NWRI Contribution No. 86-164

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by

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Short Title - Coliphage studies in N. and South America.

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ABSTRACT

Studies were undertaken to assess the potential of coliphages to be used universally as water quality indicators and more specifically as health hazard indicators for Canadian waters. Sample and data were collected from three distinct and separate water bodies, a northern Canadian River, inshore water samples from Lake Ontario and from marine beaches in Brazil. Data from this two continent, three water body study indicate (a) that within location fecal coliform and coliphages are positively correlated (b) coliphage values can be indicated or predicted by using fecal coliform MPN, fecal streptococci MF and *E. coli* MF data and (c) it would be feasible to propose a coliphage freshwater quality guideline of 20 coliphage/100 mL for recreational waters.

MANAGEMENT PRESPECTIVE

This study was undertaken to assess the potential of using coliphage as a universal water quality indicator and more specifically a health hazard indicator for Canadian waters. The simplicity, speed and economical nature of the coliphage procedure, coupled with the stability of coliphage in water samples, are major reasons for wanting to include this test in all water quality surveys. With a sufficient data base, the procedure could be used, initially, in parallel with traditional indicators (fecal coliforms, E. coli, fecal streptococci) and eventually as a replacement for these microbial indicators of fecal contaminaiton of recreational waters and drinking water sources. This work is part of the NWRI Biomonitoring Project to develop the use of microbiological assays for the assessment of water quality.

In this investigation, samples were collected from three distinct and separate water bodies, a northern Canadian River, inshore water samples from a Canadian Great Lake and marine beaches in Brazil. Data from this two continent, three water body study indicate that (a) it would be feasible to propose a coliphage freshwater quality guideline of 20 coliphage/100 mL for recreational waters (b) coliphage values can be indicated or predicted by using fecal coliform MPN, fecal streptococci MF and E. coli MF data, (c) in marine waters where pathogens are found, coliphage/pathogen ratios are smaller than fecal coliform/pathogen ratios and (d) indicator densities were not indicative of pathogen absence or presence.

La présente étude a été entreprise pour évaluer la possibilité de se servir des coliphages comme indicateur universel de la qualité de l'eau et plus particulièrement comme indicateur des dangers pour la santé publique dans les eaux canadiennes. L'épreuve des coliphages devrait faire partie intégrante de toutes les enquêtes sur la qualité de l'eau puisque c'est une technique simple, rapide et économique et que les coliphages se conservent bien dans les échantillons d'eau. Au départ, cette technique pourrait être utilisée conjointement avec les indicateurs classiques (coliformes fécaux, E. coli, streptocoques fécaux). Moyennant une base de données suffisamment élaborée, elle pourrait éventuellement remplacer ceux-ci comme indicateurs microbiens de la contaminition par les matières fécales des étendues d'eau servant aux loisirs et à la consommation humaine. Cette étude se rattache aux travaux du Projet de biosurveillance de l'INRE ayant pour but de perfectionner l'application d'épreuves microbiologiques pour évaluer la qualité de l'eau.

Aux fins de l'étude, des échantillons ont été prélevés dans trois cours d'eau situés dans deux continents différents et n'ayant absolument rien en commun, soit une rivière dans le nord du Canada, les eaux près de la rive d'un des Grands Lacs et celles d'une plage sur la côte brésilienne. Les données issues de ces échantillons ont permis de dégager plusieurs conclusions: (a) il serait plausible de fixer comme norme de la qualité des eaux servant aux loisirs un seuil de 20 coliphages par 100mL; (b) on peut calculer ou prévoir les taux de coliphages à partir du nombre le plus probable de coliformes fécaux ainsi que les streptocoques fécaux et les E. coli isolés par membrane filtrante; (c) les eaux marines dans lesquelles on trouve des agents pathogènes présentent des rapports de coliphages-agents pathogènes inférieurs à ceux des coliformes fécaux-agents pathogènes; (d) la densité des indicateurs ne permet pas de déterminer la présence ou l'absence des agents pathogènes.

SOMMAIRE

Des études ont été entreprises pour évaluer la possibilité de se servir des coliphages comme indicateur universel de la qualité de l'eau et plus particulièrement comme indicateur des dangers pour la santé publique dans les eaux canadiennes. Des échantillons ont été prélevés dans trois cours d'eau situés dans deux continents différents et n'ayant absolument rien en commun, soit une rivière dans le nord du Canada, les eaux près de la rive d'un des Grands Lacs et celles d'une plage sur la côte brésilienne. Les données issues de ces échantillons ont permis de dégager plusieurs conclusions: (a) il serait plausible de fixer comme norme de la qualité des eaux servant aux loisirs un seuil de 20 coliphages par 100mL; (b) on peut calculer ou prévoir les taux de coliphages à partir du nombre le plus probable de coliformes fécaux ainsi que les streptocoques fécaux et les *E. coli* isolés par membrane filtrante; (c) les eaux marines dans lesquelles on trouve des agents pathogènes présentent des rapports de coliphages-agents pathogènes inférieurs à ceux des coliformes fécaux-agents pathogènes; (d) la densité des indicateurs ne permet pas de déterminer la présence ou l'absence des agents pathogènes.

INTRODUCTION

Research by scientists of the Atlantic Research Corporation (1979) on the use of coliphage as an indicator of potential health hazards in water due to fecal pollution has greatly advanced the knowledge on the utility of this water quality indicator. Guelin in 1948 was the first researcher to recognize the potential of bacteriophages as indicators of fecal pollution. Since Guelin's work of the potential of bacteriophages to act as indicator systems, there have been several papers indicating the potential of bacteriophage/coliphage to act as indicators of bacterial water quality (Bosco 1963, Kuznetsova and Ostrowkaja 1963, Amin-Zade adn Poultof 1964, Kenard and Valentine 1974, Scarpino 1975, Zais 1982, Wensel, O'Neal and Kitchens 1982 and Kennedy et al. 1985) and viral water quality (Vaughn and Metcalf 1975, Kott, Ben-Ari and Vinokur 1978 and Grabow et al. 1984).

Scarpino (1975), stated that "Correlations appear to exist in fresh and marine waters between fecal bacterial pathogens such as Salmonella and Shigella species and fecal indicator bacteria such as E. coli and their bacteriophages". Then in 1984 Grabow et al. reported "coliphage counts could give a useful estimate of numbers of other microorganisms in sewage polluted water", and presented evidence showing that coliphages meet the basic requirements of an indicator for the virological safety of water.

There is also sufficient evidence to suggest that the coliphage test has many advantages over traditional bacteriological and virological tests in that the procedure is economical, simple to perform and provides results within six hours. The speed with which results can be obtained indicates that the coliphage test is a definite asset where approximate or hazard estimate data are required urgently, i.e. (1) repair of broken water mains and an indication is required on the possibility of fecal pollution entering the broken water line, (2) in cases of suspected contamination of enclosed water supplies such as onboard a cruise ship or artesian well supplying a small neighbourhood (3) in field studies to test and characterize or give priority rating to potable water sources or (4) in field studies to evaluate the extent of sewage treatment plant effluent's contamination of receiving waters.

Although a review of the literature on the coliphage test indicates that it may be an ideal test for approximation of health hazard estimation due to fecal pollution, there appears to be a reluctance to accept research implications to local waters, even though the procedure has now been tentatively accepted by North America's two major method standardization organizations, APHA and ASTM. Therefore, the conclusion one is forced to face is that it may be necessary for each area or jurisdiction considering the use of coliphage to establish coliphage relationships to coliform or fecal coliforms and other traditional indicators and pathogens. These "veting" studies for coliphage could be considered inappropriate,

are no direct numerical relationships between coliforms, fecal coliforms, E. coli and the degree of hazard as related to the incidence and infectivity rate of waterborne Salmonella, Shigella, Cholera, viruses (Dutka 1973). Also there are no consistent and obvious numerical relationships in receiving waters and drinking water between fecal coliforms, E. coli, Salmonella, Shigella, Cholera, viruses and coprostanol, the absolute indicator of fecal contamination (Dutka-El-Shaarawi 1975).

In all uses of indicator organisms we are dealing with a concept, a concept that usually works and is protective (and possibly over protective) of users of potable and natural waters. We believe that due to increasing stresses on water supplies, rising analytical costs, frequency of natural disasters which require immediate responses, e.g. earthquakes, volcano eruptions, frost upheaval of pipes, we must develop cheaper, simpler and quicker indicator systems which will reflect both bacterial and viral contamination from sewage. Coliphages appear to be one of the most obvious candidates. However, to allay the doubts of local implementers of the coliphage indicator system and those involved in guideline setting, it would be prudent to collect more local data from fresh water application sites as well as marine sites to support the use of this procedure.

Studies were undertaken to assess the potential of coliphages to be used universally as water quality indicators. In this study,

samples and data were collected from three distinct and separate water bodies, a northern Canadian river, inshore samples from one of the Canadian Great Lakes and marine beaches in Brazil. The data and their implications are presented below.

METHODS

Sample Collection

River samples were collected usually every two weeks during the September 25, 1984 to December 4, 1985 period in the Ottawa River near Lemieux Island within the city of Ottawa, Ontario. Twenty-six water samples were also collected from 26 different sites along the north shore of Lake Ontario from Kingston on the east to the Niagara R. on the west during a seven day period in June, 1985.

The following nine marine beaches on the eastern coast of Brazil were sampled over a twelve month period (1984-85), usually one sample from each beach per month: Praia Grande, Praia do Tombo, Ponta da Praia, Praia do Boqueirao, Praia do Itarare', Praia da Enseada, Praia das Pitangueiras, Praia de Bertioga and Praia de Pernambuco. In all instances the samples upon collection were cooled on melting ice and processed within 24 hours.

Microbiological Tests

Fecal coliform populations were estimated by the membrane filtration technique using mFC agar with incubation at 44.5°C for 24 hours and two MPN procedures, one using Al broth with incubation at 44.5°C for 24 hours (APHA 1985) and the other using lactose broth (35°C for 24 hours) with acid and gas positive tubes being transferred to EC broth for 24 hours at 44.5°C. *E. Coli* populations were estimated by the membrane filtration technique using mTEC agar with a two-hour resuscitation period followed by 20±2 hours at 44.5°C (Dufour *et al.* 1981). Water samples (5ℓ) for *Salmonella* were concentrated through Millipore HAWP membrane filters (0.45 μm). After filtration, membranes were transferred to Selenite Broth with novobiocin, for enrichment during 24 hour, 48 hour and 5 days at 42.5°C. Xylose-lysine desoxycholate agar and brilliant green agar were used for isolation, the incubation of both media being done at 35°C during 24 hours. *Salmonella* typical colonies in these media were transferred to Rugai Medium modified by Pessoa (1972) for a first screening of biochemical reactions. Serological identification was made using polyvalent somatic and flagellar sera.

Coliphage Test

The procedure used to estimate coliphage concentrations is that found in Section 919C, 16th ed. APHA Standard Methods (1985), with the addition of 2,3,5-triphenyl tetrazolium chloride.

Enterovirus

a) Virus concentration

40ℓ volumes of each sample were filtered through Millipore AP_{20} and HAWP (0,45 µm) MF, according to Standard Methods for the examination of water and wastewaters (1975). Reconcentration was done by organic flocculation according to Katzenelson et al. (1976).

b) Virus isolation and identification

From each sample, 1.0 ml was inoculated into each of 15 prescription bottles containing monolayers of BS-C-1 cell lines. Virus were assayed by the Dulbecco's plaque technique (1952), modified according to Hsiung and Melnick (1975). The overlay medium consisted of Eagle's Medium, 2% fetal calf serum, neutral red, $MgCl_2$, antibiotics and Difco Agar. As soon as they became clearly visible, all PFUs were inoculated into test tubes containing BS-C-1 cells in order to confirm the presence of virus

particles. Viruses were identified by NT tests against the Lim & Benyesh-Melnick enterovirus immune serum pools (1973).

Fecal Sterols

Fecal sterol analyses were performed on water using procedures described by Dutka, Chau and Coburn (1974).

Statistical Methods

The aim of the statistical analyses was to examine the association between various water quality indicators. This has been done informally by graphical methods and formally by statistical tests. Due to the large range of variability which is typical for microbiological data, the analyses are performed on the natural logs of the data.

The variety of statistical techniques used cover the following:

(1) data display for univariate and multivariate data,

(2) correlation and regression analyses, and

(3) principal component analysis.

These techniques can be found in many statistical texts.

RESULTS AND DISCUSSION

Ottawa River

In Figure 1 a box plot of all the natural logarithms of the microbiological data obtained from the Ottawa River samples are presented. The box plot provides a summary statistic using five numbers. These are the minimum, the maximum, the median, the 25th and 75th percentile. The distance between the 75th percantile and the median should be approximately equal to the difference between the median and the 25th percentile when the distribution is symmetric. It is clear from the plot that coliphage, E. coli and fecal coliforms show symmetry. Hence it is appropriate to use the log transformation.

Table 1 presents a summary of the mean densities and ratios between coliphage and the other indicator organisms. This table was prepared to highlight the mean counts for various sampling periods as well as coliphage: fecal coliform, E. coli and fecal streptococci ratios. One interesting observation illustrated in Table 1 is that depending on the enumeration technique used [MF or MPN and the media and membrane filter brand (Dutka et al. 1979)] and the time of the year the samples were collected, fecal coliform-coliphage ratios vary from a high of 9.5:1 to a low of 2.6:1, both extremes shown by the MPN technique. E. coli/coliphage ratios varied from 2.2:1 to 3.9:1 and fecal streptococci/coliphage ratios for 3.0:1 to 7.8:1. The stability

of the fecal streptococci-coliphage ratios are striking as their only common factor is their fecal origin and they are not part of each others reproductive cycles. Based on the mean fecal streptococci and fecal coliform populations found in the Ottawa River, the data are very suggestive that there should be minimal concern about human fecal pollution (Geldreich 1966) being the main contributor of microbial health indicator populations in these waters.

To study the statistical association between the various parameters of the Ottawa River study, a statistical evaluation of the data from the 55 water samples was undertaken. On each of the samples the following measurements are available: X_1=coliphage, X_2=fecal coliforms MF, X_3=*E. coli*, X_4=fecal streptococci MF and X_5=fecal coliform MPN (Fig. 1). The association between the five water quality indicators is given in the following correlation matrix.

	X_1	X_2	X_3	X_4	X_5
X_1	1.00	0.38**	0.28*	0.15	0.41**
X_2		1.00	0.89**	0.13	0.80**
X_3			1.00	0.29*	0.85**
X_4				1.00	0.37*
X_5					1.00

This matrix gives the correlation between each pair of log parameters. For example, in the first row and second column, we have 0.38 which is the correlation coefficient between log fecal coliform and log coliphage. Values marked by * and ** are significant at the 5% and 1% levels, respectively. Coliphage is highly correlated with fecal coliforms MF and MPN and correlated with E. coli and fecal coliform MPN. E. coli are highly correlated with fecal coliform MPN and correlated with fecal streptococci. Fecal streptococci are correlated with fecal coliform MPN.

To study the total variation in the Ottawa River data, principal component analyses was used to divide the total variation into five uncorrelated components. The results showed that the first two components contain 78.3% of the total variation. The summary presented below gives the explained variation for each of the five principal components:

	Principal Components				
	1	2	3	4	5
% explained variation	59.8	18.5	16.2	3.8	1.6

The first principal component PC_1 is dominated by fecal coliform MF, E. coli, fecal coliform MPN and coliphage. The second principal component PC_2 is dominated by fecal streptococci and the third principal component PC_3 is dominated by coliphage. The expressions for PC_1, PC_2 and PC_3 are:

$$PC_1 = 0.305\ \ell nX_1 + 0.530\ \ell nX_2 + 0.533\ \ell nX_3 + 0.234\ \ell nX_4 + 0.536\ \ell nX_5$$

$$PC_2 = -0.006\ \ell nX_1 - 0.306\ \ell nX_2 - 0.124\ \ell nX_3 + 0.944\ \ell nX_4 + 0.019\ \ell nX_5$$

and

$$PC_3 = 0.942\ \ell nX_1 - 0.131\ \ell nX_2 - 0.289\ \ell nX_3 - 0.073\ \ell nX_4 - 0.086\ \ell nX_5$$

Forward Stepwise Regression method was used to determine the best regression equation for representing coliphage as a function of other bacteriological parameters. The significance level for entering and deleting the variables are 0.01 and 0.10, respectively. The equation is,

ℓn coliphage = 0.8294+0.2255 ℓn fecal coliform MPN+1.1415 ℓn fecal streptococci

and R^2 = 69%, and the F statistic associated with the coefficients of ℓn fecal coliform MPN and ℓn fecal streptococci is 54.6 which is very highly significant ($p<.01$). This equation can be used to predict coliphage values from fecal coliform MPN and fecal streptococci data.

Part of these results was not unexpected as both coliphage and fecal coliform MPN are broth type measurements and the coliphage

hosts are fecal coliforms. The relationship with fecal streptococci was unexpected and illustrates their common source, feces.

Based on these data and the results of statistical analyses and the fairly consistent ratios observed (Table 1) between fecal coliforms, E. coli, fecal streptococci and coliphage and the present recreational water quality standard of 100 fecal coliforms/100 mL, it would be feasible to propose a coliphage water quality guideline of 20 coliphage/100 mL for fresh recreational waters.

Lake Ontario

To assess the association between coliphage, fecal coliforms, E. coli and the fecal sterols, coprostanol and cholesterol, the observations on these parameters were transferred to logarithms prior to analysis. The logarithm transformations provide a suitable scale for the analysis of bacteriological data, since the variance of bacterial counts increases with the observed count. Also in these analyses, due to the fact some values were not observed quantitatively but recorded as less than or greater than, these values were replaced by their cutoff point (i.e.) a value of <5 is used as 5.

Due to the great variability of the waters sampled, the data from the 26 samples were summarized and a multivariate display produced and shown in Figure 2. To obtain this data display, the coliphage values

were divided into four classes $\leq$ 5, 5-20, 20-100 and greater than 100. For each class the median of the natural logarithm bacterial counts were calculated. The X axis represents the coliphage classes, the Y axis represents ℓn fecal coliforms, E. coli is represented by a line parallel to the Y axis with length proportional to the median of the E. coli in the coliphage class and cholesterol and coprostanol data are presented similarly. The line for cholesterol estimates at the intersection of the coliphage class with ℓn fecal coliform and moves toward the left, parallel to the X axis. The same for coprostanol but the line moves to the right. From Figure 2, it appears that coliphage increases as fecal coliform and E. coli increase and the relationship appears to be non-linear. The association between coliphage and fecal sterols is not quite as consistent, as can be seen in the Figure. The same picture of the various relationships is suggested by Figure 3 which shows the existence of a strong association between ℓn E. coli and ℓn fecal coliform, a medium association between ℓn coliphage and ℓn fecal coliform and a very weak association of cholesterol with ℓn fecal coliform and ℓn E. coli and consequently with ℓn coliphage.

In a further attempt to clarify the relationship between the five parameters the following correlation matrix was prepared:

	Fecal Coliforms	E.coli	Coliphage	Cholesterol	Coprostanol
Fecal coliform	1.00	0.93**	0.60**	0.01	0.17
E. coli		1.00	0.47*	0.1	0.14
Coliphage			1.00	0.04	0.65**
Cholesterol				1.00	0.49**
Coprostanol					1.00

Values marked by * and ** are significant at the 5% and 1% levels, respectively.

Fecal coliform densities show significant correlations with E. coli and coliphage densities. Coliphage is also correlated with E. coli and coprostanol. Cholesterol and coprostanol are highly correlated in these samples.

Furthermore, principal component analysis was used to divide the total variation into five uncorrelated components. The results showed that the first two components contain 80.7% of the total variation. The percentage of explained variation is given below:

The first principal component is dominated by fecal coliform, E. coli and coliphage. The second is dominated by coprostanol and

cholesterol, while the third is dominated by coliphage and cholesterol. The first three components are respectively;

$$PC_1 = 0.55\ \ln x_1 + 0.52\ \ln x_2 + 0.52\ \ln x_3 + 0.14\ \ln x_4 + 0.37\ \ln x_5$$

$$PC_2 = 0.33\ \ln x_1 - 0.35\ \ln x_2 + 0.11\ \ln x_3 + 0.63\ \ln x_4 + 0.60\ \ln x_5$$

$$PC_3 = 0.22\ \ln x_1 - 0.33\ \ln x_2 + 0.54\ \ln x_3 - 0.67\ \ln x_4 + 0.29\ \ln x_5$$

where ln = natural log and x_1, x_2, x_3, x_4, x_5 denote fecal coliforms, E. coli, coliphage, cholesterol and coprostanol, respectively.

Finally, stepwise regression was used to model the ln coliphage using the other four parameters. The results indicate that coliphage can be modelled as a function of Fecal coliforms and E. coli. The model is

$$\ln x_3 = 1.6582 + 0.6512\ \ln x_1 - 0.3305\ \ln x_2$$

with x_1 being the first parameter to enter the regression equation. Thus, it would appear that coliphage counts provide similar indications of fecal pollution as do fecal coliform and E. coli counts. Thus the data from the Lake Ontario study are supportive of the Ottawa River data and the proposal that a recreational fresh water quality guideline of 20 coliphage/100 mL is feasible and practical and would be equally protective as present standards of 100 fecal coliforms per 100 mL.

In trying to establish the ratios between coliphage and fecal coliform and E. coli counts in Lake Ontario waters, a problem was encountered due to the tremendous variation in the samples, e.g. eutrophic waters, almost pristine waters, sewage polluted waters and toxicant laden waters. In order to organize the data it was decided to divide the samples based on fecal coliform counts. Table 2 presents a summary of ratio data. Here it can be seen that the ratios increase with increasing fecal coliform counts and that mean ratio of coliphage to the traditional indicators is similar to those observed in the Ottawa River when fecal coliform densities are less than 1000/100 mL.

Table 2 Ratios of mean fecal coliform and E. coli counts to mean coliphage counts for Lake Ontario water samples.

Fecal Coliform Count Range	Ratio	
	Fecal coliform / Coliphage	E. coli / Coliphage
1-100	1.6:1	0.64:1
101-1000	3.2:1	1.5:1
1001+	22:1	11:1

The ratio data are very supportive of the statistical findings that coliphage levels can be predicted by fecal coliform and E. coli counts.

The lack of statistical relationship between coliphage, E. coli, fecal coliforms and fecal sterols shown in this study confirms the earlier report by Dutka et al. (1974) that did not support the concept of the existance of a consistent, significant relationship between bacterial densities and fecal sterols.

Brazilian Marine Beaches

Nine coastal beaches were sampled 12 times (monthly) from June 1984 to May 1985. These beaches are: I Praia do Tombo, II Praia de Bertioga, III Praia de Enseada, IV Praia Grande, V Praia de Pernambuco, VI Praia das Pitangueiras, VII Praia do Boqueirao, VIII Ponta da Praia and IX Praia do Itarare.

Table 3 summarizes the data from this study and in Figure 4 fecal coliform MPN and coliphage data are displayed in a basic box plot. The upper part of Figure 4 shows the coliphage data and the lower part the fecal coliform data. The box plots are ordered according to the magnitude of the median of the coliphage data, so that the first box plot represents the location with the highest median, the second represents the location with the second highest median and the 9th

represents the location with the lowest median. From the graph (Figure 4), it is clear that fecal coliform counts follow the pattern of the coliphage. For example, locations with high median coliphage counts have high median fecal coliform counts, the symmetries of the box plots for the two parameters are similar and so is the spread. This indicates that the probability distributions of the two parameters are not independent so information available on one parameter provides information about the other.

Table 4 gives the correlation coefficient between the ℓn fecal coliform and ℓn coliphage. Cases marked by * are significant at the 5% level. It is clear that all the correlations are positive which indicates that a consistent pattern of association exists between coliphage and fecal coliforms.

Table 4 Correlation between ℓn fecal coliform and ℓn coliphage.

Sample Site	I	II	III	IV	V	VI	VII	VIII	IX
Correlation	.98*	.11	.57*	.66*	.79*	.94*	.74*	.33	.08

*Significant at the 5% level.

To determine if a constant ratio between coliphage and fecal coliform exists, the following equation

$Y = \alpha + \beta X + e$ is fitted to the data. Here Y refers to coliphage, X represents ℓn fecal coliform, α and β are unknown parameters and e is the random variable. Testing for a constant ratio is the same as testing the hypothesis $H_0: \beta=1$. If H_0 is accepted then the ratio i e^{α}.

For testing H_0, each data set was fitted to the above model. The estimated values of α (intercept) and β (slope) are given in Table 5. It is clear that all slopes are below 1 which casts doubt about the validity of H_0. Performing a formal test for H_0 indicates that a constant ratio is accepted for the first location. Indeed the hypothesis of a constant value for β for all data sets was not accepted at the 5% level.

Another aspect considered was to determine whether the presence of virus is associated with high levels of fecal coliforms and coliphage (Table 6) (Figure 5). Figure 5a gives the plot of the mean ℓn fecal coliform when enterovirus are present against ℓn fecal coliform when enteroviruses are absent. The same is given in Figure 5(b) for coliphage. It is clear that the presence of virus is correlated with high concentrations of fecal coliforms since all the points fall above the 45° line. In the case of coliphage, some points fall below the 45° line so the pattern is not as obvious as for fecal coliforms. In the marine beach study, maximum fecal coliform and coliphage counts were usually encountered during the middle of the summer, January and February. A secondary peak sometimes occurred during the August September period. Median and mean fecal coliform

and coliphage ratios (Table 3) showed greater variations than encountered during the two freshwater studies, Ottawa River and Lake Ontario. Praia do Itarare, Praia das Pitangueiras, Praia de Bertioga and Praia de Pernambuco showed mean fecal coliform/coliphage ratios similar to those observed in the Ottawa River. The data distribution patterns noted at these Brazilian beaches may be typical of subtropical marine waters and freshwater microbiological correlations may not be applicable to marine environments.

These marine studies have produced several interesting findings (Table 6). One of these is the finding of enteroviruses (51 per 40L) in waters that contain only 22 and 15 coliphage/100 mL (Praia de Bertioga) giving a ratio of 172 fecal coliforms to one enterovirus and 118 coliphage to one enterovirus.

Again at Praia das Pitangueiras there were 49 fecal coliforms and 10 coliphage per 100 mL and 12 enteroviruses per 40L. These counts give a fecal coliform to enterovirus ratio of 1633:1 and a coliphage to enterovirus ratio of 333:1.

At Ponta da Praia in two instances, Salmonella were found when the fecal coliform counts were 130 and 330 and the coliphage counts were 15 and 17. Assuming that only one Salmonella existed in the 5 litre sample, we find minimum fecal coliform/Salmonella ratios of 11,000,000:1, coliphage/Salmonella ratios of 77,500:1 and fecal coliform/ enterovirus ratios of 4,900,000:1 and coliphage/enterovirus ratios of 114,000:1. In all the above ratios the coliphage to pathogen ratio was always the lesser.

These widely fluctuating ratios between indicator and pathogen and the presence of pathogens in relatively unpolluted marine waters, based on fecal coliform counts, are strongly supportive of the earlier findings by Dutka (1973) in fresh water that there are no consistent ratios between indicator and pathogen and that pathogen presence cannot be predicted by indicator density. Also from those limited data, it would appear that there is a lower ratio between coliphage and pathogen presence than fecal coliform and pathogen presence.

In summary, from these three studies of a freshwater river, a freshwater lake and marine beaches, it can be stated (a) that within location fecal coliform and coliphages are positively correlated, (b) coliphage values can be indicated or predicted by using fecal coliform MPN, fecal streptococci and E. coli data, (c) it would be feasible to propose a coliphage freshwater quality guideline of 20 coliphage/100 mL for recreational waters, (d) fecal coliform or coliphage counts in marine water are not predictive of the presence of Salmonella and enteroviruses and (e) in marine water where pathogens are found, coliphage/pathogen ratios are smaller than fecal coliform/pathogen ratios.

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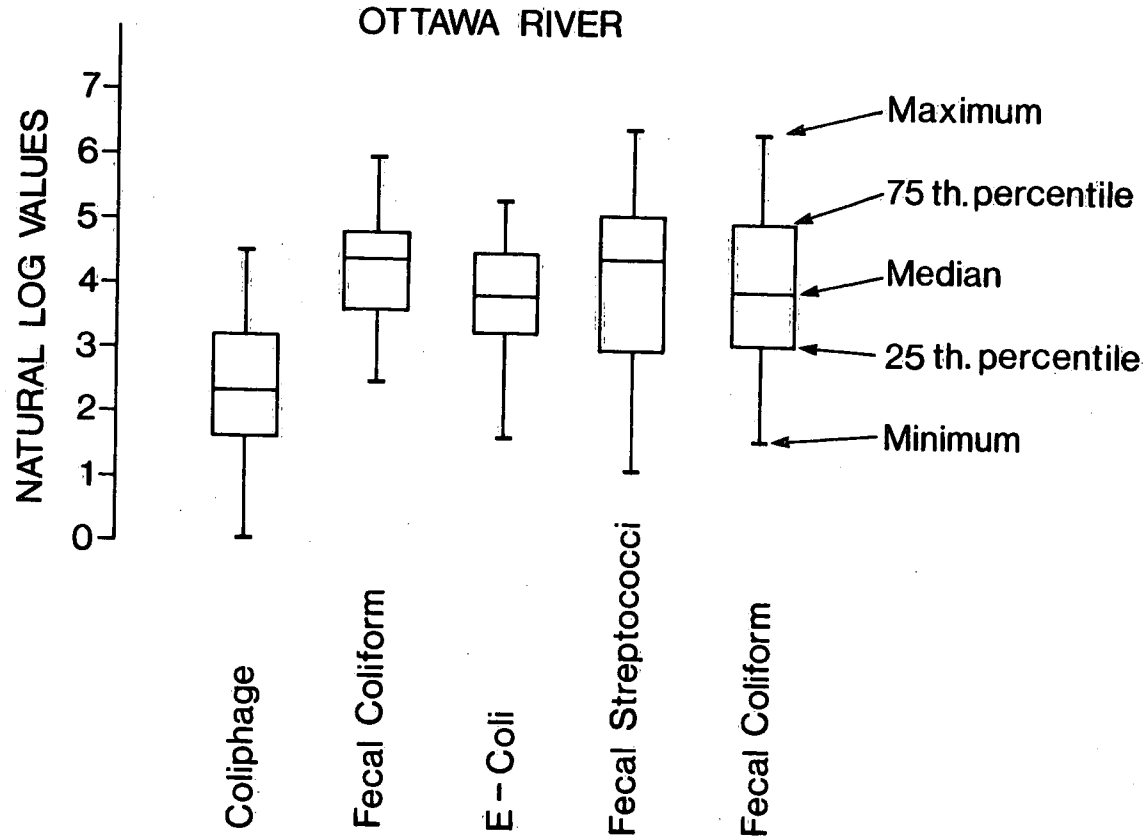

FIGURE 1. BOX PLOTS FOR OTTAWA RIVER DATA
PRESENTED AS NATURAL LOGARITHAMS
SEPT. 1984 to DEC. 1985

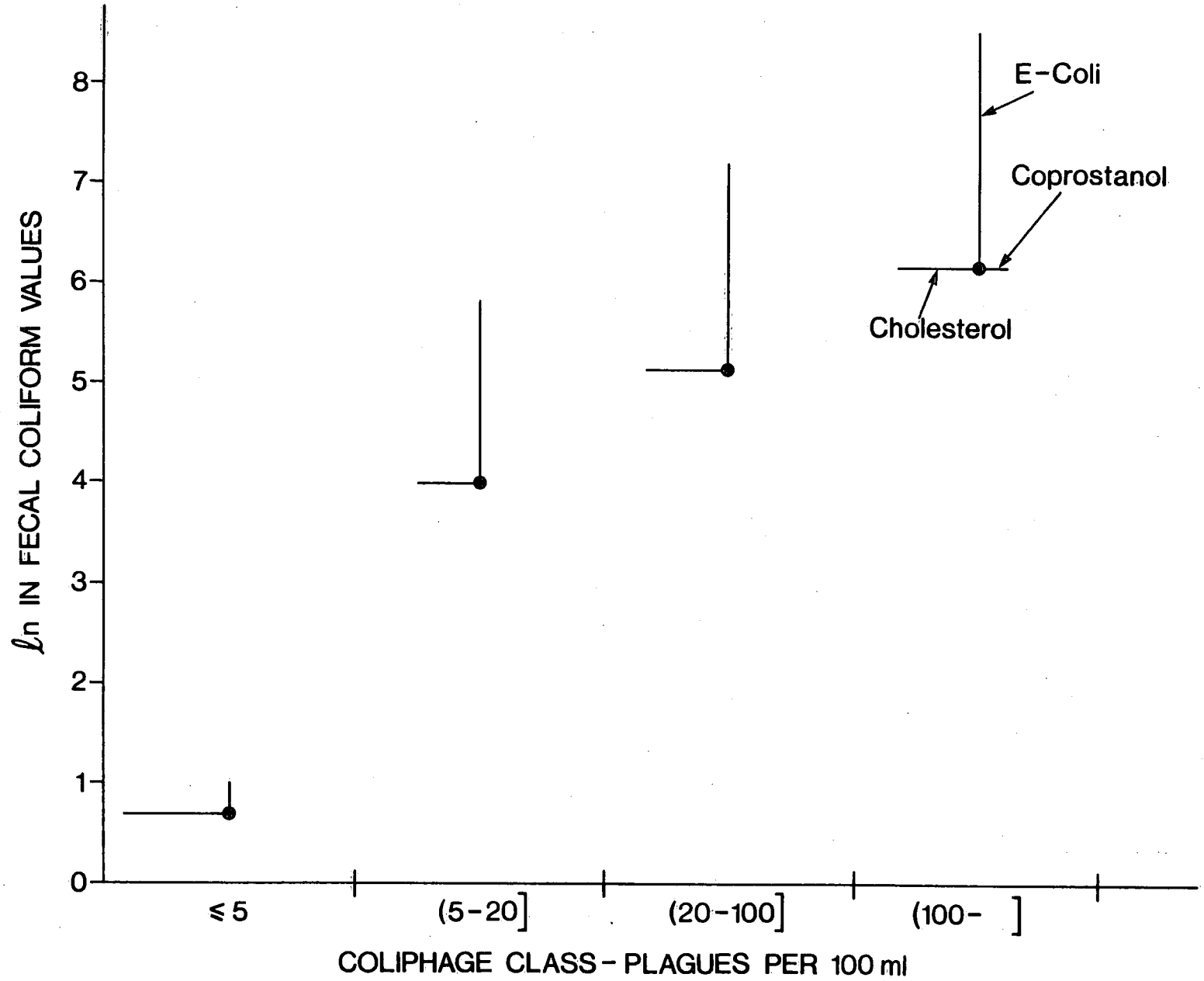

FIGURE 2. MULTIVARIATE DISPLAY OF THE ASSOCIATION BETWEEN VARIOUS INDICATORS, LAKE ONTARIO, 1985

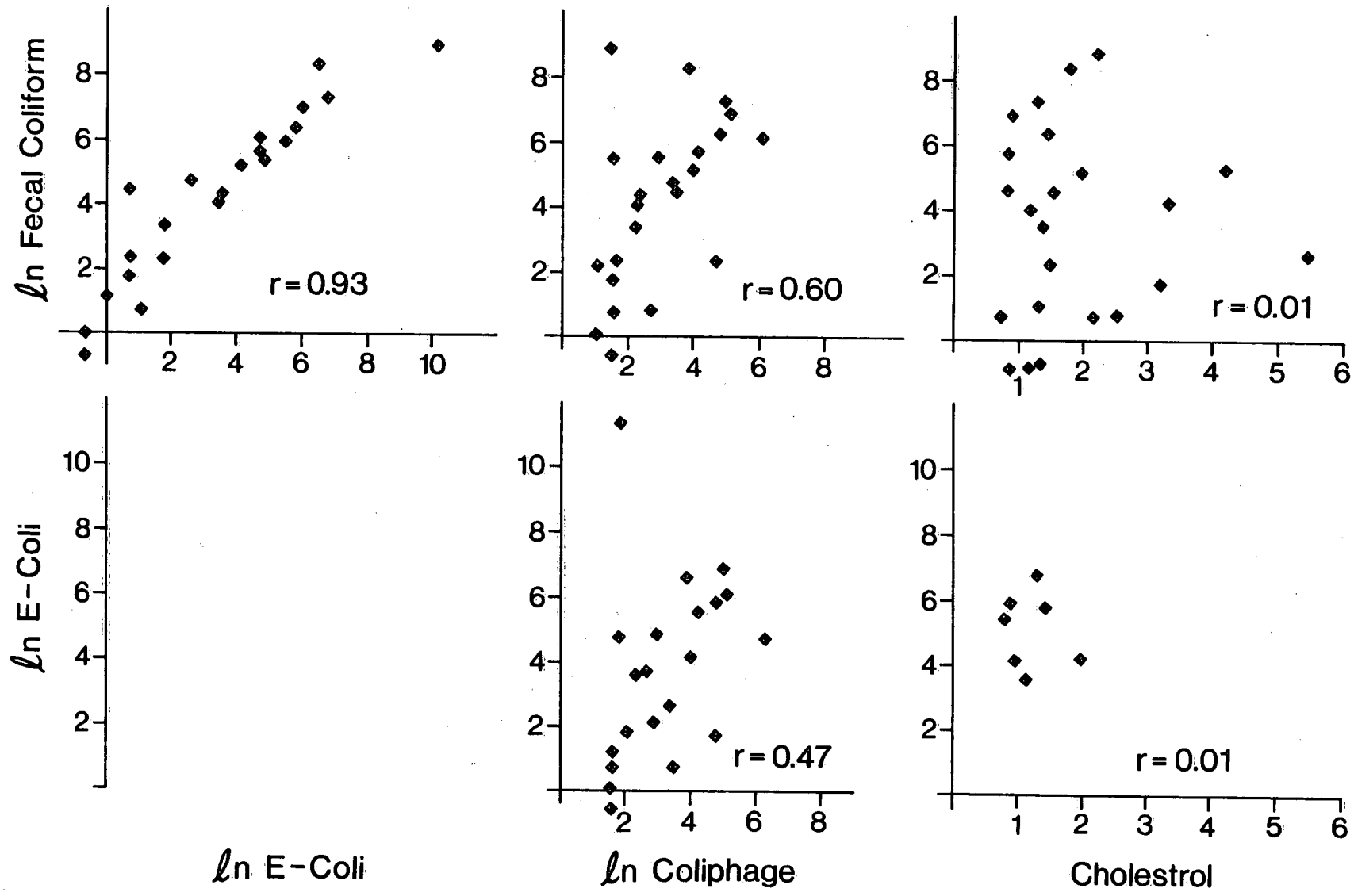

FIGURE 3. SCATTER PLOTS SHOWING ASSOCIATIONS BETWEEN VARIOUS PARAMETERS FROM LAKE ONTARIO SAMPLES 1985

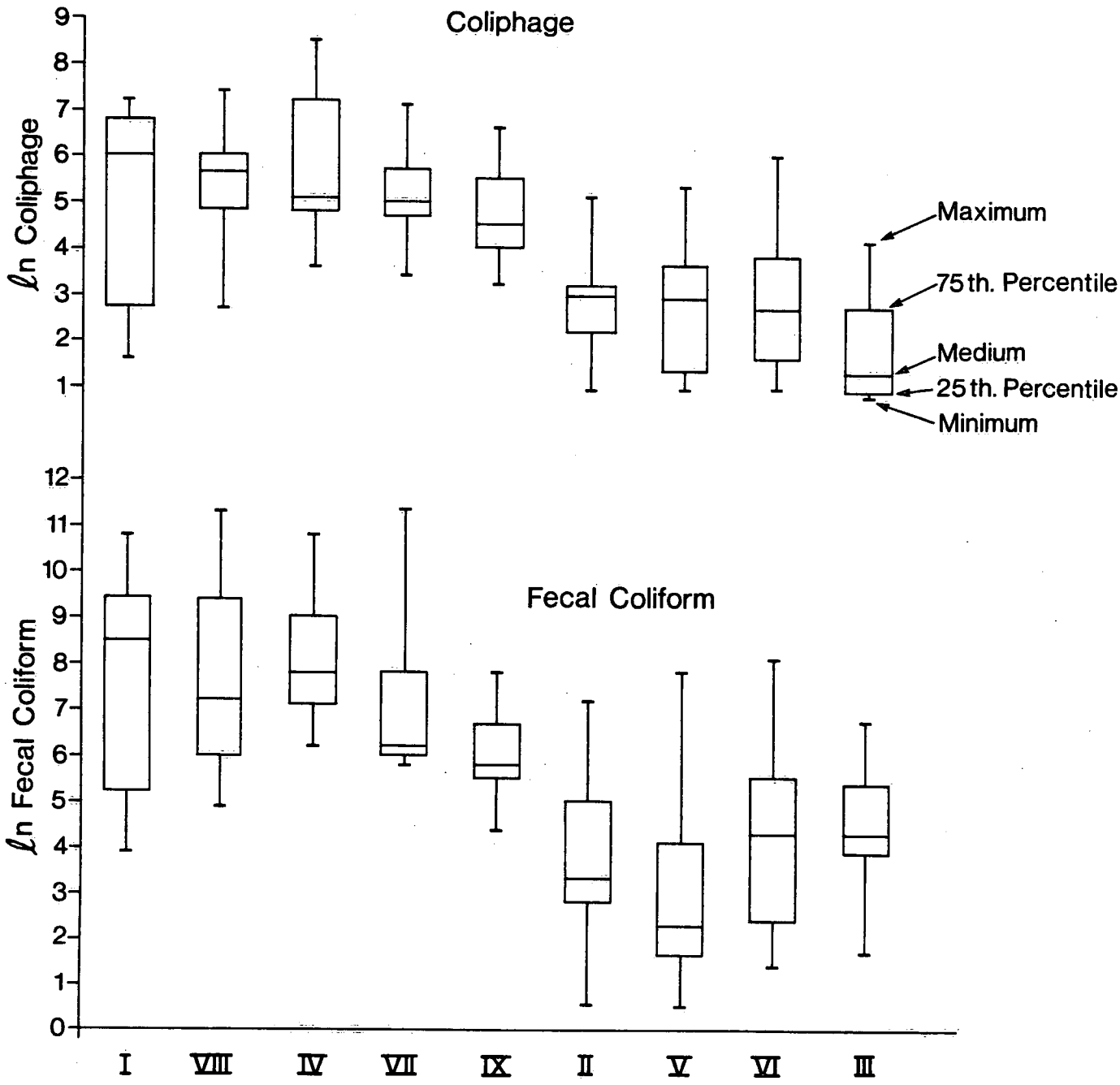

FIGURE 4. BOX PLOT FOR 9 BRAZILIAN MARINE BEACHES PRESENTED AS NATURAL LOGARITHAMS. JUNE 1984 to MAY 1985

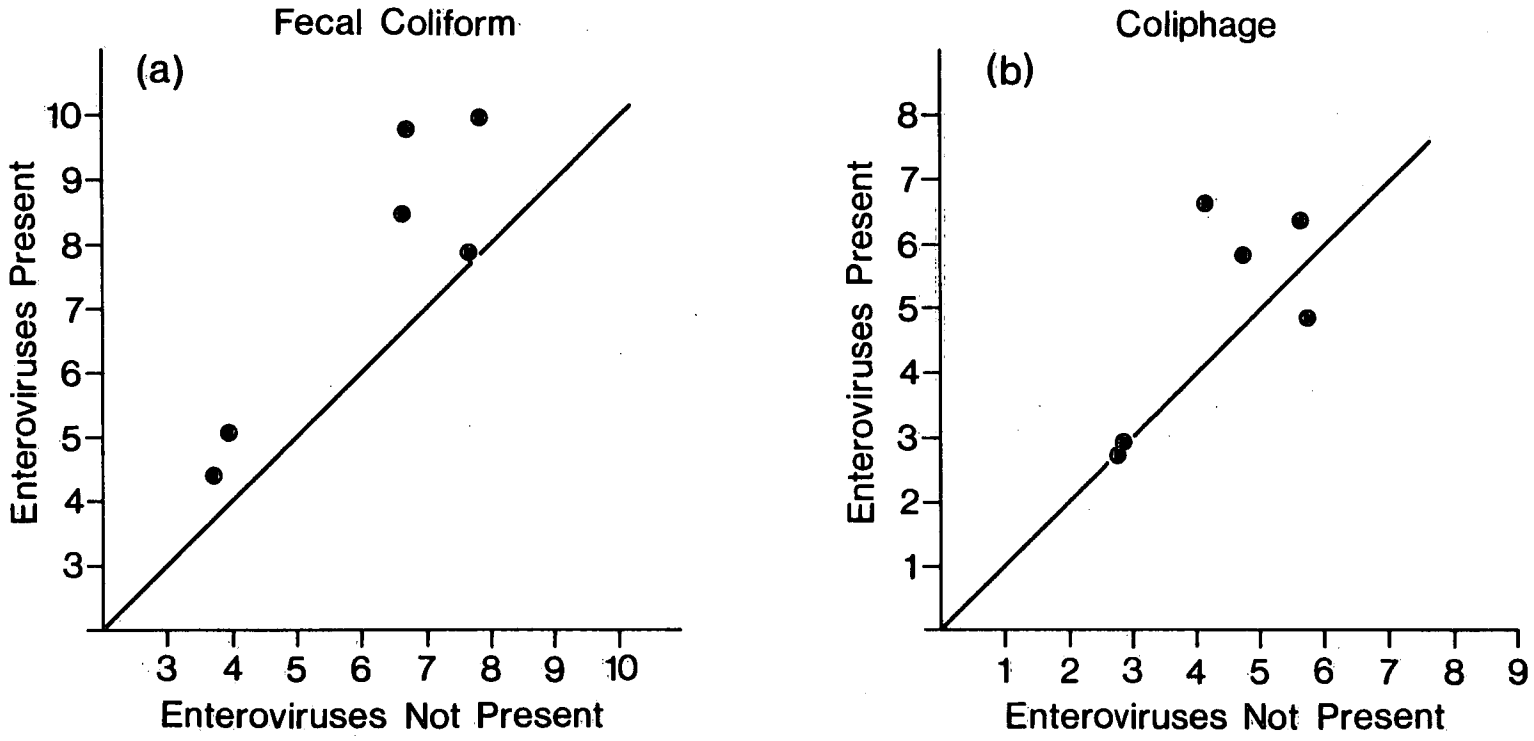

FIGURE 5. RELATIONSHIP BETWEEN MEAN ℓn FECAL COLIFORMS, MEAN ℓn COLIPHAGE AND ENTEROVIRUSES. BRAZILIAN MARINE BEACHES 1984-85

TABLE 1 **Mean microbiological densities and coliphage ratios for various periods of the year, Lemieux Island, Ottawa River, Coliphage-Coliform Study, 1984-1985**

Time Period	Coliphage /100 mL	Fecal Coliforms /100 mL MF	Ratio: Coliphage	Fecal Coliforms /100 mL MPN	Ratio: Coliphage	E. coli /100 mL MF	Ratio: Coliphage:	Fecal Streptococci MF /100 mL	Ratio: Coliphage:
Maximum Count Period Sept.-Oct.	25.7	123.4	4.8:1	188.0	7.3:1	93.8	3.6:1	199.7	7.8:1
Minimum Count Period Jan.-May	11.1	53.1	4.8:1	29.5	2.6:1	31.8	2.8:1	33.4	3.0:1
Rest of Samples	18.9	84.8	4.5:1	126.9	6.7:1	52.2	2.7:1	137.4	7.3:1
Samples exceeding 100 fecal coliform/100 mL	26.9	144.6	5.4:1	221.6	8.2:1	98.0	3.6:1	134.3	5.0:1
Duplicate Samples Sept.25-Dec.4/84	33.2	121.1	3.6:1	144.6	4.4:1	72.0	2.2:1	98.3	3.0:1
Sept. 24-Dec. 4/85	26.9	159.5	5.9:1	255.3	9.5:1	105.0	3.9:1	139.3	5.2:1
Survey Mean	18.8	85.0	4.5:1	97.3	5.2:1	57.3	3.0:1	119.6	6.4:1

TABLE 3 Summary of Brazilian Beach Study Data with Fecal Coliform/Coliphage Ratios and Presence of *Salmonella* and Enteroviruses 1984-1985

Sampling Site		No.	Fecal Coliforms MPN/100 mL Range	Coliphage Plaques/100 mL Range	Median FC/Coliphage Ratio	Mean FC/Coliphage Ratio	No. of Samples	
							Salmonella present/5L	Enteroviruses present/40L
I	Praia do Tombo	12	50-50000	5-1355	12.3:1	23.4:1	2	2
II	Praia de Bertioga	12	2-1300	<5-160	1.4:1	8.1:1	1	1
III	Praia de Enseada	12	5-230	<5-60	20:1	13.9:1	0	0
IV	Praia Grande	12	490-5000	35-4645	15.7:1	11.7:1	0	2
V	Praia de Pernambuco	12	2-2300	<5-195	0.5:1	8.2:1	0	0
VI	Praia das Pitangueiras	12	4-3300	<5-420	5.3:1	6.9:1	1	1
VII	Praia de Boqueirao	12	330-22000	30-1080	3.4:1	78.7:1	4	0
VIII	Ponta da Praia	12	130-80000	15-1550	5.4:1	33.3:1	4	0
IX	Praia do Itarare	12	79-2300	25-685	4.1:1	3.2:1	0	0

Table 5 Summary of Simple Linear Regression:
Dependent Variable: Coliphage
Independent Variable: Fecal Coliforms

Sample No.	Intercept	Slope	R^2
I	-1.41643	0.83391*	0.9543
II	2.54060	0.06584	0.0112
III	-0.26067	0.47008	0.3278
IV	0.38326	0.65706	0.4303
V	1.06505	0.51070	0.6172
VI	-0.10002	0.70065	0.8802
VII	2.49096	0.36290	0.5501
VIII	3.93815	0.19255	0.1102
IX	4.13719	0.08976	0.0063

*Not different from unit slope at the 5% level.

TABLE 6 Relationship of Salmonella presence/5 L and enterovirus presence/40L to fecal coliform and coliphage densities and ratios.

	Site	Fecal Coliforms MPN/100 mL	Coliphage Plaques/ 100 mL	Ratio FC/ Coliphage	Salmonella ±*/5L	Ratio FC/S	Ratio Coliphage/S	Entero-virus/ 40L	FC/ Entero-virus	Coliphage/ Enterovirus
I	Praia do	50000	1090	45.9:1	+	2,500,000:1	54,500:1	17	1,176,470:1	25,647:1
	Tombo	7900	420	18.8:1	-	395,000:1	21,000:1	3	1,053,333:1	56,000:1
		13000	890	14.6:1	+	650,000:1	44,500:1			
II	Praia de	22	15	1.5:1	-	1,100:1	750:1	15	172:1	118:1
	Bertioga	1100	20	55:1	+	55,000:1	1,000:1	0		
III	Praia	49000	1105	44.3:1	-	2,450,000:1	55,250:1	4	4,900,000:1	110,500:1
	Grande	8000	285	28.1:1	-	400,000:1	14,250:1	1	3,200,000:1	114,000:1
IV	Praia das	500	20	25:1	+	25,000:1	1,000:1	-		
	Pitangueiras	49	10	4.9:1	-	2,450:1	500:1	12	1,633:1	333:1
V	Praia de	490	145	3.4:1	+	24,500:1	7,250:1	-		
	Boqueirao	330	165	2:1	+	16,500:1	8,250:1	-		
		220000	1080	203:1	+	11,000,000:1	54,000:1	-		
		13000	515	25.2:1	+	650,000:1	25,750:1	0		
VI	Ponta de	330	15	22:1	+	16,500:1	750:1	0		
	Praia	130	75	1.7:1	+	6,500:1	3,750:1	0		
		80000	1550	51.6:1	+	4,000,000:1	77,500:1	0		
		11000	140	78.6:1	+	550,000:1	7,000:1	0		

*Assume one Salmonella per 5L