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Columbia University Seminar Series

Edited by
GEORGE J. HALASI-KUN

Volume XIV Part 2
1981

Pollution, Coastal Biology & Water Resources
Selected Reports

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INTRODUCTION

This volume contains articles presented by the members and contributors of the Seminar. Similarly to the previous volumes, most of them are intended to be published also in our planned Encyclopedia on Geohydrology and Water Resources.

In accordance with the topics, the articles can be grouped into:

(a) research papers of the American Academy of Ocean Sciences on coastal biology;

(b) pollution of water resources, including water quality standards and water resources planning;

(c) effects of water on vegetation; and

(d) meterological characteristics of a drought period.

The Seminar's activity for the 1980-1981 academic year will be described in Volume XIV-3 together with the contents of the Volumes I-XIV of the Proceedings.

The Editor of the Proceedings together with the Steering Committee of the Seminar wish to express their appreciation to all members and contributors of articles and lectures participating in the Seminar. Their dedicated effort made possible the compilation of this volume.

We thank L. Straka, President of Pergamon Press, Inc., for the careful preparation and printing of the Proceedings.

George J. Halasi-Kun
Chairman of the
University Seminar on
Pollution and Water Resources
Columbia University

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THE DISTRIBUTION OF *VIBRIO PARAHAEMOLYTICUS*, YEASTS AND YEAST-LIKE FUNGI, ESPECIALLY *CANDIDA ALBICANS* IN VARIOUS ENVIRONMENTS OF THE GREAT SOUTH BAY OF LONG ISLAND, NEW YORK

by

Peter P. Madri, Ph.D., and George Claus, M.D., Ph.D.
American Academy of Ocean Sciences

INTRODUCTION

Increasing levels of utilization and pollution of coastal waters and estuaries have had a dramatic effect on the animals, plants and sediments in these bodies of water. Rapid expansion of communities surrounding the estuarine areas has also stressed these environments (Teal, 1968; Odum, 1970; Inman and Brush, 1973; Goodwin and Niering, 1974). Population growth on the one hand, accompanied by increasing amounts of waste generated by man on the other hand, have increased the demands for utilization of the estuarine environments both for recreational activities and as discharge sites from homes, industry and communities.

An example of an area going through such change and increased demands is the Great South Bay of Long Island's south shore. Homeowners, industry and communities would use the bay as a waste disposal basin, recreation-minded people want to use clean water for bathing, boating and sport fishing, and conservation groups would like to see the bay left in its natural state. This conflict of interest has been discussed by Claus (1972), and he drew the conclusion that these diverse interests have created a climate of pettiness and rhetoric rather than focusing attention on solving problems of the deteriorating environment.

Geography, hydrography and history of the study area

The Great South Bay is a coastal lagoon complex on the south shore of Long Island, New York, approximately midway between New York City and Montauk Point. The bay is a shallow submerged glacial outwash plain on the southern portion of the fluvio-glacial deposits that form Long Island (Cohen, 1968). It is separated from the Atlantic Ocean by a series of barrier islands. The western portion of the bay is connected to the ocean through Jones Inlet via South Oyster Bay and the eastern section by the Fire Island Inlet.

Such a bar-built lagoon (Penn, 1968) is typified by its shallowness and restricted tidal exchange with the adjacent ocean (Pritchard, 1967). Inlets to this type of estuarine waters are small compared to the area of the embayment. Tidal currents through the inlets are usually strong even though tidal amplitude may be small. The tidal range throughout Great South Bay is quite small. At Fire Island Inlet it is approximately 3.0 feet (0.93 m) and decreases to approximately 1.5 feet (0.46 m) in western Great South Bay (Foehrenbach, 1969). Because of the small range of tidal amplitude and the shallowness of the bay, wind is the dominant mixing mechanism.

Current velocity measurements (Jamieson, 1968) established that currents flowing between South Oyster Bay and Great South Bay towards the east move 224 million cubic feet during flood tide and 217 million cubic feet west during ebbtide. This results in a 7 million cubic foot net eastward flow during each tidal cycle. The maximum velocity recorded was 1.9 knots and excursions during the incoming tide were approximately 3 miles. Claus (1972) demonstrated that the typical counter clockwise flow pattern in the bay produces a faster flow in the New York State boat channel and out Fire Island Inlet. Estimates of the flushing rate are inconclusive, but various authors (Redfield, 1961; Foehrenbach, 1969; Jamieson, 1968; Claus, 1972) believe that 14 to 48 days are required to flush the entire bay.

Foehrenbach (1969) points out that the tidal prism, the amount of water which enters and leaves the bay during each tidal cycle, is not all new or fresh sea water because a large quantity of bay water which leaves the bay during ebbtide is returned during flood tide. A dye study by the U.S. Public Health Service (1962) indicates that after three high tides the bulk of the dye remained inside Fire Island Inlet.

The shallowness of the bay, extensive eel grass (<u>Zostera marina</u>) beds, and tidal islands tend to impede flow. The exchange of water, therefore, is largely limited to the dredged channels. Thus limited circulation may be a significant factor when considering pollution of the bay. Pollutants from the north shore of the bay and motor boats may remain, with limited dilution, eventually being incorporated into the sediments.

Several investigators have studied various water quality parameters in Great South Bay and adjacent waters (Ichiye, 1966; Foehrenbach, 1969; Wagner, 1971; Claus, 1971, 1972; Hair and Buckner, 1973). Hair and Buckner (1973) demonstrated that phosphorus levels are highest along the north shore of the bay and that certain rivers, notably the Amityville Creek and Santapogue River, are contributing excess phosphate to the bay system. This study also demonstrated that nitrogen levels in the Great South Bay are approaching non-limiting concentrations, exceeding nutrient requirements, and producing excessive plant growth, resulting in enhancement of eutrophication processes.

The area has a long history of utilization by man. Before "New World" settlements of Long Island, native Indians used the shoreline and islands for fishing stations and harvested mulloscs and other shellfish from the abundant tidal flats. As settlers moved eastward on Lond Island, the Indians were displaced, villages appeared bordering the bay and the shellfishing industry began.

Although as late as 1947, the Great South Bay was considered an exceedingly clean area for the harvesting of large amounts of oysters (<u>Crassostrea virginica</u>) and hard shell clams (<u>Mercenaria mercenaria</u>) (Van Popering and Glancy, 1947). Public health problems resulting from improper shellfish handling or heavy environmental pollution led to a national policy concerning sanitary control of the industry in 1925. By the early 1930's, the local shellfishing industry began to experience difficulties, particularly concerning the growth and maturity of oysters (Van Popering and Glancy, 1947). Although inlet incursions and subsequent salinity changes were implicated in annual variations of oyster yields, major problems were noted concerning excessive water turbidity and the presence of enormous numbers of microalgae.

By 1960 the total shellfish revenue from the area had decreased to 1.5 million dollars from 50 million dollars in 1910 (Claus, 1971, 1972) and the famous Blue Point oyster could no longer be found in the confines of the bay. The final disappearance of the oyster industry in the Great South Bay left the baymen with only the hard shell clam as a source of significant income from this unique estuary.

By the early part of this decade, however, regulatory agencies have been threatening to prohibit shellfishing in various areas of the Great South Bay due to a significant decrease in water quality as ascertained by the "coliform count;" this shall be discussed later in detail. In 1976 the New York State Department of Environmental Conservation, following nationally accepted standards, attempted to close vast areas of clam beds and was met with local resistance from baymen and municipalities (Trial Transcript, 1977). To date, the problems facing the future of the shellfishing industry in the Great South Bay have not been resolved.

Scope of the present studies

This paper covers two different, but closely interrelated topics, both dealing with the public health aspects of the distribution and ecology of certain marine microbes occurring in the Great South Bay and their significance as human pathogens.

The occurrence of Vibrio parahaemolyticus in the marine milieu - The first area of investigation centered around the detection of Vibrio parahaemolyticus in Long Island (N.Y.) bay areas. This microorganism is a halophilic gram negative rod which has been implicated in human disease in Japan since 1950 (Colwell, 1975). The organism was originally reported by Fujimo and his co-workers in 1951 after investigating a food-borne epidemic which involved 272 people, 20 of whom died (Fujimo et al., 1951). Initially, much confusion existed concerning its taxonomic position but all investigators agreed upon the ability of the organism to cause moderate-to-severe gastrointestinal illness upon ingestion of contaminated seafood. By 1960 food-borne epidemics caused by V. parahaemolyticus in Japan prompted the Japanese ministry of Health and Welfare to initiate investigations of the "pathogenic halophilic bacteria" (Colwell, 1975).

As a result of some of the attempts to characterize the organism, it was found that V. parahaemolyticus strains differed not only antigenically, but also in their ability to produce hemolysis on a specific medium (Kato et al., 1965; Miyamoto et al., 1969). This hemolytic activity in Wagatsuma agar was called the "Kanagawa phenomenon" and was found to be directly related to the enteropathogenicity of the bacterium (Sakazaki et al., 1968).

Interest in V. parahaemolyticus in the United States began with its isolation from Gulf and southern Atlantic coastal sediments in 1968 (Ward, 1968). Following this report, V. parahaemolyticus was found in coastal sediments and marine animals from Puget South (Baross and Liston, 1968), marine invertebrates in the Chesapeake Bay (Krantz et al., 1969) and Gulf Coast (Vanderzant and Nickelson, 1970), Canadian shellfish (Thomson and Trenholm, 1971), and from marine animals off the coast of New Hampshire (Bartley and Slanetz, 1971) and Massachusetts (Barker et al., 1975). In August of 1971 confirmed outbreaks of food-borne illness due to V. parahaemolyticus began to become evident in the United States. Since this time the epidemiology and toxicity of the organism has been the topic of many research papers (Barker et al., 1975; Twedt and Brown, 1975).

In spite of the relatively recent attention shown to this marine pathogen there are still unanswered problems concerning the ecology, physiology and epidemiology of V. parahaemolyticus. It is generally presumed that marine animals, as well as sediments, serve as reservoirs for the organisms. Studies have indicated, however, that V. parahaemolyticus is not only commensal with the blue crab, but may also cause disease and eventual death of the animal (Krantz et al., 1969). Other investigators have found that V. parahaemolyticus was responsible for high mortality rates among brown shrimp in laboratory aquaria (Vanderzant and Nickelson, 1970). For these reasons it is difficult to classify the organism as being strictly commensal with these invertebrates. The action of V. parahaemolyticus on its vertebrate hosts is as yet unknown, although it has been shown to be capable of multiplication in the gut, tissues and mucus of fish along with other potential pathogens (Janssen, 1970).

A further problem in connection with the natural occurrence of the organism is presented by the somewhat contradictory data pertaining to its abundance in open sea water (Kaneko and Colwell, 1974). Various authors are of the opinion that V. parahaemolyticus is limited to coastal or estuarine waters (Anonymous, 1970; Colwell, 1975; Horie et al., 1963; Kampelmacher et al., 1972; Sakazaki et al., 1963), while others have failed to isolate the organism from certain coastal waters (Scheffers and Golten, 1973). V. parahaemolyticus has been isolated from pelagic fish, but Colwell ascribes this phenomenon to probable contamination after landing (Colwell, 1975). The same investigator claims that organic nutritional requirements and zooplankton-chitin relationships tend to limit V. parahaemolyticus to rather defined coastal or estuarine areas (Kaneko and Colwell, 1973). Other investigators have claimed isolation of V. parahaemolyticus from various pelagic areas, particularly in the Pacific Ocean (Aoki, 1967a; Aoki, 1967b; Yasunaga and Kuroda, 1964).

Another uncertainty concerning the occurrence of this organism is its distribution throughout the year in temperate zones. According to available data, V. parahaemolyticus is a mesophilic organism which decreases in numbers upon the falling of temperature (Baross and Liston, 1968; Kaneko and Colwell, 1973). It has been shown, however, that the organism is capable of survival in enriched ocastal waters in Alaska, and sediments and shellfish during winter months (Baross and Liston, 1968; Vasconcelos et al., 1975). Although investigators have correlated numbers of V. parahaemolyticus in shellfish with ambient temperature as a method of directly reflecting numbers of these organisms present in the water column (Baross and Liston, 1970), the fact that shellfish decrease their pumping activity as the temperature drops and may actually become dormant during the winter (Anonymous, 1968; Hamwi, 1967; Jamieson, 1973) was apparently not taken into consideration. Present data do not indicate the concentration fluctuations of V. parahaemolyticus on a seasonal basis either in appropriate host animals or open sea water.

In the present study our essentially negative results are reported concerning the occurrence and distribution of this Vibrio in Long Island Bay waters and the biota which thrive there.

Investigations concerning the usefulness of Escherichia coli and Candida albicans as indicator organisms of pollution of sea water - The second concern of the study was to further demonstrate the proposed usefulness of C. albicans as an indicator organism of pollution of human origin. For this reason, extensive weekly samplings of water, sediment and invertebrates (mainly clams) were undertaken and total fungi and C. albicans counts were established and compared with those of total and fecal coliform numbers obtained from the same areas and/or the same specimens.

Rationale for using the coliform group in water pollution studies - Both drinking and bathing waters contain a large number of microorganisms, some of which may represent a health hazard for man. At the turn of the century, it was recognized that it is not feasible to test every drinking or bathing water supply for each organism which might be present and could represent a potential human pathogen. After the pioneering studies of Zacharias in 1898, it was recognized that Bacillus coli, as he named the organism, or by its modern name, Escherichia coli, represented approximately 40-50% of the fecal matter of man, which, when acceding into the water, could be determined both on a qualitative and quantitative basis. The reasoning was developed that quantities of E. coli or other similar organisms, when discovered, could serve as indicators of the presence of fecal pollution. Furthermore, the establishing of the coliform counts was supposed to be a presumptive indicator, if exceeding a certain value, of the presence of other, more serious pathogens such as Salmonella, Vibrio, Shigella, etc., or even viruses -- organisms all occurring as contaminants of fecal origin. Even at the present time, the procedure most commonly used to indicate the presence of pathogenic microorganisms in water is the Standard Total Coliform Test described in Standard Methods for the Examination of Water and Wastewater (1976). The coliform group, as defined in the 14th edition of this manual, includes all of the aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria which ferment lactose with gas formation within 48 hours at a temperature of 35°C.

With respect to the "total coliform count" as a method for characterizing the load of pathogenic organisms transmitted by the fecal route present in waters, it may be said that sophistication of microbiological techniques have improved on the original methodology of multiple-tube fermentation, which is still regarded as the standard test for the detection of fecal pollution of water. (The results of this test are conveniently expressed as most probable number -- MPN.) Recently the membrane filter technique has been developed and is widely used to enumerate the coliform bacteria. A further development of this basic test attempts to differentiate the segment of the coliform population referred to as fecal coliforms. Coliform bacteria from the gut and feces of warm-blooded animals (including man) produce gas in a suitable lactose culture medium at 44.5 ± 0.2°C, whereas coliform bacteria from other sources do not do so. It has been claimed that this differentiation is helpful in tracing those sources of pollution which definitely originate from human feces. Unfortunately, however, there is no satisfactory test capable of differentiating human fecal coliform organisms from those originating from other warm-blooded animals. It is therefore necessary at present to consider all fecal bacteria as indicators of dangerous contamination -- whether or not this is in fact the case.

In spite of some further refinements, such as the use of enterococci or Mycobacterium as indicator organisms, the "total coliform count" still remains the accepted procedure for determining the pathogenic pollutional load of waters. Yet, despite its widespread use, this procedure does not meet the criteria for a reliable test when estuarine waters are in question for a number of reasons to be discussed below.

The survival times of eight water-born pathogens in sea water was investigated by Jamieson et al. in 1976. The organisms tested were exposed to different temperatures and salinities. The only organism which survived to the termination of the experiment was C. albicans. After the sixth day of exposure the numbers of the fungus more or less stablized and held constant to the eleventh day. Salinity had no appreciable effect on the reproduction of C. albicans, while the lowest temperature (4°C) permitted the development of stabilized populations of lower densities than the other two higher temperatures. The tests with enteropathogenic E. coli showed that the low temperature range favored the growth of the organism, while salinity had no appreciable effect on its survival. After the fourth day of sea water exposure, only those cultures which were kept at the lowest temperature showed growth, and even those died by the sixth day.

M. tuberculosis, the slowest growing organism in this report exhibited a high survival rate coupled with a reproductive ability at the lowest salinity and at the two lower temperature levels. At a higher salinity, after five days of exposure, a dramatic decline in viable cell numbers occurred and by the tenth day no growth was evident.

Extreme sensitivity to salinity was reportedly demonstrated by L. interrogans which survived for only one day after inoculation into sea water at 4°C and 5 ppth. S. typhi, group D, survived only up to six days in the tests.

As with the other coliforms, lower temperatures favored the growth of S. dysenteriae, although its survival time did not exceed the fifth day.

V. cholerae biotype El Tor did not survive day 1 at 37°C and 35 ppth. At the lowest temperature and salinity values the organism died after the fourth day.

Yersinia enterocolitica died off rapidly in the sea water tubes, surviving in small numbers at low temperatures until the fifth day.

Due to the bactericidal action of sea water as described above, coliform bacteria may disappear relatively rapidly from the waters. The 90% kill rate of the organisms varies from less than 24 hours to a maximum of 48 hours (Pramer et al., 1963). Jones (1963) actually showed that natural sea water was more inhibitory to Escherichia coli than 16 units of penicillin G per ml. of artificial sea water. Although the bactericidal action of sea water plays an important role in the decrease of coliform counts in estuaries, dilution, adsorption, sedimentation, starvation and predation also contribute to the rapid destruction of E. coli and the other members of the coliform group (Carlucci and Pramer, 1959; Jannasch, 1968; Ketchum et al., 1952). Even in fresh water studies, E. coli has been shown to be less resistant to die-off than Salmonella typhi and some enteric viruses (Coetzee and Fourie, 1965; Slanetz et al., 1972). Yet, under other conditions, such as high nutrient levels in salt water, it has been demonstrated that coliform bacteria entered a growth phase for one to three days duration before the setting in of a death phase, which then resulted in a rapid decrease of their density. Whereas the E. coli were obviously fluctuating in numbers, the total bacterial count remained essentially the same during the whole period (Savage and Hanes, 1971). These results give credence to the earlier claim that the initial growth of coliforms is proportional to the concentration of available nutrients in the environment (Orlob, 1956). Rapid increases with subsequent decreases of coliform numbers in both sewage and pure sea water cultures are a common phenomenon (Slanetz et al., 1964).

Coliform density has also been shown to exhibit diurnal fluctuations due to changes in water temperature (Mallman and Seligmann, 1950). Obviously, such sudden and rather unpredictable changes must impair any direct correlation between coliform density and the degree of organic pollution.

Another disadvantage of using the coliform group as an indicator of fecal pollution in open waters is that E. coli survives in marine sediments, and these deposits serve as reservoirs for the organism. Since members of the Enterobacteriaceae are facultative anaerobes, the deeper layers of sediments provide an adequate environment for them. Whenever bottom currents or upwellings disturb these layers, the coliforms move into the above lying waters. It is possible, therefore, that after a storm or following dredging operations one will find large numbers of coliforms in the water, even when no recent fecal contamination has taken place.

The use of the "total coliform count" as a criterion for evaluating the quality of estuarine waters is also basically unreliable because the procedure was designed to detect only intestinal pathogens. In quality testing of drinking water such a criterion may be useful, but in the case of recreational waters, such as estuaries, skin microorganisms rather than enteric pathogens are responsible for the greatest hazard to swimmers. It is commonly agreed that the majority of disorders contracted from water-borne pathogens are those of the eyes, ears, nose, throat, genitalia, and the skin (Foster et al., 1971).

In this connection, it should be obvious that the coliform count is not a suitable indicator of pollution caused by bath or laundry water. The latter contain large numbers of microorganisms originating from the skin or mucous membranes of the body rather than from the digestive tract.

If one investigates the accumulation of coliform organisms in the marine biota, especially in shellfish further problems arise. Shellfish are able to at least partially utilize coliforms in their nourishment; thus, if there is a coliform influx in their surroundings, they will concentrate the bacteria in their bodies and retain them in their mantle cavities and throughout their whole digestive system, in different stages of breakdown. Since a single clam can filter up to 120 liters of water per day (Hamwi, 1967), even a relatively small number of coliforms present in that volume will be concentrated in its body. Therefore, even if the coliform count in the surrounding water is relatively low, a certain enrichment will occur in the clams. On the other hand, if the coliform count is high in the clam's environment, the animal will utilize proportionately greater amounts of the bacteria for its feeding; thus, the numbers of undigested coliforms in the clam itself may not be greatly different in slightly or highly-polluted conditions.

It may thus be seen that coliform counts are not only unreliable as far as deciding the pollutional status of sea water is concerned when directly investigated, but they are also false indicators of the degree of pollution when their presence in shellfish is ascertained.

All of these disadvantages have demonstrated for years the need for a more reliable indicator organism than the coliform group and efforts were directed to find and characterize an "ideal" indicator organism. C. albicans seemed to conform to most requirements as a water pollution indicator for the marine milieu and the investigations presented below seem to give further credence to this supposition.

Candida albicans as a possible pollution indicator organism - According to the classification of Lodder and Kreger-Van Rij (1952), C. albicans is an asporogenous yeast belonging to the family Cryptococcaceae. It is characteristic of the genus in that it develops pseudo-hyphae. In its yeast form, the organism is round or short-oval and seldom elongate. The cell size varies considerably, but it is usually between three to eight microns wide and five to eighteen microns long. One usually finds a rich development of pseudo-mycelium or true mycelium on solid substrates. On the pseudo-mycelium, ball-like clusters of blastospores or blastoconidia are formed, whereas on the true mycelium large round chlamydospores occur, usually terminally but also intercalary. The polymorphism of the organism has given rise to considerable confusion as to its naming and taxonomic identity. No less than eighty-seven synonyms for Candida albicans appear in the literature from 1839 on (Winner and Hurley, 1964).

In the middle of the nineteenth century, it was recognized that a fungus is responsible for the widespread disease called thrush. Because of the medical importance of this discovery, researchers expended considerable effort to delineate the organism. Although a terrestrial parasitic organism, C. albicans is capable of surviving in sea water according to the latest findings. The marine environment influences its morphology in the sense that Candida will almost always occur as a yeast-like form, without myceliation or pseudo-hyphae formation. Its only known reproductive process in sea water is budding; neither blastospores or chlamydospores develop. The fact that sea-living C. albicans usually develops as a yeast means that the organism retains a relatively constant size and shape in the marine environment. Thus, it should be readily accessible to concentration through differential filtration techniques.

The pathogenic characteristics of C. albicans tend to manifest themselves when the body's natural defenses are not yet fully developed and/or weakened by other debilitating conditions. Thus, the incidence of and mortality due to thrush can be particularly high in infants and adults who are immunologically compromised. Studies of sputum, feces, vaginal mucous, and antibodies in blood serum have documented the widespread presence of C. albicans in normal, healthy adults. However, there is very little documentation of the extent of serious systemic diseases (e.g., meningitis, encephalitis) etiologically related to pathogenic strains of C. albicans. Evidence indicates that increasing occurrence of dermatomycoses seem to be associated with increased use of public swimming areas, which with mounting pollution yield high fungal cell counts.

Many varieties of thrush have been recognized during past centuries. The first case descriptions were given by Hippocrates in his Epidemics. He also delineated what we today call vaginal candidiasis.

C. albicans is a direct pathogenic organism occurring as a widespread commensal microbe on the mucous membranes or in the alimentary tract of healthy people. Thus, its pathogenic characteristics will most likely be manifested when other debilitating conditions are present or when the natural defenses of the individual are not yet fully developed, as in infants. Hence, Valleix in 1838 first noted the pediatric importance of the fungus. At the turn of the century, Concetti (1900) reported widespread epidemics of thrush in foundling hospitals in Italy, where 50% of the children were afflicted with it and those who were diseased showed a 60% mortality. Stenderup and Pedersen (1962) found that 53% of all hospitalized patients in Sweden showed manifestations of oral thrush. In newborn infants, Bret and Coupe (1958) reported a 16.3% incidence of Candida infection, while in an earlier, but more thorough study in Germany, Epstein (1924) found that 54% of infants up to six weeks of age were infected, decreasing to 38.5% in the six-year-old group. This study was confirmed by Basu et al. in 1961, who found 22.2% of healthy children infected with the fungus.

Since overt symptoms of infection are less common than the carrier state, attempts have been made to ascertain the general distribution of the fungus in seemingly healthy populations in order to delineate its epidemiological importance. Tanner et al. (Germany, 1927) found 10% of normal adults to be carriers in their mouths. The occurrence in sputum is much higher; Woolley, working in Arizona in 1938 found only a 5% infection in sputum, but in 1960 Baum reported an average of 18% in a New England population, and in 1950 Helms found that 77% of normal adults in Denmark were lung carriers. The distribution of Candida in the feces also varies considerably: in 1944 Felsenfeld reported 19.3 to 44.6% in different regions of the United States. Akrawi's studies in 1960 showed that 70% of the inhabitants of Baghdad have the fungus in their feces.

A more distressing and, therefore, more important infective locale in the human population is the female vagina. Giunchi (1958) found that between 13.7 to 35.9% of Italian women investigated were carriers while Carter, working in the United States, reported in 1940 that 43% of the female population of this country are carriers.

Further inferences as to the widespread occurrence of the fungus in the human population can be derived from serological studies. Since the antibodies for Candida apparently do not disappear from blood serum, such investigations will disclose the numbers of people who have been exposed at one time or another, even if not current carriers. The early work of Todd (1937) showed that 22.5% of sera from normal persons agglutinated C. albicans while Skobel et al. (1956) found antibodies to Candida in the serum of 80% of school-age children.

Although the most serious manifestations of candidiasis are the systemic infections, which are usually associated with other disease conditions, scattered statistics are available on the frequency of such infections. When an individual is a mouth carrier, such a simple operation as the extraction of a wisdom tooth may lead to systemic disease (Wegmann, 1954). If the fungus becomes established in the urinary tract or enters the circulation, it can cause endocarditis, meningitis, and encephalities, usually fatal diseases. The only data found in the literature about the incidence of systemic infections are from Hungary (Fejer et al., 1957) where a total of 6,945 cases were reported (out of a population of ten million people) during the year 1950. Of these cases, 2,073 originated from mycotic infections of the foot.

In general, dermatomycoses are on a continual increase and have taken the proportions of population diseases. Since 1938 to 1949 the known cases of mycotic infections rose from 4% to 17.5% in Germany (Gotz, 1952), and in the U.S. athlete's foot is one of the most common and difficult to cure among skin diseases. Every third person is afflicted with it (Montgomery and Casper, 1945). This is directly attributable to the increased usage of swimming areas: pools and beaches (Talalov et al., 1937). The worldwide trend applies to this country too; that is, when a beach community is established a sudden increase in the occurrence of dermatomycoses takes place. Whereas the open oceans carry about 200 to 300 fungla cells per liter (Fell and Van Uden, 1963), in moderately polluted beach areas ten to twenty thousand cells per liter are not unusual and in heavily polluted estuaries their numbers reach the 100,000 mark (Whalen et al., 1970).

Until recently, C. albicans was thought to be a parasite of warm-blooded animals, including humans. Thus, the search for its presence in the soil or in the air was not too successful. Ajello (Tennessee, 1955 and 1956) found in 1,039 soil samples only one which contained the fungus; while Nilsby and Nordon (1949) failed to grow it on 600 plates exposed to air. It seemed, therefore, that the route of infection must be from animal to animal. Reports about its occurrence in the marine environment were thus surprising. At first, most yeasts and yeast-like fungi in the marine milieu were attributed to terrestrial contamination, but recent evidence indicates that C. albicans can indeed survive in the marine environment. Fell and his coworkers published two important articles in 1963 reporting the isolation of C. albicans from a marine habitat. In one of their papers, Fell and Van Uden (1963) concluded that the presence of this fungus is unequivocal evidence of human contamination while, upon further investigation, the authors (Fell et al., 1963) reevaluated their position and postulated that this yeast-like organism may be a free-living inhabitant of true marine waters.

Further studies have shown that C. albicans not only survives in the sea water environment, but also that it retains its infectivity and pathogenicity even after prolonged periods of exposure to marine conditions (Madri et al., 1966). The long survival time of this fungus under artificial conditions simulating the marine environment was first demonstrated by Dzawachiswili, et al. in 1964 and under more precise conditions by Madri in 1968.

Madri et al. carried out experiments in 1966 with human pathogenic strains of C. albicans in which they exposed the fungus grown in laboratory cultures to filter-sterilized sea water for eight weeks. Care was taken not to introduce nutrient carryover with the culture medium before the cells were dispersed in the sea water. Cell numbers were determined weekly for the period of the experiment. It was found that, after a short period of initial decline in fungal concentration, there was a three-week period during which no observable change in numbers took place, followed by a gradual increase in cell multiplication, lasting to the end of the experimental period. When the cells were harvested and injected into mice, the typical pathological picture of systemic candidiasis was manifested. In further studies (Madri, 1968) it was shown that, during prolonged sea water exposure (up to twelve weeks), the fungi reproduced almost exclusively through budding.

Several natural characteristics of E. coli render it unsuitable for use as a reliable, general pollution indicator. At least seven criteria for an ideal indicator organism can be specified. Evidence indicates that human pathogenic strains of C. albicans meet all seven of these criteria, whereas E. coli or the coliform group do not.

For accurate indication of pollution, the organism should have the following characteristics:

1. It should be of uniquely human origin.

2. It should always be present in the water when pathogens from human contamination are there.

3. It should survive at least as long as the water-borne pathogens and should not be susceptible to either growth or rapid die-off during this period.

4. It should not be restricted to fecal origins alone, but should also be related to other waste effluents, such as bathing and laundry water. In other terms, the indicator should also originate from the skin of people.

5. It should not survive in deeper strata of sediments.

6. It should not be susceptible to attack by naturally occurring antibiotics in the marine environment.

7. It should be amenable to concentration and absolute identification within a short period of time and at a low cost.

The studies of several investigators and our own have shown that C. albicans meets all these criteria fully or in case of criterion (1) at least partially.

Criterion 1 - C. albicans has been found both in the feces and on the skin of several animal species besides man. It has been found to inhabit, among others, turkeys (Hinshaw, 1934), hens and pigeons (Coutelen and Cochet, 1942), ducks (Ainsworth and Austwick, 1955a & b), rabbits and rats (Coutelen and Cochet, 1942), cows (Clark and DiMenna, 1961) pigs (Parle, 1957), and monkeys (Thiry, 1913). However, since the great bulk of the pollution entering estuaries is in the form of domestic sewage, the failure to meet this criterion would not normally be detrimental to the use of C. albicans as an indicator organism. As was previously mentioned, the search for a C. albicans reservoir in the soil or in the air has been unsuccessful (Ajello, 1955, 1956; Nilsby and Nordon, 1949).

Criterion 2 - C. albicans can be expected to be in the estuarine waters whenever human pathogens are present, since it is found in both the feces and body surfaces of humans. Although it is known that C. albicans occurs as a widespread commensal microbe on the mucous membranes or in the alimentary tract of healthy people, just how widespread its occurrence is seems to vary considerably by geographic region, as point out earlier.

Criterion 3 - The long survival time of C. albicans, under artifical conditions simulating the marine environment, was first shown by Dzawachiswili et al. in 1964 and, under more precise conditions, by Madri in 1968. In the experiments of Madri et al. (1966), C. albicans was subjected to sterile sea water for a period of eight weeks and the fungi were recovered unharmed from this treatment. No appreciable growth was observed until about three weeks following inoculation, after which a gradual growth took place. However, since pure cultures of C. albicans were grown in filter-sterilized sea water, the effect of predation on the fungus could not be observed. Thus, it was considered possible that in natural estuarine waters this reported growth could be offset by predation. In further experiments it was found that the same fungi retained their infectivity and pathogenicity to mice after an exposure of eight weeks to sea water (Madri et al., 1966).

Criterion 4 - C. albicans is not confined to fecal origin alone since, as discussed above, it is found in several areas of the human body. Mucous membranes in the mouth and vagina often contain C. albicans. Bathing and washing water can be expected to carry C. albicans, and public beaches can also be contaminated with it.

Criterion 5 - *C. albicans* being an aerobe does not penetrate and survive in deeper sediments. Fell and Van Uden (1963) found that the organism is restricted to the surface layers in estuarine sediments (not deeper than two to four centimeters). Since these top few centimeters of sediments usually are subject to constant or frequent agitation by tidal currents they are not likely to have a higher fungal count than the surrounding waters. Thus, *Candida*, unlike *E. coli*, will not present a false alarm.

Criterion 6 - *C. albicans* should experience very little detrimental antibiotic action in the marine environment. There are relatively few known antibiotics that have a candidicidal action, and these are particularly rare in the marine environment. The report by Buck and Meyers (1965) concerning the isolation of a *Pseudomonas sp.*, which was able to secrete an antibiotic substance fatal to *Candida*, does not substantially detract from the general statement since the numbers of this bacterium in estuaries are not large enough to produce adequate quantities of fungicidal agents to eliminate *Candida* populations. Furthermore, the dilution of this antibiotic is so large that effective levels are not achieved, except within restricted locales such as a highly-fouled log in a harbor area (Buck and Meyers, 1965).

Criterion 7 - *C. albicans* displaying a yeast-like form in a well-defined size range can be concentrated with relative ease. The 10 to 12 micron diameter of the fungus is smaller than most of the other planktonic organisms or debris, yet it is larger than the microalgae or bacteria. Therefore, with differential filtration, it is possible to concentrate the organism on a filter pad with a relative exclusion of other cells or material. According to the studies by Fell and Van Uden (1963), there are 200 to 300 fungal cells in one liter of sea water -- even in such clean areas as the Bahamas. In polluted estuaries this number is much higher. The identification of the concentrated organisms could be easily and rapidly achieved by employing modern serological or immunofluorescent techniques (Jamieson, 1974).

Finally, as far as the utilization of *Candida* as an indicator of contamination of shellfish is concerned, the following should be borne in mind: This fungus, as has been experimentally shown with other yeasts, cannot be digested by clams, oysters or mussels (Galtsoff, 1965). Therefore, it will be rejected with other undigestible particles; and as long as the numbers of the fungus are not higher than the base level, the shellfish will be clean -- i.e., not harboring the organism. However, if the number of *Candida* per unit volume of water exceeds a certain critical value, the filtering activity of the shellfish will not be able to completely eliminate all fungal cells from the mantle cavity, and some organisms will be retained. These could be cultured and, if their presence in significant numbers is ascertained, that could serve as an indicator for relatively unhealthy conditions prevailing in the surrounding waters.

MATERIALS AND METHODS

Field Experiments

Sampling stations and procedures - Sample station locations are identified by numbers 1 through 10 as seen on Fig. 1.

All sampling was performed from the deck of a 17-foot, center console "Aquasport"^{R} fishing vessel powered by an 85 HP outboard engine.

- Description of sampling stations

Station #1 - A triangular area adjacent to the Robert Moses Causeway, south of Keith Canal and north of Seganus Thatch. This area is frequented by baymen har-

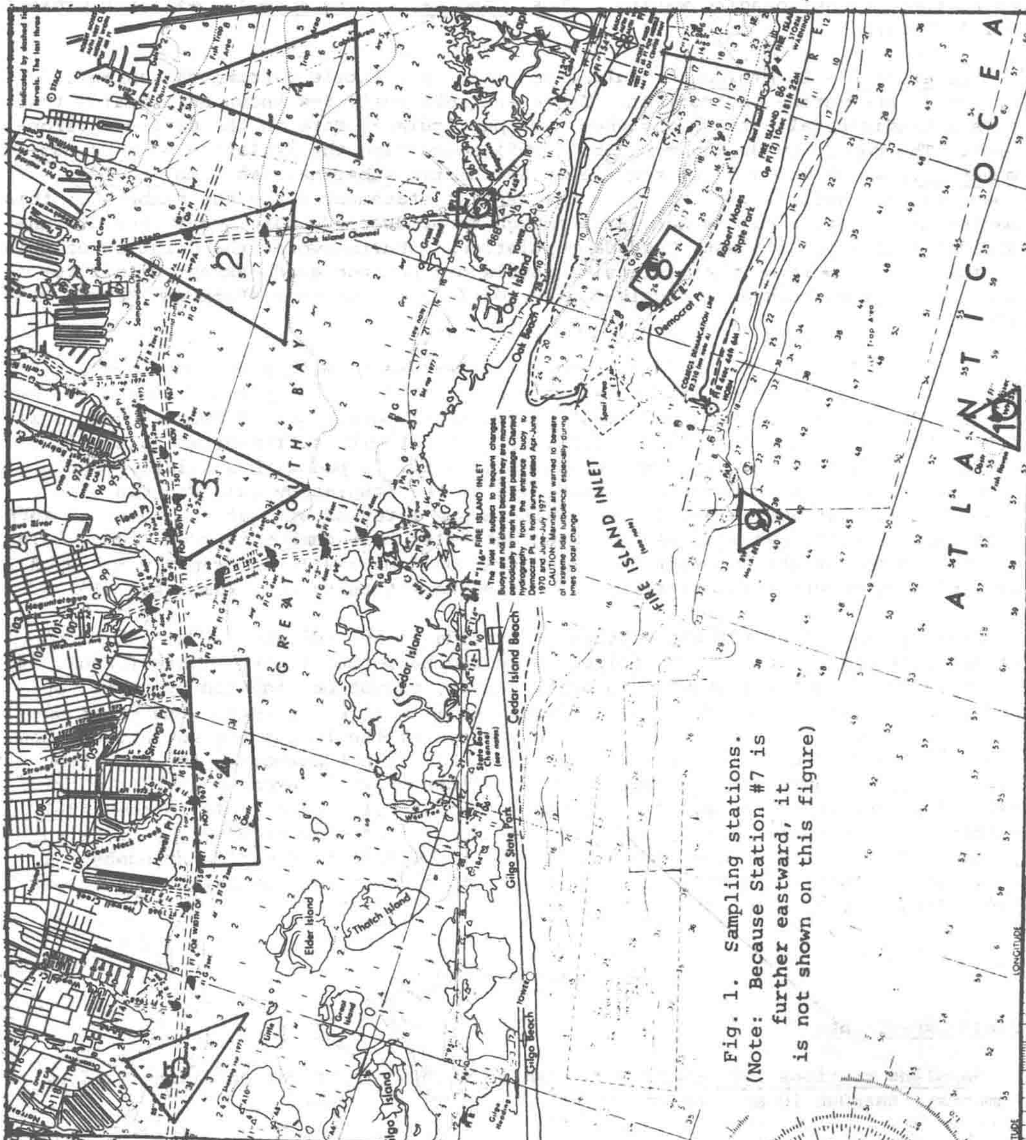

Fig. 1. Sampling stations. (Note: Because Station #7 is further eastward, it is not shown on this figure)