticase peptone	10.0
Yeast extract	5.0
Mannitol	10.0
Dipotassium phosphate	5.0
Lithium chloride	5.0
Glycine	10.0
Agar (dried)	16.0
Phenol red	25 mg.
pH 7.2	

2. This medium contains lithium chloride as an inhibitor and phenol red as an indicator of mannitol fermentation. Tellurite is added as the indicator of coagulase positive cocci, which have the ability to reduce potassium tellurite, giving the colonies their typical black color.
3. This medium is prepared according to manufacturer's instructions, and plates were stored in cardboard boxes at 4°C for a maximum of four weeks.

V. Pseudomonas aeruginosa Analysis

A. Pseudosel Agar (L 11554)

B. Formulation and preparation

1. Formula in grams per liter distilled water:

Gelysate peptone	20.0
Magnesium	1.4
Potassium sulfate	10.0
Cetrimide	0.3
Agar (dried)	13.6
pH 7.2	

2. This medium promotes *Pseudomonas* growth and pyocyanin production at the same time as the cetrimide (a quaternary ammonium compound: cetyltrimethyl ammonium bromide) inhibits other organisms.
3. This medium is prepared according to manufacturer's instructions, and plates were stored at 4°C for two weeks.

VI. β hemolytic Streptococcus Isolation Medium

A. Trypticase Soy Agar (BBL 11043)

B. Formulation and preparation

1. Formula in grams per liter distilled water:

Trypticase peptone	15.0
Phytone peptone	5.0
Sodium chloride	5.0
Agar (dried)	15.0
pH 7.3	

2. The addition of blood to this agar yields a non-inhibitory medium for the detection of hemolysis for aerobic and facultative microorganisms.
3. The medium is prepared according to manufacturer's instructions, and plates were stored under refrigeration for no longer than two weeks.

VII. Coagulase Test

A. Rabbit Plasma (BBL 40568)

- B. Plasma dispensed in 0.5 ml amounts into small test tubes. Tubes are inoculated with 24-hour cultures and incubated at 35°C for two to four hours, then observed for clots. Negative tests are incubated overnight before discarding as negative.

APPENDIX B: BACTERIOLOGICAL ASSESSMENT
OF INDIVIDUAL PONDS, 1975

TABLE B.1

Great Pond (Wellfleet)

Date	Total Coliform	Fecal Coliform	Fecal Streptococci	<u>Staphylococcus aureus</u>	<u>Pseudomonas</u>
7/2	0	0	0		
7/7	15	0	0		
7/8	1	0	3		
7/9	3	0	1		
7/14	11	0	2	1	-
7/15	0	0	20	1	-
7/16	0	0	1	1	-
7/21	11	4	1	1	+
7/22	25	70	23	20	-
7/24	16	3	1	0	-
7/27	14	6	12	0	-
7/28	790	450	26	52	-
7/30	1	0	1	0	-
AM					
7/30	3	4	5	75	-
PM					
8/4	6	1	1	0	-
8/6	0	0	1	2	-
8/11	39	14	1	0	E
8/12	3	1	2	0	++++
8/13	0	2	0	1	+
8/19	2	0	10	1	-
8/20	5	0	2	29	-
8/21	1	0	4	4	-

TABLE B.1 (Cont.)
GREAT POND (WELLFLEET)

Date	Total Coliform	Fecal Coliform	Fecal <u>Streptococci</u>	<u>Staphylococcus aureus</u>	<u>Pseudomonas</u>
8/22	1	0	2	0	-
8/25	2	0	0	0	-
9/3	1	0	1	0	-
9/4	0	0	0	0	-
9/5	0	0	1	4	-
9/10	2	0	0	3	-
9/11	0	0	3	0	-

TABLE B.2

LONG POND

Date	Total Coliform	Fecal Coliform	Fecal Streptococci	<u>Staphylococcus aureus</u>	<u>Pseudomonas</u>
7/2	10*	6	3	6	-
7/7	16*	1	1	67	-
7/8	22*	15	1	42	-
7/9	25*	18	14	2	+
7/14	9*	9	9	0	+
7/15	500*	280	10	6	+
7/16	190*	>2000	15	0	-
7/17	40*	13	2	0	++
7/21	2*	0	3	8	+
7/22	25*	25	17	2	ND
7/24	240*	85	2	56	+
7/27	10*	3	11	0	-
7/28	19*	20	42	0	+
7/30	8*	0	1	2	
AM					
7/30	11	3	9		
PM					
8/4	13*	1	8	0	+
8/6	110*	67	136	5	-
8/11	11*	3	0	0	-
8/12	5*	0	0	1	E
8/13	3*	0	1	3	-
8/19	15	2	3	0	-

* = Background count too high.

TABLE B.2 (Cont.)

Date	Total Coliform	LONG POND			
		Fecal Coliform	Fecal Streptococci	<u>Staphylococcus aureus</u>	<u>Pseudomonas</u>
8/25	3	3	1	0	-
9/3	1	0	1	0	-
9/4	1	0	1	0	-
9/5	0	0	1	3	-
9/10	0	0	0	0	-
9/11	19	9	9	0	+

TABLE B.3

GULL POND

Date	Total Coliform	Fecal Coliform	Fecal <u>Streptococci</u>	<u>Staphylococcus</u> <u>aureus</u>	<u>Pseudomonas</u>
7/2	8	0	1		
7/7	109	>2000	9		
7/8	40	6	3		
7/9	38	38	35		
7/14	80	9	45	55	-
7/15	180	140	170	200	+++
7/16	90	>2000	190	83	++
7/17	170	31	61	70	+++
7/21	19	3	4	11	++
7/22	>2000	120	36	240	+
7/24	7	100	230	153	+
7/27	71	25	56	5	+++
7/28	1150	90*	350	112	++
7/30 AM	36	7	38	123	ND
7/30 PM	76	26	37	77	+++
8/4	>2000	16	580	121	+++
8/6	>2000	31	40	83	+++
8/11	24	23	11	13	E
8/12	21	5	20	11	+++
8/13	40	3	58	28	+++
8/15	30	16	48	26	++
8/16	90	95	28	14	++

* = Background count too high.

TABLE B.3 (Cont.)

GULL POND

Date	Total Coliform	Fecal Coliform	Fecal Streptococci	<u>Staphylococcus aureus</u>	<u>Pseudomonas</u>
8/19	24	21	77	34	-
8/20	13	4	0	0	-
8/21	34	26	3	3	+
8/22	33	9	10	2	-
8/25	10	5	1	4	-
9/3	3	0	0	3	-
9/4	3	3	2	1	-
9/5	0	0	1	175	-
9/10	2	0	2	2	++

TABLE B.4

DUCK POND

Date	Total Coliforms	Fecal Coliforms	Fecal <u>Streptococcus</u>	<u>Staphylococcus</u>	
				<u>Aureus</u>	<u>Pseudomonas</u>
7/2	0	0	0		
7/7	0	0	0		
7/14	53	7	8	0	-
7/21	2	0	1	0	+
7/28	15	2	20	0	++
8/4	0	0	3	1	-
8/12	10	0	3	7	+++
8/25	0	0	3	1	-

APPENDIX C

STATISTICAL ANALYSIS

The accompanying table gives the correlations and least squares lines between the variables "total no. of people" and "mean fecal coliform," and the variables "total no. of people" and "mean fecal Strep." for Tables 1-8.

In all cases, x represents "total no. of people," and y represents "mean fecal coliform" or "mean fecal Strep."

The entries in the table are:

$$r^2 = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sum x^2 - \frac{(\sum x)^2}{n} \quad \sum y^2 - \frac{(\sum y)^2}{n}},$$

and the least squares line

$$y = a_1 x + a_0, \text{ where}$$

$$a_1 = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sum x^2 - \frac{(\sum x)^2}{n}}, \text{ and } a_0 = \bar{y} - a_1 \bar{x}.$$

All calculations are correct to two-decimal-place accuracy.

None of the correlations appear to be significant.

Total Number People
vs.
Mean Fecal Coliform

Total Number People
vs.
Mean Fecal Strep.

Table 18	$r^2 = .00$ $y = .01_x + 4.76$	$r^2 = .09$ $y = .16_x + 6.04$
Table 18A	$r^2 = .00$ $y = .07_x + 28.46$	$r^2 = .04$ $y = .10_x + 12.16$
Table 19	$r^2 = .00$ $y = .02_x + .99$	$r^2 = .04$ $y = -4.10_x + 32.60$
Table 19A	$r^2 = .03$ $y = .03_x + 3.54$	$r^2 = .07$ $y = -.15_x + 37.55$
Table 20	$r^2 = .01$ $y = .30_x + 2.97$	$r^2 = .04$ $y = 2.06_x + 8.33$
Table 20A	$r^2 = .01$ $y = -.06_x + 26.04$	$r^2 = .03$ $y = -.08_x + 24.63$
Table 21	$r^2 = .00$ $y = .10_x + 2.05$	$r^2 = .00$ $y = 1.88_x + 17.00$
Table 21A	$r^2 = .15$ $y = 2.11_x + .10$	$r^2 = .07$ $y = 4.62_x + 8.18$

APPENDIX D

KEY TO ANALYSIS OF THE TABLES*

A. Counts:

1. All counts are based on number of bacteria per 100 ml of water.
2. Each plate that had sufficient number of dispersed colonies was counted. Any plates that had confluent growth too numerous to count were designated as > 200. The maximum number of colonies counted on any individual plate was 200. A count of > 200 could represent 201 to several thousand colonies.
3. Total coliform, fecal coliform, and fecal Streptococcus counts were originally based on 10 ml of water. The counts were then multiplied by 10 to obtain the number of organisms per 100 ml of water. This accounts for the readings of > 2000.
4. Staphylococcus aureus counts were originally based on 100 ml of water. These counts therefore read as > 200.
5. Because of soluble pigments produced by Pseudomonas aeruginosa diffusing throughout the bacteriological medium, it was not possible to do quantitative studies. The following key was therefore adopted as a ranked test:
 - + = one to five pigmented colonies on a plate.
 - ++ = pigmented colonies covering about one-fourth of a plate.
 - +++ = pigment present on 50% of a plate.
 - ++++ = pigment covering more than 50% of a plate.
6. ND = No Data. Either no sampling was performed on the day specified, or the data gathered were not usable due to uncontrollable factors, such as external contamination.
7. E = Error. An error occurred during incubation of the filtered samples.

* Water Quality Standards Criteria Digest: A Compilation of Federal/State Criteria on Bacteria.

B. Interpretation:

1. Total coliform:

This count gives coliforms of both warm- and cold-blooded animal origin. Some states use a count of 2400 while others use a count of 1200 per 100 ml water as an indication of recreational water pollution.

Interpretation of the results is difficult because of the high background bacterial counts obtained.

2. Fecal coliform:

This count gives an indication of warm-blooded animal pollution. The American Public Health Association (APHA) standard for swimming waters is ≥ 200 coliform organisms per 100 ml of water.

3. Fecal Streptococcus:

This organism is used as an auxiliary indicator of water pollution. Although no specific standards have been set up, present recommendations are 100 fecal Streptococci per 100 ml of water as a base for warm-blooded animal pollution.

$$a. \text{ Ratio: } \frac{\text{Fecal coliform (FC)}}{\text{Fecal } \underline{\text{Streptococci}} \text{ (FS)}} = \text{Ratio}$$

This ratio has been established to differentiate pollution of human origin from that of animal origin (1, 16).

1. $\frac{FC}{FS} \geq 4.0$ = human pollution
2. $\frac{FC}{FS} \leq 0.7$ = livestock or poultry pollution
3. Ratio values from 1.0 to 2.0 indicate a gray area of both human and animal pollution.

4. Staphylococcus aureus:

The presence of Staphylococcus aureus in swimming water is an indication of human pollution. At present there are no standards for this organism. The organism, if coagulase-positive, is considered a human pathogen.

5. Pseudomonas aeruginosa:

An organism present both in decaying organic debris and in human or animal feces, P. aeruginosa is of concern because of its implication in ear and skin infections in humans. At present no standards are available.

C. Background count:

Number of saprophytic bacteria (nonindicator organisms) was too large.

D. Number of coliforms tolerable in water standards:

National Technical Advisory Committee Recommends:

1. Public water supplies:

- a. 20 fecal coliform per 100 ml of water desirable
- b. 100 total coliform per 100 ml of water desirable
- c. 2000 fecal coliform per 100 ml of water permissible
- d. 10,000 total coliform per 100 ml of water permissible

2. Recreational waters:

A - Primary

200 fecal coliform per 100 ml of water
400 fecal coliform per 100 ml of water not to be exceeded
in more than 10% of samples

B - Secondary

1000 total coliform per 100 ml of water - log mean
2000 total coliform per 100 ml of water not to be
exceeded in more than 10% of samples

3. Commonwealth of Massachusetts Criteria:

A - Public water supplies

50 total coliform per 100 ml of water

B - Recreational waters

1. Primary

1000 total coliform per 100 ml of water
2400 total coliform per 100 ml of water not to be
exceeded in more than 20% of samples

2. Secondary

none in concentrations that would impair usage

TABLE 1

Bacteriological Assessment for Long Pond
1975 and 1976

<u>Mean Total Coliform</u>				<u>Mean Fecal Coliform</u>	
<u>1975</u>	<u>Count</u>	<u>1976</u>	<u>Count</u>	<u>1975 Count</u>	<u>1976 Count</u>
7/2	10.00	6/19	144.25	6.00	78.25
7/7	16.00	6/18	127.50	1.00	27.50
7/8	22.00	6/19	15.00	15.00	7.75
7/9	25.00	6/21	62.50	18.00	14.00
7/14	9.00	6/22	77.00	9.00	17.50
7/15	500.00	6/25	12.00	280.00	2.50
7/16	190.00	6/26	35.00	>2000.00	10.00
7/17	40.00	6/28	11.00	13.00	0.25
7/21	2.00	6/29	56.00	0.00	7.50
7/22	25.00	7/3	29.00	25.00	10.00
7/24	240.00	7/4	12.00	85.00	4.75
7/27	10.00	7/5	65.50	3.00	14.75
7/28	19.00	7/6	21.50	20.00	5.50
7/30	9.50	7/10	28.50	1.50	3.50
8/4	12.00	7/12	57.50	1.00	20.50
8/6	110.00	7/13	82.00	67.00	4.25
8/11	11.00	7/16	17.50	3.00	3.38
8/12	5.00	7/17	9.00	0.00	6.50
8/13	3.00	7/19	5.00	0.00	4.00
8/19	15.00	7/20	9.00	2.00	8.50
8/25	3.00	7/23	-	3.00	2.00
9/3	1.00	7/24	25.50	0.00	7.00
9/4	1.00	7/26	28.00	0.00	17.00
		7/27	15.00		-
Mean	55.9		41.1	110.9	11.7
Normal Deviate = -2.1436 P = 0.032				Normal Deviate = -1.372 P = 0.17	

TABLE 1 (Cont.)

Bacteriological Assessment of Long Pond
1975 and 1976

<u>Mean Fecal Streptococci</u>				<u>Mean Staphylococcus aureus</u>	
<u>1975</u>	<u>Count</u>	<u>1976</u>	<u>Count</u>	<u>1975 Count</u>	<u>1976 Count</u>
7/2	3.00	6/15	60.50	-	-
7/7	1.00	6/18	44.50	-	0.00
7/8	1.00	6/19	5.25	-	0.00
7/9	14.00	6/21	7.50	-	1.00
7/14	9.00	6/22	11.00	6.00	0.50
7/15	10.00	6/25	10.00	67.00	2.25
7/16	15.00	6/26	87.50	42.00	2.75
7/17	2.00	6/28	7.50	2.00	-
7/21	3.00	6/29	13.50	0.00	5.00
7/22	17.00	7/3	18.25	6.00	5.75
7/24	2.00	7/4	6.50	0.00	-
7/27	11.00	7/5	7.00	0.00	-
7/28	42.00	7/6	19.63	8.00	2.50
7/30	5.00	7/10	0.50	29.00	3.25
8/4	8.00	7/12	17.50	0.00	3.75
8/6	136.00	7/13	13.13	5.00	1.13
8/11	0.00	7/16	2.00	0.00	1.25
8/12	0.00	7/17	1.88	1.00	0.25
8/13	1.00	7/19	1.50	3.00	0.00
8/19	3.00	7/20	2.88	0.00	0.25
8/25	1.00	7/23	2.38	0.00	1.75
9/3	1.00	7/24	3.75	0.00	3.50
9/4	1.00	7/26	3.13	0.00	0.00
		7/27	10.25		24.75
Mean	12.43		14.9	8.9	3.0
Normal Deviate = -2.000			Normal Deviate = 1.259		
P = 0.046			P = 0.208		

TABLE 2

Bacteriological Assessment for Gull Pond
1975 and 1976

<u>Mean Total Coliform</u>				<u>Mean Fecal Coliform</u>	
<u>1975</u>	<u>Count</u>	<u>1976</u>	<u>Count</u>	<u>1975 Count</u>	<u>1976 Count</u>
7/2	8.00	6/18	7.50	0.00	1.50
7/7	109.00	6/19	3.75	>2000.00	3.25
7/8	40.00	6/21	20.00	6.00	1.75
7/9	38.00	6/22	367.00	38.00	2.75
7/14	80.00	6.25	7.50	9.00	5.00
7/15	180.00	6/26	375.50	140.00	5.00
7/16	90.00	6.28	359.00	>2000.00	3.50
7/17	170.00	6/29	20.00	31.00	8.00
7/21	19.00	7/3	53.00	3.00	12.00
7/22	>2000.00	7/4	7.50	120.00	8.00
7/24	7.00	7/5	116.00	100.00	30.75
7/27	71.00	7/6	92.50	25.00	17.00
7/28	1150.00	7/10	222.50	90.00	3.50
7/30	56.00	7/12	192.50	16.50	79.75
8/4	>2000.00	7/13	582.50	16.00	145.50
8/6	>2000.00	7/16	43.00	31.00	5.75
8/11	24.00	7/17	30.00	23.00	9.50
8/12	21.00	7/19	83.00	5.00	64.50
8/13	40.00	7/20	19.00	3.00	5.50
8/15	30.00	7/23	26.50	16.00	2.25
8/16	90.00	7/24	237.50	95.00	21.00
8/19	24.00	7/26	51.00	21.00	6.50
8/20	13.00	7/27	38.50	4.00	-
8/21	34.00			26.00	
8/22	33.00			9.00	
8/25	10.00			5.00	
9/3	3.00			0.00	
9/4	3.00			3.00	

Mean 298

128.5

172.7

20.1

Normal Deviate = -0.7896

P = 0.43

Normal Deviate = 1.334

P = 0.184

TABLE 2 (Cont.)

Bacteriological Assessment for Gull Pond
1975 and 1976

<u>Mean Fecal Streptococci</u>				<u>Mean Staphylococcus aureus</u>	
<u>1975</u>	<u>Count</u>	<u>1976</u>	<u>Count</u>	<u>1975 Count</u>	<u>1976 Count</u>
7/2	1.00	6/18	3.25	-	1.50
7/7	9.00	6/19	9.25	-	2.50
7/8	3.00	6/21	15.50	-	0.00
7/9	35.00	6/22	6.75	-	5.00
7/14	45.00	6/25	7.50	55.00	1.35
7/15	170.00	6/26	10.25	200.00	2.50
7/16	190.00	6/28	3.50	83.00	12.50
7/17	61.00	6/29	6.50	70.00	3.00
7/21	4.00	7/3	8.00	11.00	66.50
7/22	36.00	7/4	15.25	240.00	-
7/24	230.00	7/5	29.25	153.00	-
7/27	56.00	7/6	27.00	5.00	6.00
7/28	350.00	7/10	6.50	112.00	37.50
7/30	37.50	7/12	27.00	100.00	23.75
8/4	580.00	7/13	28.88	121.00	3.88
8/6	40.00	7/16	9.50	83.00	22.63
8/11	11.00	7/17	3.00	13.00	10.25
8/12	20.00	7/19	4.00	11.00	0.25
8/13	58.00	7/20	11.13	28.00	2.75
8/15	48.00	7/23	5.13	26.00	2.50
8/16	28.00	7/24	16.75	14.00	5.00
8/19	77.00	7/26	4.75	34.00	0.00
8/20	0.00	7/27	34.50	0.00	16.50
8/21	3.00			3.00	
8/22	10.00			2.00	
8/25	1.00			4.00	
9/3	0.00			3.00	
9/4	2.00			1.00	
Mean	75.2		12.7	57.2	10.75

Normal Deviate = 1.4716
P = 0.142

Normal Deviate = -2.659
P = 0.008

TABLE 3

Bacteriological Assessment for Great Pond (W)
1975 and 1976

<u>Mean Total Coliform</u>				<u>Mean Fecal Coliform</u>	
<u>1975</u>	<u>Count</u>	<u>1976</u>	<u>Count</u>	<u>1975 Count</u>	<u>1976 Count</u>
7/2	0.00	6-15	3.75	0.00	-
7/7	15.00	6-18	377.00	0.00	0.00
7/8	1.00	6-19	95.00	0.00	18.75
7/9	3.00	6-21	0.00	0.00	1.00
7/14	11.00	6-22	1.00	0.00	1.00
7/15	0.00	6-25	6.00	0.00	0.25
7/16	0.00	6-26	11.00	0.00	0.25
7/21	11.00	6-28	4.00	4.00	1.00
7/22	25.00	6-29	6.50	70.00	0.00
7/24	16.00	7-2	16.00	3.00	2.50
7/27	14.00	7-3	105.00	6.00	13.25
7/28	790.00	7-4	42.50	450.00	8.50
7/30	1.50	7-5	19.00	2.00	1.00
8/4	6.00	7-6	56.50	1.00	1.25
8/6	0.00	7-10	35.50	0.00	3.00
8/11	39.00	7-12	50.50	14.00	6.00
8/12	3.00	7-13	75.00	1.00	0.50
8/13	0.00	7-16	22.50	2.00	1.50
8/19	2.00	7-17	12.50	0.00	0.50
8/20	5.00	7-19	2.00	0.00	1.25
8/21	1.00	7-20	9.00	0.00	2.00
8/22	1.00	7-23	25.00	0.00	4.00
8/25	2.00	7-24	0.00	0.00	1.25
9/3	1.00	7-26	9.00	0.00	3.25
9/4	0.00	7-27	20.00	0.00	
Mean	37.9		40.17	22.12	3.13
Normal Deviate = -3.304				Normal Deviate = -2.120	
P = <0.001				P = 0.034	

TABLE 3 (cont.)

Bacteriological Assessment for Great Pond (W)
1975 and 1976.

<u>Mean Fecal Streptococci</u>				<u>Mean Staphylococcus aureus</u>	
<u>1975</u>	<u>Count</u>	<u>1976</u>	<u>Count</u>	<u>1975 Count</u>	<u>1976 Count</u>
7/2	0.00	6/15	140.00	-	-
7/7	0.00	6/18	182.50	-	0.25
7/8	3.00	6/19	20.00	-	2.00
7/9	1.00	6/21	37.50	-	3.50
7/14	2.00	6/22	21.75	1.00	15.00
7/15	20.00	6/25	3.00	1.00	0.50
7/16	1.00	6/26	1.25	1.00	3.00
7/21	1.00	6/28	35.00	1.00	8.25
7/22	23.00	6/29	0.75	20.00	0.25
7/24	1.00	7/2	11.50	0.00	9.00
7/27	12.00	7/3	4.00	0.00	20.50
7/28	26.00	7/4	10.25	52.00	-
7/30	3.00	7/5	11.25	37.50	0.00
8/4	1.00	7/6	16.00	0.00	9.25
8/6	1.00	7/10	0.88	2.00	2.00
8/11	1.00	7/12	4.75	0.00	2.13
8/12	2.00	7/13	10.50	0.00	0.13
8/13	0.00	7/16	1.63	1.00	0.25
8/19	10.00	7/17	13.00	1.00	4.38
8/20	2.00	7/19	1.25	29.00	0.00
8/21	4.00	7/20	1.38	4.00	0.13
8/22	2.00	7/23	1.25	0.00	0.75
8/25	0.00	7/24	7.88	0.00	0.25
9/3	1.00	7/26	0.00	0.00	0.00
9/4	0.00	7/27	19.00	0.00	93.50
$\bar{X}$ =	4.6		22.25	7.16	7.6

Normal Deviate = -3.225
P = <0.001

Normal Deviate = -0.989
P = 0.322

TABLE 4

Bacteriological Assessment for Duck Pond
1975 and 1976
(Control Pond)

<u>Mean Total Coliform</u>		<u>Mean Fecal Coliform</u>			
<u>1975</u>	<u>Count</u>	<u>1976</u>	<u>Count</u>	<u>1975 Count</u>	<u>1976 Count</u>
7/2	0.00	6/15	5.25	0.00	0.00
7/7	0.00	6/18	10.25	0.00	0.00
7/14	53.00	6/29	45.50	7.00	5.25
7/21	2.00	6/21	17.50	0.00	1.00
7/28	15.00	6/22	7.50	2.00	0.50
8/4	0.00	6/25	5.50	0.00	0.00
8/12	10.00	6/26	48.00	0.00	2.00
<u>8/25</u>	<u>0.00</u>	6/28	63.00	<u>0.00</u>	<u>32.00</u>
Mean	10.00	6/29	4.00	1.125	0.25
SD	18.27	7/2	3.50	2.47	1.50
		7/3	45.75		11.75
		7/4	2.00		4.00
		7/5	72.50		17.75
		7/6	10.00		0.00
		7/9	13.00		
		7/10	3.00		0.00
		7/12	11.00		0.50
		7/13	90.00		0.25
		7/16	5.00		0.50
		7/17	0.00		0.75
		7/19	2.50		0.00
		7/20	3.50		0.00
		7/23	4.00		0.50
		7/24	1.00		0.50
		7/26	5.00		1.00
		<u>7/27</u>	<u>12.50</u>		
		Mean	18.875		<u>3.35</u>
		SD	25.08		7.50

TABLE 4 (cont.)

Bacteriological Assessment for Duck Pond
1975 and 1976
(Control Pond)

<u>Mean Fecal Strep.</u>				<u>Mean Staphylococcus aureus</u>	
<u>1975</u>	<u>Count</u>	<u>1976</u>	<u>Count</u>	<u>1975 Count</u>	<u>1976 Count</u>
7/2	0.00	6/15	90.00	-	-
7/7	0.00	6/18	77.50	-	0.50
7/14	8.00	6/19	2.75	0.00	0.50
7/21	1.00	6/21	15.00	0.00	0.75
7/28	20.00	6/22	7.25	0.00	0.00
8/4	3.00	6/25	2.00	1.00	0.00
8/12	3.00	6/26	1.75	7.00	1.00
8/25	3.00	6/28	3.25	1.00	1.00
Mean	4.75	6/29	1.00	1.50	2.50
SD	6.67	7/2	17.75	2.73	5.00
		7/3	14.50		12.00
		7/4	7.75		1.25
		7/5	46.50		2.00
		7/6	7.75		0.75
		7/9	1.00		0.50
		7/10	0.25		0.13
		7/12	10.25		0.50
		7/13	1.75		0.25
		7/16	0.50		0.13
		7/17	9.25		0.50
		7/19	0.38		0.00
		7/20	0.13		0.00
		7/23	2.50		0.50
		7/24	5.88		0.25
		7/26	3.50		0.00
		7/27	12.38		137.50
		Mean	13.17		6.46
		SD	22.90		28.83

TABLE 5

P Value for the Bacteriological Assessments of Individual
Ponds Comparing the Results of 1975 and 1976

Name of Pond	Total Coliform		Fecal Coliform		Fecal Streptococci		Staphylococcus aureus	
	Normal* Deviate	P Value	Normal Deviate	P Value	Normal Deviate	P Value	Normal Deviate	P Value
Long Pond	-2.1436	0.032 Sign. Dif.	-1.372	0.17 Not Sign.	-2.000	0.046 Sign. Dif.	1.259	0.208 Not Sign.
Gull Pond	-0.7896	0.43 Not Sign.	1.334	0.184 Not Sign.	1.4716	0.142 Not Sign.	-2.659	0.008 Sign. Dif.
Great Pond	-3.304 Sign. Dif.	<0.001 Sign. Dif.	-2.120	0.034 Sign. Dif.	-3.225	<0.001 Sign. Dif.	-.989	0.322 Not Sign.

$$\text{* Normal Deviate} = \frac{W_x - \bar{W}}{\sigma_w}$$

$$W_x = mn - S_x + \frac{n(n+1)}{2} \quad \text{Mann-Whitney for } X(1976)$$

$$\bar{W} = \frac{mn}{2} \quad \text{rank mean}$$

$$\sigma_w^2 = mn(m+n+1)/12 \quad \text{rank variance}$$

Reference: Wilcoxon rank sum and Mann-Whitney test.

TABLE 6
Confirmation of Isolates from
M-Endo LES Agar Plates (Total Coliform)

Type of Colony		Positive	Negative	Total
Typical (Green Sheen)	#	282	84	366
	%	77%	23%	100%
Atypical (Background)	#	28	44	72
	%	39%	61%	100%

TABLE 7
 Classification of Confirmed Coliform Isolates
 from M-Endo LES Agar Plates

Classification	# of Colonies	% of Total
Typical Colonies		
<u>Aerobacter</u>	70	50.0
<u>Klebsiella</u>	31	22.2
<u>Escherichia</u>	39	27.8
Total	140	100.0%
Background Colonies		
<u>Aerobacter</u>	59	36.6
<u>Klebsiella</u>	20	12.4
<u>Escherichia</u>	28	17.4
<u>Serratia</u>	9	5.6
<u>Citrobacter</u>	10	6.2
<u>Chromobacter</u>	5	3.1
<u>Pseudomonas</u> (?)	3	1.9
<u>Providencia</u>	2	1.2
<u>Shigella</u> (?)	2	1.2
Unclassified Enterics	9	5.6
Non-Enterobacteriaceae	14	8.7
Total	161	100.0%

TABLE 8
Confirmation of Isolated Colonies
from M-FC Agar Plates (Fecal Coliforms)

Type of Colony		E.C. Positive	E.C. Negative	Total
Typical	#	338	33	371
Colonies	%	91	9	100%
Atypical	#	5	21	26
Colonies	%	19.2%	80.8%	100%

TABLE 9
Classification of Isolated Colonies*
Obtained from M-FC Agar Plates

Classification	# of Isolates	% of Total
<u>Escherichia</u>	122	61.0
<u>Klebsiella</u>	43	21.5
<u>Aerobacter</u>	5	2.5
<u>Aeromonas</u>	2	1.0
<u>Shigella</u>	4	2.0
Unclassified	24	12.0
Total Isolates	200	100.0%

* Includes typical and atypical (background) colonies.

TABLE 10
Confirmation of Isolates from
K-F Agar Plates (Fecal Streptococcus)

Type of Colony	Positive	Negative	Total
Typical #	177	78	255
(pink) %	69	31	100%
Atypical #	10	12	22
%	45	55	100%

TABLE 11
Classification of Isolates Obtained
from K-F Agar Plates

Classification	# of Isolates	% of Total
<u>Streptococcus Faecalis</u>	13	15.1
<u>var liquifaciens</u>	26	30.2
<u>var. zymogenes</u>	6	7.0
<u>Streptococcus durans</u>	6	7.0
<u>Streptococcus faecium</u>	13	15.1
Unclassified*	22	25.6
Total Isolates	86	100.0%

*Includes 12 catalase positive isolates that are termed micrococci (coagulase negative).

TABLE 12
Confirmation of Isolates
from Vogel-Johnson Agar

Type of Colony		Coagulase Positive (<i>S. aureus</i>)	Coagulase Negative	Total
Typical* (Black)	#	160	20	180
	%	89	11	.100
Atypical (Grey)	#	0	78	78
	%	0	100	100

*Initially a discrepancy was observed between what we believed to be Staph. aureus colonies and those colonies which proved to be coagulase positive. However, a morphology was observed which proved consistently to be Staph. aureus. Low counts precluded confirmations on a regular basis, however.

TABLE 13

Sources of Clinical Samples Received
in 1975 and 1976 and Percentage of Each

<u>Source</u>	<u>1975</u>	<u>1976</u>	<u>Total</u>	<u>Percent</u>
Throat	70	51	121	73.78
Ear	13	5	18	10.98
Ear & Throat	9	6	15	9.15
Eye	1	0	1	0.61
Skin	3	0	3	1.82
Other	1	5	6	3.66
Total	97	67	164	
Percent	59.0	41.0		100.0

H0: Throat = Ear = Ear & Throat

$$\chi^2_2 = 1.35$$

Conclusion: Accept H0 - They are the same.

TABLE 14

Total Number of Clinical Samples
Received for Bacteriological Analyses
by Sex and Results, 1975 and 1976

Sex	Positive	Negative	Total	Percent
Male	14	72	86	52
Female	12	66	78	48
Total	26	138	164	100
Percent	16	84	100	

H0: Male positive = female positive
Male = Female

$$\chi^2_2 = .048$$

Conclusion: Accept H0 - no difference between number of males and females and the number of positive.

TABLE 15

Genus and Species of Organisms Isolated from Positive
Clinical Samples by Source, 1975 and 1976

Genus and Species	Ear	Throat	Ear and Throat	Skin	Eye	Total	Percent
β hemolytic <u>Streptococci</u>	1	6	2	0	0	9	37.5
<u>Staphylococcus</u> <u>aureus</u>	2	6	2	2	0	12	50.0
<u>Pseudomona</u> <u>aeruginosa</u>	3	0	0	0	0	3	12.5
Total	6	12	4	2	0	24	100
Percent	25	50	16.66	8.34	0	100	

H0: Strep. = Staph. = Pseud.

Then probability of ear isolation for Pseudomonas = $(1/4)^3 = .015625$

Conclusion:

Reject H0 - There is a difference in the isolation of Pseudomonas from the ear.

TABLE 16

Ponds Used by Patients Presenting
Clinical Symptoms, 1975 and 1976

<u>Pond</u>	<u>Positive</u>	<u>Results of Clinical Samples</u>		<u>Percent</u>
		<u>Negative</u>	<u>Total</u>	
Great(w)	10	41	51	25.0
Long	9	50	59	28.9
Gull	7	57	64	31.4
Others	5	25	30	14.7
Total	31	173	204	100
Percent	15.2	84.8	100	

H0: Great = Long = Gull = Others

$$\chi^2_3 = 1.613$$

Conclusion: Accept H0 - They are the same.

TABLE 17

Staphylococcal Phage Typings of S. aureus Isolates
from Patients and from Gull Pond

I. Patient #		Phage Type
19		53/83A/84/85/+
22		untypable
31		55/71+
32		3A
39		29/52+
44		96
46		94/96 *
86		untypable
87		52 *
88		71 *
89		D11/95

II. Pond		
Great Pond (W)		D11/85
" " "		29
" " "		95
Gull Pond		42E
" "		untypable
" "		52 *
" "		71 *
" "		untypable
" "		29
" "		187
" "		29
" "		94/96 *
" "		95
" "		6/42E/47/54/75/77/84/81
" "		

* Matching phage types

Patient #	Phage	Pond	Phage
46	94/96	Gull	94/96
87	52	Gull	52
88	71	Gull	71

TABLE 18
Bacteriological Analysis of
Gull Pond - Morning Samples

DATE 1976	WEATHER	WATER TEMP °CENTIGRADE	TOTAL NUMBER PEOPLE	MEAN TOTAL COLIFORM	MEAN FECAL COLIFORM	MEAN FECAL STREP.	MEAN STAPH. AUREUS
T6-16	P-C	20.0	0	10.0	2.5	1.5	ND
F6-18	S	21.0	0	5.0	1.0	5.0	0.0
S6-19	C	22.0	0	2.5	2.0	5.0	0.0
M6-21	H	23.5	0	15.0	2.0	5.0	0.0
T6-22	C	23.0	0	18.0	5.0	0.0	0.0
F6-25	C	24.0	0	0.0	5.0	5.0	2.0
S6-26	C	23.5	0	6.0	0.0	1.0	0.0
M6-28	C	23.5	0	4.0	0.0	2.0	6.5
T6-29	R	23.5	6	20.0	10.0	8.0	0.0
S7-3	S	25.0	6	86.0	18.0	3.0	25.5
Su7-4	S	24.0	2	5.0	2.0	15.5	ND
M7-5	S	24.0	19	6.0	4.5	10.5	ND
T7-6	S	25.5	12	30.0	19.0	10.5	0.0
S7-10	S	-	3	5.0	1.0	0.25	2.5
M7-12	C	25.0	3	20.0	4.5	7.5	1.0
T7-13	P-C	24.5	0	25.0	3.0	43.0	1.25
F7-16	S	24.0	46	48.0	2.5	2.0	6.25
S7-17	R	24.5	0	10.0	2.0	2.5	0.0
M7-19	S	25.0	46	0.0	6.0	3.0	0.0
T7-20	S	24.0	66	4.0	7.0	4.75	4.5
F7-23	S	24.5	6	18.0	2.5	1.0	0.0
S7-24	R	24.5	0	255.0	11.0	6.0	0.0
M7-26	S	24.0	79	10.0	3.0	2.0	0.0
T7-27	S	25.0	74	22.0	-	59.5	17.0

S = Sunny
 C = Cloudy
 H = Humid
 R = Rain
 P-C = Partly cloudy
 ND = No data

TABLE 18A
Bacteriological Analysis of
Gull Pond - Afternoon Samples

DATE 1976	WEATHER	WATER TEMP °CENTIGRADE	TOTAL NUMBER PEOPLE	MEAN TOTAL COLIFORM per 100 ml	MEAN FECAL COLIFORM	MEAN FECAL STREP.	MEAN STAPH. AUREUS
T6-15	P-C	20.0	27	35.0	-	25.0	-
F6-18	S	22.5	43	10.0	2.0	1.5	3.0
S6-19	P-C	23.0	60	5.0	4.5	13.5	5.0
M6-21	S	24.5	120	25.0	1.5	26.0	0.0
T6-22	S	24.5	81	716.0	0.5	13.5	10.0
F6-25	H	25.0	39	15.0	5.0	10.0	25.0
S6-26	S	25.0	27	745.0	10.0	10.5	5.0
M6-28	S	24.5	77	714.0	7.0	5.0	18.5
T6-29	H	24.0	34	20.0	6.0	5.0	6.0
F7-2	S	25.0	98	90.0	19.0	18.5	18.0
S7-3	H	25.0	30	20.0	6.0	13.0	107.5**
Su7-4	S	25.0	61	10.0	14.0	15.0	
M7-5	S	27.5	94	226.0	57.0	48.0	15.0
T7-6	S	ND	38	155.0	15.0	43.5	12.0
F7-9	H	25.0	137	980.0	>200.0*	3.5	15.5
S7-10	S	26.5	105	440.0	6.0	12.75	72.5**
M7-12	S	25.0	63	365.0	155.0	46.5	46.5
T7-13	C	25.0	28	1140.0	288.0*	14.75	6.5
F7-16	S	25.0	63	38.0	9.0	17.0	39.0
S7-17	P-C	25.5	151	50.0	17.0	3.5	20.5
M7-19	S	25.0	180	166.0	69.0	5.0	0.5
T7-20	S	25.0	168	34.0	4.0	17.5	1.0
F7-23	C	25.0	56	35.0	2.0	9.25	5.0
S7-24	S	25.0	194	220.0	31.0	27.5	10.0
M7-26	S	25.0	73	92.0	10.0	7.5	0.0
T7-27	C	24.0	65	55.0	-	9.5	16.0
F7-30	R	24.0	3	12.0	5.0	14.0	0.5
S7-31	C	24.0	13	5.0	1.0	30.5	12.0
M8-2	P-C	ND	76	35.0	6.0	2.5	3.5

TABLE 18A (Continued)

DATE 1976	WEATHER	WATER TEMP °CENTIGRADE	TOTAL NUMBER PEOPLE	MEAN TOTAL COLIFORM	MEAN FECAL COLIFORM	MEAN FECAL STREP.	MEAN STAPH. AUREUS
T8-3	P-C	24.0	59	35.0	5.0	7.5	9.5
T8-10	S	25.0	65	120.0	17.0	31.0	-
F8-13	S	25.0	156	165.0	67.0	138.0*	-
S8-14	P-C	25.0	53	80.0	72.0	13.0	-
M8-16	S	25.0	118	120.0	6.5	37.0	26.0
F8-20	S	24.5	140	202.0	17.0	5.0	25.5

S = Sunny

C = Cloudy

H = Humid

R = Rain

P-C = Partly cloudy

* = Above National Standard

** = Above Proposed Standard

TABLE 19
Bacteriological Analysis of
Great Pond (W) - Morning Samples

DATE 1976	WEATHER	WATER TEMP °CENTIGRADE	TOTAL NUMBER PEOPLE	MEAN TOTAL COLIFORM	MEAN FECAL COLIFORM	MEAN FECAL STREP.	MEAN STAPH. AUREUS
T6-15	P-C	20.0	0	5.5	1.0	260.0*	ND
F6-18	S	21.3	0	749.0	0.0	140.0*	0.5
S6-19	C	22.1	0	105.0	0.0	25.0	4.0
M6-21	H	22.5	0	0.0	1.0	40.0	6.5
T6-22	C	23.0	0	1.0	1.0	40.0	0.0
F6-25	C	24.0	0	2.0	0.0	1.0	1.0
S6-26	C	24.0	2	2.0	0.0	20.0	1.0
M6-28	C	24.0	0	8.0	2.0	5.0	1.5
T6-29	R	23.5	0	8.0	0.0	1.0	0.5
F7-2	S	-	0	2.0	1.0	5.0	3.5
S7-3	S	25.0	0	170.0	0.5	2.5	-
Su7-4	S	24.5	2	35.0	15.0	2.5	-
M7-5	S	24.0	2	0.0	1.0	5.0	-
T7-6	S	25.5	0	8.0	0.0	20.5	0.5
S7-10	S	25.0	5	6.0	0.0	0.25	0.0
M7-12	C	25.0	0	6.0	0.0	8.75	0.0
T7-13	P-C	-	0	65.0	0.0	4.0	0.0
F7-16	S	24.5	3	10.0	0.0	2.0	0.0
S7-17	R	25.0	0	0.0	0.0	14.0	0.0
M7-19	S	25.0	7	0.0	0.5	0.5	0.0
T7-20	S	25.0	3	6.0	0.0	0.25	0.0
F7-23	S	24.0	5	35.0	0.0	0.5	0.5
S7-24	R	24.5	0	-	0.0	3.0	0.5
M7-26	S	24.0	5	0.0	2.5	3.0	0.0
T7-27	S	24.0	10	10.0	0.0	31.0	115.5**

S = Sunny
 C = Cloudy
 R = Rain
 H = Humid
 P-C = Partly cloudy
 ND = No data
 * = Above National Standard
 ** = Above Proposed Standard

TABLE 19A
Bacteriological Analysis of
Great Pond (W) - Afternoon Samples

DATE 1976	WEATHER	WATER TEMP. °CENTIGRADE	TOTAL NUMBER PEOPLE	MEAN TOTAL COLIFORM	MEAN FECAL COLIFORM	MEAN FECAL STREP.	MEAN STAPH. AUREUS
T6-15	P-C	20.0	45	2.0	-	20.0	ND
F6-18	S	22.5	26	5.0	0.0	225.0*	0.0
S6-19	P-C	23.0	38	85.0	37.5	15.0	0.0
M6-21	S	23.0	68	0.0	1.0	35.0	5.0
T6-22	S	24.0	82	1.0	1.0	3.5	30.0
F6-25	H	25.0	40	10.0	0.5	5.0	0.0
S6-26	S	25.5	181	20.0	0.5	0.5	5.0
M6-28	S	24.5	87	0.0	0.0	65.0	15.0
T6-29	H	24.0	16	5.0	0.0	0.5	0.0
F7-2	S	25.0	101	30.0	4.0	18.0	14.5
S7-3	H	25.0	38	40.0	26.0	6.0	41.0
Su7-4	S	25.5	48	50.0	2.0	18.5	-
M7-5	S	25.0	199	38.0	1.0	17.5	2.0
T7-6	S	26.5	153	105.0	2.5	11.5	18.0
F7-9	H	25.0	95	45.0	3.0	1.0	3.0
S7-10	S	26.5	230	65.0	6.0	1.5	4.0
M7-12	S	26.0	177	95.0	12.0	0.75	4.25
T7-13	C	25.5	31	85.0	1.0	17.0	0.25
F7-16	S	25.5	166	35.0	3.0	1.25	0.5
S7-17	P-C	26.0	130	25.0	1.0	12.0	8.75
M7-19	S	26.0	191	4.0	2.0	2.0	0.0
T7-20	S	26.0	239	12.0	4.0	2.5	0.25
F7-23	C	25.0	106	15.0	8.0	2.0	1.0
S7-24	S	25.0	183	13.0	2.5	12.75	0.0
M7-26	S	25.0	127	18.0	4.0	8.0	0.0
T7-27	C	24.5	117	30.0	-	7.0	71.5**
F7-30	R	24.0	3	8.0	0.5	23.0	19.25
S7-31	C	24.0	0	5.0	0.5	24.5	18.25

TABLE 19A (Continued)

DATE 1976	WEATHER	WATER TEMP. °CENTIGRADE	TOTAL NUMBER PEOPLE	MEAN TOTAL COLIFORM	MEAN FECAL COLIFORM	MEAN FECAL STREP.	MEAN STAPH. AUREUS
M8-2	P-C	24.5	95	5.0	0.5	15.0	9.0
T8-3	P-C	25.0	94	35.0	1.0	1.25	5.5
T8-10	S	25.0	118	250.0	18.5	28.0	1.5
F8-13	S	26.0	245	74.0	13.0	13.5	43.5
S8-14	P-C	26.0	144	92.0	5.5	98.0	40.5
M8-16	S	25.5	149	42.0	2.5	2.0	0.5
F8-20	S	25.5	220	146.0	51.0	6.25	2.0

S = Sunny

C = Cloudy

R = Rain

H = Humid

P-C = Partly cloudy

ND = No data

* = Above National Standard

** = Above Proposed Standard

TABLE 20
Bacteriological Analysis of
Long Pond - Morning Samples

DATE 1976	WEATHER	WATER TEMP. °CENTIGRADE	TOTAL NUMBER PEOPLE	MEAN TOTAL COLIFORM	MEAN FECAL COLIFORM	MEAN FECAL STREP.	MEAN STAPH. AUREUS
T6-15	P-C	20.0	0	8.5	1.5	11.0	-
F6-18	S	22.0	0	165.0	5.0	9.0	0.0
S6-19	C	22.5	0	15.0	2.0	0.5	0.0
M6-21	H	22.5	0	5.0	1.0	5.0	0.0
T6-22	C	23.5	0	78.0	5.0	1.5	0.5
F6-25	C	24.5	0	14.0	0.0	5.0	3.5
S6-26	C	24.0	7	40.0	10.0	165.0*	5.0
M6-28	C	24.0	0	20.0	0.0	10.0	0.0
T6-29	R	24.0	0	100.0	5.0	22.5	0.0
S7-3	S	25.0	2	46.0	10.0	28.0	7.5
Su7-4	S	24.5	8	16.0	5.5	3.0	-(ND)
M7-5	S	25.0	2	5.0	1.5	4.5	-(ND)
T7-6	S	25.5	2	18.0	1.0	11.5	1.0
S7-10	S	25.5	9	22.0	1.0	0.5	1.0
M7-12	C	25.5	0	10.0	2.0	2.0	0.5
T7-13	P-C	-	0	66.0	1.5	19.25	0.25
F7-16	S	24.5	7	25.0	4.0	1.5	0.5
S7-17	R	25.5	0	8.0	6.5	3.0	0.0
M7-19	S	25.5	1	6.0	1.0	0.75	0.0
T7-20	S	25.5	10	12.0	8.5	3.25	0.0
F7-23	S	24.0	4	-	1.0	1.5	0.5
S7-24	R	24.5	0	25.0	8.0	4.25	0.0
M7-26	S	24.5	6	8.0	4.5	0.25	0.0
T7-27	S	24.5	0	12.0	-	6.5	0.5

S = Sunny
 C = Cloudy
 R = Rain
 H = Humid
 P-C = Partly cloudy
 ND = No data
 * = Above National Standard

TABLE 20A
Bacteriological Analysis of
Long Pond - Afternoon Samples

DATE 1976	WEATHER	WATER TEMP. °CENTIGRADE	TOTAL NUMBER PEOPLE	MEAN TOTAL COLIFORM	MEAN FECAL COLIFORM	MEAN FECAL STREP.	MEAN STAPH. AUREUS
T6-15	P-C	20.0	22	280.0	155.0	110.0*	ND
F6-18	S	23.0	91	90.0	50.0	80.0	0.0
S6-19	P-C	24.0	56	15.0	13.5	10.0	0.0
M6-21	S	24.5	81	120.0	27.0	10.0	2.0
T6-22	S	25.0	60	76.0	30.0	20.5	0.5
F6-25	H	25.5	37	10.0	5.0	15.0	10.0
S6-26	S	25.5	106	30.0	10.0	10.0	5.0
M6-28	S	25.0	75	2.0	0.5	5.0	-
T6-29	H	24.5	50	12.0	10.0	4.5	10.0
F7-2	S	26.0	192	82.0	46.5	13.0	2.0
S7-3	H	26.0	40	12.0	10.0	8.5	40.0
Su7-4	S	26.0	69	8.0	4.0	10.0	-(ND)
M7-5	S	26.5	138	126.0	28.0	9.5	4.0
T7-6	S	-	136	25.0	10.0	27.75	4.0
F7-9	H	26.0	145	65.0	10.0	2.5	-
S7-10	S	27.0	145	35.0	6.0	0.5	5.5
M7-12	S	26.0	142	105.0	39.0	33.0	7.0
T7-13	C	25.0	15	98.0	7.0	7.0	2.0
F7-16	S	26.0	79	10.0	2.75	2.5	2.0
S7-17	P-C	26.5	142	10.0	6.5	0.75	0.5
M7-19	S	26.0	151	4.0	7.0	2.25	0.0
T7-20	S	26.5	142	6.0	8.5	2.5	0.5
F7-23	C	25.0	53	11.0	3.0	3.25	3.0
S7-24	S	25.0	185	26.0	6.0	3.25	7.0
M7-26	S	25.5	110	48.0	12.5	6.0	0.0
T7-27	C	25.0	30	18.0	-	14.0	69.0**
F7-30	R	-	2	42.0	7.0	4.0	0.25

TABLE 20A (Continued)

DATE 1976	WEATHER	WATER TEMP. °CENTIGRADE	TOTAL NUMBER PEOPLE	MEAN TOTAL COLIFORM	MEAN FECAL COLIFORM	MEAN FECAL STREP.	MEAN STAPH. AUREUS
S7-31	C	24.0	5	8.0	0.0	10.0	4.0
M8-2	P-C	24.0	67	20.0	1.0	3.0	1.5
T8-3	P-C	24.5	79	35.0	3.0	1.5	4.0
T8-10	S	25.5	109	130.0	28.0	43.0	1.0
F8-13	S	26.0	145	35.0	16.0	6.0	12.5
S8-14	P-C	26.0	90	130.0	108.5	95.0	13.5
M8-16	S	25.5	174	20.0	3.0	2.0	1.5
F8-20	S	25.0	200	20.0	6.5	5.5	2.5

S = Sunny

C = Cloudy

R = Rain

H = Humid

P-C = Partly Cloudy

ND = No data

* = Above National Standard

** = Above Proposed Standard

TABLE 21
Bacteriological Analysis of
Duck Pond - Morning Samples

DATE 1976	WEATHER	WATER TEMP. °CENTIGRADE	TOTAL NUMBER PEOPLE	MEAN TOTAL COLIFORM	MEAN FECAL COLIFORM	MEAN FECAL STREP.	MEAN STAPH. AUREUS
T6-15	P-C	20.0	0	5.0	0.0	165.0*	ND
F6-18	S	21.5	0	10.5	0.0	50.0	1.0
S6-19	C	22.5	0	76.0	10.0	5.0	1.0
M6-21	H	22.5	0	10.0	0.0	20.0	1.5
T6-22	C	23.5	0	10.0	0.0	13.0	0.0
F6-25	C	24.5	0	6.0	0.0	2.0	0.0
S6-26	C	24.0	0	4.0	4.0	3.5	2.0
M6-28	C	23.5	0	10.0	0.0	0.0	1.0
T6-29	R	23.5	0	8.0	0.5	1.5	0.0
F7-2	S	-	0	5.0	0.5	25.0	-
S7-3	S	25.0	0	90.0	23.5	25.0	18.5
Su7-4	S	24.5	2	0.0	4.5	10.5	-
M7-5	S	24.0	2	-	0.0	31.0	-
T7-6	S	25.0	0	12.0	0.0	8.0	0.0
S7-10	S	25.0	0	4.0	0.0	0.5	0.0
M7-12	C	25.0	0	6.0	0.0	20.0	0.0
T7-13	P-C	-	0	70.0	0.5	2.5	0.5
F7-16	S	24.5	0	8.0	0.0	1.0	0.25
S7-17	R	25.0	0	0.0	1.0	13.5	0.0
M7-19	S	25.0	0	0.0	0.0	0.25	0.0
T7-20	S	25.5	0	2.0	0.0	0.25	0.0
F7-23	S	24.0	0	8.0	0.0	4.5	0.0
S7-24	R	24.0	0	-	5.0	8.75	0.0
M7-26	S	24.0	0	0.0	0.0	3.0	0.0
T7-27	S	24.5	0	10.0	-	14.75	70.5**

S = Sunny

C = Cloudy

P-C = Partly Cloudy

H = Humid

ND = No data

* = Counts above National Standard

** = Counts above proposed standard

TABLE 21A
Bacteriological Analysis of
Duck Pond - Afternoon Samples

DATE 1976	WEATHER	WATER TEMP. °CENTRIGRADE	TOTAL NUMBER PEOPLE	MEAN TOTAL COLIFORM	MEAN FECAL COLIFORM	MEAN FECAL STREP.	MEAN STAPH. AUREUS
T6-15	P-C	20.0	0	5.5	0.0	15.0	NO
F6-18	S	22.5	5	10.0	0.0	105.0	0.0
S6-19	P-C	23.0	0	15.0	0.5	0.5	0.0
M6-21	S	24.5	0	25.0	2.0	10.0	0.0
T6-22	S	24.5	0	5.0	1.0	1.5	0.0
F6-25	H	25.0	0	5.0	0.0	2.0	0.0
S6-26	S	25.0	1	92.0	0.0	0.0	0.0
M6-28	S	24.5	4	116.0	65.0	6.5	1.0
T6-29	H	24.0	0	0.0	0.0	0.5	5.0
F7-2	S	25.0	0	2.0	2.5	15.5	10.0
S7-3	H	25.0	0	1.5	0.0	2.0	5.5
Su7-4	S	25.0	0	4.0	3.5	5.0	2.5
M7-5	S	26.5	8	145.0	35.5	62.0	4.0
T7-6	S	28.0	0	8.0	0.0	7.5	1.5
F7-9	H	26.0	2	22.0	0.5	1.0	0.5
S7-10	S	26.0	4	2.0	0.0	0.5	0.25
M7-12	S	26.0	3	16.0	1.0	0.5	1.0
T7-13	C	25.5	0	110.0	0.5	1.0	0.0
F7-16	S	27.0	0	2.0	1.0	0.0	0.0
S7-17	P-C	26.0	0	0.0	0.5	5.0	1.0
M7-19	S	26.0	3	5.0	0.0	0.5	0.0
T7-20	S	27.0	1	5.0	0.0	0.0	0.0
F7-23	C	25.5	0	0.0	1.0	0.5	1.0
S7-24	S	26.5	2	2.0	1.0	3.0	0.5
M7-26	S	26.5	2	10.0	2.0	4.0	0.0
T7-27	C	25.0	0	15.0	NG	10.0	>200**

TABLE 21A (Continued)

DATE 1976	WEATHER	WATER TEMP. °CENTRIGRADE	TOTAL NUMBER PEOPLE	MEAN TOTAL COLIFORM	MEAN FECAL COLIFORM	MEAN FECAL STREP.	MEAN STAPH. AUREUS
F7-30	R	24.5	0	0.0	0.0	30.0	11.5
S7-31	C	24.0	0	5.0	0.0	13.75	8.5
M8-2	P-C	24.0	0	0.0	0.0	0.5	14.0
T8-3	P-C	24.5	0	20.0	0.0	1.5	65.0
T8-10	S	25.0	3	3.5	1.5	11.0	0.0
F8-13	S	26.5	3	0.0	0.5	4.0	0.0
S8-14	P-C	26.0	2	32.0	1.0	>200*	1.0
M8-16	S	27.0	0	12.0	1.0	1.0	0.0
F8-20	S	27.0	8	4.0	0.0	1.0	1.0

S = Sunny

C = Cloudy

H = Humid

R = Rain

P-C = Partly cloudy

* = Counts above National Standard

** = Counts above proposed standard

TABLE 22

Comparison of MPN vs. MF
Gull Pond

DATE	TOTAL COLIFORM MPN		TOTAL COLIFORM MF	FECAL COLIFORM MPN		FECAL COLIFORM MP	MPN PSEUDOMONADS	
	Index	Range		Index	Range		Index	Range
1976								
6-19	49	17-130	20	33	11-93	6.0		
7-4	79	25-190	10	11	2-25	14.0		
7-6	110	31-250	155	49	17-130	15.0	<2.0	
7-12	920	300-3200	365	540	180-1400	155.0		
7-13	1600	640-5800	1140	920	300-3200	288.0		
7-17	17	5-46	50	33	11-93	17.0		
7-19	300	35-300	116	300	35-300	60.0	<2.0	
7-24	220	57-700	220	94	28-220	31.0	2.0	<0.5-7.0
7-26	49	17-130	92	23	7-70	10.0	<2.0	
7-31	17	5-46	5.0	5.0	<0.5-13	1.0	2.0	<0.5-7.0
8-2	33	11-93	35	5.0	<0.5-13	6.0	<2.0	
8-6	11	2-25	-	7.0	1-17	-		
8-10	540	180-1400	120	33	11-93	17.0	<2.0	
8-14	79	25-190	80	33	11-93	72.0	<2.0	
8-20	27	9-80	202	27	9-80	17.0	4.0	<0.5-11

TABLE 23
Comparison of MPN vs. MF
Great Pond (W)

DATE	TOTAL COLIFORM MPN		TOTAL COLIFORM MF	FECAL COLIFORM MPN		FECAL COLIFORM MF	MPN PSEUDOMONADS	
	Index	Range		Index	Range		Index	Range
1976								
6-29	2.0		5.0	<2.0		0.0		
7-4	170.0	43-490	50.0	2.0	<0.5-7.0	2.0		
7-6	33.0	11-93	105.0	5.0	<0.5-13	2.5	<2.0	
7-12	49.0	17-130	95.0	49.0	17-130	12.0		
7-13	11.0	2.0-25	85.0	11.0	2-25	1.0		
7-17	2.0	<0.5-7	25.0	2.0	<0.5-7.0	1.0		
7-19	5.0	<0.5-13	4.0	5.0	<0.5-13	2.0	<2.0	
7-24	13.0	3-31	13.0	2.0	<0.5-7.0	2.5	<2.0	
7-26	13.0	3-31	18.0	5.0	<0.5-13	4.0		
7-31	5.0	<0.5-13	5.0	<2.0		0.5	2.0	<0.5-7.0
8-2	2.0	<0.5-7	5.0	2.0	<0.5-7.0	0.5	<2.0	
8-6	23.0	7-70	-	<2.0		-		
8-10	23.0	7-70	250.0	23.0	7.70	18.5	<2.0	
8-14	33.0	11-93	92.0	5.0	<0.5-13	5.5	<2.0	
8-20	79.0	25-190	146.0	49.0	17-130	51.0	<2.0	

TABLE 24
Comparison MPN vs. MF
Long Pond

DATE	TOTAL COLIFORM MPN		TOTAL COLIFORM MF	FECAL COLIFORM MPN		FECAL COLIFORM MF	MPN PSEUDOMONADS	
	Index	Range		Index	Range		Index	Range
1976								
6-29	8.0	1-19	12.0	8.0	1-19	10.0		
7-4	130.0	35-300	8.0	7.0	1-17	4.0		
7-6	17.0	5-46	25.0	11.0	2-25	10.0	<2.0	
7-12	130.0	35-300	105.0	79.0	25-190	93.0		
7-13	17.0	5-46	98.0	49.0	17-130	7.0		
7-17	11.0	2-25	10.0	11.0	2-25	6.5		
7-19	17.0	5-46	4.0	17.0	5-46	7.0	< 2.0	
7-24	13.0	3-31	26.0	8.0	1-19	6.0	2.0	<0.5-7.0
7-26	23.0	7-70	48.0	8.0	1-19	12.5	< 2.0	
7-31	9.0	2-21	8.0	6.0	<.5-15	0.0	8.0	1-19
8-2	2.0	<.5-7	20.0	2.0	<.5-7	1.0	< 2.0	
8-6	23.0	7-70	-	23	7-70	-		
8-10	130.0	35-300	130	49	17-130	28	< 2.0	
8-14	920.0	300-3200	130	540	180-1400	108.5	< 2.0	
8-20	79.0	25-190	20	11	2-25	6.5	< 2.0	

TABLE 25
Comparison MPN vs. MF
Duck Pond

DATE 1976	TOTAL COLIFORM MPN		TOTAL COLIFORM MF	FECAL COLIFORM MPN		FECAL COLIFORM MF	MPN PSEUDOMONADS	
	Index	Range		Index	Range		Index	Range
6-29	<2.0		0.0	<2.0		0.0		
7-4	11.0	2-25	4.0	5.0	<0.5-13	3.5		
7-6	<2.0		8.0	<2.0		0.0	<2.0	
7-12	2.0	<0.5-7	16.0	2.0	<0.5-7.0	1.0		
7-13	79.0	25-190	110.0	79.0	25-190*	0.5		
7-17	<2.0		0.0	<2.0		0.5		
7-19	<2.0		5.0	<2.0		0.0	<2.0	
7-24	2.0	<0.5-7	2.0	2.0	<0.5-7.0	1.0	<2.0	
7-26	8.0	1-19	15.0	5.0	<.5-13	-		
7-31	23.0	7-70	5.0	2.0	<0.5-7.0	0.0	<2.0	
8-2	2.0	<0.5-7	0.0	2.0	<0.5-7.0	0.0	2.0	<0.5-7.0
8-6	2.0	<0.5-7	-	<2.0		-		
8-10	23.0	7-70	3.5	5.0	<0.5-13	1.5	<2.0	
8-14	13.0	3-31	32.0	<2.0		1.0	<2.0	
8-20	5.0	<0.5-13	4.0	5.0	<0.5-13	0.0	<2.0	

