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(Résumé en français)

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Abstract

There are inherent weaknesses associated with currently used bacterial fecal pollution indicator systems. Fecal pollution indicator data would be more meaningful if supplemented with information relating to the occurrence of pathogens in recreational water. In this report we have reviewed the potential of different fungi to serve as fecal pollution indicators and the literature pertaining to the occurrence of the potentially pathogenic yeast *Candida alibcans* in the aquatic environment.

In preliminary investigations, mCA agar proved superior to Sabouraud dextrose agar in the enumeration of *C. albicans* from the aquatic environment. Different porosity membrane filters seemed equivalent in their ability to recover *C. albicans* cells from suspension. Oxgall agar proved superior to cornmeal agar with added Tween in the induction of chlamydospore formation in environmental isolates of *C. albicans.*

It has been established that the opportunistically pathogenic yeast *C. albicans* occurs in Lake Ontario beaches. The four beaches surveyed could be differentiated on the basis of bacterial fecal pollution indicator levels and numbers of the pathogens *C. albicans* and *Pseudomonas aeruginosa.* The occurrence of the two pathogens in the beach waters examined appears to be related to elevated fecal pollution indicator levels. Maximum numbers of all microbial parameters were observed in July and August in association with peak bather loads at the beaches. Where microbial population levels were adequate, a decreasing distribution pattern from the shoreline to the offshore waters was observed. In only one instance does the data suggest that a beach had been subjected to human fecal contamination. Storm water runoff is the most plausible source of the contamination in most other cases, although the possibility that the bathers themselves may contribute to the pollution level of a beach must also be considered.

Résumé

Les indicateurs bactériens de pollution fécale couramment utilisés comportent certaines lacunes. Les données fournies par ces indicateurs seraient plus significatives si elles étaient employées de pair avec des renseignements sur l'incidence des organismes pathogènes dans les eaux servant à des fins récréatives. Le présent rapport traite des différents champignons en tant qu'indicateurs de pollution fécale, ainsi que des ouvrages portant sur l'incidence dans le milieu aquatique de *Candida albicans,* levure potentiellement pathogène.

La gélose mCA s'est révélée, lors d'études préliminaires, supérieure à la gélose SDA pour la numération de *C. albicans* qui se trouve dans l'environnement aquatique. Les membranes filtrantes de diverses porosités ont été toutes aussi efficaces pour la récupération de cellules de *C. albicans* en suspension. L'induction de chlamydospores chez des isolats de *C. albicans* provenant de milieux naturels s'est réalisée plus facilement avec une gélose à la bile de boeuf qu'avec une gélose à la farine de maïs et au Tween.

Les études ont démontré que la levure pathogène facultative *C. albicans* est présente dans les baignades du lac Ontario. Ainsi, les quatre plages étudiées ont pu être caractérisées en fonction des teneurs en indicateurs bactériens de pollution fécale et en organismes pathogènes *C. albicans* et *Pseudomonas aeruginosa*. Il semble y avoir un rapport entre les incidences de *C. albicans* et de *P. aeruginosa* dans les eaux des plages étudiées et les teneurs élevées en indicateurs de pollution fécale. Les concentrations maximales de tous les paramètres microbiens ont eu lieu aux mois de juillet et d'août, périodes correspondant aux populations de baigneurs les plus importantes. Dans les cas où les données de populations microbiennes étaient suffisantes, il semble y avoir une décroissance des teneurs en fonction de la distance à la rive. Les données de l'étude n'indiquent une contamination fécale humaine que dans un seul cas. Dans la plupart des autres cas, la source la plus probable de contamination semble être le ruissellement des eaux de pluies; il ne faut cependant pas écarter la possibilité que les baigneurs puissent aussi contribuer à la pollution des eaux.

Acknowledgments

We are grateful to Dr. E.A. Belle, Director, Public Health Laboratory, Hamilton, for his co-operation and advice during the course of this project. We acknowledge the assistance of Mr. D. Stronach, Halton Regional Health Unit, who collected the swimming pool water samples.

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Introduction

With the increased public use of natural waters for recreational purposes in recent years, a need has developed to protect water recreationists from enteric, respiratory tract and skin infections which may result from contact with water or sand-borne infectious agents. As the feces of man and warm-blooded animals contain a variety of human pathogens (Geldreich, 1972), the presence of fecal contamination in bathing waters is of particular concern.

In the past, bacterial indicators such as the coliform and fecal coliform groups have been used to detect and monitor fecal contamination in water (Wolf, 1972). Apart from providing only indirect evidence of the presence of waterborne pathogens, such indicators have further drawbacks. Their use does not make allowance for the known differential survival and proliferation rates of pathogens and indicators in natural waters (Shuval *et al.,* 1973). In addition, the presence of both coliform and fecal coliform indicators has been observed in non-fecally polluted water (Wolf, 1972; Rokosh *et al.,* 1977), whereas enteric pathogens have been detected in waters containing negligible bacterial indicator levels (Dutka and Bell, 1973).

For the above reasons, an obvious need exists to develop improved and supplementary procedures for use in the detection of fecal contamination in water, preferably utilizing organisms which are both primarily found in feces and are also known pathogens.

The yeast *Candida albicans* is a known component of the body flora of humans (Beneke and Rogers, 1970), some animals (van Uden, 1960) and birds (Cragg and Clayton, 1971; Hasenclever and Kocan, 1975). As a consequence of its intestinal habitat, *C. albicans* is commonly found in both urine and feces. Because *C. albicans* is not normally associated with non-polluted waters, its presence therein may be considered an indication of fecal contamination (Meyer, 1974). *C. albicans* is also a pathogenic yeast and has long been associated with a variety of superficial (oral, vaginal and cutaneous) and disseminated mycotic infections in man (Gentles and la Touche, 1969; Emmons *et al.,* 1977). Thus, apart from its possible significance as a pollution indicator organism, *C. albicans,* when present in water, may also be a potential health hazard to water recreationists.

While the occurrence of *C. albicans* in fresh water has been reported (Cook, 1970), little attention has been devoted to its occurrence and distribution in freshwater bathing beaches. Thus, the main objectives of this study were (a) to investigate the occurrence and distribution of *C. albicans* in selected Lake Ontario bathing beaches during the summer months of 1977 and (b) to compare the numbers of *C. albicans* in these waters with the densities of the fecal coliform and fecal streptococcus bacterial fecal pollution indicators and the pathogen *P. aeruginosa.*

Literature Review

Over the past twenty years, as a consequence of increased public education and awareness, water quality has become a subject of concern to the public, local authorities and scientists alike. Also, the advent of the affluent society has resulted in increased participation in water recreational activities and also in vastly elevated bather densities at popular coastal and inland beach resorts. The risk of contracting waterborne infectious diseases is of particular concern to these bathers.

Contamination of recreational waters with fecal material is not only aesthetically offensive, but also potentially dangerous, since many organism-caused diseases which are thought to be transmitted through water are discharged from the intestines of infected persons (Wolf, 1972). However, as pathogenic microorganisms are also known to occur in the feces of both domestic and wild animals (Geldreich, 1972), fecal contamination of water caused by warm-blooded animals may also have public health significance. Bonde (1977), citing from the reports of Bonde (1962) and Craun and MacCabe (1973), lists the following diseases as being waterborne:

(a) Bacterial diseases: cholera, typhoid, paratyphoid, and infections with other pathogenic *Salmonella* types, pathogenic *Escherichia coli* types and *Vibrio parahaemolyticus;* dysentry, leptospirosis, tularaemia, brucellosis and tuberculosis.
(b) Viral diseases: hepatitis, Coxsackie virus, poliomyelitis, adenovirus and reovirus infections.
(c) Parasitic diseases: taeniasis, oxyuriasis, trichinosis, amoebiasis, lambliasis, ascariasis, and anchylostomiasis.
(d) Fungi: *Candida* and yeasts

The most common of the waterborne pathogens include strains of *Salmonella, Shigella, Leptospira,* enteropathogenic *E. coli, Pasturella, Vibrio, Mycobacterium,* human enteric viruses, cysts of *Endamoeba hystolytica* and hookworm larvae (Geldreich, 1972). *Salmonella* strains have often been detected in sewage, streams and tidal waters (Geldreich, 1972) and are considered to be a frequent cause of disease in humans (Bonde, 1977), although the symptoms are often slight.

These microbial pathogens may enter recreational waters via inadequately treated sewage discharges, urban and rural storm water runoff or animal wastes (Geldreich, 1972; 1974-1975). Possibly water recreationists themselves also contribute to the pathogen content of recreational waters.

Originally the techniques and knowledge did not exist to accurately and routinely monitor the numbers of specific pathogenic microorganisms in water samples. Consequently, groups of bacterial indicator organisms, which contain bacteria mainly found in the feces of warm-blooded animals, were delimited. When detected in natural waters in numbers that exceed established standards, these indicators indirectly suggest the potential presence of intestinal pathogens.

To fulfill their role, indicators should be:

(1) consistently associated with the source of the pathogen;
(2) present in water whenever the suspected pathogens are present;
(3) present in greater numbers than the pathogens;
(4) more resistant to disinfectants than the pathogens;
(5) readily enumerated and identifiable using rapid laboratory procedures.

In addition, the numbers of the indicator enumerated should vary in proportion to variations in the quantity of pollutant present (Bonde, 1977; Cabelli, 1977). The indicators most commonly used are total coliforms, fecal coliforms, *E. coli,* fecal streptococci and *Clostridium perfringens.*

Methodological advances involving technique refinements and the development of improved media have resulted in the increased accuracy and reliability of the bacterial indicator systems. Such advances have also broadened the information which may be deduced from available indicator data. Of particular interest in this respect is the observation of Geldreich *et al.* (1964) and Geldreich (1976) that the ratio of fecal coliforms to fecal streptococci may be used to distinguish fecal contamination of human origin

from that of animal origin. However, despite such refinements in the use of, and interpretation of, results obtained using the bacterial indicator systems, attention has been focused on certain weaknesses inherent in this essentially indirect method of monitoring the presence of pathogens in water. It has been observed (Shuval *et al.*, 1973) that coliforms and fecal coliforms can survive and reproduce in chlorinated waste waters, thus negating any established indicator : pathogen population density ratios. LeClerc *et al.* (1977) have determined that *Salmonella* can survive for 19 weeks in contaminated surface well waters, whereas *E. coli* only survives for 2 weeks. In such cases, the use of *E. coli* as an indicator may lead to false negative results. Moreover, some studies have shown that low or negligible indicator levels do not preclude the presence of enteric pathogens (Cherry *et al.*, 1972; Dutka and Bell, 1973).

A possible solution to this problem is to supplement, at least in the short term, the data obtained using bacterial indicators with information relating to the presence of known pathogens in the waters under examination. This possibility has been facilitated by the development of improved techniques for use in the isolation of pathogenic microorganisms from water. Such data could provide a basis for a more realistic assessment of the health hazards present in recreational waters. However, as pointed out by Cabelli (1977), the task of monitoring each of the pathogenic microorganisms present in municipal sewage would be an herculean one, notwithstanding the fact that enumeration methods for some of the more important pathogens have yet to be developed. Nevertheless, one feels that with judicious selection of the pathogens, mindful of the diseases causing concern in the locality and of the use to which the water is put such data would usefully complement the results of water quality assessments made using bacterial indicators.

In addition to bacteria, the microflora of feces from warm-blooded animals contains a variety of moulds, yeasts, viruses and protozoa (Geldreich, 1972; 1976; Cooke,1976). Any individual or group of these microorganisms may be considered as a potential indicator of fecal contamination provided it satisfies the previously listed conditions for bacterial indicators. Should such an organism also be pathogenic towards man, then a dual purpose would be served, that of indicating indirectly and directly the existence of a public health hazard in water.

It has been known since the fifties, mainly through Cooke's studies (1959; 1965; 1970), that fungi are present in large numbers at every stage of the sewage treatment process and occur in elevated densities in natural waters that have received an input of sewage effluent. During an intensive examination of the yeast microflora of Lake Champlain, Meyers *et al.* (1970) noted that in areas directly affected by industrial or urban effluents, yeast populations generally exceeded 300 colony-forming units (cfu)/100 ml, as opposed to baseline levels of approximately 20 cfu/100 ml. In these areas of the lake, physiologically distinct species predominated, which suggested to the authors the possibility of using various yeasts as indicators of specific water quality. Similar conclusions were drawn by Woollett and Hedrick (1970), who in a survey of 13 polluted freshwater habitats, observed that the generic composition of the predominant segment of the yeast population was dependent on the nature of the pollutants. Cook (1970) also observed higher populations of yeasts at a polluted site on Lake Champlain (predominant genera *Rhodotorula, Cryptococcus and Candida),* and on the basis of the consistent isolation of *C. albicans* near effluents from college dormitories, confirmed the previous suggestion of Ahearn *et al.* (1968) that this opportunistic pathogen could serve as an indicator of recent fecal contamination. Simard (1971) tentatively suggested the possibility of using 'pink yeasts' (especially *Rhodotorula glutinis)* as indicators of fecal pollution. However, as Meyer (1974) pointed out, the ubiquitous, oxidative yeasts such as *Rhodotorula* may not be suitable indicators of enrichment with organic materials, although local increases in numbers of these species may reflect an imbalance in the normal environment. Strongly fermentative yeasts, such as members of the genus *Candida,* which are predominant in sewage waters, are potentially more suitable as water quality indicators.

Of those species of *Candida* which have been detected in water, most attention is being directed towards *C. albicans* as a specific indicator of fecal contamination (Buck, 1977). *C. albicans* is a known component of the body flora of humans, some animals (van Uden, 1960; Winner and Hurley, 1964) and birds (Gentles and la Touche, 1969; Cragg and Clayton, 1971; Hasenclever and Kocan, 1975). Because of its intestinal habitat, *C. albicans* is commonly isolated from both urine (Ahearn *et al.*, 1966) and feces (Gentles and la Touche, 1969). Moreover, *C. albicans* has been isolated from sewage settling ponds (Ahearn *et al.*, 1968) and raw sewage (Ahearn, 1973) but not from treated effluents (Ahearn, 1973). On occasion, *C. albicans* has been isolated from soils that were contaminated with mammal and bird droppings (di Menna, 1955) and plants (van Uden *et al.*, 1956), proving that it can survive for at least an appreciable time in the terrestrial environment in the absence of an animal host. As *C. albicans* is not normally associated with non-polluted waters, its presence therein may be considered an indication of fecal contamination, an association which has been observed in the field by Cook (1970) and Ahearn *et al.* (1968). In addition, the yeast is rarely isolated from normal healthy skin (Njoku-Obi *et al.*, 1976; Aly and Maibach, 1977). In a survey of 126 hospitalized patients, Rose and Kurup (1977) observed that 36.5% of the patients carried *C. albicans* in their throats, 29.4% in their rectums and only 0.008% on their

skin. These observations emphasize that *C. albicans* is a member of the internal and not of the external body flora of humans, which adds to its significance as a potential indicator of feces-associated pathogens.

Since the earliest days of medical microbiology, the fungus *Candida* has been associated with various 'thrush' type infections in man. *C. albicans* is not only the most common species of *Candida* associated with diseases in man, but is also the most pathogenic (Gentles and la Touche, 1969). *C. albicans* is the causal agent of a variety of superficial mycotic infections such as oral and vaginal thrush, skin infections (especially in interdigital locations) and ocular infections (Beneke and Rogers, 1970; Emmons *et al.*, 1977). Systemic *Candida* infections may involve the central nervous system, the circulatory system, the respiratory system, the digestive system and the urinary system (Gentles and la Touche, 1969).

It should be stated, however, that *C. albicans* may be isolated from various sites in the human body such as the mouth, vagina and intestines where it apparently exists as a component of the body's normal saprophytic microflora (Winner and Hurley, 1964). Under certain conditions the yeast's role is altered from that of a commensal to a pathogen. While the factors which trigger this transformation have yet to be elucidated, it appears that infection generally occurs in hosts whose resistance has been altered by a predisposing factor such as obesity or diabetes (Emmons *et al.*, 1977). Trauma caused by surgical operations or environmental conditions which cause skin maceration and debilitating diseases are also important factors contributing to infection. With the increasing use of broad spectrum antibiotics and oral contraceptives, *C. albicans* is becoming increasingly important as a cause of mycoses in man. *C. albicans* has been associated with the following clinical conditions in humans: intertriginous candidiasis, paronchyia, generalized cutaneous candidiasis, vulvovaginal candidiasis, oral thrush, ocular candidiasis, urinary tract infections, broncho-candidiasis, pulmonary candidiasis, endocarditis and meningitis (Taplin, 1976; Emmons *et al.*, 1977; Montgomerie and Edwards, 1978; Kozinn *et al.*, 1978).

Indeed, the importance of *Candida* as a disease agent is illustrated by the fact that 22% of all mycotic deaths recorded in the United States in 1966 were attributed to *Candida* (Emmons *et al.*, 1977 – citing Vital Statistics for the U.S.A.). Although the normal mode of infection is probably by person-to-person contact or through a change in the status of the host's native *C. albicans* flora, the possibility of infection from a previously contaminated environment cannot be eliminated at the moment, particularly when a danger of dermal maceration exists either through occupational (housewives, fruit canners) or recreational (bathers) hazards.

Although few studies have been made of the presence of pathogenic fungi in freshwater beaches, the occurrence in marine beaches of fungi potentially pathogenic to man has been more frequently examined. Dabrowa and co-investigators (1964) detected *C. albicans* in samples of crabs and bird droppings obtained from tide-washed coastal areas of southern California. These investigators suggested, albeit prematurely, that the intertidal zone may constitute a reservoir of potentially pathogenic fungi and that certain fungus infections may be acquired by exposure to these areas. During an examination of beach sands in Hawaii, Kishimoto and Baker (1969) isolated *C. albicans* at five separate locations which had high and medium bather loads. From a comparative survey of selected beaches in the Tampa Bay, Florida area, Bergen and Wagner-Merner (1977) reported the isolation of *Candida* spp. from beach sands. However, these investigators did not identify the *Candida* isolates to species level. Buck (1976) examined water, sediment and sand from recreational and other areas in the southern Biscayne Bay region for the presence of indicator and potentially pathogenic bacteria and yeasts. This study recorded high yeast populations in both sands and sediments. The highest yeast density in water was found in association with high bather levels. *C. albicans* has also been cultured from swimming pool waters which had a free chlorine content of <0.35 mg/l (Kraus and Tiefenbrunner, 1975).

While the occurrence of *C. albicans* in recreational waters has yet to be directly and conclusively associated with outbreaks of candidiasis among bathers, Brisou (1975) recently commented on the increased instances of vaginal infections caused by *Candida* among females frequenting marine beaches contaminated with intestinal fungi. Consequently, Brisou (1975) recommended that the sanitary surveillance of beaches should include systematic monitoring of intestinal yeasts as supplementary evidence of fecal pollution. In addition, Kishimoto and Baker (1969) stressed the epidemiological significance of pathogenic fungi as potential foci of infection for people using public beaches.

Because of the paucity of information on the occurrence of pathogenic fungi in recreational fresh water, a study of four selected bathing beaches in Lake Ontario was undertaken to establish whether or not *C. albicans* occurs in these waters. It was intended to ascertain the potential of *C. albicans* to complement the traditional fecal pollution indicators by determining its distribution patterns in relation to the fecal coliform and fecal streptococci indicators and the pathogenic bacterium *Pseudomonas aeruginosa*.

Materials and Methods

SAMPLE COLLECTION AND BEACH LOCATIONS

On June 13, July 4, August 8 and September 12, 1977, the following beaches on Lake Ontario, Canada, were surveyed.

Beach	Military Grid Reference No.	Town Served
Hamilton Beach	978 944	Burlington
Confederation Park	012 890	Hamilton
Lakeside Park	408 848	St. Catharines, Port Dalhousie
Weller Park	444 871	St. Catharines, Port Dalhousie

Each of the above beaches is a designated public bathing area, located in the Hamilton/St. Catharines region of Ontario (Fig. 1) and is in continual use throughout the summer. Hamilton Beach is located next to the Burlington ship canal and is probably subject to considerable pollution because of the proximity of the heavily industrialized Hamilton Harbour. Confederation Park beach is situated in a popular picnic and recreational area serving the City of Hamilton (pop. 498,500). Both Lakeside Park and Weller Park beaches are adjacent to St. Catharines (pop. 303,400). Lakeside Park is close to the outflow from Martindale Pond into which the old Welland Canal and Twelve Mile Creek flow. Weller Park beach is adjacent to the busy Welland ship canal.

At each beach, samples were collected on a transect from a central point in the bathing area; a typical transect is illustrated in Figure 2. With the exception of Hamilton Beach, the lifeguard stands were used as the base points because maximum bather loads were observed to occur in their vicinity. At all beaches, the surface sand was sampled 3 m from the water's edge and water samples were taken at 1-m, 10-m and 20-m intervals from the shore.

The surface sand samples (0-2 cm depth) were collected within a 0.5 m^2 quadrat using a presterilized wide-

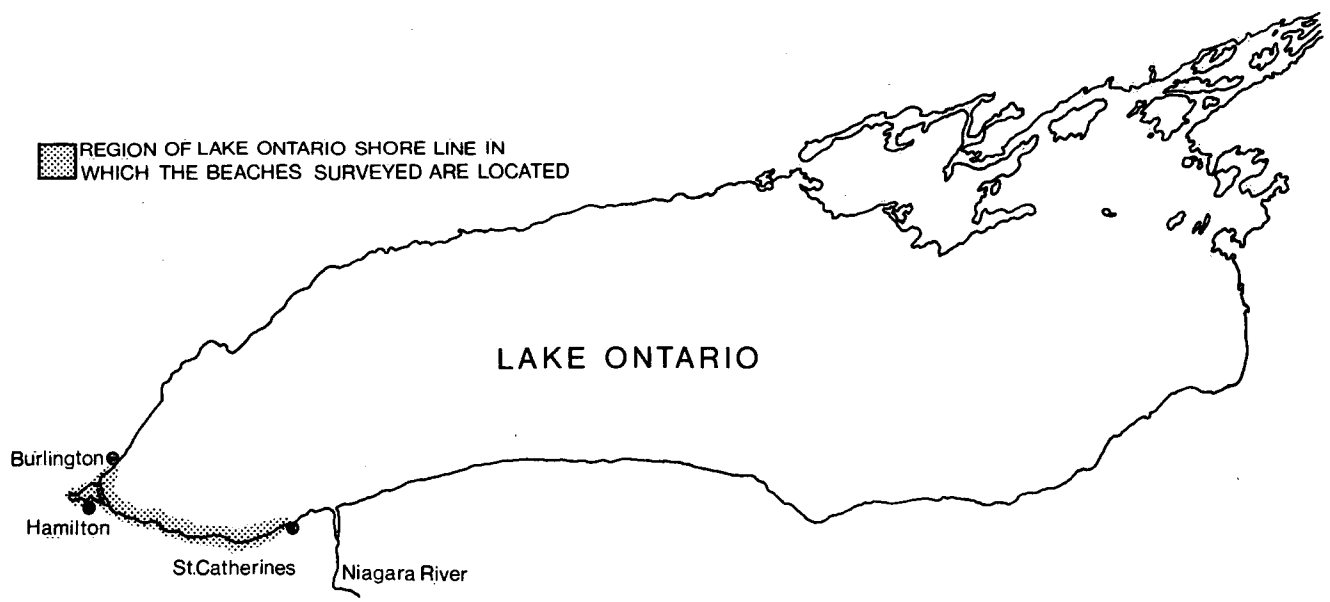

Figure 1. Location of beaches surveyed.

mouthed container. At each of the other sampling stations, surface water samples (0-5 cm depth) were collected by scooping into a one-litre sterile sampling bottle. One-metre depth and bottom water samples were collected using the methods described by Dutka (1976). The 10-m and 20-m stations were sampled from a small dinghy, the distance from the shore being determined by means of a precalibrated cable.

The surface sand temperature was determined by means of a thermometer placed 1 cm below the beach surface. The temperature of the water samples was determined immediately after collection. All samples were transferred to sterile containers and stored on ice during transportation to the laboratory. The pH of the water samples was determined at the laboratory using a Radiometer pH meter (Model 29).

SWIMMING POOLS

In addition to the bathing beaches, a number of freshwater swimming pools in the Halton regional health area were surveyed. The exact location of these pools cannot be presented in this report so they are identified by code numbers (Table 1). At each pool, water samples were aseptically collected from the water surface adjacent to the pool skimmer and at a 1-m depth near the pool main drain.

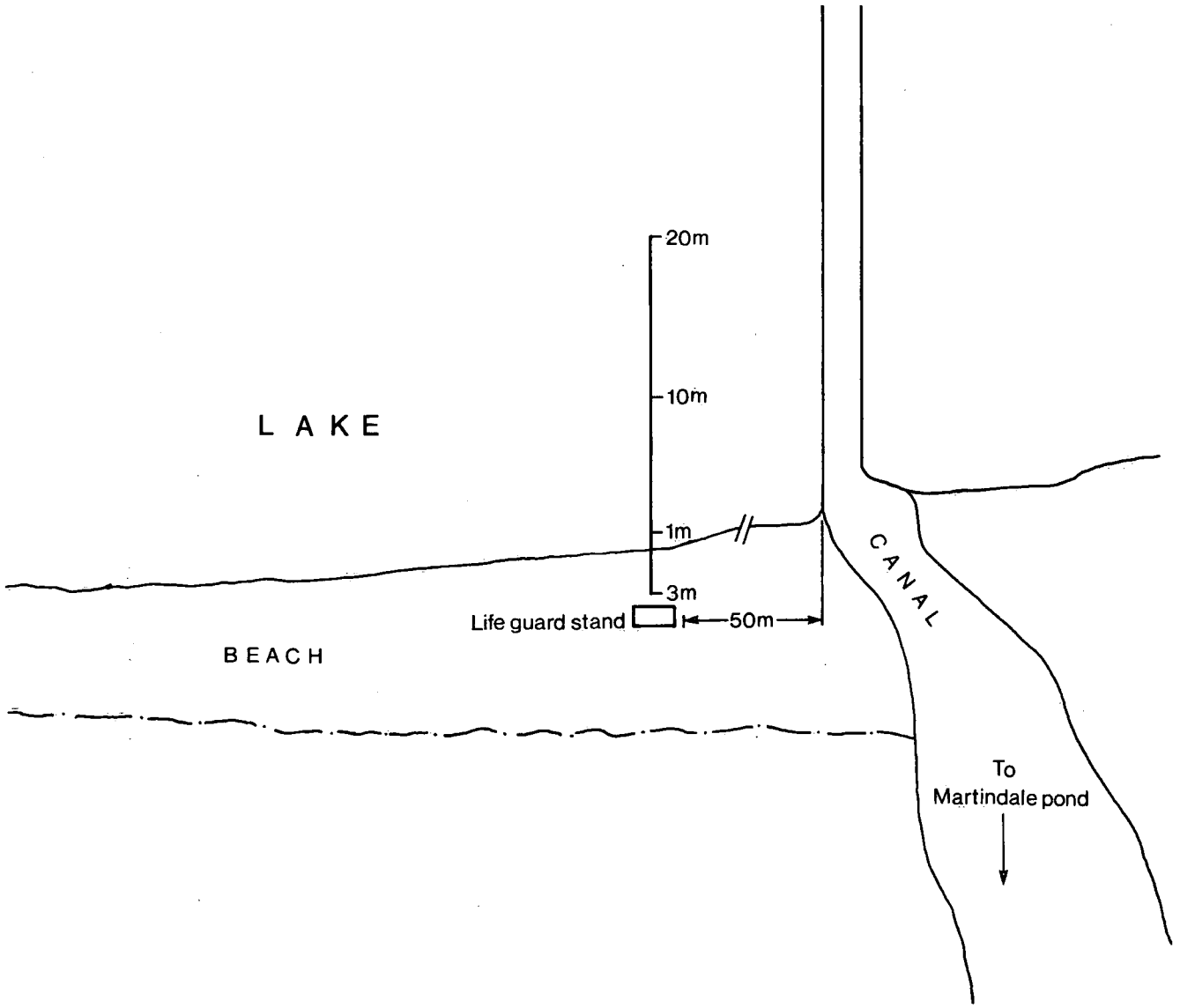

Figure 2. Location of sampling transect on Lakeside Park beach near Port Dalhousie.

Table 1. Description of Swimming Pools Surveyed, July-September, 1977

Pool Number	Class	Description	Date Surveyed (1977)	Sampling Time (approx.)	T (°C)	pH	Chlorine (ppm)	Bromine (ppm)
1	–	Whirlpool	July 27	Morning	38	7.0	0	–
			August 24	Midday	41	7.2	> 2	–
2	B	Indoor	July 27	Morning	21.1	7.2	–	0.1
3	B	Outdoor	July 27	Morning	21.1	7.6	1.5	–
			August 24	Midday	19	7.4	2.0	–
4	A	Outdoor	July 27	Morning	20	7.4	1.0	–
			August 24	Midday	21.1	7.2	2.0	–
			September 20	Afternoon	28.0	7.6	1.0	–
5	B	Indoor	September 20	Afternoon	29.0	8.0	2.0	–
6	B	Indoor Algal growth on pool bottom	September 20	Afternoon	28.0	7.2	0	–

In addition, a swab sample was taken from the interior surface of the pool skimmer. The temperature, pH and free residual chlorine or bromine content of the water were determined at each pool (Table 1).

All beach and pool water samples were analyzed for the following parameters: fecal coliforms, fecal streptococci, *P. aeruginosa* and *C. albicans*. The swab samples were analyzed for the presence of *P. aeruginosa* and *C. albicans*.

MICROBIAL ANALYSES

All samples were processed within 24 hr of collection. Both water and sand samples were prepared for analysis as described by Dutka (1976). To facilitate the membrane filtration of the required volumes of water it was sometimes necessary to prefilter those samples which were heavily contaminated with algae through a double layer of sterile gauze. This procedure may have resulted in the removal of some microorganisms from the water samples, but it was considered necessary to avoid obscuration of the membrane surface with a layer of algae.

Candida albicans

Enumeration Procedure

Five hundred millilitre aliquots of lake water were filtered through presterilized membrane filters (Millipore Ltd.; porosity 1.2 μm; diameter 47 mm), which were then placed onto the surface of prepoured plates of mCA agar. Medium mCA (Table 2) was developed by Dr. J. Buck for use in the enumeration of *C. albicans* in natural waters. After 3 days' incubation at 37°C, all raised, dome-shaped, chocolate brown colonies were counted as potentially positive isolates. This enumeration technique, which takes advantage of the resistance of *C. albicans* to high concentrations of cycloheximide (1500 μg/ml) and the ability of the yeast to grow at 37°C, is largely selective for *C. albicans* (Buck and Bubucis, 1978).

Table 2. Constituents and Preparation of mCA Agar

Constituent	g/90 ml Distilled Water
Glycine	1.0
Maltose	3.0
Bismuth ammonium citrate	0.5
Sodium sulphite	0.3
Chloramphenicol (Sigma)	0.05
Cycloheximide (Sigma)	0.15

Note: The above mixture is warmed slightly and the pH is adjusted to 7.1. One gram of agar (Oxoid purified) is added and the mixture is brought slowly to the boiling point and boiled gently for 1 min. It is then cooled to 45-50°C, and 10 ml of filter-sterilized 10X concentration yeast nitrogen base is added. The final pH of the medium should be 6.7-6.8. The medium is dispensed into sterile 60 mm x 15 mm plastic dishes and stored at 5°C before use.

One-millilitre aliquots of the sand suspensions were spread onto the surface of predried plates (41.5°C for 1 hr) of mCA agar which were incubated and examined as described above.

Because of the lack of information on both the selectivity of mCA agar and the occurrence of *C. albicans*

and closely related species in Lake Ontario inshore waters, it was considered necessary to confirm the identity of all potentially positive colonies. *C. albicans* may be identified by its production of germ tubes and chlamydospores under controlled conditions (Beneke and Rogers, 1970). As these diagnostic characteristics of *C. albicans* may possibly be altered owing to environmental stress in the aquatic environment (Crow *et al.*, 1977), it was decided to test each isolate for both germ tube and chlamydospore production.

Confirmatory Procedures

Prior to confirmation, all suspected *C. albicans* colonies were streaked onto plates of Sabouraud dextrose agar containing 500 mg/l cycloheximide and 50 mg/l chloramphenicol and were then incubated for 48 hr at 28°C. These cultures were used as the source of inoculum for the confirmatory tests.

(a) Germ tube production – Suspensions of yeast cells in 0.5-ml aliquots of fetal calf serum (Grand Island Biological Company) were incubated at 37°C for 3-5 hr. The cells were then examined at a magnification of x400 for evidence of germ tube formation. Germ tube production, in the absence of a constriction at the juncture of the filament and yeast cell, is considered indicative of *C. albicans* (Fell and Meyer, 1967). However, as cells of *C. stellatoidea* are also reported to produce germ tubes on incubation in serum (Mackenzie, 1962; Fell and Meyer, 1967; Bowman and Ahearn, 1975), this test does not permit conclusive identification of *C. albicans.* Some isolates of *C. tropicalis* are also reported to be capable of forming germ tubes (Tierno and Milstoc, 1977), however, this yeast should not grow on agar containing cycloheximide.

(b) Chlamydospore production – Using a sterile heavy-gauge needle, inocula from yeast colonies growing on Sabouraud dextrose agar, were cut into the surface of (i) cornmeal agar (Oxoid) plates containing 1% (V/V) Tween 80 or (ii) oxgall agar plates (Fischer and Kane, 1968). Each cut was covered with a sterile cover slip. The cultures were then incubated at 28°C in the dark to prevent photoinhibition of chlamydospore production (Andrieu *et al.*, 1977). The cultures were examined after 3 and 5 days and, if necessary, after prolonged incubation for the presence of characteristic chlamydospores (Lodder, 1970).

Three species of *Candida* are known to form chlamydospores in culture: *C. albicans, C. stellatoidea* and *C. tropicalis* (Emmons *et al.*, 1977). However, *C. tropicalis* forms thin-walled chlamydospores, which are produced after abnormal incubation conditions (Hasenclever, 1971). Also, *C. tropicalis* is sensitive to cycloheximide (Blair *et al.*, 1970) and thus should not grow on mCA agar. *C. stellatoidea* differs from *C. albicans* by producing only occasional chlamydospores (Fischer and Kane, 1968), which tend to develop in chains (Gentles and la Touche, 1969).

(c) Sugar assimilation – If necessary, *C. albicans* and *C. stellatoidea* may be further differentiated on the basis of their sugar assimilation patterns. *C. albicans* assimilates sucrose, whereas *C. stellatoidea* does not (Beneke and Rogers, 1970; Lodder, 1970).

In this study, all suspected isolates of *C. albicans* were tested for their ability to assimilate dextrose, lactose, maltose and sucrose in order to determine the probability that *C. stellatoidea* may cause false positive results with the previous two confirmation methods. Sugar-free yeast cell suspensions were prepared according to the method of Bailey and Scott (1970). One-millilitre aliquots of cell suspension were surface spread onto predried plates of yeast nitrogen base (Difco) agar (Bailey and Scott, 1970). When the agar surfaces had dried, discs impregnated with the test sugars were placed on the surface of each plate. The cultures were incubated at 28°C for 3 days and were then examined for growth around the discs, which indicated sugar assimilation.

(d) Agglutination test – While not adopted for routine use, the following slide agglutination test for *C. albicans* was tested during the initial investigations. Heavy suspensions of the suspected cultures were prepared in 0.85% (V/V) NaCl containing 0.5% (V/V) phenol. These suspensions were filtered through gauze to remove clumps of cells or pseudo-hyphal elements. One drop of *Candida albicans* antiserum (Difco) was added to a drop of cell suspension on a microscope slide. A control drop was prepared using NaCl solution (0.85%). After adequate mixing, the droplets were examined at a magnification of 400 for evidence of agglutination.

Bacterial Parameters

Numbers of fecal coliform, fecal streptococcus and *Pseudomonas aeruginosa* in water samples were determined using the membrane filtration techniques described by Dutka (1976). Most probable number tube techniques were used to determine bacterial numbers in the sand samples (Dutka, 1976).

Replication

Because of the extensive workload involved in the microbiological analyses, only single samples were collected at each sampling point. All *C. albicans* determinations were made in triplicate and the bacterial parameters were determined in duplicate. All results are expressed as arithmetic mean values.

Results

PRELIMINARY INVESTIGATIONS

Before field work began, mCA and Sabouraud dextrose agar (SDA) containing cycloheximide (500 μg/ml) and chloramphenicol (200 μg/ml) were examined for their suitability in the enumeration of *C. albicans* in natural waters. Water samples from the Burlington ship canal (100 and 500 ml) and samples of unchlorinated effluent (1, 5, 30 and 70 ml) and raw sewage (30 ml) from the Burlington sewage treatment plant were processed using a standard membrane filtration technique (Dutka, 1976).

Although Sabouraud dextrose agar with added antibiotics is routinely used in the isolation of *C. albicans* from clinical material (Emmons *et al.*, 1977), it proved inadequate in the enumeration of *C. albicans* in natural water. Contaminant fungi, some of which overgrew the membrane filters, were observed on the agar plates when more than 100 ml of canal water was analyzed. Common moulds such as *Aspergillus* spp., *Geotrichum* spp. and *Cephalosporium* spp. were among the more frequent contaminants. Numerous cream-coloured yeast colonies also grew on Sabouraud dextrose agar; however, of 36 *Candida*-like colonies examined, none were identified as *C. albicans.*

A single *C. albicans* isolate was recovered on mCA agar from one of six separate canal water samples collected in April, 1977. In contrast with Sabouraud dextrose agar, contaminant moulds were not a problem on mCA agar. Water samples collected from the Burlington ship canal during the summer months of 1977 showed similar low levels of *C. albicans,* which may be due to the presence of insignificant numbers of the yeast in the canal water. The low levels could also indicate that the techniques used did not recover those cells present, possibly because of a medium limitation, or that factors such as environmental stress or a waterborne toxic effect may have affected the revivability of the yeast. *C. albicans* was not detected in the unchlorinated sewage effluent. However, on another occasion, a small number of *C. albicans* isolates (9 cfu/100 ml) were recovered from unchlorinated sewage effluent. It was not possible to repeat this recovery with consistency.

A total of 73 potentially positive colonies, including colonies which even slightly resembled *C. albicans,* were isolated from the raw sewage samples. Of these, 19 were identified as *C. albicans* using the germ tube, chlamydospore and sugar assimilation tests. However, no fewer than 60 isolates gave positive agglutination reactions, an indication that this test is unsuitable for use in the identification of environmental isolates of *C. albicans.* Yeasts which gave false positive agglutination reactions included *Trichosporon* spp.; budding, non-pseudomycelium-forming yeasts; and species of *Candida* other than *C. albicans.* All of the confirmed isolates formed germ tubes under the test conditions, while a single isolate failed to produce chlamydospores even after repeated transfers on cornmeal agar containing Tween 80. This isolate assimilated sucrose, eliminating the possibility of its being *C. stellatoidea.* Fell and Meyer (1967) also observed the reluctance of a small number of *C. albicans* isolates to form chlamydospores.

The fact that only 19 of 73 presumptive isolates proved positive is principally because even weakly positive colonies were subcultured in order to test the reliability of the identification techniques. Indeed, 90% of the positive isolates were found in the initial set of colonies selected from mCA. Thus, mCA agar is capable of isolating *C. albicans* from raw sewage, an environment with a rich and varied microflora, without excessive overgrowth of the membrane filters by contaminant fungi.

Before proceeding with the field work, some further questions were considered:

(i) The effect of membrane filter pore size and isolation medium on the recovery of C. albicans cells from aqueous suspension.

Because of the suspected low levels of *C. albicans* in lake waters, it was planned to process relatively large volumes (500 ml) of water during the beach surveys. To facilitate these filtrations it was intended to use membrane filters of greater than 0.45 μm pore size. To avoid a possible underestimation of the numbers of *C. albicans* in the water samples, the effect of membrane filter pore size on the recovery of *C. albicans* from standard cell suspensions was examined. For the same reason, a comparison of mCA and SDA agars was incorporated into this experiment.

Using a conventional Neubauer counting chamber technique, *C. albicans* cell suspensions were prepared in phosphate buffer (pH 7.0) (Dutka, 1976) from shake cultures of the yeast grown at 35°C for 24 hr in glucose-yeast, extract-peptone-water (GYPW). (GYPW contained glucose, 20 g; Bacto peptone, 10 g; Bacto yeast extract, 5 g; H_2O to 1 litre.) A suspension density of approximately 100 cells/ml was used in these tests. Care was taken when counting the yeast cells to distinguish between yeast buds which had actually separated from the parent cells and those which were still attached; the latter were considered as single cells.

Cell suspensions of both an environmental (isolated from canal water) and a clinical isolate of *C. albicans* were filtered through the membrane filters listed in Table 3. The membranes were then plated onto prepoured plates of mCA agar and Sabouraud dextrose agar with antibiotics. Colonies were counted after 3 days' incubation at 37°C. The numbers of viable cells filtered were determined by means of a spread plate technique on SDA.

Table 3. Effect of Membrane Filter Pore Size and Isolation Medium on the Recovery of *C. albicans* Cells from Aqueous Suspension

Filter Manufacturer	Pore Size (μm)	Number of Cells Recovered* Environmental Isolate mCA Agar	Environmental Isolate SDA	Clinical Isolate mCA agar	Clinical Isolate SDA
Gelman	0.45	115	105	107	101
Gelman	0.8	108	102	95	100
Millipore	0.45	105	100	95	110
Millipore	0.8	118	106	103	99
Millipore	1.2	120	100	95	102

* Results are expressed as a percentage of the number of viable cells filtered. Each value is a mean of five replicates.

As may be seen from the results presented in Table 3, there was little apparent difference between the recoveries of both test organisms on the different pore size membrane filters. This is fortuitous as the larger pore size (1.2 μm) membrane filters facilitate the filtration of large volumes of lake water, a task which is difficult, if not impossible, using 0.45 μm pore size membrane filters because of clogging of the filter pores with algae, silt and detrital material. Both media and each of the membrane filters tested gave excellent recoveries of *C. albicans.* The environmental *C. albicans* isolate gave apparently slightly higher counts on mCA agar than on SDA, however, this was not the case with the clinical isolate. These results suggest that mCA agar is at least as effective as the less selective SDA in resuscitating cells of *C. albicans* from aqueous suspension.

(ii) Effect of the source of bismuth ammonium citrate on the efficiency of mCA agar.

Immediately before the field work began, City Chemical Corporation, New York, the manufacturers of the bismuth ammonium citrate used in the preparation of mCA agar, ceased to manufacture this chemical. Therefore, an experiment was designed, using the techniques described above, to determine the effect, if any, of an alternative supply of bismuth ammonium citrate from K and K Laboratories Incorporated, New York, on the ability of mCA agar to resuscitate membrane-filtered cells of *C. albicans.* Table 4 shows that there was clearly no effective difference between the batches of mCA prepared using the two supplies of bismuth ammonium citrate. However, colonies grown on the medium prepared with bismuth ammonium citrate from City Chemical Corporation seemed to grow more strongly and were more characteristic of *C. albicans* in appearance.

Table 4. Effect of the Source of Bismuth Ammonium Citrate on the Efficiency of mCA Agar

Filter Manufacturer	Pore Size (μm)	Number of cells recovered* Environmental Isolate CCC†	Environmental Isolate KK‡	Clinical Isolate CCC	Clinical Isolate KK
Gelman	0.45	115	120	107	97
Gelman	0.8	108	117	95	98
Millipore	0.45	105	108	95	97
Millipore	0.8	118	115	103	98
Millipore	1.2	120	103	95	98

* Results are expressed as a percentage of the number of viable cells filtered. Each value is a mean of five replicates.
† The mCA agar was prepared using bismuth ammonium citrate. Manufactured by City Chemical Corporation, New York.
‡ The mCA agar was prepared using bismuth ammonium citrate. Manufactured by K and K Laboratories, Incorporated, New York.

Table 4 also shows, as does Table 3, that the environmental isolate of *C. albicans* gave apparently slightly higher recoveries than the clinical isolate. Again, there was no consistent difference in the numbers of cells recovered using the low and high pore size membrane filters. For the rest of this study, bismuth ammonium citrate from K and K Laboratories was used in the preparation of mCA agar.

Conclusions from Preliminary Investigations

Following these encouraging results, it was decided (i) to filter all water samples for *C. albicans* determination through Millipore 1.2-μm membrane filters, and (ii) to use mCA agar to enumerate *C. albicans* in our planned survey of Lake Ontario bathing beaches.

BEACH SURVEY

Water and sand samples were collected on the previously indicated dates. Table 5 shows that the water temperature at the beaches showed an overall increase from approximately 9-18°C in June to a maximum of 16-27°C in July and August. In September, the water temperatures at Hamilton and Confederation Park beaches remained static, whereas at both Lakeside Park and Weller Park beaches, a distinct decrease in water temperature, from an overall range of 22-26°C to one of 17-20°C, was observed. The water at Lakeside Park beach was usually warmer than the water collected from the other beaches, possibly because the water at this beach was quite shallow; at 20 m from the shore the water was less than 1 m deep. No obvious trends were discernible in the water temperature patterns within each beach. Table 5 also shows that the temperature of the sand samples followed a similar trend to those of the water samples. In July and August, the surface sand tended to be warmer than in June and Spetember. However, the July sand temperatures at Hamilton and Confederation Park beaches were lower than the June temperatures, probably due to the slightly overcast sky during the July survey.

As may be seen in Table 6, the pH values of the water samples did not fluctuate to any great extent and, with the exception of the values (8.1-8.3) determined for Hamilton and Confederation Park beaches in July, showed no real differences between the beaches.

Table 5. Temperature of Lake Ontario Beach Water and Sand Samples, June-September, 1977

Beach	Distance From Shore (m)	Sample	Sample Temperature, (°C) June	July	August	September
Hamilton	–	Sand	33	26.5	36	20
	1	Surface	10.5	16	16	18
	1	Bottom	9.5	16	17	18
	10	Surface	9.5	16	17	18
	10	Bottom	9	16	17	19
	20	Surface	9	16	17	19
	20	Bottom	9.5	16	18	19
Confederation Park	–	Sand	35	33.5	37	25
	1	Surface	12	17	19	20
	1	Bottom	10	16	20	20
	10	Surface	10	17	20	20
	10	1 m	9.5	16	20	20
	10	Bottom	9.5	16	19	20
	20	Surface	13	17	20	20
	20	1 m	10	16	21	20
	20	Bottom	11	16	21	20
Lakeside Park	–	Sand	25	35	30	20
	1	Surface	18.5	27	25	20
	1	Bottom	18.5	27	25	20
	10	Surface	17	27	26	20
	10	Bottom	16.5	26	26	19
	20	Surface	17	25	25	19
	20	Bottom	16	21	24	–
Weller Park	–	Sand	21	36	32	30
	1	Surface	17	24	22	20
	1	Bottom	17	24	22	20
	10	Surface	17	24	22	19
	10	Bottom	17	23	21	17
	20	Surface	17	24	22	–
	20	Bottom	17	23	22	–

Table 6. pH of Lake Ontario Beach Water Samples, June-September, 1977

Beach	Distance From Shore (m)	Sample	Sample pH June	July	August	September
Hamilton	1	Surface	8.4	8.1	8.5	8.4
	1	Bottom	8.4	8.1	8.6	8.5
	10	Surface	8.5	8.1	8.5	8.3
	10	Bottom	8.5	8.2	8.5	8.5
	20	Surface	8.5	8.1	8.6	8.5
	20	Bottom	8.5	8.1	8.6	8.5
Confederation Park	1	Surface	8.4	8.1	8.4	8.5
	1	Bottom	8.6	8.1	8.4	8.5
	10	Surface	8.6	8.2	8.5	8.5
	10	1 m	8.7	8.3	8.4	8.5
	10	Bottom	8.7	8.2	8.5	8.5
	20	Surface	8.6	8.1	8.4	8.5
	20	1 m	8.6	8.1	8.5	8.5
	20	Bottom	8.7	8.2	8.5	8.5
Lakeside Park	1	Surface	8.2	8.6	8.5	8.3
	1	Bottom	8.1	8.6	8.4	8.3
	10	Surface	8.3	8.6	8.4	8.3
	10	Bottom	8.3	8.5	8.5	8.4
	20	Surface	8.3	8.6	8.5	8.4
	20	Bottom	8.3	8.5	–	–
Weller Park	1	Surface	8.5	8.6	8.2	8.3
	1	Bottom	8.5	8.6	8.0	8.2
	10	Surface	8.7	8.6	8.3	8.4
	10	Bottom	8.6	8.6	8.4	8.3
	20	Surface	8.5	8.6	8.4	–
	20	Bottom	8.5	8.5	8.3	–

Weather Conditions

As the weather conditions prevailing at the beaches prior to and during sample collection may have had a bearing on the results presented in this report, they are summarized as follows.

(i) June survey – No wind, no rain, calm water at each beach.

(ii) July survey – No wind, no rain, calm water at each beach.

(iii) August survey – A rainfall occurred the previous night and a slight wind prevailed during sample collection at Hamilton and Confederation Park beaches. An abrupt change in the weather occurred during sampling at Lakeside Park beach, when a strong onshore wind developed and there was a 30-min duration rainfall immediately after the collection of the 1-m water samples. The remainder of the water samples were collected when the rain ceased. At Weller Park beach there was also a strong onshore wind, and a heavy rainfall occurred during sample collection at this beach.

(iv) September survey – It rained intermittently during the 3 days preceding this survey. At Hamilton and Confederation Park beaches the water was calm and there was a slight offshore breeze. At Lakeside Park and Weller Park beaches, however, there was a strong onshore wind, which resulted in rough water and breaking waves within the 20-m sampling transect.

MICROBIAL PARAMETERS

The results of the bacterial analyses of the water and sand samples are listed in Tables 7-9. The fecal coliform and fecal streptococcus data (Tables 7 and 8) show that the numbers of both these indicators were highest in the samples collected from Lakeside Park and Weller Park beaches. At these two beaches, the values for the two indicators ranged from negligible counts to a maximum of 3200 fecal coliforms/100 ml at Weller Park beach in July and 6600 fecal streptococci/100 ml, also at Weller Park beach in June. In contrast, at Hamilton and Confederation Park beaches, the maximum fecal coliform levels in the water samples were always less than 100 colonies/100 ml.

The maximum number of fecal streptococci enumerated was also less than 100 colonies/100 ml at Hamilton Beach, while at Confederation Park beach a maximum count of 310 fecal streptococci/100 ml was detected in June. These results may be interpreted as an indication that Lakeside and Weller Park beaches were more heavily contaminated with fecal material than Hamilton and Confederation Park beaches. The data presented in Tables 7 and 8 suggest that there is no consistent difference between the numbers of indicator organisms in the surface and in bottom waters.

Other investigators have referred to the elevated numbers of microorganisms recovered from the surface microlayer as opposed to the subsurface water of freshwater bodies (Dutka and Kwan, 1978). The fact that such a pattern is not apparent in our data may be because we collected integrated microlayer and 0-5 cm depth water samples rather than microlayer samples. Such a sampling protocol could mask any elevated microlayer microbial populations through a dilution effect. It is also apparent from Tables 7 and 8 that the levels of fecal coliforms and fecal streptococci in the sand samples were not remarkably high; all values lay within the range of 0-160 colonies per gram of sand (fresh weight).

Examination of Table 9 suggests that the *Pseudomonas aeruginosa* population densities may also be used to differentiate Lakeside and Weller Park beaches from those at Hamilton and Confederation Park. Although such a differentiation is probably less reliable than one based on the more numerous coliform and streptococcus indicators, it may have additional significance, since *P. aeruginosa* is an opportunistic pathogen of man, commonly associated with outer ear and skin infections.

Table 7. Fecal Coliform Data for Selected Lake Ontario Beaches, June-September, 1977

Beach	Distance From Shore (m)	Sample	Fecal Coliforms (colonies/g fresh weight sand; colonies/100 ml water) June	July	August	September
Hamilton	–	Sand	0	<1	160	5
	1	Surface	10	35	55	9
	1	Bottom	10	9	42	9
	10	Surface	5	1	8	14
	10	Bottom	1	2	46	15
	20	Surface	1	4	6	13
	20	Bottom	1	2	27	11
Confederation Park	–	Sand	0	0	0	7
	1	Surface	18	11	19	2
	1	Bottom	14	10	10	3
	10	Surface	12	5	5	3
	10	1 m	1	3	4	1
	10	Bottom	1	4	6	5
	20	Surface	4	2	3	1
	20	1 m	2	1	1	2
	20	Bottom	1	4	4	*
Lakeside Park	–	Sand	3	35	35	160
	1	Surface	95	3200	630	93
	1	Bottom	91	2000	380	72
	10	Surface	3	660	200	11
	10	Bottom	4	710	170	15
	20	Surface	1	90	37	7
	20	Bottom	1	300	*	*
Weller Park	–	Sand	1	35	92	54
	1	Surface	26	11	2000	600
	1	Bottom	25	17	1300	930
	10	Surface	18	9	1200	500
	10	Bottom	9	46	860	650
	20	Surface	7	13	400	†
	20	Bottom	22	14	210	†

* Samples lost in laboratory accident.
† Samples not collected due to rough water.

Table 8. Fecal Streptococcus Data for Selected Lake Ontario Beaches, June-September, 1977

Beach	Distance From Shore (m)	Sample	Fecal streptococci (colonies/g fresh weight sand; colonies/100 ml water) June	July	August	September
Hamilton	–	Sand	< 1	14	28	<1
	1	Surface	27	16	55	9
	1	Bottom	27	43	61	5
	10	Surface	15	11	7	5
	10	Bottom	6	6	24	5
	20	Surface	1	13	3	2
	20	Bottom	1	5	19	1
Confederation Park	–	Sand	2	13	160	35
	1	Surface	310	64	14	< 1
	1	Bottom	180	48	7	0
	10	Surface	110	160	6	18
	10	1 m	16	30	4	12
	10	Bottom	11	27	3	9
	20	Surface	27	26	3	7
	20	1 m	4	1	1	9
	20	Bottom	3	17	< 1	*
Lakeside Park	–	Sand	54	5	8	17
	1	Surface	730	3900	600	32
	1	Bottom	830	3500	520	30
	10	Surface	48	1300	210	280
	10	Bottom	46	630	250	31
	20	Surface	9	260	70	20
	20	Bottom	11	340	*	*
Weller Park	–	Sand	35	54	160	28
	1	Surface	5000	18	1500	41
	1	Bottom	6600	44	850	34
	10	Surface	61	16	870	18
	10	Bottom	12	39	440	17
	20	Surface	2	21	370	†
	20	Bottom	3	21	110	†

* Samples lost in laboratory accident.
† Samples not collected due to rough water.

The major source of *P. aeruginosa* in water seems to be fecal wastes of man and animals associated with man (Hoadley, 1977). The highest levels of *P. aeruginosa* were observed in the samples taken from Lakeside Park beach (10-100 colonies/100 ml) in July, which coincides in time with the highest levels of both fecal coliforms and fecal streptococci determined for this beach (Table 11). Clearly, on the dates sampled, the numbers of *P. aeruginosa* in the waters at Hamilton and Confederation Park beaches were not of any great significance.

Thus, on the basis of the above bacterial fecal pollution indicator parameters, Lakeside and Weller Park beaches appear to be the more heavily contaminated of the beaches examined.

Table 10 shows the distribution patterns for the potential indicator organism *C. albicans;* the values given represent confirmed isolates of *C. albicans.* Again, the highest frequencies of isolation were observed from the water samples collected at Lakeside and Weller Park beaches. The numbers of *C. albicans* isolated at these two beaches varied from the occasional isolate to a maximum of 25 cfu per litre. Few isolates were recovered from the sand samples, which may be an indication that the sand is probably not the main source of inoculum for the beach waters. As with *P. aeruginosa,* the numbers of *C. albicans* in the waters under investigation are considerably less than the numbers of fecal coliforms and fecal streptococci, however, their occurrence may have added significance for bathers owing to the potential pathogenicity of this yeast.

Table 9. *Pseudomonas aeruginosa* Data for Selected Lake Ontario Beaches, June-September, 1977

Beach	Distance From Shore (m)	Sample	Numbers of *Pseudomonas aeruginosa* (Colonies/g fresh weight sand; colonies/100 ml water) June	July	August	September
Hamilton	–	Sand	< 1	2	< 1	< 1
	1	Surface	0	< 1	0	0
	1	Bottom	1	< 1	0	0
	10	Surface	1	< 1	1	0
	10	Bottom	0	0	0	0
	20	Surface	< 1	0	0	0
	20	Bottom	0	0	< 1	0
Confederation Park	–	Sand	< 1	1	1	6
	1	Surface	0	< 1	0	0
	1	Bottom	1	< 1	0	< 1
	10	Surface	0	< 1	0	0
	10	1 m	0	< 1	0	0
	10	Bottom	0	< 1	< 1	0
	20	Surface	0	0	0	0
	20	1 m	0	0	0	0
	20	Bottom	1	0	0	*
Lakeside Park	–	Sand	< 1	11	< 1	< 1
	1	Surface	8	70	0	3
	1	Bottom	2	100	0	0
	10	Surface	1	50	0	0
	10	Bottom	< 1	15	0	2
	20	Surface	0	10	0	< 1
	20	Bottom	0	10	*	*
Weller Park	–	Sand	< 1	2	1	3
	1	Surface	8	0	0	2
	1	Bottom	4	0	6	1
	10	Surface	0	< 1	4	0
	10	Bottom	0	0	8	0
	20	Surface	0	< 1	16	†
	20	Bottom	< 1	< 1	2	†

* Samples lost in laboratory accident.
† Samples not collected due to rough water.

DISTRIBUTION OF INDICATORS WITH TIME

It is recognized that the data contained in this report do not warrant a definitive statement on the fluctuations of indicator levels in the beach waters during the summer months. Nevertheless, examination of Table 11, in which the months of maximum mean population densities for each of the microbial parameters are listed, does suggest a possible trend. With the exception of the fecal streptococcus data for Confederation and Lakeside Park beaches, the highest mean numbers of all four indicators occurred in July and August. This observation gains some significance when considered in conjunction with the fact that maximum bather loads were observed at Lakeside Park beach (300-400)[1] and Weller Park beach (200-300) in July, with only slightly reduced numbers in August. It is possible that the rainfalls before and during the August surveys may have had an influence on the above results, probably by causing increased microbial densities in the beach waters through storm water runoff from the adjoining land. This possibility shall be considered further in relation to the origins of the detected pollution indicators.

[1] Visual estimation at time of sample collection.

MICROBIAL DISTRIBUTION PATTERNS WITHIN BEACHES

The distribution patterns of the microbial indicators in the waters of Lakeside Park and Weller Park beaches are plotted in Figure 3 for each of the sampling months. Examination of these plots suggests a decreasing distribution of the indicators from the shoreline to the offshore waters.

Table 10. *Candida albicans* Data for Selected Lake Ontario Beaches, June-September, 1977

Beach	Distance From Shore (m)	Sample	Numbers of *Candida albicans* (cfu/g fresh weight sand; cfu*/100 ml water) June	July	August	September
Hamilton	–	Sand	0	0	0	0
	1	Surface	2	0	1	0
	1	Bottom	<1	0	1	3
	10	Surface	1	0	5	1
	10	Bottom	0	0	0	1
	20	Surface	0	0	1	0
	20	Bottom	0	0	0	0
Confederation Park	–	Sand	0	0	0	3
	1	Surface	2	0	1	0
	1	Bottom	0	0	1	1
	10	Surface	1	0	1	0
	10	1 m	0	0	0	0
	10	Bottom	0	0	0	0
	20	Surface	0	0	1	0
	20	1 m	0	0	0	0
	20	Bottom	0	0	0	†
Lakeside Park	–	Sand	0	0	4	3
	1	Surface	3	17	18	3
	1	Bottom	1	9	13	2
	10	Surface	0	7	3	0
	10	Bottom	0	6	14	0
	20	Surface	0	3	2	0
	20	Bottom	0	7	†	†
Weller Park	–	Sand	0	0	1	0
	1	Surface	1	0	10	1
	1	Bottom	0	0	15	1
	10	Surface	2	0	25	0
	10	Bottom	0	1	17	1
	20	Surface	0	0	5	‡
	20	Bottom	0	1	3	‡

* cfu = colony forming units.
† Samples lost in laboratory accident.
‡ Samples not collected due to rough water.

Table 11. Month of Maximum Mean Indicator Population Densities in Selected Lake Ontario Beach Waters, June-September, 1977

Beach	Fecal Coliform	Fecal Streptococcus	*Pseudomonas aeruginosa*	*Candida albicans*
Hamilton	August (31)	August (28)	–	–
Confederation Park	–	June (83)	–	–
Lakeside Park	July (1166)	July (1667)	July (43)	August (10)
Weller Park	August (990)	June (1943)	August (6)	August (13)

Note: Values in parentheses represent arithmetic mean indicator densities in water (colonies per 100 millilitres for bacteria; colony forming units per litre for *C. albicans*). Dashes indicate values too low to warrant classification under this scheme.

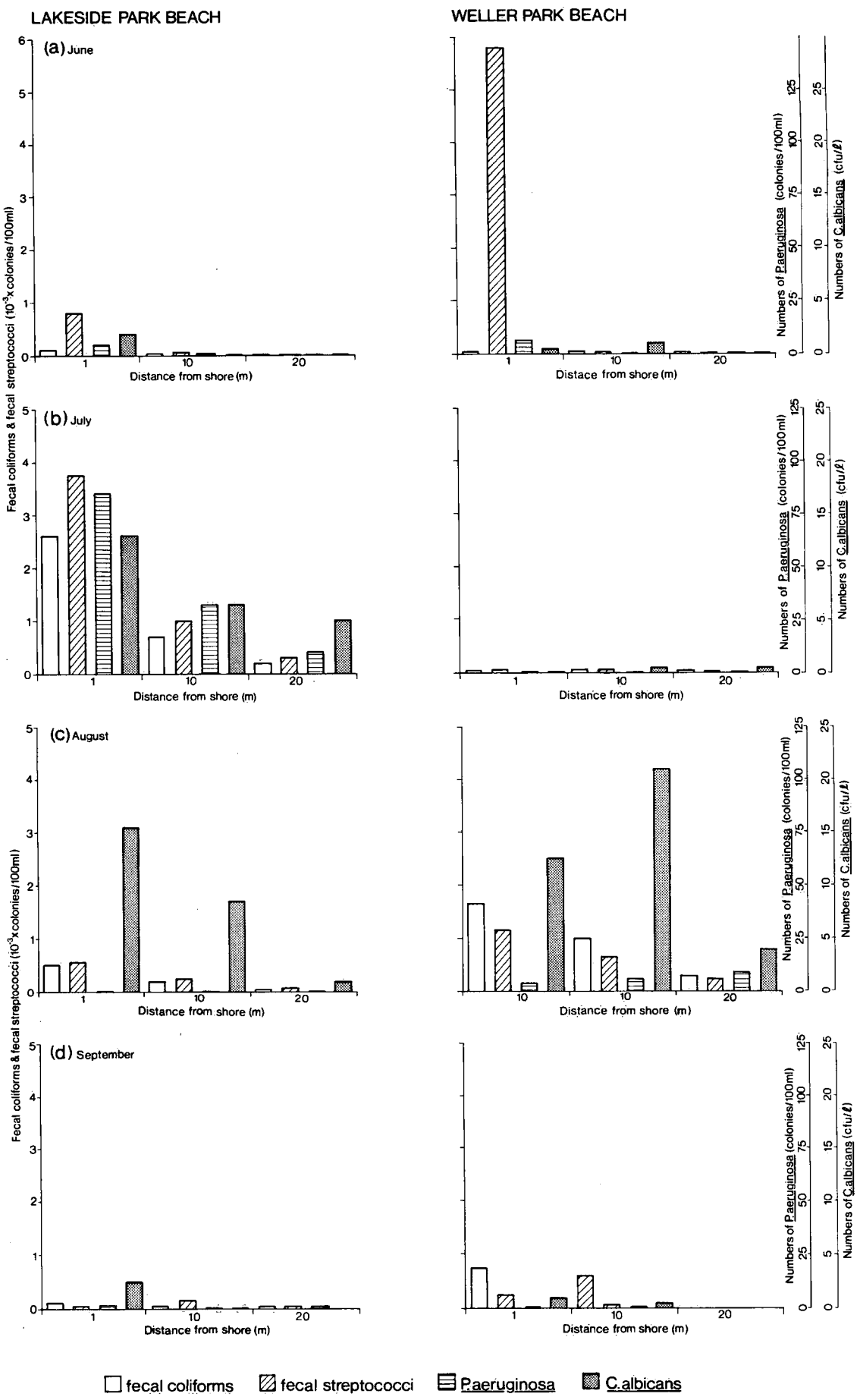

Figure 3. Distribution of indicator organisms within Lakeside Park and Weller Park beaches, June-September, 1977. Values plotted are means of indicator levels in surface and bottom waters.

Table 12. Differential Distribution Patterns of Microbial Indicators in Shoreline and Offshore Waters at Lakeside Park and Weller Park Beaches

Beach	Distance From Shore (m)	Fecal Coliforms (colonies/100 ml)	Fecal Streptococci (colonies/100 ml)	*P. aeruginosa* (colonies/100 ml)	*C. albicans* (cfu/l)
Lakeside Park	1	825 (15.3)	1274 (14)	23 (7.6)	9 (4.5)
Lakeside Park	20	54	89	3	2
Weller Park	1	614 (5.5)	1754 (20)	3 (1)	4 (2)
Weller Park	20	111	89	3	2

Note: In columns 3-6, values represent arithmetic mean of accumulated monthly determinations for June through September, 1977, of inshore and offshore data (mean of surface and bottom values). Values in parentheses indicate magnitude of inshore/offshore differential ratio.

This distribution gradient is more obvious when the beach water microbial populations are high, as at Lakeside Park beach in July. However, there are some exceptions to the trend, such as the distribution of *C. albicans* at Weller Park beach in August (Fig. 3c). Nevertheless, the presence of the pattern is reinforced by the abstracted data presented in Table 12, which show that with one exception, the mean inshore microbial population densities were higher for the sampling season than the mean offshore values. The magnitude of this difference, expressed as an inshore : offshore ratio, ranged from 2 to 20 (Table 12). Obviously, this pattern is most clearly exemplified by the fecal coliform and fecal streptococci data, which have numerically greater values in the waters under examination than the potentially pathogenic microorganisms *P. aeruginosa* and *C. albicans.*

It is possible, although not conclusively shown, that the fecal coliform and fecal streptococcus distributions do, in fact, illustrate the same trends as the two pathogenic microorganisms under observation but that this is not always apparent because of the low population densities of the pathogens. A number of factors may contribute to the distribution patterns illustrated in Figure 3. The prevailing wind, which was generally onshore, may have caused accumulations of detrital material with associated microorganisms close to the shoreline; wave action could also cause such inshore accumulations. The possibility of leaching of microorganisms from the shore cannot be discounted, although the low indicator densities in the beach sands do not substantiate this explanation. Such leaching, if caused by rainwater or stormwater runoff, could logically result in a nearshore to offshore population distribution gradient such as presented in Figure 3. However, the most perfect inshore-offshore distribution gradient was obtained in July at Lakeside Park (Fig. 3b) when there was no associated rainfall.

The observed numbers of the disease-causing microorganisms *C. albicans* and *P. aeruginosa* close to the shoreline may have a possible health significance, especially for young children who spend most of their bathing time in the shallow water close to shore.

There is a danger that these pathogenic microorganisms may adhere to the skin of bathers who must pass through the contaminated inshore waters as they leave the water. Should some of these bathers have previously macerated or damaged skin, a not unusual occurrence among bathers, or be otherwise disposed to infection, they risk contracting a superficial *Candida* or a *Pseudomonas* infection. However, as shall be pointed out in the discussion, bathing in contaminated natural waters has not yet been conclusively related to incidences of *Candida* or *Pseudomonas* infections. On the other hand, Seyfried and Fraser (1978) have established a relationship between cases of otitis externa in bathers and the occurrence of *P. aeruginosa* in swimming pool waters.

ORIGIN OF CONTAMINATION IN BEACH WATERS

The ratio of fecal coliforms: fecal streptococci (FC:FS) may be used to distinguish human from non-human fecal contamination of natural water (Geldreich *et al.,* 1964). A ratio of 4 or greater indicates that the contamination may be of human origin, whereas a ratio of 0.6 or less would suggest non-human fecal contamination, probably via a source such as stormwater runoff. Geldreich (1976) has cautioned against the indiscriminate use of such ratios because of the ubiquitous presence of *S. faecalis* var. *liquifaciens* in natural waters. Counts of less than 100 fecal streptococci/100 ml are of doubtful significance in characterizing low levels of fecal contamination because of the predominance of this organism at such low counts. Consequently, Geldreich recommends that only fecal streptococcus counts above 100 colonies/100 ml be used to determine the FC:FS ratios in natural waters. This recommendation is adhered to in this report, with the exception of the Weller Park September samples where the fecal coliform counts significantly exceeded the fecal streptococcus counts and therefore the ratios obtained should be legitimate.

The ratios presented in Table 13 strongly suggest that the fecal contamination in the months of June and July was of non-human origin. In August and September a number of the ratios were in the intermediate range of 0.6-4.0 and the contamination in these samples would be of an inconclusive origin. As indicated by the FC:FS ratios, the water contamination of Weller Park beach in September was quite probably of human origin, whether from sewage discharge or from some other source. However, Tables 9 and 10 show that the counts of *C. albicans* and *P. aeruginosa* were quite low for these samples, an observation which does not reinforce the argument that the contamination has a human origin.

If the contamination of Weller Park beach was of human origin, it might have originated from the nearby sewage treatment plant, which discharges effluent into the Welland ship canal. As the Welland ship canal flows into Lake Ontario adjacent to Weller Park beach, it is conceivable that a strong onshore wind, such as was prevalent during the September survey, could blow any fecal contaminants back towards the shoreline and consequently onto Weller Park beach.

Table 10 shows that the highest numbers of *C. albicans* were recovered from the August water samples. The rainfalls that occurred during the collection of the August water samples at Lakeside Park and Weller Park beaches may have influenced these results, possibly by causing increased leaching from the shore. However, it is also possible that other factors such as the month of sampling or a pollutant input may have been as important. Also, the isolates of *C. albicans* may have originated from the bathers themselves, in which case the question of whether a beach which is overloaded with bathers may become a health hazard must be considered.

Swimming Pool Surveys

The swimming pool water samples were surprisingly free from microbial contamination. Although the July

Table 13. Fecal Coliform : Fecal Streptococcus Ratios for Selected Lake Ontario Beach Waters, June-September, 1977

Beach	Distance from Shore (m)	Sample	Month	Fecal Coliform:Fecal Streptoccus Ratio
Confederation Park	1	Surface	June	0.06
	1	Bottom	June	0.08
	10	Surface	June	0.11
	10	Surface	July	0.03
Lakeside Park	1	Surface	June	0.13
	1	Bottom	June	0.11
	1	Surface	July	0.82
	1	Bottom	July	0.57
	10	Surface	July	0.51
	10	Bottom	July	1.13
	20	Surface	July	0.35
	20	Bottom	July	0.88
	1	Surface	August	1.05
	1	Bottom	August	0.73
	10	Surface	August	0.95
	10	Bottom	August	0.68
	1	Surface	September	2.91
	1	Bottom	September	2.40
	10	Surface	September	0.04
Weller Park	1	Surface	June	0.01
	1	Bottom	June	0.004
	1	Surface	August	1.33
	1	Bottom	August	1.53
	10	Surface	August	1.38
	10	Bottom	August	1.95
	20	Surface	August	1.08
	20	Bottom	August	1.91
	1	Surface	September	14.63
	1	Bottom	September	27.35
	10	Surface	September	27.78
	10	Bottom	September	38.24

whirlpool samples had a zero chlorine content, none of the microbial parameters tested for were detected. A single isolate of *P. aeruginosa* was recovered from pool No. 3 water containing 1.5 ppm chlorine (Table 14). The most interesting results were obtained at pool No. 6 in September (Table 14) where in the presence of zero chlorine content, small numbers of fecal coliforms, fecal streptococci and *P. aeruginosa* were detected. The other water samples and all of the swab samples gave negative results. *C. albicans* was not recovered from any of the samples. These results indicate that with two exceptions, the pools tested were apparently free from microbial contamination. *P. aeruginosa* was detected on two occasions, once in the presence of low levels of fecal coliforms and fecal streptococci—an indication that in this case, at least, the bacterial indicators fulfilled their functions.

METHODOLOGICAL ASPECTS OF SURVEY RESULTS

Some aspects of the survey results will now be considered in relation to the accuracy of mCA agar and the reliability of the confirmatory tests used in the enumeration of *C. albicans.*

Accuracy of Enumeration Method

The selective medium (mCA agar) used in the enumeration of *C. albicans* proved satisfactory and appears to be suitable for use in systematic investigations on the occurrence, distribution and survival of *C. albicans* in fresh water. Because of the relatively large volumes (500 ml) of lake water that were filtered for the *C. albicans* determinations, it was considered necessary to use 1.2 μm pore size membrane filters. Studies are now in progress in this laboratory to determine the efficiency of membrane filters of different pore sizes from a range of manufacturers on the recovery of stressed cells of *C. albicans* from water. Table 15 shows that in the first survey the enumeration method gave an overall accuracy of 47%. In the second and third surveys, when the numbers of potentially positive isolates were considerably greater, a higher level of accuracy was observed; 93% and 97% of the potentially positive isolates proved positive in the respective surveys. However, in the fourth survey, the accuracy of the enumeration technique decreased to 50%, probably because of the low numbers of *C. albicans* enumerated in September. It was also noticed that increased numbers of false positive isolates, up to 70%, were enumerated when the 37°C incubation period was extended beyond 5 days.

Reliability of Confirmatory Tests

There was generally good agreement between the number of isolates confirmed using the germ tube and chlamydospore tests (Table 15). A total of 16 isolates (5% of germ tube positive isolates) failed to produce chlamydospores following at least two successive incubations on oxgall agar. Each of these isolates had a sugar assimilation pattern characteristic of *C. albicans* and thus could not be identified as *C. stellatoidea.* A single isolate of *C. albicans* from the second survey and two isolates from the third survey failed to produce germ tubes in calf serum even after prolonged incubation (6 hr).

Table 14. Levels of Microbial Parameters in Swimming Pool Water Samples

Pool Number	Fecal Coliforms (colonies/100 ml)	Fecal Streptococci (colonies/100 ml)	*P. aeruginosa* (colonies/100 ml)	*C. albicans* (cfu/litre)
1	0	0	0	0
	0	0	0	0
2	0	0	0	0
3	0	0	1*	0
	0	0	0	0
4	0	0	0	0
	0	0	0	0
	0	0	0	0
5	0	0	0	0
6	10†	4†	2†	0
	3*			

* 1-m depth sample.
† Surface sample.

Table 15. Accuracy of Isolation Method and Reliability of Confirmatory Methods Used in the Enumeration of *C. albicans* in the Aquatic Environment

Survey	Potentially Positive Isolates	Number of Positive Isolates			Confirmed Isolates (% of Potentially Positive Isolates)
		Germ Tube Test	Chlamydospore Test	Sugar Assimilation Test	
1	34	16	16	16	47
2	81	74	75	75	93
3	210	202	193	204	97
4	30	15	10	15	50

Table 16. Ability of Cornmeal Agar and Oxgall Agar Media to Induce Chlamydospore Formation in Environmental Isolates of *C. albicans*

Number of Isolates Tested	Number of Isolates (% of total tested) Which Formed Chlamydospores			
	Cornmeal Agar		Oxgall Agar	
	Surface Streak	Covered Inoculation Cut	Surface Streak	Covered Inoculation Cut
74	59	76	23	100

Crow *et al.* (1977) recovered nine atypical isolates of *C. albicans* from sea water; these isolates failed to produce chlamydospores on cornmeal agar with Tween 80, and germ tube production was negligible in bovine serum. After four months in culture, a small percentage of the cells of each isolate formed stunted germ tubes. On the basis of this observation these investigators suggested that a thorough systematic evaluation might be required for the identification of *C. albicans* from nature, because of possible alterations in its characteristics owing to environmental stress.

The results of the present survey, in which we recovered a total of 19 atypical isolates of *C. albicans*—3 germ tube negative and 16 chlamydospore negative isolates—would tend to support this viewpoint. However, as the number of atypical isolates detected in this study amounted to only 6% of the total number of confirmed isolates, it would probably be unrealistic to incorporate a systematic evaluation of all isolates when determining the *C. albicans* content of natural waters on a routine basis. In this study, isolates which proved negative under the germ tube and chlamydospore tests (Table 15) were not identified any further. Thus, the possibility exists that some of these isolates may have been strongly atypical environmentally altered isolates of *C. albicans,* similar to those detected by Crow *et al.* (1977). Such isolates could escape detection under the enumeration and identification scheme used in this study.

Consequently, it is suggested that both germ tube and chlamydospore formation tests be used to confirm all suspected isolates of *C. albicans.* Such a procedure should eliminate the possibility, albeit slight, of falsely enumerating *C. tropicalis* or *C. stellatoidea* as *C. albicans* and may also prevent the false negative assessment of isolates which, because of environmental stress or some other factor, do not readily form germ tubes or chlamydospores. The sugar assimilation test may be used to resolve possible contradictions between these two tests or to confirm the presence of *C. stellatoidea,* if suspected. It should not be necessary, however, to employ the time-consuming sugar assimilation test on all isolates.

C. tropicalis and *C. stellatoidea* are both human-associated yeasts and are known to be occasionally pathogenic (Beneke and Rogers, 1970; Emmons *et al.,* 1977). Thus their occurrence in water may have a similar significance to that of *C. albicans.*

Comparison of the Abilities of Oxgall Agar and Cornmeal Agar to Induce Chlamydospore Formation in Environmental Isolates of C. albicans

A total of 74 environmental isolates of *C. albicans* were inoculated onto plates of cornmeal agar containing 1% (V/V) Tween 80 and oxgall agar. A surface streak and an inoculation cut were made on each plate. The inoculation cuts were covered with sterile coverslips and the cultures were incubated for 4 days at 28°C. All of the test organisms formed chlamydospores on oxgall agar when the inoculum was covered, but only 76% of the isolates formed chlamydospores on cornmeal agar in the same incubation period (Table 16). Thus, oxgall agar is apparently more suitable than cornmeal agar for use in the routine confirmation of environmental isolates of *C. albicans*. Fischer and Kane (1968) made a similar observation for clinical isolates of *C. albicans*. Table 16 also shows that a reduced number of isolates formed chlamydospores when incubated aerobically on the surface of both media.

Discussion

In this study, we have established that the opportunistically pathogenic yeast *C. albicans* occurs in the inshore waters of selected bathing beaches on Lake Ontario. While *C. albicans* has been previously recovered from both offshore (Fell, 1967; Crow *et al.,* 1977) and inshore (Ahearn *et al.,* 1968) marine waters and on occasion from fresh water (Cook, 1970), this is, we believe, the first documented report of its recovery from one of the Great Lakes.

Although Cooke (1976) has commented on the apparent absence of *C. albicans* from sewage sludge and also from waste stabilization ponds (Cooke and Matsura, 1963), Ahearn *et al.* (1968) have recovered this yeast from fresh raw sewage but not from treated effluent. We have recovered *C. albicans* from both raw sewage and non-chlorinated effluent from the Burlington sewage treatment plant. However, we did observe that the instances of recovery from the non-chlorinated effluent were erratic. The numbers of colonies of *C. albicans* enumerated varied from none to a few depending on when the samples had been collected. It is possible that the sewage had been improperly treated on the two occasions that *C. albicans* was recovered from the unchlorinated effluent. On the other hand, we have consistently isolated *C. albicans* from raw sewage samples.

Unquestionably, the development of mCA agar (Buck and Bubucis, 1978) greatly facilitated the enumeration of *C. albicans* in the waters surveyed in this study. The highly selective nature of this medium favoured the detection of relatively small numbers of *C. albicans* from the large volumes of lake water analyzed, despite the fact that these inshore waters contained a rich and varied mycoflora. Furthermore, mCA agar, when used in conjunction with the described methodology, gave a fairly consistent and high level of working accuracy. In the second and third surveys, 93 and 97% respectively of the potentially positive isolates were identified as *C. albicans.*

We have noticed that it is necessary to pay particular attention to the pH of mCA agar at the different stages of its preparation, and we have found it advantageous to incorporate a simple quality control test as a routine measure in the preparation of this medium. Our preliminary results suggest that membrane filter pore size may not have a deleterious effect on the recovery of non-stressed cells of *C. albicans* from suspension. However, it remains to be determined whether membrane filters from different manufacturers give equivalent results. Although the bismuth ammonium citrate obtained from City Chemical Corporation gave more typical and characteristic colonies when incorporated in mCA agar, there was apparently no real difference in the enumerative capabilities of media prepared using either this compound or bismuth ammonium citrate obtained from K and K Laboratories Incorporated.

When confirming the identity of possible isolates of *C. albicans,* we obtained a higher number of positive results using the germ tube test than the chlamydospore test. Fell and Meyer (1967) observed that each of 120 isolates of *C. albicans* examined formed germ tubes when incubated in serum at 37°C. Environmental stress can, however, apparently inhibit this diagnostic characteristic of *C. albicans* (Crow *et al.,* 1977). Only three isolates of *C. albicans* failed to form germ tubes in this study. While the chlamydospore test gave a higher number of negative results than the germ tube test, it may still prove useful in the identification of those *C. albicans* isolates which fail to produce germ tubes. In addition, the chlamydospore test may be used to confirm the identity of isolates that give weak or inconclusive germ tube reactions when incubated in serum. In confirmation of the work of Fischer and Kane (1968), who used clinical isolates of *C. albicans,* we observed that environmental isolates of *C. albicans* formed chlamydospores more consistently and more rapidly on oxgall agar than on cornmeal agar containing Tween.

C. stellatoidea, also a human-associated yeast, is the only other species of *Candida* that is capable of producing both germ tubes and chlamydospores under the test conditions used in this study. Jannach and Fell (cited in Fell and Meyer, 1967), in a survey of yeasts isolated from clinical material, found that 0.8% of all germ tube positive isolates could be identified as *C. stellatoidea.* Nevertheless, it is felt that the sugar assimilation test should be retained for use in resolving any contradictory or atypical results which may emerge from the germ tube and chlamydospore tests.

It should be mentioned that the taxonomic status of *C. stellatoida* is in some doubt. Some authorities (Fell and

Meyer, 1967) feel that the inability to assimilate sucrose is not a sufficient diagnostic characteristic to warrant the maintenance of a distinct species and have suggested instead a varietal rank. There is no unanimity on this topic, however, and Lodder and Kreger-van Rij (1952) and Lodder (1970) have maintained *C. stellatoidea* as a distinct species.

The survey results presented in this report show quite clearly that the bathing beaches examined may be differentiated using the levels of both bacterial indicators and of the potential pathogens *C. albicans* and *P. aeruginosa.* Higher levels of these microbial parameters were consistently observed at Lakeside and Weller Park beaches than at Hamilton and Confederation Park beaches. The numbers of fecal coliforms enumerated ranged from minimal to 3200/100 ml. The position regarding recommended fecal coliform standards for recreational waters is confused, to say the least. In the absence of adequate epidemiological data, the standards which have been established are somewhat arbitrary. Geldreich (1974-1975) states that the fecal coliform standards used in various countries have been based either on a geometric mean of 200 organisms/100 ml or an upper limit of 1000 organisms/100 ml. The European Economic Community directive of December, 1975, concerning the quality of bathing waters prescribes that 95% of bathing water samples must have fecal coliform counts below 2000 organisms/100 ml. Their more stringent guide levels state that 80% of samples shall have less than 100 fecal coliforms/100 ml. On the basis of these criteria, the condition of the bathing water at Lakeside Park in July and August and at Weller Park in August and September should give cause for concern. It is recognized that the sampling protocol observed in these surveys was probably not intensive enough to justify a conclusion as to whether these two beaches should have remained operational during the months in question. However, the results do suggest that the water at both Lakeside and Weller Park beaches should be monitored for the presence of microbial fecal pollution indicators on a routine basis during the summer months.

The numbers of fecal streptococci enumerated ranged from negligible counts to a maximum of 6600/100 ml. Again, the indicator densities at Lakeside and Weller Park beaches were suggestive of contaminated water.

Most isolates of the opportunistic pathogens *C. albicans* and *P. aeruginosa* were also recovered from Lakeside and Weller Park beaches. Although the numbers of both these organisms were low in comparison to the fecal coliform and streptococci indicators, their presence in the waters surveyed may have added significance because of their disease-causing potential.

To our knowledge, there are no reports in the literature on the distribution of *C. albicans* in freshwater bathing beaches. The presence of *C. albicans* has been reported from crabs (Dabrowa *et al.,* 1964) and in the sand (Kishimoto and Baker, 1969) at marine bathing beaches. We also isolated *C. albicans* from sand samples collected at Lakeside and Weller Park beaches in August when bather densities were high and when levels of *C. albicans* in water samples were at a maximum.

In this study, we have attempted to associate the occurrence of *C. albicans* with that of the fecal coliform and streptococci indicators and of the bacterial pathogen *P. aeruginosa.* The data presented only justify such an association in the case of Lakeside and Weller Park beaches.

At first glance, Table 11 suggests that there is little relationship between the month of maximum mean population density of *C. albicans* and of the fecal coliform and fecal streptococcus indicators. However, closer examination of the primary data tabulations (Tables 7-10) from which Table 11 was abstracted, may suggest a possible relationship. In August, the numbers of *C. albicans* were highest, whereas the numbers of fecal coliforms and fecal streptococci were, with one exception, below peak level. The levels of both indicators, however, could certainly be described as being elevated. The numbers of fecal coliforms were at a maximum in August at Weller Park beach and in all other instances, the levels of the bacterial indicators were the second highest obtained at the respective beaches. The highest numbers of *P. aeruginosa* were recovered from the Lakeside Park beach in July in association with peak levels of fecal coliforms and fecal streptococci.

Thus, while the numbers of both pathogenic microorganisms were not always associated with maximal indicator populations, their occurrence was apparently related to the presence of elevated bacterial indicator levels. Conversely, we always coincidentally detected *C. albicans* and/or *P. aeruginosa* in samples from those beaches which had elevated indicator populations.

There is a good relationship between the temporal distribution patterns of *C. albicans* and *P. aeruginosa.* Their peak populations coincided at Weller Park beach, and at Lakeside Park beach relatively high numbers of *C. albicans* were enumerated in association with the maximum *P. aeruginosa* levels during the July survey.

Some contradictions are apparent in the intermicrobial numerical relationships. In June, when relatively high numbers of fecal streptococci were enumerated from the inshore water samples, the fecal coliform counts were low. This phenomenon may have been caused by the leaching or scouring, by wave action, of animal fecal wastes from

the beach sand. Fecal wastes from the large flocks of seagulls which had gathered at Lakeside and Weller Park beaches in June could also have contributed to the high fecal streptococcus levels in the inshore waters.

At beaches where the microbial numbers warranted the examination of spatial distribution patterns, we generally found a decreasing distribution gradient from the shoreline to the offshore waters. Also, when the survey season averages were calculated for each microbial parameter, it became apparent that the mean inshore (1 m) values tended to be greater than the mean offshore (20 m) values. This result is probably not caused by a water temperature effect, as the temperature data do not indicate an inshore-offshore differential pattern. Foster *et al.* (1971) postulated that increased indicator numbers in shallow beach waters may be caused by higher bather numbers in such waters. In the present study, inshore-offshore differences for fecal coliform and streptococcus populations were observed at some beaches in June and September when the bather loads were negligible, which suggests that the bather load distribution may not be the sole influencing factor. Other environmental factors which should be considered in relation to the above phenomenon are the effect of wave action in scouring the beach sand and in accumulating contaminant materials close to the shore. Stormwater, either from storm sewer input or land drainage runoff may have an effect both on the indicator and pathogen distributions in the beach waters. Whatever the explanation, the observed occurrence of higher inshore indicator populations may be of some significance to bathers, such as young children, who spend the majority of their time in the shoreline waters.

C. albicans was not detected in any of the swimming pools examined and only small numbers of the bacterial fecal pollution indicators were isolated. Kraus and Tiefenbrunner (1975) cultured *C. albicans* from open air swimming pools with a free chlorine content of 0.35 ppm. The bacterial quality of swimming pool water is considered to be a measure of the adequacy of the disinfection process (Mood, 1977). This may also be the case for *C. albicans,* as with two exceptions the free residual chlorine content of the pools examined in this study (1-2 ppm) was greater than in those examined by Kraus and Tiefenbrunner (1975) (0.35 ppm). However, we also failed to detect *C. albicans* in the samples taken from the pools which had a zero free chlorine content.

The numbers of *P. aeruginosa* and the fecal pollution indicators in the pool samples, while low, are nonetheless worthy of some discussion, especially in view of recommendations (Public Health Laboratory Service Sub-Committee of the United Kingdom, 1953) that no swimming pool water samples should contain any coliform organisms in 100 ml of water. In North America, it has been recommended by the Joint Committee on Swimming Pools, which was established in 1964 by the American Public Health Association, that pool waters contain not more than one coliform organism per 50 ml, as determined using the membrane filtration test.

In our survey, pool No. 5 contained 10 fecal coliforms/100 ml in the surface water and three fecal coliforms/100 ml in the bottom water, which suggests that the pool was unfit for use at the time of sampling. Interestingly, this swimming pool had a zero free chlorine content, which substantiates observations by other investigators that coliforms were seldom isolated from samples of pool water with free residual chlorine content in excess of 0.10 ppm (Public Health Laboratory Service Sub-Committee, 1953). The isolation of *P. aeruginosa* from this pool (two organisms per 100 ml) may have public health significance, as this bacterium is a causal agent of otitis externa (Hoadley, 1977) which may be transmitted through the medium of swimming pool water (Mood, 1977). Favero *et al.* (1964) recommend that the isolation of *P. aeruginosa* from 100 ml or less of swimming water should warrant closure of the pool until an adequate chlorine residual could be maintained.

Although supporting evidence is not presented, it is probable that the bacteria detected in the swimming pools had been shed by the bathers. Strains of *P. aeruginosa* may also be transferred to pools from the adjacent environment or from urine discharges by bathers.

The consensus of opinion in the literature suggests that *C. albicans* is not a normal component of the aquatic microflora (Buck, 1977) and thus, the source of isolates recovered from the natural aquatic environment must be extraneous. Examination of the survey data indicated that in only the single case of Weller Park beach in September, could the water be assessed as being contaminated with human fecal material. This conclusion is based on an interpretation of the fecal coliform and fecal streptococcus density ratios in the water samples (Geldreich, 1976). Although the reliability of this ratio has been questioned (Al-Diwany and Cross, 1978) on the basis of the different survival patterns of the coliform bacteria and the enterococci in water (McFeters *et al.,* 1974), it may still have value in the determination of the nature of recent fecal pollution (Geldreich and Kenner, 1969). Of the samples that we assessed according to Geldreich (1976) 39% contained fecal contamination of an apparently non-human origin, 12.9% of apparently human origin and the data for the rest of the samples were inconclusive.

The most plausible source of the non-human fecal contamination of the beach waters is stormwater runoff from the adjacent land. Even though the numbers of fecal

coliforms and fecal streptococci detected in the sand samples were low, their presence indicates the existence of a potential source of contamination. Before accepting such a conclusion, it should be recognized that substantial numbers of each of the microbial parameters were recovered in July in the absence of an associated rain storm, but in the presence of maximum bather loads at Lakeside and Weller Park beaches.

The observation that maximum counts of fecal coliforms, fecal streptococci and *P. aeruginosa* were obtained at Lakeside Park in the presence of maximum bather loads may be more than coincidental. It is possible that the bathers may have affected the numbers of microorganisms in the water either through the shedding of dermal-borne microorganisms, urine or fecal discharges or by the accidental transport of animal fecal wastes from the shore to the water.

Similar considerations may apply to the distribution patterns of *C. albicans*. In August, the month of maximum occurrence, *C. albicans* was detected in the sand samples and thus could have been leached into the water via storm-water runoff. As *C. albicans* is known to occur in feces of seagulls (Cragg and Clayton, 1971), the possibility that scavenging gulls, plentiful in the locality, may have contaminated the nearshore sand and water has to be considered. Again, bathers may have contributed to the observed levels of *C. albicans* in August, but were probably not the sole source of input, as this yeast was also detected in lower numbers in the absence of bathers. As previously mentioned, Kishimoto and Baker (1969) isolated *C. albicans* from sand samples collected at beaches with medium and high bather loads.

Should the above considerations be justified, the question of excessive numbers of bathers contributing to the pathogen content of a water body must be considered. Obviously, this would present most serious problems at shallow beaches in small lakes or ponds.

One must be very cautious when interpreting the data in this report for public health purposes. There have been few or no properly documented epidemiological studies linking incidences of bather infections with visits to fecally contaminated recreational waters. The importance of undertaking such studies must be stressed, as only on the basis of such data can realistic microbiological standards be established for bathing beaches. Existing standards have been formulated with the primary objective of preventing further deterioration in the environmental quality of beaches.

Bather infections resulting from contact with, or ingestion of, pathogenic microorganisms of fecal material which contain such microorganisms may result in diseases of the following types:

a) *Enteric infections*

These are most often symptomized by stomach upsets, nausea and often affect the victim for only a brief time. Such infections are not usually reported to medical practitioners. To become infected, a bather must ingest an effective dose of the pathogen. As a result, this type of infection would be difficult to analyze in terms of an epidemiological study.

b) *Eye, ear and upper respiratory tract infections*

These often cause relatively long term symptoms and have been associated with *P. aeruginosa* (Hoadley, 1977). As superficial immersion, or partial ingestion of, contaminated water may be sufficient for infection, this organism is more ideally suited for correlation with incidences of bather infections than are the enteric pathogens. Seyfried and Fraser (1978) used serotyping and phage typing to establish that *P. aeruginosa* from swimming pool water was the causal agent of incidents of otitis externa in bathers.

The many problems relating to the design and execution of such epidemiological studies and the associated establishment of bacterial water quality standards have been discussed by Foster *et al.* (1971) and Cabelli (1977).

c) *Vaginal and dermal infections*

C. albicans is a causal agent of skin (Tachibana, 1976), oral and vaginal (Emmons *et al.*, 1977) infections. Again, there are no conclusive data available to link instances of *Candida* infections with exposure to a contaminated environment. Winner and Hurley (1964) mention a condition called "surfers foot" in which *C. albicans* infects macerated skin. Tachibana (1976) cites references to support the opinion that since *C. albicans* is a member of the normal flora of the gastrointestinal tract, most cutaneous infections may be of fecal origin. We consider that skin maceration, a not uncommon condition among bathers, would strongly predispose towards *Candida* infections and feel that studies should be undertaken to determine whether this is a problem condition among bathers.

Brisou (1975) has commented on the increasing occurrence of vaginal infections from *Candida* in women who vacation at the seaside and frequent beaches which are polluted with intestinal yeasts.

yeasts. Brisou's study, which involved cooperation with medical practitioners, utilized both clinical observations and epidemiological studies to establish instances of *Candida* vaginal infections in women who had frequented polluted beaches. While Brisou (1975) did not present data to justify any possible relationship between yeast numbers and instances of infection, he did conclude that the relationship between the pollution of littoral waters, beaches and sands, and the occurrence of intestinal yeasts can no longer be placed in doubt. Brisou recommends systematic research on the occurrence of intestinal yeasts as an excellent complementary method of monitoring the quality of bathing waters.

While we are inclined towards these conclusions, we feel that further substantiation and research is required before conclusions may be drawn concerning the health significance of *C. albicans* in beach waters.

CONCLUSIONS

We have established that the opportunistically pathogenic yeast *C. albicans* occurs in Lake Ontario beaches. The four beaches surveyed could be differentiated on the basis of bacterial fecal pollution indicators and numbers of the pathogens *C. albicans* and *P. aeruginosa*. The occurrence of *C. albicans* and *P. aeruginosa* in the beach waters examined appears to be related to elevated fecal pollution indicator levels. Maximum numbers of all microbial parameters were observed in July and August in association with peak bather loads at the beaches. Where microbial population levels were adequate, a decreasing distribution pattern from the shoreline to the offshore waters was observed. In only one instance does our data suggest that a beach had been subjected to human fecal contamination. Storm water runoff seems to be the most plausible source of the contamination in most other cases, although the possibility that the bathers themselves, may contribute to the pollution level of a beach must also be considered.

We failed to detect *C. albicans* in any of the swimming pools surveyed. In the one pool where we did detect *P. aeruginosa* in association with fecal coliforms and fecal streptococci, the pool's free chlorine content was at a zero level.

Future Investigations

The development of mCA agar and the proven reliability of the identification techniques for *C. albicans* have provided a useful tool with which to expand our knowledge concerning the distribution of *C. albicans* in the aquatic environment. One aspect of the methodology requiring further consideration is the effect of different pore sized membrane filters, from a range of manufacturers, on the recovery of stressed cells of *C. albicans* from lake water.

Investigations of the distribution patterns of *C. albicans* in relation to bather load at heavily utilized beaches should provide an insight into whether bathers contribute to the *C. albicans* content of beach water. The survivability of *C. albicans* and its relation to environmental parameters such as temperature, water source and microbial population density may affect both the usefulness of *C. albicans* as a potential fecal pollution indicator and its public health significance.

Beach surveys before and after rainfalls and examination of storm sewer waters, rivers and creeks may give further insight into potential sources of *C. albicans* input into the lake ecosystem.

Of prime importance is the necessity, although it is unquestionably a difficult task, to conduct properly designed epidemiological studies which would provide reliable data relating to the incidences of infections from *Candida* among bathers frequenting beaches that are contaminated with *C. albicans.*

Finally, we shall again stress the importance of employing a wide range of microbial pollution indicators to determine the quality of recreational waters and emphasize the advantage of routinely monitoring pathogen levels to complement such data.

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