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A Water Quality Study of Lake Matoaka

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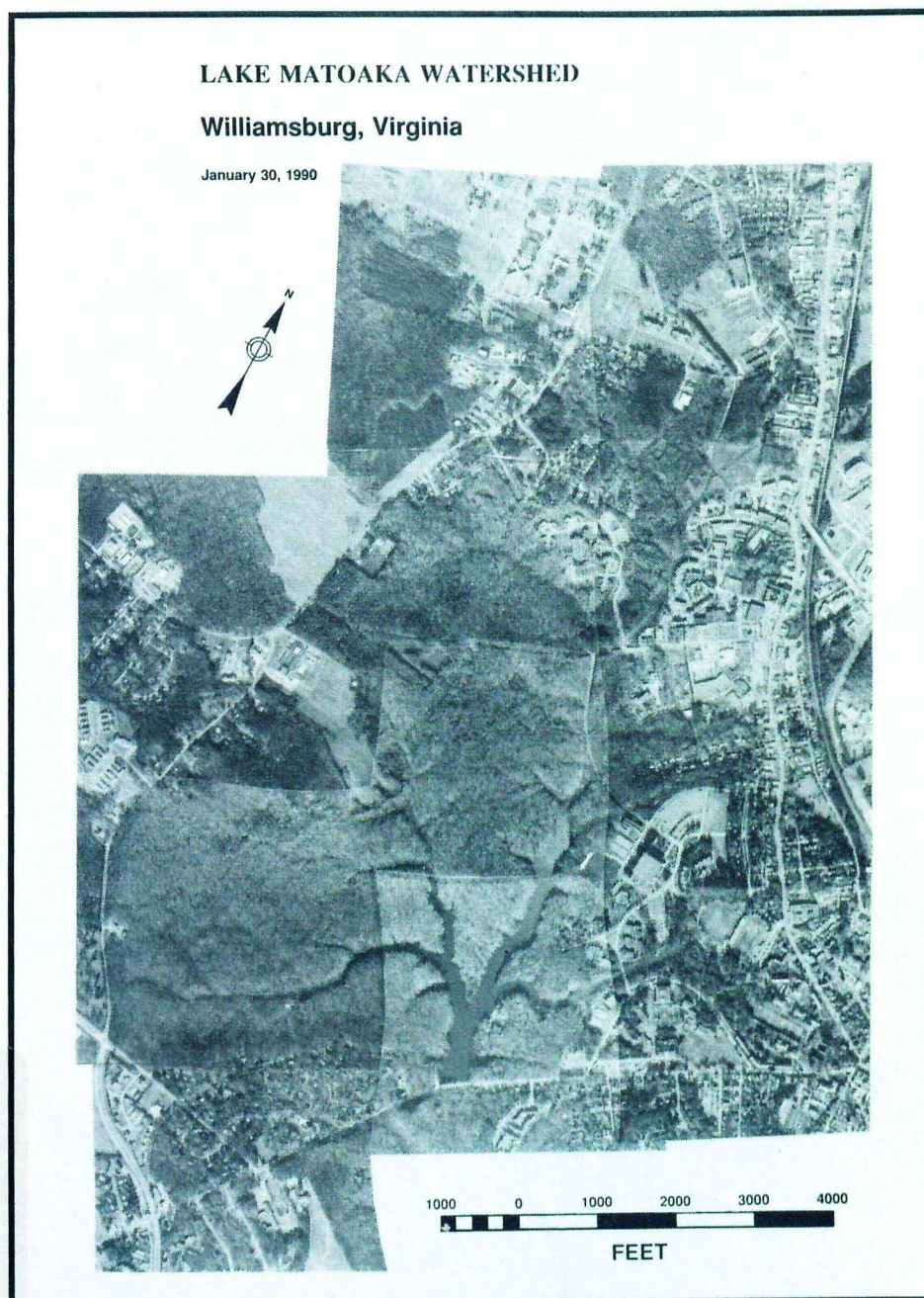
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ABSTRACT

Concern over health risks apparently related to the poor water quality of Lake Matoaka prompted a comprehensive two year study to measure nutrient and microbiological parameters in the lake. Based on total phosphorus, chlorophyll a and Secchi depth measurements, the lake is hypereutrophic. Dissolved oxygen depletion below the thermocline occurs in the spring, yielding oxygen values at 3 m of <4 mg/l, a critical level for higher forms of aquatic life. Mean bacterial indices of fecal contamination, i. e., fecal coliforms or *Escherichia coli*, met the recreational water criterion in the absence of sewage spills but exceeded acceptable limits following storm events.

Densities of mesophilic aeromonads in Lake Matoaka were comparable to those observed in Virginia lakes in transition from mesotrophy or eutrophy to more advanced stages of eutrophication. Based on a multilake dataset, aeromonad densities were inversely related to total dissolved nitrogen and showed no significant relationship to total phosphorus or chlorophyll a. *Aeromonas sobria* and *A. hydrophila*, potential human pathogens, were the dominate biotypes isolated and showed a seasonal occurrence with the former most frequently isolated from Lake Matoaka and three other tidewater lakes at

temperatures $>20^{\circ}\text{C}$. Hemolysis and autoagglutination, phenotypic characteristics associated with virulence, were observed for aeromonad isolates from Lake Matoaka.

Although Lake Matoaka is subject to relatively rapid natural aging because of its geographic location, hydrology, and large ratio of watershed to lake area, cultural factors associated with urban development (construction, sewage spills, unmanaged or improperly managed stormwater runoff) are accelerating the process of nutrient enrichment and infilling.

INTRODUCTION

Lake Matoaka is a man-made lake bounded on one side by the College of William and Mary campus and on the opposite side by a forested and relatively undisturbed area known as the "College Woods". Although popular for aesthetic and recreational reasons, Lake Matoaka and College Woods provide for the conservation of rare and endangered species and for the study of a variety of topics within the environmental and natural sciences.

During recent years concern has been growing within the college community that the lake ecosystem was being subjected to excessive stresses associated with urbanization and development. The 596 hectare drainage basin for Lake Matoaka is situated in the City of Williamsburg and James City County. Located within the watershed are approximately 800 residences (including apartments), 14 major institutions, 3 shopping centers, 27 businesses and 5 sewage pump stations. As development within the watershed has intensified, the lake has experienced algal blooms and dense growths of emergent macrophytes which have restricted access to shallow portions of the lake. Deltaic areas in two arms of the lake reflect significant sediment deposition derived from construction activities both on- and off-campus. Numerous sewer line breaks and pump station failures have resulted in raw sewage entering the lake. Such occurrences heightened concern that Lake Matoaka had become eutrophic, i.e., nutrient enriched.

In the fall of 1989, several students collecting biological specimens by wading in shallows around Lake Matoaka developed skin infections that required antibiotic therapy and hospitalization of one student. Lake Matoaka was subsequently implicated as the source of these infections because only the students exposed to lake water developed skin lesions. Bacteriological analyses conducted in immediate response to this outbreak

showed fecal indicator levels exceeded state or federal water quality criteria for recreational usage. Furthermore, high densities of bacteria from the *Aeromonas hydrophila* group were detected in lake areas where the affected students had sampled. These microorganisms are of particular concern as they are the dominant causative agent of freshwater wound infections (Dufour, 1986), are present at high densities in sewage, (Miescier and Cabelli, 1982; Monfort and Baleux, 1990; Poffe and Op de Beeck, 1991) and have been proposed as bacterial indicators of trophic status for freshwaters (Rippey and Cabelli, 1980, 1989).

Consequently, the College's Office of Administration and Finance authorized the Virginia Institute of Marine Science to conduct comprehensive water quality studies of Lake Matoaka and its watershed. During the first year of study the lake was characterized with respect to densities of bacterial indicators of fecal wastes and mesophilic aeromonads and tests were conducted to determine its trophic status. Aeromonad densities in Lake Matoaka were compared with other local lakes of similar trophic condition and representative isolates biotyped. Comparative studies were expanded during the second year to test the hypothesis that aeromonad densities were directly related to trophic status, i.e., higher levels indicate greater eutrophication.

This report, in addition to discussing the observed results, provides an extensive review of relevant literature to highlight the variety of environmental and public health issues surrounding the eutrophication of Lake Matoaka. The document is designed to present a comprehensive report on the status of Lake Matoaka which will have broad utility to members of the administrative, academic, and scientific communities at the College.

MATERIALS AND METHODS

Study area

Table 1 summarizes various characteristics of Lake Matoaka and the other study areas. All lakes are located in the tidewater area of Virginia except Holliday Lake which lies within the piedmont counties of Appomattox and Buckingham.

Sampling procedure

Samples were collected from Lake Matoaka on 14 dates from October 1989 to December 1990. Samples for microbiological analyses were collected in sterile containers by grab sampling at 0.3 to 0.5 m depth except in shallow streams or shoal areas where bottles were immersed immediately beneath the surface. Samples for nutrient and chlorophyll a analyses were similarly collected in acid-washed plastic containers (APHA, 1989). On several occasions, samples were also collected from feeder streams to the lake and from other lakes in the Williamsburg area. Lake Matoaka sampling sites are shown in Fig. 1.

Lakes Matoaka, Burnt Mills, and Holliday were each sampled on six occasions in 1991. Sampling was not initiated if precipitation had occurred during the prior 72 h because stormwater runoff would affect biological and chemical results. One survey was conducted at each site during the winter with remaining surveys conducted from late spring through fall when surface water temperatures exceeded 16°C. Samples were collected about 0.5 m below the surface at six sites located away from the shoreline in each lake as recommended by Rippey and Cabelli (1989). Samples were transported in

insulated containers at ambient temperatures to the laboratory. Transport times ranged from 0.5 h for Lake Matoaka to 4 h for Lake Holliday.

Additional sampling of Lake Matoaka was conducted in the spring and summer of 1992 to provide isolates for virulence testing.

Environmental parameters measured on site included temperature, dissolved oxygen, pH and Secchi depth. Chemical, bacteriological and turbidity determinations were performed in the laboratory.

Fecal coliform and E. coli enumeration

Fecal coliforms were enumerated using a five-tube MPN procedure (APHA, 1989) with lactose broth (Difco Laboratories, Detroit, MI) as the presumptive medium and EC medium (Difco) as the confirmatory medium. A fluorogenic substrate (Feng and Hartman, 1982) methylumbelliferyl- β -D-glucuronide (Sigma, St. Louis, MO) was incorporated into the EC medium to determine *E. coli* densities.

Enterococci enumeration

Enterococci were enumerated by the mE method (Levin et al., 1975) as modified by Dufour (1980) to incorporate 0.75 g indoxyl- β -D-glucoside, 0.24 g nalidixic acid, and 0.15 g tetrazolium chloride per l medium. Plates were resuscitated for 2-3 h at room temperature for samples collected at water temperatures below 20°C. Incubation periods were extended from 24 h (Dufour, 1980) to 48 h to maximize colony and color development.

Mesophilic aeromonad enumeration and characterization

Mesophilic aeromonads are those species which have an optimum growth temperature within the range of 20°C to 45°C. The membrane filter method of Rippey and Cabelli (1979) was used to enumerate mesophilic aeromonads and plates resuscitated as for enterococcal analyses. Selected colonies were isolated and biotyped using the schemes of Popoff (1984) and Janda et al. (1984). *Aeromonas caviae* (ATCC 15468), *A. hydrophila* (ATCC 7966), and *A. sobria* (ATCC 43979) were included as reference cultures. Biochemical tests were performed according to accepted protocols (BAM, 1984; ASM, 1985) and included fermentation of lactose, mannitol, trehalose, inositol, arabinose and salicin; gas production from glucose; production of lysine and ornithine decarboxylases, arginine dihydrolase, DNase and oxidase; esculin hydrolysis; production of acetylmethylcarbinol; and motility. All media were incubated at 35°C and observed after 1 and 2 days and randomly thereafter for 2 weeks. β -hemolysin activity was determined by streaking isolates onto tryptic soy agar plates (Difco) containing 5% sheep erythrocytes (Daily et al., 1981) and examined for clearing zones after 24 and 48 h incubation at 35°C.

Autoagglutination of aeromonad isolates was determined according to the method of Janda et al. (1987). Brain heart infusion broth cultures grown overnight at 35°C were examined for evidence of self-pelleting and pelleting induced after boiling for 1 h. The relative degree of precipitation (RDP) was calculated by determining the difference in absorbance (A_{540}), before and after boiling.

Extraction of S-layer proteins was performed according to the method of Ford and Thune (1991) modified by increasing the pH of the glycine hydrochloride extraction buffer from 2.2 to 4.0. S-layer extracts were examined by sodium dodecyl sulfate-

polyacrylamide gel electrophoresis (SDS-PAGE) according to the procedure of Laemmli (1970) using 4% stacking and 12% resolving gels. Gels were stained with 0.125% Coomassie blue solution R250 and destained with 50% methanol in 10% acetic acid. Appropriate mid-range molecular markers, 14-98 kDa (Promega, Madison, WI) were used to reference the 52-54 kDa aeromonad S-layer (Kokka et al., 1990; Ford and Thune, 1991).

Droplets of S-layer extract were spotted onto 200 mesh Formvar-coated grids and allowed to settle for 15-20 min until almost dry. The film surface was immediately stained with 1% sodium phosphotungstate 0.5M ammonium acetate for 1 min, blotted, and air dried. Grids were examined for the presence of S-layers using a Zeiss CEM 902 electron microscope (Carl Zeiss Inc., Thornwood, NY.).

Appropriate S-layer positive (*A. sobria* ATCC 9071) and negative (*A. hydrophila* ATCC 14715) controls were included in autoagglutination, SDS-PAGE and electron microscopy procedures.

Chemical analyses

Chlorophyll a concentrations for 1989-90 samples were determined by a standard spectrophotometric method (Strickland and Parsons, 1972). Samples were filtered onto Whatman GF/F filters (Whatman Ltd., Hillsboro, OR), placed in a 90% acetone solution and ground with a Teflon grinder. Filters were steeped for 12 h at 4°C in the dark, centrifuged, and extracted chlorophyll a determined at appropriate wavelengths with a scanning spectrophotometer (Model 1201, Milton Roy, Rochester, NY). A trichromatic equation using readings at three wavelengths was used to calculate chlorophyll a concentrations. During 1991 samples were processed using a solvent mixture composed

of 45% dimethyl sulfoxide (DMSO), 45% acetone, and 10% distilled water. A 5 ml sample was filtered through a 2.5 cm Whatman GF/F filter and the filter transferred to 8 ml solvent in an opaque tube and sealed. After a minimum of 24 h the sample was analyzed according to standard fluorometric protocol (APHA, 1989) using a Turner Designs Fluorometer, Model 10 (Turner Designs, Mountain View, CA).

Particulate phosphorus (PP) was determined by filtration using Whatman GF/F filters, ashing the filters in a muffle furnace at 550°C for 2 h, cooling to room temperature, and extracting overnight with 1N HCl (Aspila and Chau, 1976). Ammonium molybdate and antimony potassium tartrate reacted in the acid medium to form a blue-colored antimony-phospho-molybdate complex which was assayed using a continuous flow analyzer (Orion Research Inc., Boston, MA). Total dissolved organic and inorganic nitrogen (TDN) and phosphorus (TDP) were determined by autoclaving the filtered sample in the presence of alkaline potassium persulfate (D'Elia et al., 1977). Following digestion, the sample was buffered and analyzed for nitrate plus nitrite and orthophosphorus using a continuous flow analyzer. Total phosphorus (TP) was equal to the sum of particulate and dissolved fractions.

Physical parameters

Temperature and dissolved oxygen were measured using a YSI Model 57 dissolved oxygen meter (Yellow Springs Instrument Co., Yellow Springs, OH). pH was determined on site with an Orion SA 250 pH meter (Orion Research Inc., Boston, MA). Secchi depth measurements were performed according to Standard Methods (APHA, 1989). Turbidity was determined in the laboratory using a turbidimeter (Cole Parmer, Chicago, IL).

Statistical analyses

Nonparametric tests were used for statistical analyses as the data did not follow a normal distribution (Kolmogorov-Smirnov goodness of fit test; Zar, 1984). Comparisons of measured parameters between multiple sites or sampling dates were determined using the Kruskal-Wallis test and significant differences identified by a nonparametric Tukey-type multiple comparison (Zar, 1984). Comparisons of two sample data sets were analyzed using the Mann-Whitney U test. Correlation analyses were performed on two variables using the Spearman rank correlation test.

RESULTS

Bacterial parameters

Mean bacterial densities at sampling sites in Lake Matoaka, Crim Dell and the Ice House Cove feeder stream are shown in Table 2. Significant differences between lake sites occurred only for aeromonad densities (Kruskal- Wallis test, $P=0.0002$) which were higher at sites A and B in the uppermost "delta" area of the major lake arm and at site F (Ice House Cove) compared with site J located in the mainstem. Bacterial indicators of fecal pollution were significantly correlated (Spearman's rank test, $P<0.05$) with each other and with mesophilic aeromonad densities, with the exception of enterococci with aeromonad densities ($r = 0.22$, $P=0.07$) (Table 3). Densities of fecal bacterial indicators in Crim Dell were elevated compared with values in the lake but aeromonad levels were comparable. Mean values for all bacterial groups were highest in the Ice House Cove feeder stream. These were elevated in part due to sampling on one occasion during a sewage spill. Omission of spill data reduces the mean values for all bacterial parameters in the feeder stream by one-half.

Significant differences occurred between dates for all bacterial parameters (Kruskal-Wallis test, $P < 0.01$). When the data were examined using the modified Tukey test, differences appeared to be associated with rainfall or the entry of sewage into the lake. Further analysis showed that fecal indicator and mesophilic aeromonad concentrations in lake samples collected 4-6 days after sewage spills were significantly larger (Wilcoxon test, $P < 0.03$) than values observed in the absence of sewage spills. In general, fecal indicator densities were significantly ($P < 0.05$) increased by rainfall although the amount of rainfall did not necessarily correlate with bacterial concentrations (Tables 4, 5). Rainfall did not appear to increase aeromonad densities. On one occasion, March 18, 1991, campus storm drains that feed the stream to Ice House Cove were sampled during a rain event that produced extensive runoff. Mean fecal coliform and *E. coli* MPN values were 2,400/100 ml ($n=11$) with values ranging from 20 to 79,000/100 ml.

Densities of bacterial fecal indicators were inversely related to temperature, dissolved phosphorus, and chlorophyll a (Table 5). In contrast, numbers of mesophilic aeromonads were not correlated with temperature and positively correlated with dissolved nitrogen and particulate phosphorus. All bacterial groups were positively correlated with turbidity and inversely correlated with Secchi depth.

Chemical and physical parameters

There were no significant differences between lake sampling sites for the chemical and physical indices of eutrophication summarized in Table 6. Mean phosphorus and nitrogen concentrations were approximately five to ten-fold higher in the House Ice Cove feeder stream (site K) than in the lake. Stream nutrient concentrations were high, e.g.

total phosphorus = 830 mg/m³ and total nitrogen = 540 mg/m³, even when data from a sampling concurrent with a sewage spill was omitted.

Differences between sampling surveys were highly significant ($P < 0.0001$) for all parameters. Analysis (modified nonparametric Tukey's test) revealed that phosphorus concentrations were usually highest during surveys conducted from June through October. No seasonal pattern could be distinguished for total dissolved nitrogen. These results are consistent with the strong correlation (Spearman rank) between phosphorus concentrations and temperature (Table 7). Significant correlations occurred between the latter two parameters and chlorophyll a. Secchi depth was inversely related to temperature and dissolved nitrogen, particulate and total phosphorus, and chlorophyll a.

Based on summer mean values of chlorophyll a, total phosphorus and Secchi depth, Lake Matoaka is classified as hypereutrophic using the Walker trophic index ($I = 83$, Table 8). Seasonal examinations of dissolved oxygen concentrations as a function of depth are shown in Fig. 2. Oxygen depletion was rapid in the spring with concentrations decreasing to < 2.5 mg/l at 3 m by mid-June.

Other area lakes were examined on one to three occasions during the summer of 1990 and observations compared with those of Lake Matoaka for the same time period (Table 9). Water quality data showed that Little Creek Reservoir was borderline between mesotrophy and eutrophy ($I = 50$); Lake Waller Mill was eutrophic ($I = 54$); and Lake Powell ($I = 78$) resembled Lake Matoaka in showing signs of advanced eutrophication. Aeromonad densities in Lake Waller Mill were more than tenfold higher than those in Little Creek Reservoir.

Comparative lake studies

Because our observations of local lakes suggested there was no relationship between trophic status and aeromonad levels, we decided to test this hypothesis through intensive monitoring of three lakes of contrasting water quality in 1991. Results for lakes Holliday, Burnt Mills and Matoaka are summarized for January-February and April-October surveys in Tables 10 and 11, respectively. Statistical analyses of the entire dataset showed that there were significant differences (Kruskal-Wallis, $P < 0.05$) between the lakes for all parameters measured at 0.5 m except temperature, turbidity and dissolved oxygen. Lake Matoaka data from surveys at warm temperatures were compared to those from lakes Holliday or Burnt Mills using the Mann-Whitney U test (Table 11).

Densities of bacterial indicators of fecal contamination were highest in Lake Matoaka (Tables 10, 11). Although all surveys were conducted following a three-day period of no precipitation, the cumulative rainfall (7.2 cm) which occurred for an eight day period prior to the January Lake Matoaka survey probably contributed to the elevated bacterial densities. There were no reported sewage spills on- or off-campus during December or January. Lake Matoaka also showed the highest concentrations of mesophilic aeromonads among the three lakes sampled during winter. At warm water temperatures, aeromonad populations in Lake Matoaka were significantly higher than in Lake Burnt Mills, but lower than in Lake Holliday.

Based on the dataset for all lakes, indicator bacterial densities tended to be inversely related to temperature and Secchi depth and were positively correlated with turbidity (Table 12). Dissolved nitrogen, dissolved and particulate phosphorus, and chlorophyll a concentrations were positively correlated with fecal bacterial indicators. Indicator bacteria in Lake Matoaka showed similar correlations except for the lack of correlation

with particulate phosphorus or chlorophyll a (Table 13). Considering the entire multilake dataset, mesophilic aeromonads were inversely correlated with dissolved oxygen and dissolved nitrogen. Relationships of temperature and nutrients with aeromonad densities varied on an individual lake basis. Thus, in Lake Matoaka aeromonad densities were inversely correlated with temperature and particulate and total phosphorus. In Lake Holliday, aeromonads were positively correlated with temperature ($r=0.40$, $P<0.05$, $n=35$) and inversely correlated with total phosphorus ($r=-0.40$, $P<0.05$, $n=35$). Mesophilic aeromonads in Lake Burnt Mills showed no correlation with temperature or the preceding nutrient parameters ($P>0.05$, $n=36$).

Although Lake Matoaka was characterized by significantly higher total phosphorus and chlorophyll a concentrations and lower Secchi depths, total dissolved nitrogen concentrations were higher in Burnt Mills. During the Lake Burnt Mills winter survey (Table 10), total dissolved nitrogen and chlorophyll a concentrations exceeded the mean values observed at temperatures $>16^{\circ}\text{C}$ (Table 11). Winter chlorophyll a observations in Lake Burnt Mills were equivalent to mean values in Lake Matoaka during warm water temperatures.

In general, nutrient parameters correlated with each other and chlorophyll a, and were inversely correlated with Secchi depth values (Table 14). Lake Matoaka data showed a similar pattern except relationships between particulate and total phosphorus with dissolved nitrogen were not significant (Table 15). Particulate and total phosphorus concentrations were positively correlated with temperature, particularly in Lake Matoaka.

Based on data from year 2 of our study, Lake Matoaka is hypereutrophic. Values of the Walker trophic index for lakes Holliday and Burnt Mills suggest transitions from mesotrophy to eutrophy, and eutrophy to hypereutrophy, respectively (Table 16).

Characterization and distribution of Aeromonas spp. biotypes

Based on an evaluation of 273 *Aeromonas* spp. isolates from Lake Matoaka and other area lakes, *A. sobria* was the dominant biotype recovered (54%) followed by *A. hydrophila* (29%) and *A. caviae* (10%). Differentiation of biotypes is summarized in Table 17. Dominance by *Aeromonas* spp. biotypes varied seasonally in Lake Matoaka (Table 18), with *A. hydrophila* the most abundant at temperatures <20°C whereas *A. sobria* dominated at higher temperatures. The latter biotype was also the most frequently encountered biotype in other area lakes when water temperatures >20°C (Table 19).

Hemolysin assays of 46 *Aeromonas* isolates from Lake Matoaka or the Ice House Cove stream showed that 70% were β -hemolytic and that an additional 10% produced weak or delayed (48 h) hemolysis of sheep red blood cells.

Recent as well as archived aeromonad isolates (stored frozen in 30% glycerol at -18°C) were examined for autoagglutination. Of the 500 strains isolated during 1989-90 surveys, only 15% could be resuscitated, and of these 42% (31/73) autoagglutinated. In contrast, 15% (13/85) of aeromonad isolates collected in 1992 were positive for this virulence characteristic. Biotyped isolates that autoagglutinated were dominated by *A. sobria* (56%) followed by *A. hydrophila* (26%) and *A. caviae* (19%).

S-layer proteins were not detected using SDS-PAGE on fourteen aeromonad isolates positive for autoagglutination. The absence of S-layers was confirmed by electron microscopy of glycine extracts from five isolates negative by SDS-PAGE analysis.

DISCUSSION

The major objective of this study was to characterize the bacteriological and nutrient water quality of Lake Matoaka. Two years of observations confirmed the lake to be severely nutrient enriched, i. e., hypereutrophic, and at times to manifest levels of fecal indicator bacteria exceeding regulatory standards for recreational usage. These findings were indicative of gross insults by raw sewage and improperly managed stormwater runoff, both of which have had serious implications to public health and lake trophic status. The following discussion will consider complex interrelated issues that include: (1) the trophic status of Lake Matoaka and associated lake and watershed management concerns, and (2) the sanitary microbiological quality of the lake and its public health implications.

Trophic Status

General considerations

"Lakes are the geological fireflies" (Moore, 1987) which naturally tend to fill in and become wetlands, a process occurring over a time period of hundreds to thousands of years. In the southeastern United States, the primary factors and processes that favor rapid aging, (e.g., climate, rainfall, topography, soils, and geology) are also conducive to eutrophication (USEPA, 1990). Man exacerbates rates of natural aging through diverse activities (e.g., land clearing, poorly managed stormwater runoff, failure to implement best management practices at construction sites, agricultural practices, industrial and domestic wastewater discharge) which introduce sediment and nutrients from surrounding watersheds. These activities, often referred to as cultural eutrophication, can shorten a lake's lifespan to tens of years. Lake Matoaka is a relatively small shallow lake

with a large watershed to lake area ratio (596:16 hectares) and extended hydraulic residence time (73 days; Neilson et al., 1990), intrinsic factors which predispose lake trophic status toward eutrophication (USEPA, 1990). Consequently, it is expected that Lake Matoaka would be extremely sensitive to cultural eutrophication caused by disturbances to its watershed.

Lakes are typically classified according to trophic states that reflect a gradation of increasing lake productivity (USEPA, 1990):

Oligotrophic -	nutrient-poor, biologically unproductive
Mesotrophic-	intermediate nutrient availability and biological productivity
Eutrophic-	nutrient-rich, highly productive
Hypereutrophic -	"pea-soup" conditions, the extreme end of the eutrophy

This categorization is a continuum without sharply defined boundaries between trophic states (Carlson, 1977). Figure 3 summarizes the casual pathways in the development of a trophic state index (Walker, 1979). Briefly, primary productivity as assessed by chlorophyll a concentration is a function of total phosphorus concentration (when nitrogen is not limiting) and will be inversely related to water transparency (Secchi depth). Chlorophyll a, which is considered less sensitive to nonbiological factors (color and suspended solids), is the basis for the widely used Walker classification system (Walker, 1979). Regression analyses relating chlorophyll a to phosphorus and transparency values in 30 lakes were used to develop phosphorus and transparency based indices. The average of the three component indices yields the Walker trophic state index.

Trophic status of Lake Matoaka

According to the Walker index (Walker, 1979), the transition zone between eutrophy and hypereutrophy is not sharply defined but occurs approximately between 65 and 70 index units. The 1989-90 and 1991 index values of 83 and 70 for Lake Matoaka indicated hypereutrophy. This trophic designation was confirmed when lake values were compared to those values EPA (USEPA, 1990) considers characteristic of hypereutrophic conditions, i. e., chlorophyll a $>25 \text{ mg/m}^3$ and transparency $<1 \text{ m}$. Each of the three component indices indicated slightly more eutrophic conditions during the first year of study when sewage spills were more frequent and of greater magnitude. However, it is possible that the lower value obtained during year 2 was affected by the year 2 sampling design, which precluded sampling for lake comparisons if a rain event or sewage spill preceded a sampling date. Continued monitoring is needed to assess if the reduced index value is indicative of improvement.

The Walker trophic state index can also be used to predict lake summer oxygen status (Walker, 1979). Decomposition of organic matter derived from high biological productivity associated with nutrient enrichment can deplete the dissolved oxygen in the hypolimnion, the cooler and denser bottom layer which forms during the summer. Areal hypolimnetic oxygen depletion rates calculated using Walker trophic indices are 1.15 to 1.86 g O₂/m²/day, rates larger than the 0.55 g O₂/m²/day cited for eutrophic lakes (Mortimer, 1941). Using these areal rates, an approximate mean hypolimnion depth of 3 m, and an O₂ concentration at spring turnover of 12 g/m³, anoxic conditions in Lake Matoaka would be anticipated within 19 to 31 days after spring turnover. At open lake sites in mid-June, oxygen concentrations at 2-3 m below the surface of about 2 mg/l is consistent with Walker's model of rapid oxygen depletion under hypereutrophic conditions. Oxygen concentrations below 2 m continued to be less than 4 mg/l into

September, a critical level representing a "marginal environment for survival, growth, and reproduction" (Moore, 1987).

Sediment/nutrient transport

Jones and Holmes (1985) reviewed the significance of urban land use with respect to hydrologic changes and water quality. Urban development, accompanied by increased impervious surface area, and simplification of drainage networks, contributes to increased runoff and rapid funneling of water downstream contributing to channel erosion and sediment transport. Under conditions of urban development, significantly more sediment is exported than from rural or forested areas and the rate of sediment export is directly proportional to the intensity of development. Similarly, loadings of phosphorus and nitrogen are significantly larger under conditions of urban development compared with other land use practices and correlate with landuse intensity. Randall et al. (1978) noted that TOC and BOD loadings were greater in urban runoff than in effluents with the same flow from secondary sewage treatment plants. Geldreich (1978) considered urban stormwater discharged through separate systems as "another form of domestic sewage." These observations emphasize that lake remediation programs must include stormwater runoff control in watershed pollution abatement programs, particularly in urbanized watersheds.

Aerial photographs of Lake Matoaka revealed marked evidence of sediment transport into the upper reaches of the central or main lake arm. This area receives runoff from the most developed portion of the watershed (Monticello Avenue, Richmond Road and Ironbound Road). Sediment transport from this area has partially or totally blocked two of three culverts under Monticello Avenue and Compton Drive. Construction of William and Mary Hall and the nearby Intramural Sports facility also contributed large

amounts of sediment to this portion of the lake. Another area of infilling is Ice House Cove which receives combined stormwater from a major portion of the campus. Field observations made during a storm in March 1991 revealed many examples of uncontrolled runoff and sediment transport to the lake. Storm drains elevated too far above grade have produced severe erosion. In one instance erosion was of such severity that the soil supporting a large storm sewer pipe had been totally washed away, causing the unsupported part of the pipe to break.

Nutrient loading

Relationships between phosphorus and nitrogen export with land use have been reviewed by Rast and Lee (1983) and are summarized in Table 20. Export of phosphorus is predicted to increase ten-fold as forested land becomes urbanized. Using these export coefficients (Rast and Lee, 1983) and Vollenweider's (1975) model, which relates phosphorus loading to summer lake characteristics such as mean chlorophyll a concentration, Secchi depth, and hypolimnetic oxygen depletion rate, Neilson et al. (1990) calculated a theoretical phosphorus loading based on landuse in the Lake Matoaka watershed. This analysis suggested inputs of phosphorus would have to be reduced by about 80% to reach "acceptable" levels. The Vollenweider model appeared to underestimate lake responses in that 1990 chlorophyll a and Secchi depth observations indicated a more eutrophic state than the model predicted. Possible explanations for this disparity are that there are other sources of phosphorus loading to the lake or the export coefficients as applied or developed are inaccurate.

A major potential source of phosphorus that the Vollenweider model does not consider is internal loading or translocation from bottom sediment, a source of particular importance in shallow eutrophic lakes (Moore et al., 1984; Souza and Koppen, 1984;

Sondergaard, 1990; Sondergaard et al., 1990). Phosphorus can be translocated from sediment via two principal mechanisms, macrophyte pumping and diffusion, and is mobilized under anaerobic conditions because of its increased solubility (Mawson et al., 1983). Low or zero oxygen values were routinely observed below 4m in Lake Matoaka during the summer months. The mean total phosphorus concentration from 11 Lake Matoaka sediment samples (collected by Dr. G. Capelli and analyzed at VIMS) was 350 mg/kg dry sediment, exceeding concentrations reported by Souza and Koppen (1984, 186 mg/kg) and Willenbring et al. (1984, 270 mg/kg) to produce significant internal loading. Internal loading of sediment phosphorus frequently limits restoration efforts in shallow eutrophied lakes (USEPA, 1990; Rossi and Premazzi, 1991) even when external loading is reduced. If phosphorus recycling from sediment is significant, in-lake treatment procedures such as dredging, chemical treatment, and artificial aeration should be considered (Chapra and Canale, 1991). A comprehensive review of these techniques (USEPA, 1990) and example case studies (USEPA, 1984; NALMS, 1985) are available.

Bacteriological significance of nutrients

Survival of enteric bacteria in aquatic environments is influenced by many factors including nutrient concentration and competition with indigenous bacteria for nutrients (Sinclair and Alexander, 1984; Rozak and Colwell, 1987). Verstraete and Voets (1976) studied *E. coli* survival in lake waters subject to or relatively free of fecal and organic pollution and demonstrated enhanced persistence to be associated with elevated nutrients. Promotion of *E. coli* survival in natural lake water containing synthetic sewage is attributed primarily to the nitrogenous components and is proportional to the concentration of the nitrogen amendment (Lim and Flint, 1989). These findings indicate the potential of nutrients derived from sewage spills or nonpoint source inputs to promote survival of indicator and pathogenic bacteria in Lake Matoaka.

Although an association between elevated nutrient concentrations and high aeromonad densities in environmental waters has also been recognized (Shotts et al., 1972; Biamon and Hazen, 1983; Hazen and Esch, 1983; Rippey and Cabelli, 1989), the relationship between *Aeromonas* spp. densities and trophic status remains unclear. Rippey and Cabelli (1980, 1989) reported that aeromonad densities are strongly correlated with lake trophic status and provide an early discriminatory index in the oligotrophic through mesotrophic range. Based on data from 68 lakes and ponds, they proposed the following mean aeromonad density ranges (aeromonads/100 ml) for trophic classification: (1) mesotrophic = 65–325; (2) mesoeutrophic = 326–575; (3) eutrophic = 576–3400; and (4) hypereutrophic = >3400. In our study we tested the hypothesis that mesophilic aeromonad densities reflect trophic status by determining aeromonad concentrations in three lakes classified by conventional indices as oligotrophic, mesotrophic, and hypereutrophic. Lakes of contrasting trophic classifications were selected on the basis of information and recommendations provided by the Virginia State Water Control Board. Unfortunately, information used to select Lake Holliday as an "oligotrophic" lake was four years old and our recent data showed increased nutrient enrichment. The mean aeromonad density in Lake Holliday, which may be in transition from mesotrophy to eutrophy, was 8700 aeromonads/100 ml and was statistically higher than in hypereutrophic Lake Matoaka. Lake Burnt Mills, representing a trophic status intermediate between Holliday and Matoaka, exhibited the lowest aeromonad densities. According to Rippey and Cabelli (1989) aeromonad densities should have been as much as ten-fold lower in Lake Holliday than in Lake Matoaka. Our observations suggest that their method of trophic classification, based on data obtained almost exclusively from the lakes in the north and northeastern United States, may not be valid for freshwater lakes in a mid-Atlantic state such as Virginia. Upon closer examination of their data, we noted that for nine lakes in the southeast, i. e., in Georgia and North Carolina, oligotrophic or

southeast, i. e., in Georgia and North Carolina, oligotrophic or mesotrophic lakes exhibited aeromonad densities from 2–170/100 ml and were located in mountainous regions. Mesotrophic to hypereutrophic lakes were located in the piedmont or coastal zone and had aeromonad densities of 724–1621/100 ml. Because these levels are not unlike those we observed in similar geographic regions in Virginia, we conclude that aeromonad levels are poor predictors of trophic status when lakes with dissimilar regional geographies are compared. A discussion of the relationship of aeromonad densities with specific water quality parameters will be presented in the next section.

Bacteriological Water Quality

Although public health safety concerns regarding Lake Matoaka focused specifically on *Aeromonas* spp., other microorganisms are capable of producing disease via ingestion or exposure to environmental waters (Table 21). Most of the causative agents listed are shed in the urine and/or feces of infected animals and humans, and enter receiving waters in sewage or stormwater, but some are also indigenous to soils and freshwater (e.g., *Aeromonas* spp., *Chromobacterium violaceum*, *Legionella* spp., *Mycobacterium* spp., *Pseudomonas* spp., and *Yersinia enterocolitica*).

Bacterial loadings

Although mean densities of bacterial indicators used to assess the public health safety of recreational waters were acceptable in Lake Matoaka from a regulatory perspective (i.e., fecal coliforms <200/100 ml; *E. coli* <126/100 ml; and enterococci <33/100 ml), samples collected after sewage spills and rainfall frequently exceeded these criteria. From the fall of 1989 through 1991 there were 11 documented sewage overflows into the lake, six of which occurred at the college's lift station. These spills resulted in the

introduction of indicator organisms and possibly bacterial, protozoan and viral pathogens. The elevated densities of aeromonads in the lake following spills is consistent with their reported densities in raw sewage (i.e., $10^{5-8}/100$ ml) (Miescier and Cabelli, 1982; Monfort and Baleux, 1990; Poffe and Op de Beeck, 1991) and our own observations during a lift station overflow (i.e., $6 \times 10^7/100$ ml). Araujo et al. (1989) observed that mesophilic aeromonad and fecal coliform densities correlated in sewage contaminated waters but not in nonpolluted freshwater. The correlation of fecal coliforms and aeromonad populations in our study is therefore indicative of sewage as the major source of aeromonads to Lake Matoaka. In addition to sewage inputs, stormwater is a significant source of fecal indicator bacteria (Geldreich, 1978). In rural and wilderness areas, fecal contamination is attributed to domestic and wild animals with less frequent contributions from inadequate on-site domestic sewage disposal systems. The impact on receiving waters is a function of ground cover, frequency, intensity of rainfall, and other environmental conditions affecting microbial survival. Pet wastes are primary contributors of fecal pollutants in urban areas. The daily load of dog feces deposited on the streets of New York City is estimated at 150,000 pounds or 68,027 kilograms (Feldman, 1974). Reported fecal coliform concentrations in urban stormwater of 10^3 - 10^5 per 100 ml (Geldreich, 1978; Randall et al., 1978) are similar to those observed in stormwater samples collected from the watershed surrounding Ice House Cove. Although our data do not implicate stormwater as a significant source of aeromonads, surveys now underway should provide more conclusive information as sampling will be closely coupled to storm events and contrasted to baseline surveys.

Relationship with physical/chemical parameters

For our data set which included three lakes of different trophic characteristics, densities of fecal bacterial indicators were positively correlated with total dissolved

nitrogen and phosphorus as well as particulate phosphorus. These findings probably reflect the combined result of differences in bacterial loadings as functions of land use within the three contrasting watersheds as well as differential bacterial persistence as a function of nutrient availability. Thus, urban runoff to Lake Matoaka would result in high densities of indicator bacteria as well as nutrients which promote bacterial survival. This explanation is consistent with the observations of significant nutrient (Randall et al., 1978) and bacterial loadings from urban stormwater (Geldreich, 1978; Schillinger and Gannon, 1985), and the enhancement of indicator survival in waters enriched in nutrients as demonstrated by in situ experiments (McFeters and Stuart, 1972; Dutka and Kwan, 1980).

Aeromonad densities in the lakes examined were not significantly correlated with either total phosphorus or chlorophyll a, two parameters identified by Rippey and Cabelli (1980, 1989) as strongly predictive of aeromonad concentration. As mentioned before, this observation appears to be the result of a preponderance of northern lakes in their database as well as their examination of an entire spectrum of trophic conditions with end members ranging from oligotrophy to hypereutrophy. Additionally, Rippey and Cabelli (1985) observed that the numerical relationship between aeromonads and total phosphorus or chlorophyll a is parabolic, such that large changes in aeromonad numbers occur over relatively small increases in total phosphorus and chlorophyll a concentrations. Being parabolic, the relationship is reversed during more advanced stages of eutrophication. Hazen (1983) observed significant positive correlations between densities of *A. hydrophila* and total Kjeldahl nitrogen, orthophosphate and total phosphorus and developed a model to predict its abundance in freshwater and estuarine environments of Albemarle Sound. He postulated that small increases in nitrogen and phosphorus lead to large increases in phytoplankton production and "leaky" phytoplankton provide nutrients for growth of *A. hydrophila*. In an investigation of North

regression analysis showed aeromonad densities were directly related to chlorophyll a and therefore would be theoretically related to phosphate, nitrate, and total organic carbon (Hazen and Esch, 1983). However, correlation analysis showed a significant positive relationship only for total Kjeldahl nitrogen and aeromonad concentrations.

In situ diffusion chambers studies of *A. hydrophila* indicated that ammonium contributed to decreased survival and was negatively correlated with aeromonas densities (Hazen and Esch, 1983). Laboratory studies showed that low concentrations of ammonium significantly increased the generation time of *A. hydrophila* (Rivera and Hazen, unpublished results). Differences in ammonium concentrations may offer a possible explanation to the lower aeromonad values observed in Lake Burnt Mills which had the highest total dissolved nitrogen concentrations. Samples collected during several late summer surveys (data not presented) showed ammonium concentrations two to three times higher in Lake Burnt Mills than in the other two lakes.

Densities of bacterial fecal indicators tended to be higher during periods of low temperature which is consistent with field observations (Nuttal and Parry, 1987; Bergstein-Ben and Stone, 1991) and in situ survival studies (Mitchell and Chamberlin, 1978). No relationship between aeromonad densities and temperature during 1989-90 surveys of Lake Matoaka was observed, possibly due to masking by the occurrence of sewage or rain events. The seasonal cycle of aeromonads is inversely related to that of fecal coliforms in both sewage treatment ponds and brackish receiving waters (Monfort and Baleux, 1990; 1991). The relationship of temperature and aeromonad densities varied for the different lakes examined, i.e., a strong inverse correlation in Lake Matoaka, a positive correlation in Lake Holliday, and no significant correlation in Lake Burnt Mills. Although aeromonad levels in the southeastern United States have been both inversely (Hazen, 1979) or not significantly correlated (Hazen and Esch, 1983) with

Esch, 1983) with temperature, they have shown a seasonality characterized by a spring maximum, a summer decline, and a fall resurgence. This pattern parallels seasonal changes in phytoplankton densities suggesting an association between populations of aeromonads and planktonic primary producers. In contrast, seasonal sampling of Rhode Island ponds revealed that maximum densities from early summer through early fall were followed by dramatic declines of 10^{2-3} cells/100 ml during the winter (Rippey and Cabelli, 1980). Although aeromonad densities correlated with parameters which reflect phytoplankton biomass, their observation that marked changes in aeromonad densities could occur with little change in these parameters in oligotrophic to mesotrophic lakes suggests an weak coupling between aeromonad densities and phytoplankton.

In addition to seasonal variations in aeromonad concentrations in the lakes studied, there were also differences in the biotypes recovered with *A. sobria* dominating at temperatures $>20^{\circ}\text{C}$. Montfort and Baleux (1990, 1991) observed dominance by *A. sobria* in the summer and *A. caviae* in the winter in sewage effluent and estuarine receiving waters. In our study, the preponderance of *A. sobria* in eutrophied waters during warm temperatures suggests this biotype is better adapted for multiplication in response to ambient nutrients. Studies of aeromonad biotypes in sewage treatment ponds, accompanied by diffusion chamber survival experiments (Montfort and Baleux, 1990, 1991), support the hypothesis that among the mesophilic aeromonads *A. sobria* multiplies best in eutrophied waters.

Densities of fecal bacterial indicators were inversely correlated with Secchi depth. Sunlight-induced mortality is known to be an important factor affecting enteric bacterial survival in aquatic environments (Barcina et al., 1986; Rhodes and Kator, 1990) and possibly aeromonad persistence (Montfort and Baleux, 1990). Secchi depth, which is also considered indicative of aeromonad levels (Rippey and Cabelli, 1989), showed a

significant inverse correlation with aeromonad concentrations in Lake Matoaka in 1989-90 but not in the 1991 comparative lake or Lake Matoaka datasets. The absence of a relationship in the latter surveys may have resulted from the preponderance of samples collected during late spring to fall when solar radiation as well as biological productivity are highest. Aeromonad levels in Lake Matoaka were frequently lower during intense macrophyte blooms which are coincident with decreased nutrient levels and increased Secchi depth which permits greater sunlight transmittance. Our results suggest caution relating aeromonad levels to Secchi depths, the latter varying seasonally within a lake as a function of the dominant primary producer as well as between lakes of different trophic status and water chemistry.

Our observations and those of others demonstrate the difficulty of discerning unique causative factors which control mesophilic aeromonad populations in aquatic environments. Many of the possible factors are interrelated in a complex fashion and their relative importance may vary seasonally. Experiments designed to address specific factors under controlled conditions are necessary to evaluate direct and indirect effects of environmental variables.

Mesophilic aeromonads as pathogens

During the last 10 years the medical and scientific communities have expressed increased interest and awareness of the pathogenicity of the *A. hydrophila* group (Holmberg et al., 1986; Janda and Duffey, 1988). California became the first state to mandate reporting of infections caused by these organisms in 1988 (Werner and Rutherford, 1990). Besides being pathogenic to aquatic vertebrates and invertebrates, this group of organisms can cause diverse pathologies in humans ranging from localized to invasive infections (von Graevenitz, 1985; Janda and Duffey, 1988; Rolston, 1988).

Acute gastroenteritis, typically self-limiting and resulting in profuse watery diarrhea, is the most common presentation of aeromonad infection. Wound infections, the second most prevalent form of disease, can range in severity from mild cellulitis to a fulminant myonecrosis (death of muscle tissue) leading to limb amputation. Other but less frequently reported aeromonad diseases include septicemia (a severe condition in which large numbers of bacteria are found in the blood), pneumonia, ocular infections, peritonitis, and meningitis. Aeromonad septicemia originating from invasive infections of the gastrointestinal tract, wounds or possibly the biliary tree or respiratory tract exhibit an overall mortality rate equal to 50% (Janda and Duffey, 1988).

Janda and Duffey (1988) reviewed factors predisposing to the development of aeromonad infections. Risk factors associated with gastrointestinal infection include underlying gastrointestinal or hepatic disease, recent antimicrobial therapy or a compromised immune system due to disease or immunosuppressive therapy (e. g., radiation, chemotherapy). Traumatic injury has been identified as a significant predisposing event leading to skin and soft tissue infection. Typical injuries include lacerations or abrasions during recreational or occupational activities, e. g., diving injuries, fish hook wounds, fish fin cuts or punctures, animal or insect bites, etc. (Dufour, 1986; Werner and Rutherford, 1990). The clinical manifestation of extraintestinal aeromonad infections may be related to the host's immune status, the site and size of dose, and the virulence properties of the infecting strain. However, wound infections resulting in aeromonad gas gangrene or septicemia have been reported to occur in healthy adults (Janda and Duffey, 1988; Wolff et al., 1980) but are believed to be rare. Although septicemia, which is the most invasive disease produced by *Aeromonas* spp. was thought to occur mostly in association with hepatic, biliary, or pancreatic disease or with malignancy (von Graevenitz, 1985), it has now been reported in non-immunocompromised persons and in all age groups (Janda and Duffey, 1988).

Significance of Aeromonas spp. densities

There is no water quality criterion for the *A. hydrophila* group in recreational or other waters. Although these organisms are recognized as the dominant pathogens of freshwater wound infections (Dufour, 1986), limited surveillance data suggests that aeromonad wound infections occur at a relatively low incidence, i.e., 0.7 per million population (Werner and Rutherford, 1990). The degree of health risk associated with aeromonad densities in natural waters is unknown (Centers for Disease Control, personal communication), but presumably is directly related to *A. hydrophila* densities. The only report in the literature relating density and aeromonad infection is one which describes a severe aeromonad infection in a young healthy male following a head injury in waters shown subsequently to contain 500 *A. hydrophila*/100 ml (Hanson, 1977). The only known published study involving challenge trials demonstrated diarrhea in 2 of 57 healthy volunteers challenged with high doses (10^4 - 10^{10} cells) using *A. hydrophila* strains possessing several virulence properties (Morgan et al. 1985).

Overview of Aeromonas spp. virulence factors

Aeromonas spp. produce a variety of extracellular proteins, i.e., proteases, nucleases, enterotoxins, hemolysins, and cytotoxins, all of which are potential virulence factors. Cahill (1990) reviewed numerous studies to relate the occurrence of these extracellular products with phenotypic markers and pathogenicity. She concluded that single toxins could have multiple activities, e. g., enterotoxicity, cytotoxicity, etc. Furthermore, different strains produce unique variations of these toxins, indicating that future research using purified proteins is needed to clarify the controversy surrounding the role of these virulence factors in vitro and in vivo. Taxonomic complexities, strain

specific virulence characteristics, and lack of an appropriate animal model have also contributed to difficulties in determining factors regulating pathogenicity (Kokka et al., 1990).

The complicated task of assessing virulence factors is compounded by the myriad of aeromonad infections described above. Enterotoxigenic strains can usually be isolated from patients with acute gastroenteritis and identified by a biochemical classification scheme of Burke et al. (1982). The biotyping scheme is based on a positive Voges-Proskauer test, a negative arabinose fermentation, and positive hemolysin assay. These investigators contended that 97% of enterotoxigenic isolates could be identified using these phenotypic markers. Kirov et al. (1986) confirmed the validity of this scheme and added two additional features, a positive lysine decarboxylase test and growth at 43°C. They concluded that isolates which were toxigenic (by the suckling mouse assay) possessed three or more of the five preceding criteria. Adhesion factors which enable bacteria to attach to the mucosal surface of intestinal epithelial cells are considered a criterion of a successful enteric pathogen (Doyle and Padhye, 1989). Agglutination of erythrocytes, a facile method for detecting adhesive (or colonization) factors has been exemplified by human diarrheal and enterotoxigenic strains of *Aeromonas* spp. (Atkinson and Trust, 1980). There appear to be various attachment mechanisms including lectins which recognize sugars on receptor cell surfaces and pili. Conflicting data exists regarding the role of pili as a virulence factor (Daily et al., 1981; Kirov et al., 1986) and there is evidence that pili production and hemagglutination are independent characteristics (Atkinson et al., 1987). Fucose-resistant hemagglutination (FRHA) of human group O blood cells is the pattern most frequently shown by *Aeromonas* spp. from patients with diarrheal illness (Burke et al., 1984c). Further work has shown that FRHA strains which agglutinate human, horse, rat and guinea pig erythrocytes are the predominant group isolated from diarrheal stools (Burke et al., 1986). Adhesion of

Aeromonas spp. to HEp-2 cells also has been proposed as a model for investigating enteropathogenicity (Carrello et al., 1988).

Invasive aeromonad strains producing systemic infections in humans (Janda et al., 1987) and in fish (Dooley and Trust, 1988) possess common phenotypic markers: autoagglutination in liquid media, possession of a common somatic antigen (0:11), and a crystalline surface array protein known as the S layer. Kokka et al. (1990) noted that although agglutination was useful for screening for S-layer containing strains, this marker was not restricted solely to S-layer-positive virulent strains. They speculated that the surface layer may provide protective or selective advantages during infection. Serogroup 0:11 strains were most commonly isolated from blood or wound infections, the latter almost always due to *A. hydrophila* infection of a trauma injury to an extremity and associated with exposure to an aquatic environment. Although aeromonad isolates exhibiting S layers are more virulent to mice than S-layer-negative isolates (Janda et al., 1987; Kokka et al., 1990), analysis of data presented by Kokka and Janda (1990) shows that only 27% of clinical isolates were serogroup 0:11, all of which were either *A. hydrophila* or *A. sobria*. This observation suggests that additional virulence or host factors are involved in the development of invasive aeromonad infections.

Invasiveness is also associated with the presence of cytotoxic enterotoxins which produce dysentery-like symptoms (Cahill, 1990). Cytotoxic activity is frequently assayed by detection of cytopathic effects in cell cultures (Barer et al., 1986, Lye and Dufour, 1991). Invasiveness, as assessed by multiplication of *Aeromonas* spp. in HEp-2 cell monolayers, appears to be associated with dysenteric symptoms (Watson et al., 1985).

An evaluation of biotypes with respect to virulence characteristics indicates that *A. sobria*, and to a lesser extent *A. hydrophila*, are the most pathogenic strains. Virulence

characteristics such as invasiveness (based on HEp-2 assays; Watson et al., 1985), hemagglutination (Burke et al., 1986), presence of an S-layer (Janda et al. 1987), and production of cytotoxin (Daily et al., 1981; Barer et al., 1986) and enterotoxin (Daily et al., 1981; Watson et al., 1985; Kirov et al., 1986) are almost exclusively manifested by strains representing these biotypes.

Clinical data support the pathogenic role of *A. sobria* and *A. hydrophila* in vivo. Janda et al. (1984) noted a selective distribution of aeromonad biotypes with respect to body sites. Although gastrointestinal tract isolates were equally represented by the three biotypes, *A. hydrophila* and *A. sobria* accounted for almost 90% of all bacteremic isolates, suggesting that they are more invasive than *A. caviae*. Wound isolates were represented by all three biotypes, but *A. hydrophila* was most commonly recovered. Other investigators (Daily et al., 1981; Watson et al., 1985) have similarly noted *A. sobria* and *A. hydrophila* as the most frequently observed clinical isolates of *Aeromonas*, with the former predominate. Although *A. caviae* is frequently considered less enteropathogenic than *A. hydrophila* and *A. sobria*, it is regarded as a pediatric enteric pathogen in formula-fed infants and children with altered stool flora associated with underlying disease, severe malnutrition, or antibiotic use (Moyer, 1987; Namdari and Bottone, 1990 a, b). *A. caviae* has also been attributed an attenuated virulence based on fish pathogenicity (Wakabayashi et al., 1981).

Virulence of environmental aeromonads

Of primary public health concern is the issue of virulence or pathogenicity of environmental strains of *Aeromonas* spp. Enterotoxigenic strains from diverse aquatic environments have been reported to occur in 15 to 70% of the isolates examined (Burke et al., 1984a; Kirov et al., 1986). Hemagglutination patterns characteristic of clinical

strains occurred in 33% of domestic water isolates (Burke et al., 1986), whereas the virulence factor associated with adhesion and common to clinical strains, L pili production, was absent in environmental samples (Carrello et al., 1988). Data presented by Kokka and Janda (1990) show that 12% of environmental isolates examined belonged to serogroup O:11, an important group of highly pathogenic *Aeromonas* strains. Some investigators concluded that the presence of environmental isolates possessing virulence characteristics indicates the environment as a reservoir. There is an increased frequency of isolation of enterotoxigenic isolates from feces and a peak of gastroenteritis cases during the summer and fall months (Burke et al., 1984b; Agger et al., 1985; Moyer, 1987), when *Aeromonas* spp. are most abundant in water supplies (Burke et al., 1984b) and surface waters (Fliermans et al., 1977; Hazen et al., 1978).

Results of the present study show that the potentially more invasive biotypes, *A. sobria* and *hydrophila*, were dominant in Lake Matoaka as well as in other Virginia lakes. Although the majority (70%) of aeromonad isolates from Lake Matoaka were β -hemolytic, the significance of this virulence characteristic is controversial (Cahill, 1990). Our observation of a large difference in the recovery of autoagglutinating strains in 1989-90 (42%) compared to 1992 (15%) suggests a hypothesis that this difference was related to the frequent occurrence of sewage spills during the first period. Another plausible explanation for the observed difference is that storage conditions selected for autoagglutinating strains having a surface characteristic imparting some degree of cryoprotection. Our finding that none of the autoagglutinating strains from the lake possessed S-layers by SDS-PAGE or electron microscopy concurs with a previous observation for animal isolates that autoagglutination is not restricted to serogroup O:11 (Kokka et al., 1990).

Finally, *A. sobria* was the dominant aeromonad biotype isolated under warm temperature conditions from Lake Matoaka as well as from other lakes of contrasting

trophic classification. This finding suggests that temperature may favor selective multiplication of this biotype under conditions of nutrient enrichment as suggested by Monfort and Baleux (1991). This hypothesis should be tested in Lake Matoaka and other area lakes using in situ persistence techniques. From our perspective, the occurrence of *A. sobria* in eutrophied waters coincident with peak periods of recreational usage is a public health concern that should be addressed.

RECOMMENDATIONS

The following recommendations are based on the recognition that Lake Matoaka is hypereutrophic. The lake's status is the combined result of intrinsic lake and watershed characteristics which contribute to natural aging processes and cultural eutrophication involving stress factors associated with urbanization.

1. The College must identify and develop a plan-of-action to address sources of sediment and pollutants to Lake Matoaka. Immediate lake protection efforts must address processes in watershed as well as on campus and should focus on prevention of sewage spills, identifying improper or inappropriate development, and insistence on enforcement of best-management-practices (BMPs) during construction activities in the watershed. The College should participate in the development and implementation of a comprehensive stormwater management plan within the watershed. The serious deterioration of the lake necessitates adoption of a philosophy that actions which may satisfy local or state ordinances may be inadequate and more stringent protection is necessary.
2. Potential restoration approaches should be evaluated with respect to feasibility, technical validity, cost, and potential negative environmental impacts.

Hypolimnetic withdrawal may be an effective technique to remove nutrient rich anoxic water and could perhaps be coupled to the watering of campus grounds. The hypothesis that lake sediments are an internal phosphorous source should be validated prior to considering an expensive remediation strategy involving sediment removal.

3. A technical committee composed of college and local and state regulatory officials having expertise in environmental sciences and watershed management should be formed to address issues 1 and 2. This committee should advise and seek advice from the college's Landscape, Environment and Energy Committee and the City of Williamsburg. Considering the various interest groups which impact the lake throughout the watershed, it might be prudent to employ a consultant with experience in lake restoration and watershed management techniques to provide an independent and unbiased review of options and recommendations.

4. Water quality and bacteriological monitoring of Lake Matoaka and its major feeder streams should continue to assess changes in water quality and the affects of development and construction activities in the watershed.

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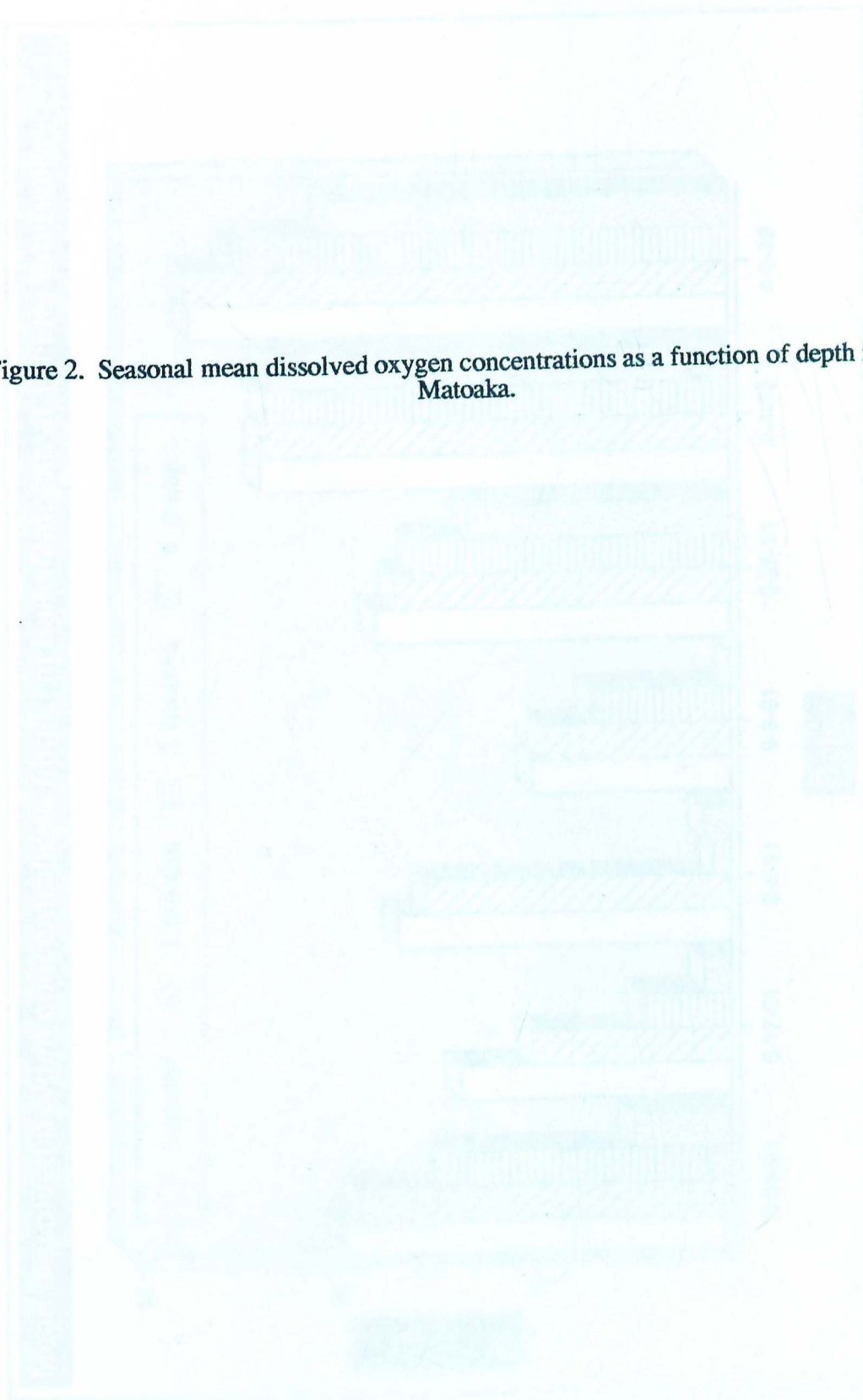
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Figure 1. Lake Matoaka sampling sites.



Figure 2. Seasonal mean dissolved oxygen concentrations as a function of depth in Lake Matoaka.



Mean dissolved oxygen concentrations (mg/L) in Lake Matoaka at 1 meter intervals

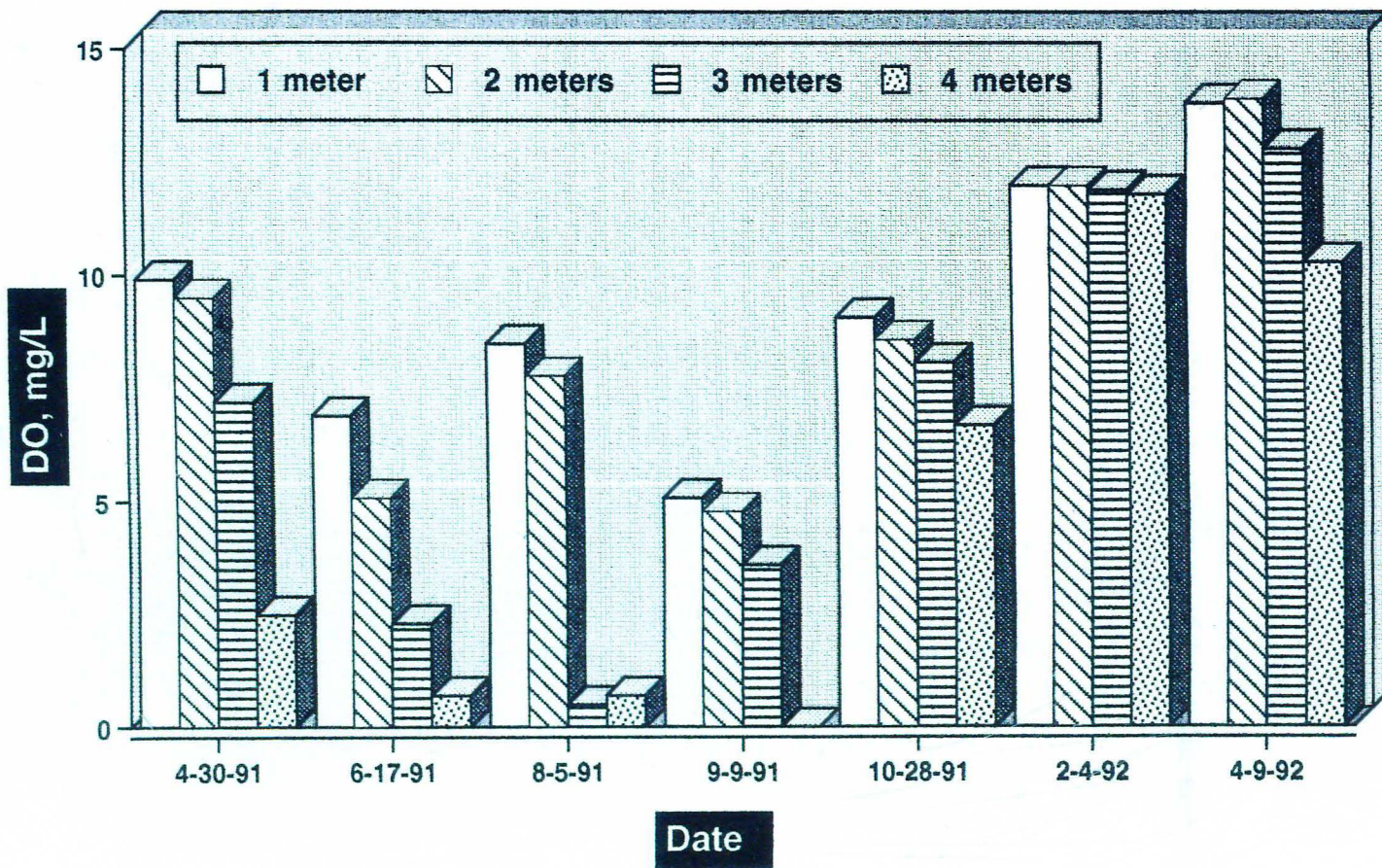
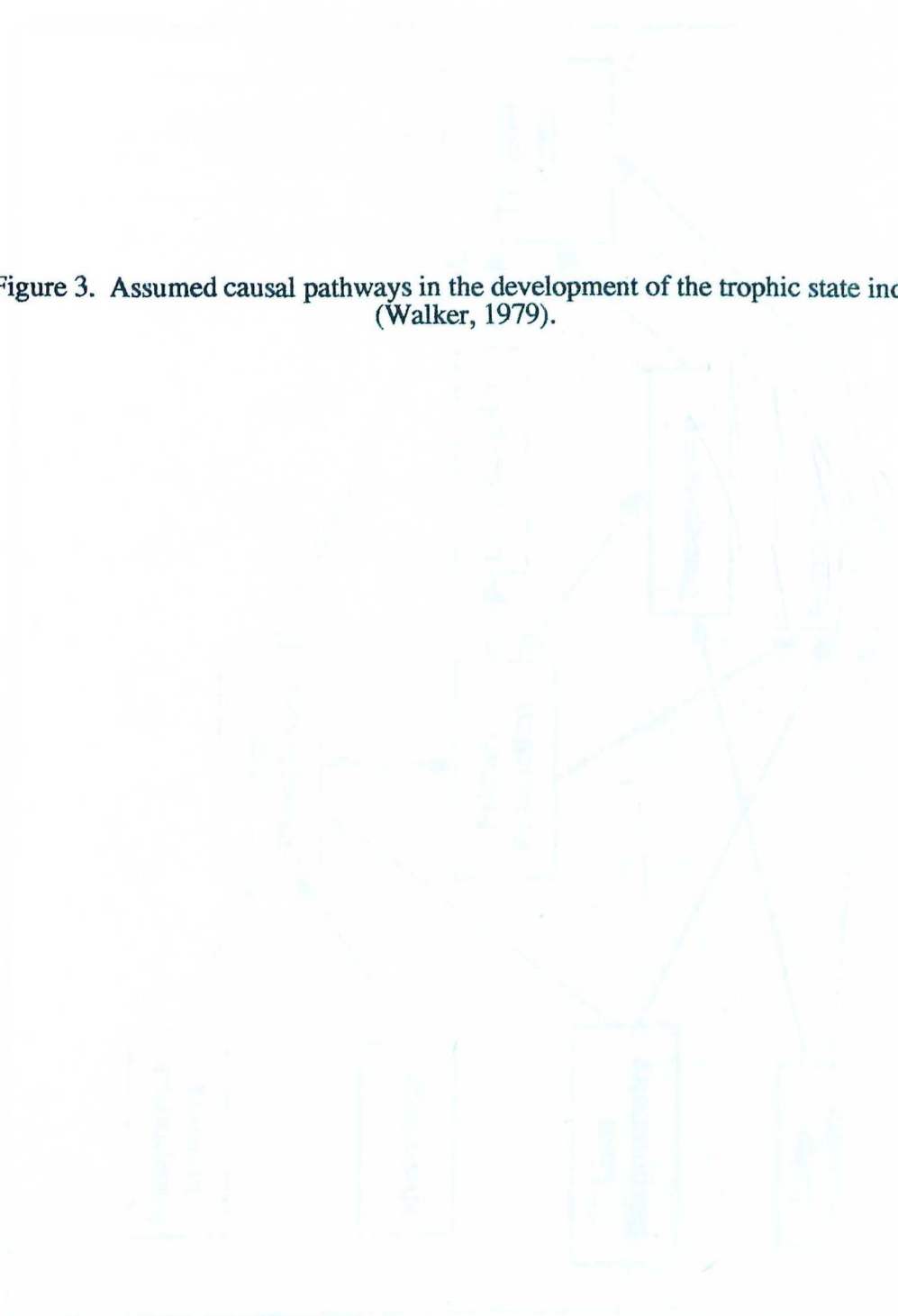


Figure 3. Assumed causal pathways in the development of the trophic state index (Walker, 1979).



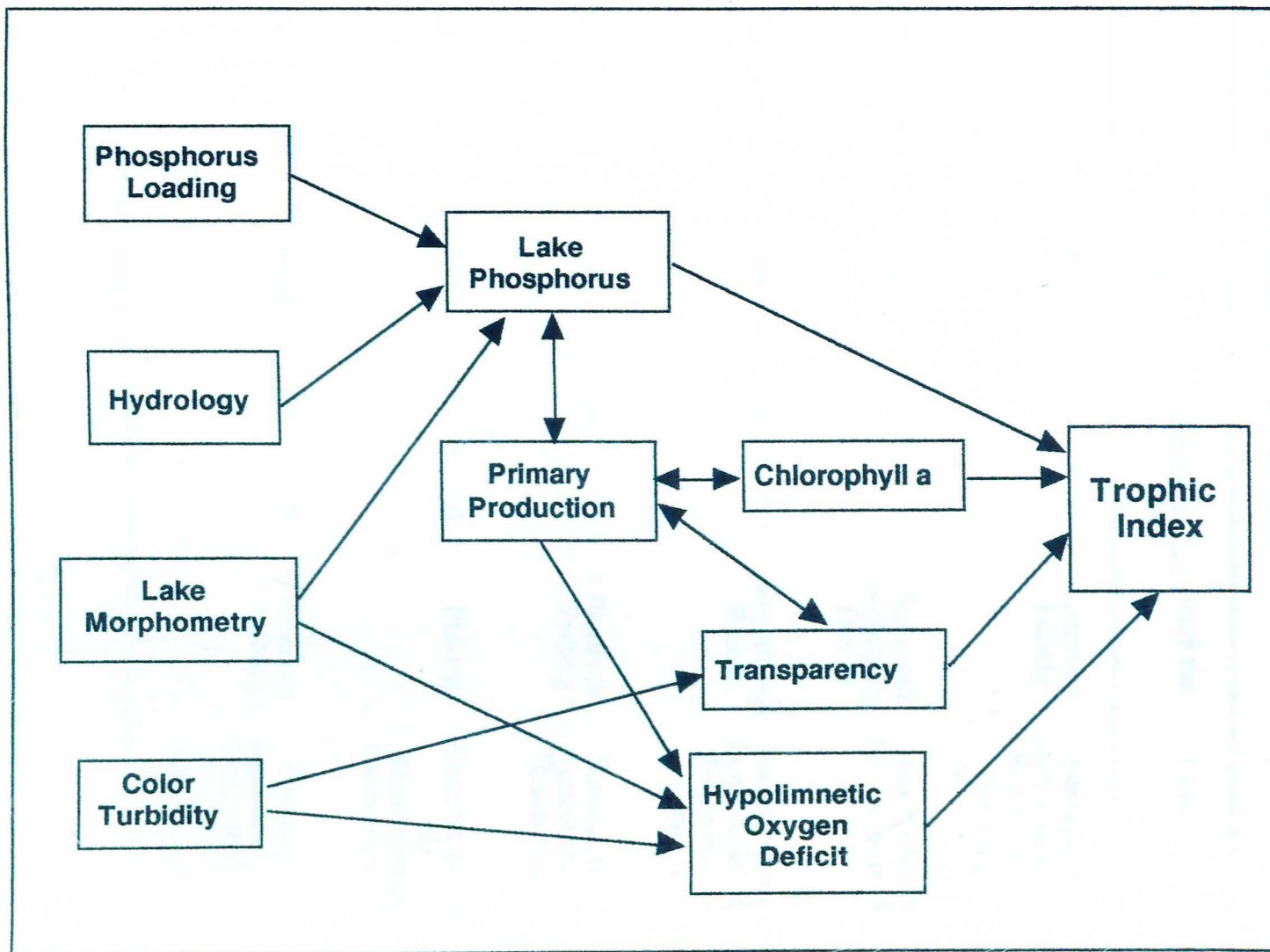


Table 1. Characteristics of lakes and their watersheds

Lake	Area (hect)		Depth (m)		Lake use	Land use (%)
	lake	watershed	mean	max.		
Burnt Mills	247	4060	10	11	Reservoir Fishing	Forested (50) Agricultural (40) Residential (5) Wetland (5)
Holliday	59	3643	3.7	9	State park Swimming Fishing	Forested (80) Recreational (20)
Matoaka	16	596	2.5	5.2	Instructional Boating	Forested (65) Commercial (23) Residential and Rural (12)
Little Creek Reservoir	371	1090	7.3	17.7	Reservoir Fishing	Forested (n.a.) Agricultural (") Residential (")
Powell	33	1340	3.1	4	Fishing	Commercial (ca. 75) Residential and Forested (ca. 25)
Waller Mill	127	1796	4.5	9	Reservoir Fishing	Forested (60) Agricultural (10) Commercial (10) Industrial (10) Residential (10)

Table 2. Mean densities of microbial parameters in Lake Matoaka, Ice House Cove feeder stream, and Crim Dell: 1989-90.

Sampling site	Count per 100 ml water			
	Fecal coliforms	<i>Escherichia coli</i>	Enterococci	Mesophilic aeromonads
A	120 (12) ^a	110 (12)	6 (8)	11,000 (10)
B	95 (11)	95 (11)	6 (8)	10,000 (9)
C	120 (12)	120 (12)	_b	-
CX	66 (6)	70 (5)	2 (5)	5,400 (6)
D	56 (12)	57 (11)	-	-
E	51 (13)	51 (13)	4 (10)	4,000 (12)
F	60 (12)	60 (12)	8 (10)	13,000 (11)
G	50 (13)	43 (12)	5 (5)	5,400 (7)
H	34 (12)	32 (12)	3 (9)	3,300 (10)
I	52 (12)	44 (11)	5 (10)	3,500 (11)
J	33 (13)	32 (13)	3 (10)	2,700 (12)
K (Feeder stream)	1,400 (12)	1,700 (11)	75 (12)	60,000 (11)
L (Crim Dell)	360 (6)	330 (6)	66 (6)	5,900 (4)

^aNumber of samples.

^b- Not determined.

Table 3. Significant ($P < 0.05$) Spearman rank correlation coefficients for bacterial densities^a measured in 1989-90 in Lake Matoaka.

Microbial parameter	Fecal coliforms	<i>Escherichia coli</i>	Enterococci	Mesophilic aeromonads
Fecal coliforms		0.98 (124) ^b	0.62 (76)	0.43 (91)
<i>Escherichia coli</i>	0.98 (124)		0.63 (73)	0.41 (88)
Enterococci	0.62 (76)	0.63 (73)		
Mesophilic aeromonads	0.43 (91)	0.41 (88)		

^aLog₁₀ bacterial density.

^bNumber of samples.

Table 4. Comparison of bacterial and nutrient parameters in the absence and presence of major sewage spills and/or rainfall events^a.

Date	Sewage spill	Rainfall (cm)	Geometric mean (count/100 ml)			Mean concentration (mg/m ³)		
			Fecal coliforms	<i>Escherichia coli</i>	Mesophilic aeromonads	TDN	TDP	TP
10-12-89	+	0.00	340 (10) ^c	290 (10)	10,000 (10)	389 (10)	10 (10)	51 (10)
11-13-90	+	1.55	140 (5)	120 (5)	13,000 (5)	— ^d	—	—
1-9-90	-	2.69	400 (10)	400 (10)	4,100 (7)	—	15 (7)	40 (7)
7-16-90	-	4.31	72 (11)	72 (11)	5,500 (9)	312 (8)	19 (8)	81 (8)
Reference surveys ^b	-	0.00	36 (71)	35 (67)	4,600 (47)	368 (30)	100 (44)	138 (44)

^aSewage – sewage spill occurred (+) or did not occur (-) within 7 days prior to sampling. Rainfall – amount within 3 days prior to sampling.

^bReference surveys – 10-17-89, 11-28-89, 2-27-90, 4-10-90, 6-18-90, 10-30-90, 12-3-90.

^cNumber of samples.

^dNot determined.

Table 5. Significant ($P < 0.05$) Spearman rank correlation coefficients for bacterial densities^a and environmental parameters measured in Lake Matoaka: 1989-90.

Microbial parameter	Rainfall		Temperature	Turbidity	Secchi depth	Total dissolved		Particulate phosphorus	Chlorophyll a
	3d	7d				nitrogen	phosphorus		
Fecal coliforms			-0.24 (128) ^b	0.40 (126)	-0.47 (54)		-0.31 (87)		-0.32 (67)
<i>Escherichia coli</i>			-0.23 (124)	0.43 (122)	-0.53 (51)		-0.28 (84)		-0.32 (64)
Enterococci		0.32 (76)	-0.23 (76)	0.32 (76)	-0.40 (44)		-0.26 (71)		
Mesophilic aeromonads	-0.23 (91)			0.32 (91)	-0.45 (48)	0.42 (60)		0.26 (81)	

^aLog₁₀ bacterial density.

^bNumber of samples.

Table 6. Mean values of chemical and physical indices of eutrophication in Lake Matoaka, Ice House Cove feeder stream, and Crim Dell: 1989-90.

Sampling site	Total phosphate (mg/m ³)	Total dissolved nitrogen (mg/m ³)	Chlorophyll a (mg/m ³)	Secchi depth (m)
A	83 (10) ^a	42 (8)	21.47 (8)	_b
B	51 (6)	312 (5)	18.00 (4)	-
C	37 (3)	277 (2)	-	0.76 (5)
CX	144 (5)	365 (4)	34.83 (5)	0.87 (6)
E	73 (11)	324 (8)	24.88 (9)	1.04 (9)
F	115 (10)	370 (7)	27.09 (8)	-
G	116 (7)	347 (6)	38.39 (5)	1.01 (8)
H	210 (11)	398 (8)	24.37 (9)	1.23 (8)
I	78 (11)	317 (8)	26.66 (9)	1.11 (8)
J	91 (11)	358 (8)	24.50 (9)	1.07 (9)
K (Feeder stream)	937 (9)	2,270 (5)	1.59 (9)	-
L (Crim Dell)	85 (2)	511 (2)	9.99 (2)	0.50 (2)

^aNumber of samples.

^b- Not determined.

Table 7. Significant ($P < 0.05$) Spearman rank correlation coefficients for physical and nutrient parameters measured in Lake Matoaka: 1989-90.

Parameter	Total dissolved		Particulate phosphorus	Total phosphorus	Chlorophyll a	Dissolved oxygen	Secchi depth	Temperature
	nitrogen	phosphorus						
Total dissolved nitrogen		0.45 (66) ^a		0.35 (66)		-0.47 (66)	-0.39 (28)	
Total dissolved phosphorus	0.45 (66)		0.48 (87)	0.77 (87)	0.59 (67)	-0.38 (87)		0.53 (87)
Particulate phosphorus		0.48 (87)		0.90 (87)	0.66 (67)	-0.41 (87)	-0.59 (43)	0.57 (87)
Total phosphorus	0.35 (66)	0.77 (87)	0.90 (87)		0.72 (67)	-0.47 (87)	-0.43 (43)	0.64 (87)
Chlorophyll a		0.59 (67)	0.66 (67)	0.72 (67)		-0.50 (67)	-0.51 (43)	0.87 (67)
Secchi depth	-0.39 (28)		-0.59 (43)	-0.43 (43)	-0.51 (43)	0.62 (54)		-0.60 (54)

^aNumber of samples.

Table 8. Trophic classification of Lake Matoaka^a.

Parameter	Survey date			Summer mean	Component index	Trophic status
	6/90	7/90	9/90			
Chlorophyll a (mg/m ³)	35	53	59	49	76	HE
Total phosphorus (mg/m ³)	545	81	125	250	95	HE
Secchi depth (m)	1.1	0.6	0.9	0.9	77	HE
I					83	HE

^aLake classification based on Walker's trophic index I (Walker, 1979). Index (I) components are: $I_B = 20.0 + 33.2 \log_{10} B$, $I_P = -15.6 + 46.1 \log_{10} P$, and $I_T = 75.3 + 44.8 \log_{10} (1/Z_S - 0.04 \text{ m}^{-1})$ where B is the chlorophyll a concentration, P is the total phosphorus concentration, and Z_S is Secchi depth. Walker's trophic index $I = (I_B + I_P + I_T)/3$. The approximate transition zone between oligotrophy and mesotrophy occurs between 25–30 index units; mesotrophy to eutrophy between 45–50 index units; and eutrophy to hypereutrophy (HE) is arbitrarily set between 65–70 index units.

Table 9. Comparison of 1990 summer values for water quality parameters determined for Lake Matoaka with those of other local lakes.

Parameter ^a	Little Creek Reservoir	Lake Waller Mill	Lake Powell	Lake Matoaka
Dissolved oxygen (mg/l)	8.9	5.9	7.8	7.8
pH	8.3	8.1	7.9	8.3
Secchi depth (m)	2.5	2.0	1.0	0.8
Turbidity (NTU)	3.0	5.5	6.2	7.2
Temperature (°C)	28	25	25	27
Total dissolved phosphorus	12	15	187	195
Particulate phosphorus	10	<8	29	56
Total phosphorus	22	<23	216	250
Chlorophyll a	7	12	26	49
Fecal coliforms	<2	6	5	30
<i>Escherichia coli</i>	<2	6	5	29
Enterococci	<1	<1	<1	<3
Mesophilic aeromonads	300	8,300	5,140	4,790

^aUnits of measurement are mg/m³ for nutrients and chlorophyll a and cells/100 ml for bacterial parameters, NTU = Nephelometric Turbidity Units. Number of observations for Little Creek Reservoir and Lake Waller Mill = 6; Lake Powell = 12-18; and Lake Matoaka = 18.

Table 10. Mean values for parameters measured during 1991 winter surveys in lakes Holliday, Burnt Mills, and Matoaka.

Parameter ^a	Holliday Lake	Lake Burnt Mills	Lake Matoaka
Dissolved oxygen (mg/l)	11.1	11.2	9.2
pH	6.9	6.8	8.1
Secchi depth (m)	1.6	1.3	0.8
Turbidity (NTU)	8.2	_b	9.5
Temperature (°C)	6.0	8.0	7.6
Total dissolved phosphorus	9	15	18
Particulate phosphorus	8	8	24
Total phosphorus	17	23	42
Total dissolved nitrogen	263	823	477
Chlorophyll a	4	35	13
Fecal coliforms	<2	19	430
<i>Escherichia coli</i>	<2	5	490
Enterococci	<1	<2	460
Mesophilic aeromonads	380	910	11,000

^aUnits of measurement are mg/m³ for nutrients and chlorophyll a and cells/100 ml for bacterial parameters, NTU = Nephelometric Turbidity Units. Number of observations for each lake = 6.

^b- Not determined.

Table 11. Mean values for parameters measured in 1991 for lakes Holliday, Burnt Mills, and Matoaka at temperatures >16°C.

Parameter ^a	Holliday Lake	Lake Burnt Mills	Lake Matoaka
Dissolved oxygen (mg/l)	7.8	7.6	7.6
pH	7.4 ^b	8.1	8.0
Secchi depth (m)	2.5 ^b	1.6 ^b	1.2
Turbidity (NTU)	4.5	4.4	5.1
Temperature (°C)	24	26 ^b	23
Total dissolved phosphorus	9 ^b	15 ^b	17
Particulate phosphorus	6 ^b	17 ^b	41
Total phosphorus	15 ^b	32 ^b	61
Total dissolved nitrogen	297 ^b	427 ^b	362
Chlorophyll a	6 ^b	22 ^b	38
Fecal coliforms	<3 ^b	<3 ^b	<32
<i>Escherichia coli</i>	<2 ^b	<3 ^b	<32
Enterococci	<1 ^b	<2	<2
Mesophilic aeromonads	8700 ^b	1700 ^b	5300

^aUnits of measurement are mg/m³ for nutrients and chlorophyll a and cells/100 ml for bacterial parameters, NTU = Nephelometric Turbidity Units. Number of observations for each lake = 18-30.

^bSignificantly different (P<0.05) from values for Lake Matoaka.

Table 12. Significant ($P < 0.05$) Spearman rank correlation coefficients for bacterial densities^a and environmental parameters measured for lakes Burnt Mills, Holliday and Matoaka: 1991.

Microbial parameter	Temperature	Dissolved oxygen	Turbidity	Secchi depth	Total dissolved		Particulate phosphorus	Chlorophyll a
					nitrogen	phosphorus		
Fecal coliforms	-0.21 (101) ^b		0.28 (72)	-0.62 (107)	0.36 (107)	0.49 (107)	0.60 (106)	0.58 (107)
<i>Escherichia coli</i>	-0.19 (101)		0.30 (72)	-0.61 (107)	0.30 (107)	0.48 (107)	0.65 (106)	0.57 (107)
Enterococci			0.37 (72)	-0.36 (107)	0.35 (107)	0.24 (107)	0.35 (106)	0.23 (107)
Mesophilic aeromonads		-0.30 (101)			-0.30 (107)			

^aLog₁₀ bacterial density.

^bNumber of samples.

Table 13. Significant ($P < 0.05$) Spearman rank correlation coefficients for bacterial densities^a and environmental parameters measured for Lake Matoaka: 1991.

Microbial parameter	Temperature	Turbidity	Secchi depth	Total dissolved		Particulate phosphorus	Total phosphorus	Chlorophyll a
				nitrogen	phosphorus			
Fecal coliforms	-0.42 (35) ^b	0.81 (24))	-0.35 (36))	0.52 (36)	0.29 (36)			
<i>Escherichia coli</i>	-0.37 (35)	0.77 (24)	-0.36 (36)	0.52 (36)	0.35 (36)			
Enterococci		0.66 (24)		0.39 (36)	0.61 (36)			
Mesophilic aeromonads	-0.77 (35)					-0.64 (35)	-0.60 (35)	

^aLog₁₀ bacterial density.

^bNumber of samples.

Table 14. Significant ($P < 0.05$) Spearman rank correlation coefficients for physical and nutrient parameters measured for lakes Burnt Mills, Holliday and Matoaka: 1991.

Parameter	Total dissolved		Particulate phosphorus	Total phosphorus	Chlorophyll a	Dissolved oxygen	Secchi depth	Temperature
	nitrogen	phosphorus						
Total dissolved nitrogen		0.47 (108) ^a	0.32 (107)	0.43 (107)	0.53 (107)		-0.45 (108)	
Total dissolved phosphorus	0.47 (108)		0.47 (107)	0.77 (107)	0.47 (107)	-0.25 (102)	-0.46 (108)	
Particulate phosphorus	0.32 (107)	0.47 (108)		0.90 (107)	0.69 (106)	-0.21 (101)	-0.70 (107)	0.22 (106)
Total phosphorus	0.43 (107)	0.77 (107)	0.90 (107)		0.69 (106)	-0.25 (101)	-0.68 (107)	0.23 (106)
Chlorophyll a	0.53 (107)	0.47 (107)	0.69 (106)	0.69 (106)		-0.25 (101)	-0.77 (107)	
Secchi depth	-0.45 (108)	-0.46 (108)	-0.70 (107)	-0.68 (107)	-0.77 (107)			

^aNumber of samples.

Table 15. Significant ($P < 0.05$) Spearman rank correlation coefficients for physical and nutrient parameters measured for Lake Matoaka: 1991.

Parameter	Total dissolved		Particulate phosphorus	Total phosphorus	Chlorophyll a	Dissolved oxygen	Secchi depth	Temperature
	nitrogen	phosphorus						
Total dissolved nitrogen		0.43 (36) ^a			0.36 (36)	-0.39 (36)	-0.67 (36)	
Total dissolved phosphorus	0.43 (36)		0.58 (35)	0.67 (35)	0.35 (36)	-0.48 (36)	-0.46 (36)	
Particulate phosphorus		0.58 (35)		0.97 (35)	0.54 (35)	-0.67 (35)		0.71 (34)
Total phosphorus		0.67 (35)	0.97 (35)		0.52 (35)	-0.68 (35)	-0.34 (35)	0.68 (34)
Chlorophyll a	0.36 (36)	0.35 (36)	0.54 (36)	0.52 (35)		-0.85 (36)	-0.57 (36)	
Secchi depth	-0.67 (36)	-0.46 (36)		-0.34 (35)	-0.57 (36)	0.57 (36)		

^aNumber of samples.

Table 16. Comparison of trophic classification of Lakes Burnt Mills (BM), Holliday (H) and Matoaka (M).^a

Parameter	Mean value			Component index		
	H	BM	M	H	BM	M
Chlorophyll a (mg/m ³)	6	22	38	46	65	72
Total phosphorus (mg/m ³)	15	32	61	39	54	68
Secchi depth (m)	2.5	1.6	1.2	55	65	71
I				47	61	70

^aLake classification based on Walker's trophic index I (Walker, 1979). Index (I) components are: $I_B = 20.0 + 33.2 \log_{10} B$, $I_P = -15.6 + 46.1 \log_{10} P$, and $I_T = 75.3 + 44.8 \log_{10} (1/Z_S - 0.04 \text{ m}^{-1})$ where B is the chlorophyll a concentration, P is the total phosphorus concentration, and Z_S is Secchi depth. Walker's trophic index $I = (I_B + I_P + I_T)/3$. The approximate transition zone between oligotrophy and mesotrophy occurs between 25–30 index units; mesotrophy to eutrophy between 45–50 index units; and eutrophy to hypereutrophy (HE) is arbitrarily set between 65–70 index units.

Table 17. Phenotypic characterization of environmental isolates of mesophilic *Aeromonas* spp.

Test	Percent positive		
	<i>A. caviae</i>	<i>A. hydrophila</i>	<i>A. sobria</i>
Esculin hydrolysis	100 (27) ^a	100 (78)	5 (148)
Fermentation of arabinose	93 (27)	91 (78)	3 (148)
Fermentation of salicin	56 (27)	67 (78)	0 (148)
Gas from glucose	0 (25)	86 (71)	99 (144)
Lysine decarboxylase	4 (27)	92 (77)	95 (148)
Voges-Proskauer	6 (16)	98 (54)	100 (141)

^aNumber of isolates tested. The biotype of seven percent (20/273) of isolates examined could not be determined.

Table 18. Percent occurrence of *Aeromonas* biotypes isolated from Lake Matoaka at contrasting temperature ranges.

Temperature range	Percent occurrence of biotype		
	<i>A. caviae</i>	<i>A. hydrophila</i>	<i>A. sobria</i>
<20°C	19 (14) ^a	69 (50)	11 (8)
>20°C	3 (3)	17 (16)	78 (74)

^aNumber of isolates.

Table 19. Percent occurrence of *Aeromonas* biotypes in tidewater lakes.

Lake	Percent occurrence		
	<i>A. caviae</i>	<i>A. hydrophila</i>	<i>A. sobria</i>
Little Creek Reservoir	0	17 (4)	83 (20)
Matoaka	3(3)	17 (16)	78 (74)
Powell	26 (10)	5 (2)	69 (27)
Waller Mill	0	24 (6)	76 (19)

^aNumber of isolates.

Table 20. Watershed nutrient export coefficients (Rast and Lee, 1983).

Watershed landuse	Watershed export coefficient (g/m ² /yr.)	
	Total phosphorus	Total nitrogen
Urban	0.1	0.5
Rural/agriculture	0.05	0.5
Forest	0.01	0.3
Rainfall and dry fallout	0.02	2.4

Table 21. Waterborne diseases caused by ingestion of or contact with freshwater^a.

Disease	Primary agent(s)
Amebiasis	<i>Entamoeba histolytica</i>
Campylobacteriosis	<i>Campylobacter jejuni</i>
Cholera	<i>Vibrio cholerae</i>
Ear infections	<i>Pseudomonas aeruginosa</i> , <i>Staphylococcus</i> spp., <i>Streptococcus</i> spp.
Enterotoxigenic <i>Escherichia coli</i> gastroenteritis	<i>Escherichia coli</i>
Tularemia	<i>Francisella tularensis</i>
Giardiasis	<i>Giardia lamblia</i>
Hepatitis	Hepatitis A
Leptospirosis	<i>Leptospira</i> spp.
Mycobacteriosis	<i>Mycobacterium marinum</i>
Pneumonia	<i>Pseudomonas putrefaciens</i> , <i>Aeromonas</i> spp., <i>Legionella</i> spp.
Primary amebic meningoencephalitis	<i>Naegleria fowleri</i>
Schistosome dermatitis	Parasitic flatworm cercaria
Septicemias	<i>Aeromonas</i> spp., <i>Chromobacterium violaceum</i>
Shigellosis	<i>Shigella</i> spp.
Typhoid fever and Salmonellosis	<i>Salmonella</i> spp.
Viral gastroenteritis	Various enteroviruses, Norwalk virus, rotaviruses
Wound infections	<i>Aeromonas</i> spp.
<i>Yersinia enterocolitica</i> infections	<i>Yersinia enterocolitica</i>

^aTable adapted from information presented in Harris (1986) and Dufour (1986).

