STUDIES ON BACTERIAL INDICATORS AND PATHOGEN *VIBRIO PARAHAEMOLYTICUS* IN COCHIN BACKWATER

THESIS SUBMITTED TO
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
MICROBIOLOGY

PRADEEP, R.

DIVISION OF MARINE BIOLOGY, MICROBIOLOGY AND BIOCHEMISTRY
SCHOOL OF MARINE SCIENCES
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
COCHIN - 682 016

JULY - 1986

In memory of

my brother

late Mr. Pramod. R.

DECLARATION

I hereby declare that this thesis entitled 'Studies on Bacterial Indicators and Pathogen *Vibrio parahaemolyticus* in Cochin Backwater' has not previously formed the basis of the award of any degree, diploma or associateship in any University.

Pradeep

Cochin-16

28.7.1986

(PRADEEP. R)

CERTIFICATE

This is to certify that this thesis is an authentic record of research carried out by Mr. Pradeep. R, under my supervision and guidance in the School of Marine sciences, Cochin University of Science and Technology, in partial fulfilment of the requirements of the Ph.D. Degree of Cochin University of Science and Technology and no part thereof has been presented before for any other degree in any University.

Cochin - 16

28.7.1986

Dr. P. Lakshmanaperumalsamy
Reader in Microbiology
School of Marine Sciences
Cochin - 682 016.

ACKNOWLEDGEMENT

I wish to express my sincere appreciation and gratitude to:

Dr. P. Lakshmanaperumalsamy, Reader in Microbiology, School of Marine Sciences, Cochin University of Science and Technology, Cochin-16, my teacher and supervisor, for the scientific freedom, magnanimous support in my research, constant encouragement, valuable advise during preparation of the thesis and his genial understanding which made project, both enjoyable and productive.

Dr. R.R. Colwell, University of Maryland, U.S.A., Dr. T. Mori, Research Institute for Microbial Diseases, Osaka University, Japan and Dr. I. Karunasagar, Associate Professor and Head, Department of Fishery Microbiology, University of Agricultural Sciences, College of Fisheries, Mangalore for donating type and reference strains of Vibrio parahaemolyticus.

Dr. G.J. Bonde, Aarhus University, Denmark, Dr. V.J. Cabelli, University of Rhode Island, Kingston, U.S.A., Dr. B.J. Dutka, National Water Research Institute, Ontario, Canada, Dr. A.L.H. Gameson, Water Pollution

Research Laboratory, Essex U.K., Dr. E.E. Geldreich, Environment Protection Agency, Ohio, U.S.A., Dr. L.R. Beuchat, University of Georgia, U.S.A., Dr. R.R. Colwell, University of Maryland, U.S.A., Dr. M. Fishbein, FDA, Washington, U.S.A., Dr. R. Sakazaki, National Institute of healtH, Tokyo, Japan and Dr. R.M. Twedt, FDA, Ohio, U.S.A. for providing me with a voluminous supply of valuable reprints, books and monographs.

Professor and Head, Department of Computer science, Cochin University of Science and Technology, Cochin-22 for providing the computer facilities and to Miss. V. Suma and Miss. Annie Joseph, M. Tech Students, Cochin University of Science and Technology for their help in statistical analysis of the data.

Director, CIFT, Cochin, for providing necessary library facilities and for the supply of chitin.

Authorities of Cochin University of Science and Technology for providing necessary facilities to carry out the work.

Shri. Subramanian, Fisherman, for helping me in the collection of fish and prawn samples.

Mr. G. Mohan, Mr. K.N. Balakrishnan and Mrs. M. Usha Devi for their arduous task in typing the manuscript neatly.

M/s. Coastal Impex and M/s. Pickwick's copy centre for various technical assistance in preparation of this thesis.

Mr. Balan Panicker, Artist, for drawing the figures neatly in time.

Miss.K.R. Sreekumari, Mrs. and Mr. Bright Singh, Dr. M. Chandrasekharan, M.P. Nandakumar, Johny Joseph, S. Geethamony and Dr. N. Rajendran for helping me in various ways in completion of this work.

Indian Council of Agricultural Research, New Delhi, and Cochin University of Science and Technology for providing me Fellowships during my research period.

Professor C.V. Kurian for going through the manuscript and his valuable suggestions.

Finally to my parents, wife and son for their love, understanding, patience and encouragement.

CONTENTS

1 INTRODUCTION

1.1 PREAMBLE

The coastal areas of any nation are a valuable resource for food and recreation. During recent years the use of marine and estuarine ecosystems for the disposal of sanitary and industrial wastes has increased greatly. The self purification power of the sea is overloaded with the increasing input of wastes. As a result, these aquatic ecosystems are getting eutrophied giving ample chances for the survival and growth of sewage borne microorganisms. They include a large number and variety of infectious and toxigenic agents such as viruses, bacteria, fungi, protozoan and metazoan parasites and biotoxins produced by several species of bacteria and dinoflagellates. In tropical situation, the range of hazard is wide including infections arising directly from water contact or from consuming uncooked or under-cooked sea foods.

The range of presence of bacterial indicator organisms such as total coliforms, faecal coliforms, Escherichia coli and faecal streptococci in an aquatic

environment determines the extent of sewage pollution and portrays the suitability of the environment for recreational or fishing activities. Disease outbreaks due to Salmonella, Vibrio, Shigella, Leptospira, Pasteurella, Mycobacterium and enteropathogenic E.coli through the use of polluted estuarine and coastal environment for food and recreation has increased. Therefore in determining water quality primary emphasis is given to health hazards. Through harvesting fish and prawn from polluted waters, disease outbreaks can be initiated by pathogenic microorganisms of autochthonous to the habitat or by the allochthonous ones brought in through the sewage input.

Vibrio parahaemolyticus has been recognised as an estuarine bacterium, which is responsible for numerous out-breaks of seafood-borne gasteroenteritis. Besides, it has been found to infect fish and prawns.

Cochin, a major commercial and industrial city (seafood landing and exporting centre also) on the south-west coast of India, supports a population of about 6.5 lakhs. The sewage from the Cochin city and neighbouring areas find their way into Cochin backwater which is the northern end of the Vembanad lake. The six major rivers emptying into Vembanad lake also brings with them large amount of enteric pathogens along with silt and sewage. This water body forms

a good nursery and fishing ground for finfish and shellfish. Also many commercial fish and prawn hatcheries and culture farms have been established to raise commercially esteemed species around the backwater system. Hence, it is appropriate to monitor faecal bacteria and pathogenic bacteria like V. parahaemolyticus in this environment to understand the ecology, annual cycle and level of faecal coliforms and V. parahaemolyticus associated with freshly harvested seafoods.

1.2 REVIEW OF PREVIOUS WORK

1.2.1 Distribution of faecal indicator bacteria

From time immemmorial, contamination of water and food with human faeces were considered to be dangerous. The use of bacteria as an indicator of water quality originated in 1880 when von Fritsch described Klebsiella pneumonia and K. rhinoscleromatis as microorganisms of human contamination. In 1885, Escherich found out a bacterium of human faecal origin, Bacillus coli and postulated that such microorganisms could be used to indicate faecal contamination of water. He put forth Bacillus coli as an indicator of faecal pollution and at the beginning this was used in the sanitary evaluation of water bodies. Within two decades, these coliforms were isolated from the intestinal contents of fresh water fishes (Amyot, 1901;

Johnson, 1904). Subsequent studies confirmed that coliform bacteria are not usually associated with the normal intestinal flora of fish and hence their presence in fish indicate recent faecal contamination (Gibbons, 1934 a,b). It was also suggested that fishes, if they acquire pathogenic organisms, could become carriers of such bacteria and pollute distant unpolluted areas (Geldreich and Clarke, 1966).

The use of streptococci as an indicator of water quality could be traced back to 1900 (Houston, 1900). He reported that faecal streptococci were present in polluted water and absent in unpolluted water. For half a centuary interest in the field of streptococcal indicators was slowed down. However in 1950s a renewed interest was seen (Hajna 1951; Allen et al., 1953; Litsky et al., 1955; Slantz and Bartley, 1957). With development of KF Streptococcus agar and broth (Kenner et al., 1961), studies on streptococcal indicator were accelerated. A detailed review on streptococcal indicators has already been made earlier (Kenner, 1978; Kibbey et al; 1978).

Geldreich and Kenner (1969) proposed a faecal coliform to faecal streptococci ratio in differentiating human faecal pollution from faecal pollution of other animal origin. In the faeces of man faecal coliform to faecal streptococci ratio was always greater than 4 while in other farm animals, cats, dogs and rodents this ratio was below 0.7. Though this concept was

originally designed for streams, a number of workers applied this to estuarine and coastal waters with varying degrees of success (Sayler et al., 1975; Carney et al., 1975; Gore et al., 1979 a,b).

1.2.1.1 Estuarine and coastal waters

Studies on bacterial indicators in coastal waters were initiated in 1950 (Stevenson, 1953; Moore, 1954; Nusbaum and Garver, 1955; Orlob, 1956; Moore, 1959) and significance of coastal bacterial pollution was recognised throughout the world subsequently. As a result, numbrous reports on the distribution of bacterial indicators in estuarine and coastal waters of different countries have appeared. These reports also included survival studies of these bacterial indicators in coastal waters and critical evaluations of them in indicating the presence of pathogenic microorganisms.

Extensive studies on the distribution of indicator bacteria in coastal waters of Texas were carried out (Gerba et al., 1977; Goyal et al., 1977). Seasonal occurrence and distribution of bacterial indicators and pathogens in Chesapeake Bay (Sayler et al., 1975; Carney et al., 1975) and the bacteriological pollution of the Long Island Sound were also worked out in detail (Bireley and Buck, 1975; Dudley et al., 1977). Similar works on Californian coast (Kim, 1975),

Biscayne Bay beaches (Buck, 1976), coastal waters of Puerto Rico (Grimes et al ., 1984) and New York Bight (Babinchak et al., 1977) were also reported from U S A.

The bacteriological quality of Canadian coastal recreational waters were assessed by Robertson (1984). Hashimoto et al (1976) and Yoshikura et al (1980) studied the distribution of faecal indicator organisms in coastal sea waters of Fukuyama and Osaka respectively in Japan. Similar studies from Shizuoka region were also reported earlier (Ogawa, 1973).

Bacterial pollution of the Bristol Channel (Ware et al., 1972), estuaries of Mersey (Dillon and Sellers, 1984) and River Lagen (Parker et al., 1979) were studied in United Kingdom. Investigations on bacterial indicators in Italian coastal waters were many (Lombardo, 1973; Parvis et al., 1975; Boeddu et al., 1977; Izzo et al 1983, Volterra et al., 1985). Similarly, there were a number of reports from French coast also (Leclerc, 1971; Moreau et al., 1971) ; Oger et al., 1974; Breittmayer and Gauthier, 1978) . Yoshpe and others had done extensive studies on the hygienic quality of coastal waters of Israel (Yoshpe and Shuval, 1972; Yoshpe, 1981, 82).

Reports on the distribution of bacterial indicators in coastal environment of other countries include Burgess (1974)

from Australia; Papadakis (1972) from Greece; Kueh (1974) from China; Sales (1976) from Chile; Fernandez (1973) from Venezuela; Owens (1978) from Malaysia; Thayib and Suhadi (1979) from Indonesia; Hirn et al (1980) from Finland; Kim and Chang (1981) and Kim (1983) from Korea; Velescu (1982) from Romania; Pinon and Pijck (1972) and Yde et al (1980) from Belgium coast.

1.2.1.2 Association with seafoods

Isolation of coliform bacteria from fishes dates back to the beginning of this centuary (Amyot, 1901; Johnson, 1904). They were isolated from marine fishes (Gibbons, 1934 a,b) and oysters and oyster waters (Perry and Bayliss, 1936) . Retention of E. coli in the intestine of trout upto fourteen days after giving food and water dosed with E.coli was reported (Glantz and Krantz, 1965). In an elaborate study on the occurrence , distribution and persistence of coliforms, faecal coliforms and faecal streptococci in the intestine of various fishes, Geldreich and Clarke (1966) came to the conclusion that the composition of the intestinal flora is related to the level of contamination of water and food in the environment. Microbiology of aquatic organisms are influenced to a great extend by their feeding habits. Because of their bottom dwelling and detritus (rich in microorganisms) feeding nature, they contain high loads of microorganisms than planktivore fishes (Natarajan et al., 1979).

Most of the microorganisms associated with freshly harvested seafoods are eliminated by chlorination and other treatments during processing. Hence from the commercial and public health point of view extensive works on bacterial indicators were done in processed seafoods (Larkin et al.,1956; Fujiwara et al ., 1972; Nickerson and Pollak, 1972; Chang et al., 1975; Baross et al., 1977; Wood et al., 1983; Greenwood et al., 1985). Informations on bacterial indicators associated with live or freshly harvested estuarine and coastal organisms are mostly confined to filter-feeding organisms such as shellfishes. Because of their unique feeding mechanisms, they accumulate large number of microorganisms including pathogens during their feeding process (Cabelli et al., 1970; Plusquellec et al., 1983; Timoney and Abston, 1984; Kelly and Dinuzzo, 1985). Bacterial indicators associated with shellfishes were reported from USA (Zapatka and Bartolomeo, 1973), UK (Ayers, 1975, Al-Jebouri and Trollope, 1981), Canada (Bernard, 1973; MacLean, 1978), China (Leung et al., 1975; Morton and Shortridge, 1976), Nigeria and Ghana (Ottunola et al., 1983), Florida (Hood et al., 1983), Chile (Tello et al., 1970), Tokyo (Kakubo et al., 1978) and Italy (Volterra et al., 1984).

Studies on bacterial indicators and specific pathogens in freshly harvested finfishes were reported from various geographic locations. Andrews et al (1977) surveyed three

hundred and thirty five fresh and three hundred and fourty two forzen samples of catfish (Ictalurus punctatus) for faecal indicators and pathogens such as Salmonella, Arizona, Shigella and Edwardsiella. Faecal coliform values for 70.7% of fresh and 92.4% of frozen samples were 400 organisms per g.132 raw foods and 593 ready-to-eat foods were evaluated by Miskimin et al (1976) for the presence of faecal indicator bacteria and food borne pathogens like Staphylococcus aureus, Clostridium perfringens and Salmonella. Significant corelations were found between the pathogens and the indicator ranges. Different genera of enteric bacteria present in kindneys and intestines of 192 carp (Cyprinus carpio) and 49 white suckers (Catostomus commersoni) were examined by Souter et al (1976). They found that Proteus and Enterobacter were the dominant genera. Enterobacter was found to be dominating among the coliform flora of migrating sockeye salmon, Oncorhynchus nerka (Strasdine and Dubetz, 1974). Alimentary tracts of teleisotei fished along the coast of Bari were found to harbour differing levels of E. Coli (Marano et al., 1974). Bacterial densities in total plate counts, coliforms and V. parahaemolyticus in Chromis notatus were found to be higher in August and September (Ahn and Hwang, 1975). Nuhi and Khorasani(1981) investigated faecal coliform and faecal streptococci densities in four species of fishes captured from Amir - Kolayeh Lagoon.

1.2.1.3 Environmental factors influencing the distribution and growth of coliforms.

Survival and growth of coliform bacteria in estuarine and coastal waters are influenced by a number of physicochemical and biological factors. Extensive works have been carried out on the response of coliforms to these factors.

Solar radiation was reported to have detrimental effect on coliform bacteria (Gameson and Saxon, 1967; Gameson et al., 1971, 73; Gameson and Gould, 1975; Chamberlin and Mitchell, 1978; Grigsby and Calkins, 1980; Fujioka et al., 1981; Kapuscinski and Mitchell, 1981; 83; Fujioka and Narikawa, 1982). This effect was primarily attributed to the UV fraction of the solar radiation. McCambridge and McMeekin (1981) and Rhodes and Kator (1984) were of opinion that the decline of E. coli in estuarine waters were greater when microbial predators and solar radiation act together than their independent action.

In estuarine and coastal waters, coliforms are acted upon by protozoan predation (Roper and Marshall, 1978; McCambridge and McMeekin, 1979, 80a) and viruses parasitic on bacteria (Carlucci and Pramer, 1960 b). The removal of E. coli from estuarine water by lytic bacteria and indigenous protozoa were reported (Enzinger and Cooper, 1976; Roper and Marshall,1977).

McCambridge and McMeekin (1980 b) observed that protozoan predation on Salmonella typhimurium and E.coli had an optimum temperature of 15-20°C whereas bacterial predation increased with temperature. Mitchell (1971) described the imbalances induced in autochthonous microbial predators by the entry of allochthonous microorganisms.

Pike et al (1970) reported that mortality of coliform bacteria increased with temperature. McFeters and Stuart (1972) also observed a sharp decline in E.coli population when temperature was increased from 5 to 25°C. Similar effects of temperature on coliform bacteria were also reported (Faust et al; 1975; Alton and Rakhno, 1979a; Oragui and Mara, 1983 and Rao and Boopathy, 1985).

Carlucci and Pramer (1960 a) reported that sea water usually has a p^H of 8.0 and it does not favour survival of E.coli. McFeters and Stuart (1972) observed that optimum p^H for survival of E.coli was between 5.5 and 7.5 and they rapidly declined below and above these values.

The detrimental effects of salinity on coliforms and E.coli were already reported (Carlucci and Pramer, 1960 a, Pike et al., 1970; Faust et al., 1975; Anderson et al., 1979). The degree of injury was proportional to salinity.

Faust et al (1975)observed survival of E.coli cells directly proportional to dissolved oxygen concentration. Freshwater isolates of E.coli showed a negative relationship with dissolved oxygen (Hanes et al., 1964).

Availability of nutrients was an advantage for coliforms in nullifying the adverse effects of some environmental factors. Nutrients like phosphorous and nitrogen, and organic substances of sewage origin were reported favouring survival and growth of E.coli (Carlucci and Pramer, 1960 a). However, Moebus (1972 a) observed that in synthetic and filter sterilized natural sea water the inactivation of coliform bacteria increased by addition of ZoBell's broth, peptone or glucose. He postulated that actively metabolizing cells are sensitised much faster than resting cells. Burke and Baird (1931), Vaccaro et al (1950), Orlob (1956), Savage and Hanes (1971) and Bethoux and Montegut (1976) were of opinion that addition of organic nutrients decreased bactericidal effect of sea water.

The response of coliforms to an environmental stress also depends on the duration and intensity of the stress. Scarpino and Pramer (1962) observed a linear relationship between death of E.coli in sea water and time. Similar observations were also reported by Bianchi et al (1976) and

Dawe and Penrose (1978).

Anticoliform activity of Skeletonema costatum was reported by Sieburth and Pratt (1962). During 1969 and 1970, the antibacterial actions of North Sea water were found to be positively correlated with the life cycle of several diatom species (Moebus, 1972b). Acrylic acid produced by certain unicellular algae were inhibitory to coliforms (Brown et al., 1977). In the Gulf of Finland, Hirn et al (1980) did not find any significant relationship between phytoplankton and coliform bacteria.

Survival of coliform bacteria associated with bottom sediment was investigated extensively (Grimes, 1975; Simmann and Rheinheimer, 1975; Gerba and McLeod, 1976; Babinchak et al, 1977; Chan et al.,1979; Edenborn and Renwick, 1981; Hood and Nes , 1982; Izzo et al., 1983; Volterra et al., 1985). Sediments with their micro-environment provide protection from predator organisms. An inverse relationship was found between grain size and bacterial density (Chan et al., 1979)

Other factors related to the survival of coliforms in estuarine and coastal waters are suspended particulate matter (Moebus, 1972c; Bitton and Mitchell, 1974; Faust et al., 1975; Ogawa, 1977,a,b) and pollution of various origin

(Gerasimenko, 1977; Figueiredo et al., 1978; Rao and Boopathy, 1985).

1.2.2 Distribution of Vibrio parahaemolyticus

History of V. parahaemolyticus can be traced to the "Shirasu food poisoning" which occurred in Osaka prefecture, Japan on October 21, 1950. Bacteriological investigation of this outbreak was carried out by Fujino and he isolated a hemolytic, fat, rod shaped and bipolar staining bacterium which he named as Pasteurella parahaemolytica (Fujino et al., 1953). Another food borne outbreak occurred on August 21, 1955 at Yokohama National Hospital involving 120 cases. Takikawa isolated a halophilic bacterium as the etiological agent and named it as Pseudomonas enteritis. Later, this was found to be very similar to Pasteurella parahaemolytica. Through the years, the nomenclature and systematic position of this bacterium underwent several modification and Sakazaki et al (1963) proposed the name V. parahaemolyticus on the basis of several morphological, cultural and biochemical charactors.

After the discovery of V. parahaemolyticus in 1950 (Fujino et al., 1951) extensive works on the clinical, epidemiological and ecological aspects of the bacterium were

carried out in Japan. For about one and a half decade these works were mostly confined to Japan because V. parahaemolyticus initiated - gastroenteritis was thought to be a problem confined to Japan and the Far East. However, in the years after 1966 interest on the study of V. parahaemolyticus was aroused in a number of countries and this resulted in accumulation of a vast body of literature from ecological to molecular aspects of this bacterium. The distribution of V. parahaemolyticus to estuarine and coastal environment and in association with seafoods has been reviewed by Natarajan et al (1978), Abraham (1981) and Nair (1981). Reports on this bacterium in coastal waters after 1980s are reviewed here.

1.2.2.1 Estuarine and coastal waters

During summer season V. parahaemolyticus was isolated in large numbers from Chesapeake Bay water and sediment (Colwell et al., 1981) and a high correlation was observed between salinity and population size of this species. Larson et al (1981) also isolated V. parahaemolyticus from Danish coast during summer months. Occurrence of this bacterium in marine organisms, water and sediment were reported from Kenya (Binta et al., 1982) and Egypt (El-Sahn et al., 1982). Other reports from coastal water bodies were from

Julu Harbor (Kim and Oh, 1982), Puget Sound (Weagant and Kaysner, 1982); Nova Scotian coastal waters (Robertson and Tobin, 1983); Rhode Island (Watkin and Cabelli, 1985), Jakarta Bay (Molitoris and Joseph, 1985) and Japan (Shinoda et al., 1985).

1.2.2.2 Association with seafoods

Among the one hundred and seventy seafood samples examined in Lebanon, Abdelnoor and Roumani (1980) isolated V. parahaemolyticus from two fishes and one crab samples. This bacterium was found to be pathogenic to a snail, Biomphalaria globrata (Ducklow et al., 1980). Population of V. parahaemolyticus in Malaysian shrimp was estimated through various stages from catch to frozen product (Cann et al., 1981) and it varied from nil to 4 x 10^4/g. 4.5% of seafish and 5.5% of shellfish collected from Kenya were positive for V. parahaemolyticus (Binta et al., 1982) 88% of the hemolymph of the blue crab Callinectes sapidus collected from Galveston Bay contained various Vibrio spp and their density varied from 10^3 to 10^5 per ml. V.parahaemolyticus was the most prevalent of the pathogenic Vibrio spp and it was detected in 23% of the hemolymph samples (Davis and Sizemore, 1982). Low levels of V. parahaemolyticus were observed in sea urchin, clams and wedge shells collected along Egyptian

coast (El-Sahn et al., 1982). Cells of E.coli were depurated faster than V. parahaemolyticus and V. harveyi by the hardshell clam (Greenberg et al., 1982). In the Long Island oysters tested 12 of 36 samples contained V. parahaemolyticus at a range of 3.6 to 23 cells per gram (Tepedino, 1982). Growth of V. parahaemolyticus in opened and unopened Sydney Rock oysters were studied by Eyles et al (1985). An outbreak of gastro-enteritis and wound infection were reported by Nolan et al (1984) and in another instance a kanagawa negative strain of V. parahaemolyticus was isolated from a wound infection (Johnson et al., 1984).

1.2.2.3 Effect of physico-chemical parameters on V.parahaemolyticus

Effect of physico-chemical parameters such as temperature (Matches et al., 1971; Goldmintz et al., 1973; Johnson and Liston, 1973; Johnson et al., 1973; Thomson and Thacker, 1973; Bradshaw et al., 1974; Goatcher et al., 1974), salt concentration (Nelson and Potter, 1976; Ro and Woodburn, 1976) and various proteins (Beuchat and Jones, 1979) on growth and survival of V. parahaemolyticus in various kinds of seafoods and tissue homogenates were reported. Vanderzant and Nickelson (1972), Ermolina and Shikalov (1975) and James (1983) also studied the interaction of temperature, p^H and NaCl concentration on growth and survival of this bacterium in seafoods or tissue

homogenates. However, the effect of enviornmental factors on the growth and survival of V. parahaemolyticus in semi-synthetic media are limited. In the light of the present study, the review is restricted to reports on the effect of enviornmental factors on V. parahaemolyticus in semisynthetic media such as nutrient broth or tryptic soy broth.

Growth of V. parahaemolyticus is adversely affected by lower and higher temperatures. Asakawa (1967) reported that inactivation of this bacterium was higher at - 10°C than at - 20°C whereas survival was more at 0°C. NaCl has a protective effect on survival of this bacterium of lower as well as higher temperatures (Covert and Woodburn, 1972; Beuchat, 1973, 74, 75; Jackson, 1974). Beuchat and Worthington (1976) observed a change in the ratio of saturated to unsaturated fatty acid in V. parahaemolyticus when temperature was increased. Membrane damage took place when these bacteria were exposed to 2°C (van den Brock and Mossel, 1977).

Growth of V. parahaemolyticus was observed at p^H4.8 at 5°C (Beuchat, 1973). At p^H 7 the organism exhibited least sensitivity to heat treatment (Goldmintz, 1974; Beuchat, 1975). Formation of lateral flagella was inhibited under an alkaline p^H whereas monotrichous flagellation was not affected (Kimura et al., 1979).

Ions usually have a protective effect on *V. parahaemolyticus* when exposed to extremes of temperature tolerance (Covert and Woodburn, 1972; Beuchat, 1973,74,75). In experimental studies with sodium, potassium and lithium ions, this bacterium did not grow in the absence of sodium ions (Rottini *et al.*, 1974) but Palasuntheram (1981) did not find any such specific sodium ion requirement. Magnesium and potassium ions were found to assist in recovery from thermal injury (Heinis *et al.*, 1977). Similarly, recovery of chill - stressed *V. parahaemolyticus* was enhanced by the presence of magnesium and iron salts (Lin and Beuchat, 1980).

Among the biological agents acting against *V. parahaemolyticus* in estuarine and coastal waters, *Bdellovibrio* is the most prominant one. These are not host specific and act on other Vibrio spp also. (Miyamoto and Kuroda, 1975). From eight of nine sampling stations this parasite was isolated in Chesapeake Bay (Williams *et al.*,1980). Among the marine bacterial flora *Vibrio* and *Pseudomonas* were found to be highly affected by *Bdellovibrio* (Horie and Kobayashi, 1981). Apart from the *Bdellovibrio*, marine phytoplankton also were found to inhibit growth of *V. parahaemolyticus* in coastal waters (Nakayama and Ohno, 1981). Goatcher and Westhoff (1975) observed repression of this bacterium by a *Pseudomonas* sp.

Growth of V. parahemolyticus was found to be inhibited by irradiation (Matches and Liston, 1971), glycerine (Chun et al., 1972), distilled water (Lee, 1972), water activity (Beuchat , 1974, 75), hydrostatic pressure (Schwarz and Colwell, 1974), spices and organic acids (Beuchat, 1976; Robach and Hickey, 1978), and iodophor (Gray and Hsu, 1979; Chandramohan et al 1980). Chitin was reported to be a major factor favouring the growth of V. parahaemolyticus in estuarine and coastal waters (Kaneko and Colwell, 1975).

1.2.2.4 Antibiotic and metal sensitivity of V. parahaemolyticus

A number of reports on the antibiotic sensitivity pattern of V. parahaemolyticus are available. Sakazaki et al (1963) observed that among a large number of V. parahaemolyticus strains tested, all were sensitive to tetracycline and chloramphenicol. The concentrations of these antibiotics were 100 and 40 mcg/disc respectively. In the studies of Chatterjee et al (1970) this bacterium was found sensitive to tetracycline, chloramphenicol, streptomycin, kanamycin and polymyxin-B. Apart from these antibiotics Sanyal et al (1973) found sensitivity of this bacterium to neomycin and gentamycin also. V. parahaemolyticus isolated from gastroenteritis cases and sea foods in Jakarta when subjected to antibiotic tests (Bonang et al., 1974) exhibited sensitivity to doxycycline,

tetracycline and bactrim but less susceptible to erythromycin and josamycin. Joseph (1974) reported the most inhibitory action of chloramphenicol, gentamycin, nalidixic acid and tetracycline on V. parahaemolyticus and the highest resistance to ampicillin.

In clinical and epidemiological studies on V. parahaemolyticus in Calcutta, Sircar et al (1976) and Sen et al (1977) reported gentamycin and chloramphenicol as most effective against this bacterium whereas ampicillin, kanamycin and streptomycin were ineffective. Apart from chloramphenicol, Kaneko and Colwell (1978) observed high sensitivity of V. parahaemolyticus to furacin, furoxone, neomycin, novobiocin and kantrex. James (1983) reported chloramphenicol and dihydrostreptomycin as most effective against this bacterium. Karunasagar and Karunasagar (1985) reported resistance of V. parahaemolyticus to 0/129 compound.

Bacterial interaction with various metals have been reported from different countries. Bacterial resistance to mercury and their role in mercury transformations were reported (Olson and Cooper, 1974; Walkar and Colwell,1974; Olson et al., 1979; Gauthier et al., 1985a). Kurata et al (1977) observed high incidence of nickel tolerant bacteria in water and sediments of the sea of Aso and they attributed this to the industrial waste containing high concentrations of nickel

dumped into this area. Transmission of arsenic resistance in Enterobacteriaceae by conjugation and through phage was reported (Smith, 1978). In a comparative study of media, for their ability to neutralize the bacteriostatic effect of silver, Tilton and Roseberg (1978) found out that tryptone glucose agar and tryptic soy agar were more neutralizing than eosin methylene blue agar. In chlorine free water distribution systems, low levels of copper were found to injure majority of coliform bacteria (Domek et al., 1984). Copper was reported to be more toxic to Vibrio alginolyticus in anaerobic culture than aerobic culture (Schreiber et al., 1985). Role of bacteria in reduction of copper toxicity by formation of complexes were reported (Rho, 1984). Apart from this, there are a number of reports on association of antibiotic resistance with heavy metal resistance (Allen et al., 1977 ; Austin et al., 1977; Marques et al., 1979., Devanas et al., 1980; Sjogren and Port, 1981; Calomiris et al., 1984; Pujol et al., 1980; Timoney et al., 1978; Kadri and Salem, 1985).

1.2.3 Indian works on faecal indicator bacteria

Importance of bacterial indicators has been recognized in India as early as 1939 (Raghavachari and Iyer, 1939). However, such studies were mostly confined to freshwater habitat, clinical environment and processed foods. Due to

increasing urbanization and discharge of untreated and primarily treated sewage into estuarine and coastal waters, there developed a basic need for the study of the bacterial indicator organisms of sewage origin in estuarine and coastal waters of India.

1.2.3.1 Estuarine and coastal waters

Bacterial pollution studies in inshore waters of Kerala and Madras were carried out by Sreenivasan (1964). The coastal fishing villages of Madras coast was commented as a source of faecal pollution and highlighted the role of tidal water in the flushing of the estuary (Azariah and Subramaniam, 1982). Sastry et al (1969) observed that enterococci were not detected in the absence of coliforms and Sen and Ghosh (1970) found enterococci index superior over coliform index in assessing water quality.

Incidence of pollution in coastal waters of Bombay was reported to be of recurring nature and the primary source of this pollution was raw or improperly treated sewage (Dwivedi and Abidi, 1977). A low level of bacterial pollution was noticed in Mandovi and Zuari estuarine waters at Goa (Row, 1981). Coliform densities in these waters ranged between 0 and 1100/100 ml. Raveendran et al (1978) conducted a seasonal study on faecal

pollution of Cherai beach and on the basis of FC/FS ratio the faecal pollution was found to be from non-human source. Gore *et al* (1979 a,b; 80) also reported faecal pollution of Cochin backwater and a few beaches in Kerala. In a six month period study in Cochin backwater, Lakshmanaperumalsamy *et al* (1981) reported the occurrence of faecal indicator bacteria, *Salmonella*, *Staphylococci*, *Pseudomonas*, *V. parahaemolyticus* *Aeromonas* and *Clostridium*. From an ecophysiological point of view, Chandrika (1983) demonstrated the seasonal variations of faecal indicator bacteria at several stations in Vembanad lake and other estuarine systems in south-west coast of India.

1.2.3.2 Association with sea foods.

Venkataraman and Sreenivasan (1953) studied the coliform and streptococcal groups of bacteria occurring in the intestinal tract of various fishes. Quantitative and qualitative studies on the bacterial flora of fresh sardines and marine fishes and prawns were reported by Karthiayani and Iyer (1967, 750). Different IMViC types of coliforms were isolated by Iyer and Pillai (1971) from various processed fishery products. Rao and Gupta (1978) isolated enteropathogenic *E. coli* from marine fishes along the coast of Kakinada. This was the first reported

case of enteropathogenic E. coli in sciaenids and cat fish from India. The significance of faecal indicator bacteria in seafood was reviewed and preventive measures were suggested by Iyer (1979). High incidence of faecal coliform and E.coli in finfish and shellfish from Vellar estuary was reported by Sivakumar et al (1980) and Lakshmanaperumalsamy et al (1986). Occurrence of E.coli, faecal streptococci, and coagulase positive staphylococci in Perna indica cultured at Vizhinjam was reported by Pillai (1980). In Tuticorin area, Durairaj et al (1983) observed a low incidence of faecal coliforms, and absence of Salmonella, V. cholerae, coagulase positive staphylococci and faecal streptococci.

1.2.4 Indian works on V. parahaemolyticus

Though V. parahaemolyticus was described as an etiological agent of seafood borne gastroenteritis in Japan as early as 1951 (Fujino et al., 1951), the first clinically confirmed cases with this bacterium from India was reported in 1970 (Chatterjee et al, 1970; Neogy et al; 1970). This was followed by a series of clinical reports in the succeeding years (Sakazaki et al; 1971, Chatterjee and Neogy, 1972a;

Chatterjee and Sen, 1974; Deb et al., 1975; Sircar et al., 1976 and Huq et al ., 1979). Apart from seafoods, transmission of V. parahaemolyticus mediated through flies was reported by Chatterjee et al (1978). In a six month period observation, 2 out of 74 gastroenteritic cases admitted in the GB Pant hospital, Port Blair were reported to be due to V. parahaemolyticus (Lall et al., 1979).

The mechanism of pathogenicity and toxin production in V. parahaemolyticus was explained (Bhattacharya et al., 1971; Guhathakurta et al., 1978). When the production of hemolysin was established, attention was branched off to isolate and purify the hemolysins. Serine and glutamic acid were found to be essential for the production of hemolysin and a chemically defined medium was described for production of hemolysin (Karunasagar, 1981). Further a factor present in lysed erythrocytes was reported to bring down the 50% lethal dose considerably in mice (Karunasagar et al., 1984). They also demonstrated the same effect with ferric ammonium citrate and manganous sulfate.

Experimental and human volunteer studies were conducted on selected strains of V. parahaemolyticus. Sasmal et al(1973), while making comparative studies on biochemical aspects of different Vibrio spp , demonstrated differences in amounts of RNA, DNA, polysaccharides and total aminoacids between V. cholerae and V. parahaemolyticus. Though kanagawa

negative strains of V. parahaemolyticus were occasionally isolated from gastroenteritic cases, Sanyal and Sen (1974) in their human volunteer studies demonstrated the kanagawa negative strains were unlikely to produce gastroenteritis in man. In survival studies of V. parahaemolyticus in sterile and nonsterile coastal and sea water V. parahaemolyticus survived longer in sea water than in coastal water, and this was attributed to low level of biological and chemical pollution in sea (Sinha and Doctor, 1983).

Antibiotic sensitivity on clinical isolates of V. parahaemolyticus were conducted by Sanyal et al (1973) and Sen et al (1977). All isolates were found to be sensitive to streptomycin, tetracycline, chloramphenicol, neomycin, kanamycin, gentamycin and polymyxin - B (Sanyal et al.,1973) while in a later study gentamycin and chloramphenicol were found to be effective (Sen et al., 1977). Among 1787 isolates of V. parahaemolyticus tested, 36 numbers were found to be resistant to 0/129 compound (Karunasagar and Karunasagar, 1985).

Apart from the clinical and experimental fields, studies on V. parahaemolyticus had further extended to aquatic environments and products of aquatic origin. These ecological studies were mostly confined to Calcutta and Porto Novo environments. Soon after the first clinically confirmed

reports on V. parahaemolyticus from Calcutta, this bacterium was isolated from cold blooded animals and non-marine fishes around this city (Chatterjee and Neogy, 1971a,72b; Sarkar et al., 1985). It was also detected in slime and gut of prawns and in seawater off Nagapattanam (Chandrabose and Chandrasekaran, 1976). In a survey conducted at Calcutta environment 57% of crabs, 35.2% of pomfrets, 32.5% of shrimps and 28.8% of tangra were contaminated with V. parahaemolyticus (De et al.,1977). Isolation of this bacterium in freshwater plankton from this area was also reported (Sarkar et al., 1983). Ecological variability was found among V. parahaemolyticus serotypes isolated from hydrobiological by dissimilar aquatic environments (Nair et al., 1985a).

Occurence of V. parahaemolyticus in Porto Novo environment was reported (Manavalan et al., 1977). Detailed investigations on the presence of the bacterium in this environment were reported in the succeeding years. In detritus feeders the incidence of V. parahaemolyticus was high (56.3%) (Natarajan et al., 1979 a). In planktivore fishes, the incidence of V. parahaemolyticus was high in gills while in other groups, it was in faecal samples. A similarity of 94.6% was observed between freshwater and estuarine strains of V. parahaemolyticus in characterization experiments (Natarajan et al; 1979b). Studies on the distribution of this bacterium and allied vibrios in backwater and mangrove biotopes at Porto Novo showed that a large percentage of animals harboured this pathogen and their

survival was enhanced through sediments.

Reports on the ecology of V. parahaemolytus in Porto Novo environment were made (Abraham et al., 1980; Nair et al.,1980 a,b; Natarajan et al.,1980a,b; Abraham, 1981; Nair, 1981) and the significance of V. parahaemolyticus in seafood industry was highlighted by Natarajan et al (1980 b, c). James (1983) reported incidence and level of V. parahaemolyticus in freshly harvested and market samples of commercially important fin fishes and shellfishes in and around Cochin,. In his studies, 45.1% of fresh samples and 43.7% of market samples were positive for V. parahaemolyticus. He also reported higher incidence of this bacterium in shellfishes than in fin fishers. Survival of V. parahaemolyticus during various processing conditions were also exemplified by him.

A few reports on isolation of this bacterium are also available from Bombay (Bandekar et al., 1982; Joshi et al., 1985) and Mangalore coast (Karunasagar and Mohankumar, 1980). Direct plating on TCBS agar was reported to be superior to MPN technique when V. parahaemolyticus counts in the sample were high (Venugopal et al., 1985).

1.3 OBJECTIVES OF THE PRESENT INVESTIGATION

Microbiological studies on the incidence, behaviour, activity and ecological implications of marine micro-organisms, particularly microbial pathogens in coastal waters and estuaries exhibit the increasing concern and awareness of environmental impacts on health and wealth. Marine microbiologists have been active in investigating on the distribution, kinds of organisms and their activity in the environment. However, informations on the effect of environment on the ecology or on the distribution (spatial/temporal) of microbial community and competition among groups inhabiting the ecosystem are sparse. Estuarine environment are complex with respect to diversity of habitats, variation in physico-chemical parameters and contamination by terrestrial bacterial species.

Being the organisms of public health significance, ecological studies on total coliforms, faecal coliforms, faecal streptococci, E. coli and V. parahaemolyticus have great relevance as studies of these types would provide a wealth of information to environmentalists and to fishery industry. In order to evaluate the status, role and significance of potentially hazardous bacterial species in natural environment,

it is necessary to monitor the ecology of such organisms systematically in relation to physico-chemical parameters.

A survey on relevant literature would reveal that most of the investigations are confined to qualitative distribution of faecal coliforms and V. parahaemolyticus. However, very few quantitative studies on these bacteria have been carried out . Except Colwell and collaborators in Chesapeake Bay and Thompson and Vanderzant (1976) in Galveston Bay, no extensive ecological studies on faecal coliforms and V. parahaemolyticus over extended period of time in a predetermined site are carried out.

Ecology of indicator bacteria present in water and sediment off Cochin backwater, southwest coast of India, has been carried out (Gore et al, 1979a; Chandrika, 1983). Ecology and annual cycle of V. parahaemolyticus in Porto Novo coastal zone, southeast coast of India were carried out (Abraham, 1981; Nair, 1981). Literature reviewed in sections 1.2.3. and 1.2.4 show that to date no attempt has been made to study the ecology of both faecal indicator bacteria and V. parahaemolyticus in an estuarine system in India. The distribution and extent of survival of micro-organisms in an ecosystem depend on various environmental factors including antagonism and pollutants. Information on a) annual cycle of faecal

coliforms and V. parahaemolyticus b) effect of temperature, p^H and salinity on the growth of V. parahaemolyticus individually and collectively and c) heavy metal and antibiotic sensitive/resistant V. parahaemolyticus in an estuarine ecosystem are not available. Hence, it is imperative to conduct a detailed investigation to bridge the prevailing gap in our knowledge on the ecology, annual cycle and relationship of faecal indicator bacteria and V. parahaemolyticus in Cochin backwater, a tropical estuarine ecosystem. The objectives of the present study were thus drawn out asfollows:

1) To monitor the population dynamics of bacterial indicators such as total coliforms, faecal coliforms, E. coli and faecal streptococci in water, sediment, zooplankton, fish and prawn over a period of one year.

2) To monitor the population dynamics of the seafood borne pathogen Vibrio parahaemolyticus and allied organisms in the above mentioned samples.

3) To ascertain the influence of the hydrobiological parameters on the seasonal distribution of the above mentioned indicators and pathogen at three selected stations in Cochin backwater.

4) To confirm identity with the aid of reference cultures and to outline the characteristics and intergroup relationships of V. parahaemolyticus.

5) To study the effect of various physico-chemical parameters (individually and collectively) on growth of V. parahaemolyticus

6) To find out the sensitivity/resistance pattern of V. parahaemolyticus to various antibiotics and heavy metals.

1.4 DISCRIPTION OF THE STUDY AREA

Vembanad lake is one of the largest tropical estuary in the south-west coast of India ($9^{o}28'$ and $10^{o}10'$ N and $76^{o}13'$ and $76^{o}30'$ E). It spreads over an area of about 300 Sq.km and has a length of about 90 km. Vembanad lake has two permanent openings into Arabian sea, one at Cochin and another at Azhicode. The major six rivers emptying into this backwater are Muvattupuzha, Manimala, Meenachil, Pamba, Achancoil and Periyar. During monsoon season these rivers bring large quantities of flood waters carrying nutrients and silt.

At Cochin, Vembanad lake joins with Arabian sea through a narrow opening of about 450 m width. The depth of the channel varies from 6 m to 14 m. Bottom of the Cochin backwater is generally muddy. The major rainfalls of this area are from south-west and north-east monsoons, the total amounting to Ca 300 cm. Of this more than 75% of the fall accounts from south-west monsoon. Air temperature of this region varies between 25 and 35^{o}C. During monsoon months large quantities of the water fern Salvinia auriculata spreads over the backwater. Mixed semidiurnal type of tides are present through out the year.

2 ECOLOGY OF FAECAL INDICATOR BACTERIA

2.1 MATERIAL AND METHODS

2.1.1 Sampling Stations

Three different stations were selected in Cochin backwater for the present study (Figure 1). Station 1 is situated opposite to School of Marine Sciences. This is least disturbed by sewage outfall.

Station 2 is situated at the bar mouth. This is characterized by marine conditions. Frequent dredging operations are carried out here for shipping purposes.

Station 3 is situated opposite to fishing harbour in the Mattancherry channel. This is the major fish landing site at Cochin. A number of fish processing industries situated nearby flush their waste into this area. Moreover there is a sewage channel opening into this area.

2.1.2 Collection of samples

Water, sediment, zooplankton, fish (Etroplus suratensis, Bloch) and prawn (Metapenaeus dobsoni Miers) were collected at monthly intervals for a period of one year (March 1982 - February 1983). Research vessel R.V. Nautilus was made use of for the collection of samples. Collections

were made between 8 A.M. and 11 A.M. Fish and prawn were caught by cast net operation from a canoe.

2.1.2.1 Water

Water samples for bacteriological analyses were aseptically collected into sterile Mc Cartney bottles about 50cm below the water surface. Bottles were pre-wrapped with filter paper to minimise contamination through handling. Water was collected in oxygen bottle and in a clean carboy for estimations of oxygen, salinity and other physico-chemical parameters.

2.1.2.2 Sediment

Sediment samples were collected using a Peterson grab. From the central portion of the collected sediment sample, about 100 g was transferred aseptically into unused polyethylene bags for bacteriological analyses. A portion of the sediment was dried in shade for estimation of organic carbon, total nitrogen and total phosphorus.

2.1.2.3 Zooplankton

Zooplankton samples were collected by towing a plankton net (mesh size 0.076 mm) horizontally from the boat at constant slow speed for 10 minutes. The net was rinsed at the end of each haul to consolidate the adhering

material and transferred into two sterile wide mouthed glass bottles. To one of the bottles 10% neutralised formalin was added and preserved for the identification of major groups of zooplankton. Plankton in the other bottle was used for bacteriological analyses.

2.1.2.4 Fish (*Etroplus suratensis* Bloch)

Fishes were caught from the three stations using castnet from a canoe. After collection individual specimens were kept in sterile polyethylene bags.

2.1.2.5 Prawn (*Metapenaeus dobsoni* Miers)

Prawns were also caught during the collection of fishes and individual specimens were kept in sterile polyethylene bags.

2.1.3 Transportation of samples

Water, sediment, zooplankton, fish and prawn thus collected were kept in a portable ice box and transported to the laboratory. Processing and inoculation of the samples for bacteriological analyses were completed within 3-5 hours after collection. Aseptic procedures were strictly adopted.

2.1.4 Estimation of physico-chemical parameters

Physico-chemical parameters monitored in the present study were, temperature, pH, salinity, dissolved oxygen and particulate organic carbon of water and temperature, pH, total organic carbon, total nitrogen and total phosphorus of sediment.

Temperature of water and sediment were recorded using a mercury bulb thermometer with 0.5°C accuracy. Salinity and dissolved oxygen content of water were determined by Mohr's argentimetric titration (Harvey, 1955) and Winkler's titration (APHA, 1980) respectively. pH of water and sediment were measured using a pH meter (Elico L1 - 10). Particulate organic carbon of water was determined spectrophotometrically (Strickland and Parsons; 1968).

Method of el Wakeel and Riley (1956) was followed for the determination of organic carbon of sediment. Total phosphorus was determined by digesting a small portion of the sediment by the method of Rochford (1951) and estimating the orthophosphate by the method of Murphy and Riley (1962). Total nitrogen was determined by microkjeldahl technique as described by Barnes (1959).

2.1.5 Bacteriological methods

There are wide differences in characters and enumeration methodology of coliforms and V. parahaemolyticus. Hence each sample brought to laboratory was divided into two batches, one batch was used for enumeration of indicator bacteria (coliforms and streptococci) while the other batch for enumeration of V. parahaemolyticus and allied organisms.

2.1.5.1 Preparation of sample for enumeration of faecal indicator bacteria

a) Water

Water sample (100 ml) was blended at low speed in a sterilized electric blending jar for 5 minutes to separate aggregates of bacterial cells. It is then mixed with an equal volume of sterile phosphate buffer solution.

b) Sediment

Generally bacteria are seen adsorbed on to the substrata and may be separated from the substrata by simple mixing before proceeding for analysis. Sediment (50 g) was aseptically weighed and transferred into a sterilized 1000 ml beaker. 450 ml of sterile phosphate buffer solution was

added and mixed for 10 minutes using a magnetic stirrer. This helps in separation of attached bacteria. In order to prevent aerial contamination, the mouth of the beaker was covered with an aluminium foil during the mixing process.

c) Zooplankton

After decanting the excess water, zooplankton was separated by centrifugation a slow speed (3000 g) for 10 minutes in a refrigerated centrifuge. The supernatent was discarded and the resulting zooplankton pellet was aseptically weighed. It is mixed with sterile phosphate buffer solution to yield a 1:10 dilution and then homogenised.

d) Fish

Fishes were washed individually with sterile phosphate buffer solution to remove adhering sand and detritus. Using sterilized scissors and forceps, 50 g portion containing sufface tissue, gills and gut were aseptically comminuted from 8 - 10 fishes and transferred into a sterile electric blender jar containing 450 ml of sterile phosphate buffer solution. The mixture was blended at high speed for 2 minutes and allowed to settle. The supernatent solution was used for enumeration of indicator bacteria.

e) Prawn

Prawns were also washed with sterile phosphate buffer solution. Cephalothorax was discarded and 50 g portion containing exoskeleton, appendages, flesh, gill and intestine were weighed out and transferred aseptically into a sterile electric blender jar containing 450 ml of sterile phosphate buffer solution. The mixture was blended, allowed to settle and the supernatent used for enumeration of indicator bacteria as in the case of fish.

2.1.5.2 Enumeration of indicator bacteria

Indicator bacteria such as total coliforms (TC), faecal coliforms (FC), Escherichia coli (EC) and faecal streptococci (FS) were enumerated by Most Probable Number (MPN) method. Decimal dilutions of water, sediment, zooplankton, fish and prawn prepared as detailed in section 2.4.1 were made using sterile phosphate buffer solution.

a) Total coliform (TC)

Presumptive test:

Three tube MPN method was employed for the enumeration of indicator bacteria. From each decimal dilutions of the sample 10 ml portions were inoculated in triplicate into screw cap bottles containing 10 ml of sterile double strength Lauryl tryptose broth.

Similarly, 1 ml and 0.1 ml of the samples were also inoculated into 10 ml of sterile single strength lauryl tryptose broth tubes. All tubes contain Durham's tube, placed inverted and sunken fully in the medium. Inoculated tubes were shaken gently for mixing and then incubated at 35± 0.5°C. Any air bubble trapped in the Durham's tube before incubation were removed by inverting the tubes. After 24± 2 hr each tube was gently shaken and examined for gas production. If no gas was observed the tubes were re-incubated and re-examined at the end of 48± 3 hr. Formation of gas in any amount in the inner fermentation tubes within 48± 3 hr was considered as a positive presumptive test.

Lauryl Tryptose Broth (APHA 1980)

Tryptose	:	20.00 g
Lactose	:	5.00 g
K_2HPO_4	:	2.75 g
KH_2PO_4	:	2.75 g
NaCl	:	5.00 g
Sodium lauryl sulphate	:	0.10 g
Distilled water	:	1000 ml
p^H	:	6.8

Confirmed test:

Using a standard loop (3 mm diameter),one loopful of culture from all positive presumptive tubes were transferred into 10 ml of sterile brilliant green lactose bile broth tubes (BGLB) containing inverted Durham's tube.

Brilliant Green Lactose Bile broth(APHA 1980)

Peptone	:	10.0 g
Lactose	:	10.0 g
Oxgall	:	20.0 g
Brilliant green	:	0.0133 g
Distilled water	:	1000 ml.
p^H	:	7.2

The tubes were then incubated at 35$\pm$ 0.5°C for 48$\pm$ 3 hr. Formation of gas in the inverted Durham's tube of the BGLB broth by the end of this incubation period constituted a positive confirmed test for TC. From the appropriate MPN table, TC density was then computed.

b) Faecal coliforms (FC)

Eijkman's elevated temperature test separates the organisms of the coliform group into those of faecal origin and non-faecal origin. A loopful of culture from all positive presumptive tubes for TC were transferred into 10 ml of sterile EC medium containing inverted Durham's tube.

EC medium (APHA 1980)

Tryptose	:	20.0 g
Lactose	:	5.0 g
Bile salts No.3	:	1.5 g
K_2HPO_4	:	4.0 g
NaCl	:	5.0 g
KH_2PO_4	:	1.5 g
Distilled water	:	1000 ml
pH	:	6.9

The inoculated tubes were incubated at 44.5 ± 0.2°C for 24± 2 hr.Gas production by the end of incubation period is considered as a positive reaction indicating coliforms of faecal origin. FC density was then computed from the MPN table.

c) Escherichia coli (EC)

A loopful of culture from all positive EC medium tubes were streaked onto MacConkey agar, Eosin Methylene Blue (EMB) agar and Tergitol - 7 (T7) agar plates.

MacConkey Agar (HI - Media)

Peptone	:	17.0 g
Proteose peptone	:	3.0 g
Lactose	:	10.0 g
Bile salts	:	1.5 g
NaCl	:	5.0 g
Neutral red	:	0.03g
Crystal violet	:	0.001g
Distilled water	:	1000 ml
Agar	:	15.0 g
p^H	:	7.1 $\pm$ 0.2

EMB Agar (HI - Media)

Peptone	:	10.0 g
Lactose	:	10.0 g
K_2HPO_4	:	2.0 g
Eosin Y	:	0.4 g
Methylene blue	:	0.065 g
Agar	:	15.0 g
Distilled water	:	1000 ml
p^H	:	7.1

Tergitol -7 Agar (HI - Media)

Sodium heptadecyl sulphate	:	0.1 g
Polypeptone	:	5.0 g
Yeast extract	:	3.0 g
Lactose	:	10.0 g
Agar	:	15.0 g
Bromothymol blue	:	0.025 g
Distilled water	:	1000 ml
p^H	:	6.9± 0.2

Smooth, round, red colonies on MacConkey agar; flat, deep purple colonies with metallic sheen on EMB agar and round yellow colonies on T-7 agar are considered to be E. coli. Two such colonies from each petri plate were isolated, purified on the same medium and subjected to indole, methyl red, Voges Proskauer and citrate tests (APHA 1980). They were once again inoculated into lauryl tryptose broth and gas production was confirmed. Those isolates giving positive reactions for indole and methyl red tests and negative reactions for Voges - Proskauer and citrate utilization tests were confirmed as E. coli type 1. Their density in the original sample was computed from the MPN table. All experiments were carried out in duplicate.

d) Faecal Streptococci (FS)

Presumptive test:

Sterile 10 ml double strength azide dextrose broth tubes were inoculated in triplicate with 10 ml decimal dilutions of the samples.

Similarly, a series of 10 ml single strength azide dextrose broth were inoculated in triplicate with 1 ml and 0.1 ml decimal dilutions of the samples. Inoculated tubes were incubated at 35± 0.5°C. Tubes were examined for turbidity at the end of 24± 2 hr and 48± 3 hr.

Azide Dextrose Broth (APHA 1980)

Beef extract	: 4.5 g
Tryptone	: 15.0 g
Glucose	: 7.5 g
NaCl	: 7.5 g
Sodium azide	: 0.2 g
Distilled water	: 1000 ml
p^H	: 7.2

Confirmed test :

A portion of growth from each positive azide dextrose broth tubes were streaked onto Pfizer Selective Enterococcus (PSE) agar plates and incubated at 35± 0.5°C for 24± 2 hr.

Pfizer Selective Enterococcus (PSE) Agar

(APHA, 1980)

Peptone C	:	17.0 g
Peptone B	:	3.0 g
Yeast extract	:	5.0 g
Bacteriological bile	:	10.0 g
NaCl	:	5.0 g
Sodium citrate	:	1.0 g
Esculin	:	1.0 g
Ferric ammonium citrate	:	0.5 g
Sodium azide	:	0.25 g
Agar	:	15.0 g
Distilled water	:	1000 ml
p^H	:	7.1

Brownish-black colonies with brown halos confirm the presence of FS. Density of FS was then computed from the MPN table.

2.1.6 Statistical analysis

Simple linear correlation (Yamane, 1967) was carried out using a computer (PSI Omni system) to findout significant relations between all parameters.

2.2 RESULTS

2.2.1 Seasonal variation of physico-chemical parameters in water

Variations in physico-chemical parameters monitored in water are given in Figure 2 and in Appendix Table 1. Temperature varied from 26 to 32°C. Higher temperatures were recorded in summer months (March-May) and lower temperatures during south west monsoon period. Variations in temperature at station 3 was unique. It showed a decreasing trend from March onwards and this trend continued upto August.

During the study period salinity in water varied from 1.5 to 33.3 °/₀₀. Highest salinity was recorded at station 2 in December and lowest at station 1 in August. During southwest monsoon period low salinity was recorded at all stations. In most of the months salinity was higher at station 2 than in other stations.

Seasonal variations of p^H in water ranged from 6.3 to 8.15. At station 3 a narrow range of fluctuation was noticed (6.55 to 7.9). At station 1 lower p^H was recorded in March-April and July-August, at station 2 in June, July and November and at station 3 in April, July, September and

October. Except in these months slightly higher p^H was observed at all stations throughout the study period. At all stations highest p^H was recorded in February.

Dissolved oxygen of water varied between 1.1 and 4.55ml/l. High oxygen values were observed in postmonsoon months at all stations whereas lower values were recorded from January to May. Lowest value of dissolved oxygen was recorded at station 3 in February and highest value at station 2 in August.

Particulate organic carbon (POC) in water varied from 0.72 to 5.13 mg/l. Compared with stations 2 and 3, range of POC fluctuation were lower at station 1. Similarly, at station 3 POC values varied around 1.75 from July to December. Highest POC value was recorded in May at station 2 and lowest at station 1 in January.

Simple linear correlation between environmental parameters monitored in water are given in Table 1. Temperature showed significant negative correlations with dissolved oxygen at stations 1 and 2. Except for a single positive correlation ($P < 0.05$) with salinity at station 1, p^H did not show any other significant correlations with other physico-chemical parameters. Dissolved oxygen showed significant negative correlations with salinity at stations 2

($P < 0.05$) and 3 ($P < 0.01$). Particulate organic carbon did not show any significant correlations with any other physico-chemical parameters.

2.2.2 Seasonal variations of physico-chemical parameters in sediment

Variations in physico-chemical parameters monitored in sediment are given in Figure 3 and in Appendix Table 2. Temperature of sediment varied from 26 to 32^{0}C. This was same as recorded in water column. Higher temperatures were observed from March to May and lower temperatures during southwest monsoon period.

p^{H} of sediment varied from 6.7 to 8.02 during the study period. Highest and lowest values at station 1 and at station 2 were recorded in February and November respectively. As in water column slightly lower p^{H} in sediment was observed during monsoon rain period at all stations while in rest of the months p^{H} was slightly higher.

Total organic carbon of sediment ranged from 10.7 to 28.1 mg/g. At station 1 it increased from March to August and showed a trough in November. After a minor peak in January it again decreased. At station 2 also an increasing

trend in total organic carbon was observed from March to September. After showing a trough in December it again showed an increasing trend. At station 3 increase in organic carbon was shown from March to July. Then, after minor fluctuations, it reached a maximum of 23.7 mg/g in January and then showed a sharp decline.

Total nitrogen in sediment varied between 0.91 and 4.21 mg/g. Lowest value was recorded at station 1 in March and highest value in October at station 3. Fluctuation pattern in total nitrogen was similar to that of total organic carbon at station 1. Peaks in total nitrogen coincided with the two monsoon rains at station 2. At station 3 an increasing trend in total nitrogen was observed from summer months and it recorded a peak value of 4.21 mg/g in October. It showed a decreasing trend in the rate of the months.

Range of fluctuation of total phosporus in sediment was from 0.62 to 2.3 mg/g. The lowest value was recorded at station 1 in June and the highest value at station 2 in September. At station 1 it showed an increasing trend from the onset of southwest monsoon (June-July) till the end of December. At station 2 total phosphorus showed an increasing trend from March to June. Till October it varied around 2.25 mg/g and then decreased in rest of the months. Variations

in total phosphorus was around 1.5 mg/g in most of the months at station 3. A peak value of 2.07 mg/g was recorded in November at this station.

Simple linear correlation between environmental parameters monitored in sediment are given in Table 2. Temperature showed significant negative correlations ($P < 0.05$) with total organic carbon (stations 2 and 3) and total phosphorus (stations 1 and 2). p^H did not show any significant correlation with any other physico-chemical parameters in sediment. Total organic carbon showed significant positive correlation with total nitrogen at station 1 ($P < 0.05$) and 3 ($P < 0.01$). It also showed a significant negative correlation ($P < 0.05$) with total phosphorus at station 1 and another significant positive correlation ($P < 0.01$) with total phosphorus at station 2. Total nitrogen and total phosphorus also correlated positively at significant levels ($P < 0.05$) at stations 2 and 3 .

2.2.3 Seasonal variation of zooplankton

Plankton collected during the study period were mostly constituted by zooplankton and copepods dominated in them. ,Occurrence of dominant zooplankton in different seasons are as below:

Monsoon (June - September)	Pseudodiaptomus annandalei, P.binghami, Acartiella biloba, A. gravelyi, A. keralensis, Acartia plumosa, Centropages tenuiremis and Diaptomus cinctus.
Post Monsoon (October - January)	Centropages orsinii, C. trispinosus, Eucalanus subcrassus, Temora turbinata, Acrocalanus simillis, Pseudodiaptomus serricaudatus, P. jonesi and Candacia bradyi.
Summer (February - May)	Pseudodiaptomus mertoni, Labidocera pectinata L. kroyeri, Eucalanus subcrassus, Centropages furcatus, C. trispinosus, Tortanus gracilis, Acartia centrura, A. spinicauda and A. erythraea.

2.2.4 Seasonal variation of faecal indicator bacteria

2.2.4.1 Water

Seasonal variations of indicator bacteria in water are given in Figure 4 and Appendix Table 3. The values are presented per 100 ml of water. TC in water ranged between 0.71×10^4 and 239.88×10^4. The highest and lowest values were recorded at station 1 in November and April respectively. Range of fluctuation of TC at station 2 was narrow when compared with stations 1 and 3. From March to August TC fluctuation was similar at all stations. TC value was decreasing from

March to April and recorded the lowest value at all stations during this month. It was then increasing till June. After recording another trough in July it again increased till August. A peak at all stations were recorded in November.

FC in water column varied between 0.13 x 10^3 and 138.04x10^3. The maximum was recorded at station 3 in October and minimum at station 1 in January. An initial decrease in FC was observed in April at all stations. This was continued till May at station 2 whereas in other stations FC population was increasing after April. At station 1 the increase in FC was till August where it recorded the maximum for the year at this station. Then it decreased till January at station 1. Compared with station 1, fluctuation of FC at other stations were more erratic. A major trough in FC at station 2 and 3 were recorded during September.

Fluctuation of EC was similar in water column at all stations. EC ranged between 3.02 and 1122 per 100 ml and showed a bimodal trend of fluctuation. From March to June EC values showed a decreasing trend at stations 1 and 2. At station 3 this trend was seen only upto May from where it increased. After this initial trough in all stations EC was increasing till August where it recorded a major peak at stations 1 and 2 and a minor peak at station 3. In September another trough was recorded at all stations. EC population

then increased during the suceeding months and recorded a second peak in November at all stations. This peak was the highest one recorded at station 3. EC was then decreasing and recorded lower values during the following months. The highest value of EC (1122) in water column was recorded at station 1 in August and lowest value (3.02) at station 2 in September and January.

Compared with stations 1 and 3, fluctuation of FS at station 2 was more erratic. FS variation ranged from 0.13×10^3 to 109.65×10^3 . Their density was comparatively less at station 1. Unlike the coliforms FS population was increasing from March to April. At station 3 this increase was continued till May and then slowly decreased till July, whereas at stations 1 and 2 an initial trough was recorded in May from where it increased till July. At station 1 FS density was decreasing slowly till October and then increased during the suceeding months. At station 2 changes in FS density from July was more erratic. At station 3 after the initial trough in July a peak was recorded in August. Then onwards it followed the fluctuation pattern of FS at station 1 showing a trough in October and then continuously increasing during the suceeding months. In most of the months FS population was between FC and EC populations at station 1. Except at station 3 in November FS was always above EC population.

Faecal index (FC/FS ratio) was higher than 4 showing human faecal contamination in water in a number of months (Table 3). Except in months of April and July, faecal indices were > 4 at station 1 from March to November. However from December to February they were < 4 at this station. From March to September faecal index at station 2 exceeded 4 intermittently. They were > 4 in March, June and August only. From October to December faecal indices were recorded > 4 at stations 2 and 3. At station 3 apart from these months faecal index exceeded 4 in March and June only.

Simple linear correlation between environmental parameters and faecal indicator bacteria in water column showed a few significant relations at stations 1 and 2 (Table 4). FC at station 1 showed significant negative correlation with p^H ($p < 0.05$) and salinity of water ($p < 0.01$). TC at stations 1 and 2 showed significant positive correlation with dissolved oxygen ($p < 0.01$).

Among bacterial parameters in water column significant correlations were not observed at stations 1 and 2 (Table 5). FC at station 3 showed significant positive correlation with TC (< 0.01).

2.2.4.2 Sediment

Seasonal variations of indicator bacteria in sediments are given in Figure 5 and in Appendix Table 4. The values are presented per g dry weight of sediment. TC ranged between 50.12×10^5 and 0.03×10^5. The highest value of TC recorded at station 1 in August and the lowest value at station 2 in April. There was no similarity in fluctuation patterns of TC among the three stations. At station 1 a major peak in August was noticed along with two minor peaks in April and January. TC was recorded lowest in February at stations 1 and 3 while it was in April at station 2.

FC in sediment fluctuated between 1819.70×10^2 and 1.26×10^2, the highest being recorded at station 3 in December and lowest at station 2 in May and September. A bimodal trend was observed in FC fluctuation at station 1. A major peak in July and a minor peak in November were recorded at this station. At station 2, a narrow range of fluctuation in FC was noticed with two major peaks in August and November. During summer (March-May) and in post monsoon months (December-February) FC value remained around 10^2. At station 3 three peaks in FC population was noticed. During the initial study period FC population decreased from 575.44×10^2 through the summer months to a low value of 3.16×10^2 in June. By the onset

of south-west monsoon in June, it increased to a higher value of 794.33×10^2 in August, and showed another trough in September. By the onset of north-east monsoon it again increased till December where it recorded the highest peak value.

EC was fluctuating around 10 in most of the months and it increased in two or three months at different stations. Fluctuation pattern was similar at all stations. They followed the fluctuation pattern of FC. A maximum EC population of 467.74 was recorded at station 1 in July and a minimum of 3.02 at all stations in a number of months. Peaks in EC population coincided with the end of two monsoon seasons (ie July-August and November-December).

Fluctuations in FS was more erratic at stations 1 and 3. It remained between 100 and 10000. Highest FS population (955×10^2) was recorded at station 3 in April and the lowest (1.12×10^2) at station 2 in March. FS density exceeded 10^4 in a number of months at stations 1 and 3 while at station 2 this level was reached only in September.

Compared with water, faecal index in sediment showed human faecal contamination to a lesser extent (Table 6). The indices were > 4 at all stations in August and November only. Apart from these higher values (> 4) of faecal index were recorded in March and July at station 1, in October at station 2 and in March and December at station 3.

Correlation coefficients between environmental parameters and indicator bacteria in sediment are given in Table 7. Significant correlations were observed only at station 1. TC, FC and FS showed significant negative correlations ($P < 0.05$) with temperature of sediment at this station. Total organic carbon in sediment also correlated with TC negatively at the same level.

Among bacterial parameters in sediment (Table 8) FC correlated positively with EC at all stations ($P < 0.01$). None of the other indicator groups showed any significant relations among them at all stations.

2.2.4.3 Zooplankton

Seasonal variation of indicator bacteria in association with zooplankton are given in Figure 6 and Appendix Table 5 . The values are expressed per g wet weight of the plankton.

Population of TC in zooplankton ranged between 741.31×10^5 and 0.08×10^5. Variation in TC population at station 1 was unique. From a population of 0.22×10^5 in March it was increasing continuously till September when it reached a maximum density of 295.12×10^5. Then it started decreasing and fluctuated erratically. TC at stations 2 and 3 followed a common pattern of fluctuation. After an

initial drop in April it was increasing till June. These drops in April ($0.76x10^5$ at station 2 and $0.08x10^5$ at station 3) were the lowest value of TC recorded at these stations. After a minor fall in TC density in July (at stations 2 and 3) it again increased at these stations and recorded a maximum population of $741.31x10^5$ and $239.88x10^5$ at stations 2 and 3 respectively in August. Then it started decreasing till October. TC again started increasing till December and then decreased.

Variations in FC ranged from $0.16x10^3$ to $6456.5x10^3$. From March to July FC variations resembled at stations 1 and 3. Population of FC was maximum at station 1 ($5370.30x10^3$) and station 2 ($6456.30x10^3$) in October and September respectively. From July onwards FC populations were above 10^5 at station 3 till December.

EC density ranged between 3.02 and 1148.20. Population of EC was comparatively low at station 1 during the study period. At this station it reached a peak value of 45.71 in August. At station 2 EC density was around 1000 in July - August. It showed another peak in December at this station. Similar to station 1 a single major peak in August was noticed at station 3. This peak was the highest value of EC (1148.2) recorded in zooplankton samples during the study period.

FS population in zooplankton ranged from 0.40×10^3 to 457.09×10^3. The highest and lowest values were recorded at station 3 during August and October respectively. Variations of FS at each station was unique. From March to April FS population was increasing at station 1 and 2. Then it was decreasing till June. It showed another peak in July and again started decreasing at these stations. This decrease in FS density was continued till November at station 2. After this months FS was showing an increasing trend at station 2 and 3 whereas at station 1 this increase started only in December. Population of FS was above 30×10^3 at station 2 and 3 during south-west monsoon period (ie: June - September).

In zooplankton samples, higher faecal indices were found in the months of October to December (Table 9). In March stations 2 and 3 recorded faecal indices above 4. Similarly in August at station 1 and in September at station 2 higher value in faecal indices were recorded.

Significant relations between indicator bacteria in zooplankton and physico-chemical parameters in water were as follows (Table 10)). EC showed significant negative correlation ($P < 0.01$) with salinity at stations 1 and 3 and p^H at station 1. At station 3 it recorded a significant

positive relationship with dissolved oxygen ($P<0.05$). TC correlated positively with dissolved oxygen at all stations. The level of significance were $P<0.01$ at stations 2 and 3 and $p<0.05$ at station 1. Apart from these, temperature of water showed significant negative correlation with TC ($P<0.01$) at station 1 and FS ($P<0.05$) at station 3.

Correlation studies among bacterial indicators in zooplankton showed very few significant relations (Table 11). FC showed significant positive correlation with TC at station 2 ($P<0.01$) and station 3 ($P<0.05$). Similarly EC at station 2 also correlated positively ($P<0.05$) with FC.

2.2.4.4 Fish

Seasonal variations of indicator bacteria in fishes are presented in Figure 7 and in Appendix Table 6. The numerical values are given per 100g of fish. TC in fishes fluctuated between 181.97 $x10^6$ and 0.19$x10^6$. Fluctuation pattern of TC was unique at each station. At station 1 from a lowest value of 0.43$x10^6$ in March TC increased to 10.96$x10^6$ in April and without much considerable decrease maintained this level till August. Through September and October they shoot up to a maximum value of 120.23$x10^6$. At station 2 TC was present

around 10^6 and increased considerably in June, September - October and December months. Lower counts were recorded in summer months (March and May). At station 3 TC was oscillating widely from March to July. From 6.17×10^6 in July they decreased through the succeeding months to 1.32×10^6 in October. In November TC shot up to a maximum of 120.23×10^6 decreased considerably through the succeeding months and reached a lower value 0.56×10^6 in February.

FC fluctuations at stations 1 and 2 were similar. From July they followed fluctuations of TC closely at both these stations. Lowest FC were recorded in May. By the onset of monsoon rains in June it increased at all stations. Wider oscillations in FC were observed at station 3.

Variations in EC were also similar at station 1 and 2 . The counts were low during summer months and recorded a lowest value in May in all the stations. In June EC shot up to peak values of 2951.2×10^2 and 11220×10^2 at stations 1 and 2 respectively. At these stations EC then showed a downward trend till October - November. At station 1 EC was slowly increasing from October till February while at station 2, after showing a small peak in December, it was decreasing. EC fluctuation from March - July at station 3 resembled those at stations 1 and 2. From July, unlike at stations 1 and 2, EC was increasing and recorded a maximum

population ($21.38x10^4$) in September. After a trough in October it further increased in November and December before coming to a value close to those recorded in the beginning of the study period.

FS population in fishes varied between $0.13x10^3$ and $10.97x10^5$. Highest count was recorded at station 3 in May and lowest at station 1 in November. Fluctuations were similar at stations 1 and 3. FS density was increasing in summer months (March - May) and recorded maximum density in May at all stations. They decreased in June and maintained around 10^3 and 10^4 at all stations till October. At station 2 this level was maintained in rest of the study period, while at stations 1 and 3 it dropped to a value around 10^2 in November. It was then increasing at both these stations in rest of the months. By February FS reached values close to those recorded in the beginning of the study period.

Except in the months of April and May, faecal index in fishes were >4 at all stations (Table 12). This showed the presence of human faecal contamination on fishes collected from these stations. Even in March faecal indices were higher at stations 1 and 2.

Indicator bacteria present on fishes showed a number of significant correlations with physico-chemical

parameters in water (Table 13). FC at all stations showed negative correlation ($P < 0.05$) with particulate organic carbon. Similarly FS at all stations correlated with particulate organic carbon ($P < 0.05$) positively. At station 3 EC showed significant positive correlation with dissolved oxygen and negative correlation with particulate organic carbon ($P < 0.05$). Dissolved oxygen correlated positively at the same level with FC at station 1 also. FC showed a negative correlation with temperature at station 1 ($P < 0.01$) and station 2 ($P < 0.05$).

Correlation studies among bacterial parameters in fishes also showed a number of significant relations (Table 14). FC showed significant positive correlation ($P < 0.05$) with TC at the three stations. Similarly EC correlated with FC positively at the same level at stations 1 and 3. FC showed negative correlation ($P < 0.05$) with FC and EC at station 3. Another significant positive correlation ($P < 0.05$) was observed between EC and TC at station 2.

2.2.4.5 Prawn

Seasonal variation of indicator bacteria in prawn are given in Figure 8 and Appendix Table 7. The values are presented per 100 g of prawn. TC in prawn ranged between 0.16×10^6 and 162.18×10^6. Highest count of TC was recorded

at station 1 in December and lowest count at station 3 in April. TC variations at stations 2 and 3 were similar. At station 2 after marking a lowest value in April, TC increased during the succeeding months and recorded a peak value (95.5×10^6) in August. Except a small trough in July TC at station 3 also increased in the same manner and recorded a highest value (120.23×10^6) in August. A trough followed by a peak were recorded in September and October respectively at both these stations. At station 3 this maximum number was found till November. In the rest of the months TC fluctuated around 10^6 and 10^7 at stations 2 and 3. At station 1 TC was increasing from March to May. From June onwards an increase and decrease in number were found between months. In January and February lower values were recorded.

Except in the summer months FC was fluctuating close to TC in prawns at the three stations. FC values were low during the summer months, increased by the onset of monsoon rains in June and reached a peak value in August at all stations. This peak was the highest FC value recorded in prawns at station 3. During the rest of the months FC varied around 10^6 at this station. At station 1 a bimodal trend was observed in FC fluctuation. The peak in August was followed by a trough in September. FC again rose in the succeeding months and attained a highest value of 1202.3×10^6 in December. FC then showed a sharp decrease and maintained around 10^6 in January and February.

EC in prawn varied between 1.51×10^3 and 11.22×10^5. At station 1 a bimodal trend was observed. In summer months EC density was around 10^3 and 10^4 at all 3 stations. It increased through June and recorded a maximum density (11.22×10^5) at station 1 in July. EC then decreased till September and again increased to reach another peak (10.97×10^5) in December. During the succeeding months EC showed a sharp decline at this station. At station 2, after recording a peak value 79.43×10^4 in June EC dropped considerably and fluctuated around 10^3 and 10^4 in the succeeding months. At station 3 an initial peak in EC in June was noticed after which it fluctuated around 10^4 in the rest of the months.

Variations of FS in prawn was unique at all stations. Wider oscillations were observed at station 1 while it fluctuated around 10^4 in other stations from July onwards. Peak densities of FS in prawn were observed in the month of May at stations 1 and 3, and in June at station 2. The highest (45.7×10^4) and lowest (0.14×10^3) FS densities were recorded at station 1 in May and November respectively.

Faecal index in prawn was higher than 4 at all stations from June onwards (Table 15). Unlike in fishes they were below 4 during summer months (March-May).

Indicator bacteria present in prawn showed a number of significant correlations with physico-chemical

parameters in water such as temperature and dissolved oxygen (Table 16). At stations 2 and 3 TC and FC showed significant negative correlations with temperature ($p < 0.05$ and $p < 0.01$ respectively). FC and temperature showed significant negative correlation at station 1 ($p < 0.05$). Dissolved oxygen showed significant positive correlations ($p < 0.05$) with TC, FC and EC at station 2 . FC at station 1 also showed significant positive correlation ($p < 0.01$) with dissolved oxygen. At station 3 FC correlated negatively ($p < 0.05$) with particulate organic carbon. FS showed significant negative correlation with dissolved oxygen and positive correlation with particulate organic carbon.

Among bacterial indicators in prawns significant positive correlations ($p < 0.01$) were observed between TC and FC at all stations (Table 17). Apart from this no other significant correlations were found among bacterial indicators in these samples.

2.3 DISCUSSION

2.3.1 Ecology of faecal indicator bacteria in water

The population dynamics of faecal indicator bacteria in estuarine environment is influenced by a number of physico-

chemical and biological factors. The survival pattern of the allochthonous faecal bacteria in the presence of autochthonous estuarine bacteria deserves great attention. This is primarily because the presence of faecal bacteria reflect the sanitary quality of the environment. If they are eliminated faster then enteric pathogens through competition, predation or other physico-chemical parameters the validity of faecal bacteria as indicators of sanitary quality will be lost. In fresh water bodies such as rivers and lakes the environmental stress on faecal bacteria are less when compared to the very dynamic estuarine environment. Major factors affecting survival of coliform bacteria in estuarine and marine environments are solar radiation, salinity, temperature, p^H, particulate matter and suspended solids, availability of nutrients, dissolved oxygen, phytoplankton and predator organisms (references cited in 1.2.1.3).

In the present study indicator bacteria such as TC, FC, E.coli ahd FS were monitored in every month in water. Marked seasonal variations in TC were not observed in all stations. It varied from 70×10^2 to 23.9×10^5 per 100 ml of water. Carney et al (1975) reported that TC in water column in a sub-estuary of Chesapeake Bay varied from 0 to 28×10^3 per 100 ml. Sayler et al (1975) observed TC population verying from a few cells to nearly 10^4 cells per 100 ml in

upper Chesapeake Bay. Compared with these values, TC population in Cochin backwater was high. The possibile reason may be a higher input of coliform bacteria into this backwater through rivers and sewage discharge. Moreover, though temperature had a detrimental effect on coliform survival (Alton and Rakhno, 1979a) in natural water, the increased amount of organic matter and nutrients had a protective effect on coliforms at adverse environmental conditions (Carlucci and Pramer, 1960 a). Apart from this, disturbances in sediments by dredging resuspended sediment-bound coliforms resulting in an apparent rise in their population in water.

Compared with TC, variations in FC were more pronounced at stations 2 and 3. A decrease in TC and FC population in water at all stations was observed in April. This may be due to higher influx of solar radiation and increased salinity in summer months. Fujioka et al (1981) demonstrated that in the absence of sunlight faecal coliforms and faecal streptococci survived for 3 days in sea water while they were inactivated within 3 hours in the presence of sunlight. They also found out that the UV light spectrum of sunlight was primarily responsible for this. Harvey (1955) reported that inorganic salts are the most potentially toxic substances in the sea and adversely influence coliforms by a general osmotic effect or by specific ion toxicity.

A well marked uniformity and seasonality was observed in the fluctuation of E. coli with peaks in August and November, it established a definite bimodal fluctuation. Highest peak was in August at stations 1 and 2 whereas at station 3 it was in November. During the premonsoon months (March - May) population of E. coli was very low. The peaks in E. coli density coincided with zooplankton blooms in Cochin backwater. However, influence of zooplankton of faecal indicator bacterial population was not investigated so far. The live zooplankton (copepods) excrete or secrete growth promoting or chemical attractant compounds specific for bacteria. Some bacteria produce extracellular surface polymers if the bacteria attach via electrostatic force. Such substances may favour prolonged stable attachment (Fletcher and Marshall, 1982). However such a relationship has to be worked out in detail, for zooplankton and E. coli.

During monsoon period, large quantity of fresh water loaded with nutrients and minerals are empted into the Cochin estuary. Carlucci and Pramer (1960 a) reported that E. coli fail to compete successfully with autochthonous bacterial flora in utilization of organic nutrients in sea water. They also reported that inorganic nutrients like phosphorus and nitrogen accelerate growth of E. coli in sea water. The increase in E. coli during late monsoon periods (August and November) may be a similar effect due to nutrient

and mineral rich flood water. It must be remembered that the six major rivers emptying into this backwater also bring with them a large quantity of microbial load including coliforms. This can also effect an increase in the coliform density.

A definite significant correlation pattern was not observed between indicator bacteria and physico-chemical parameters monitored in this study. The deterimental effect of salinity (Carlucci and Pramer, 1960 a; Faust et al., 1975) on coliform bacteria was reflected by a few significant negative correlations with salinity.

A pronounced seasonality in fluctuation of FS in water column was not observed in this study. Chamberlin and Mitchell (1978), Fujioka et al (1981) and Fujioka and Narikawa (1982) reported that in natural environment FC die off faster than FS. They suggested higher sensitivity of FC to environmental factors such as solar radiation, temperature and salinity as responsible factors for their faster die off rate. This may be the reason for the occurrence of FS population higher than coliforms in the month of April when solar radiation and salinity were also high. In all other months FS density was always lower than TC density.

From August till December, FC/FS ratio was higher than 4 in water. With this qualitative index of faecal

pollution (Geldreich and Kenner, 1969) water in these stations should be considered as polluted with human faecal wastes. Since these higher values frequently occurred following monsoon rain, the human faecal waste might have come along with the flood water.

2.3.2 Ecology of faecal indicator bacteria in sediment

In sediment TC population varied from 10^3 to 10^6 at stations 1 and 3 whereas station 2 was from 10^3 to 10^5 per gram of sediment. Compared with water, sediments marked a distinct seasonal fluctuation in FC and E.coli population. A clear bimodal fluctuation pattern was observed in FC and E. coli population in sediment and their peaks coincided with the peaks of E. coli density in water. However at station 1 when FC and E. coli population in sediment showed a peak density in July, peak density of E. coli in water was found in August. At station 3, population of FC and E.coli were higher in March. Moreover the peak in these bacterial parameters observed in November at stations 1 and 2 was shifted to December at station 3.

The number of faecal indicator bacteria in sediment (per gram) were higher than those in water column (per ml). Baross et al (1975), Gerba and Mcleod (1976), Chan et al (1979) and Hood and Ness (1982) also reported higher number and

survival of faecal bacteria and enteric pathogens in sediment than in water column. The high organic content and fine soil particles of sediment were reported to be the factors assisting the survival of these organisms in sediment.

Indicator organisms did not show any definite significant correlation with any of the physico-chemical parameters monitored in sediment. However at station 1, TC, FC and FS showed significant negative correlations with sediment temperature. Among the bacterial indicators in sediment FC and E. coli showed significant positive correlations This suggest that density of E. coli in sediment had definite influence on FC population.

Survival of faecal bacteria and other enteric pathogens in sediment has got further ecological implications. Cochin backwater is subjected to frequent water transport and dredging operations, whereby sediment bound microorganisms are resuspended in the water column. Grimes (1975) also reported an increase in FC density in the immediate vicinity of a dredging operation in Mississippi river navigation channel. He attributed this to the disturbance and relocation of bottom sediments by dredging and concomitant release of sediment bound FC. Such release of sediment bound bacteria has got definite impact on recreational and fishing activities.

Babinchak et al (1977) observed sewage sludge material contaminating sediment upto a total distance of 48 km from the side of discharge in New York Bight. Edenborn and Renwick (1981) reported that microbial indicator organisms and metals were higher in New Jersey estuarine sediments near urbanized areas. Urbanization around Cochin has increased tramendously in the last decade. This might have been the reason for the detection of higher number of indicator organisms in the present study. Gore et al (1979 a) reported coliform densities varying from $7x10^3$ to $260x10^3$/ml of water and $2x10^3$ to $136x10^3$/g of sediment in Cochin backwater. In the report of Chandrika (1983) these values were $12x10^3$ to $210x10^3$/ml of water and $16x10^3$, $250x10^3$/g of sediment . E.coli and FS were not detected in a number of months at different stations (Gore et al., 1979 a). Similarly very low counts of FC (2/ml in water and 14/ml of sediment) and FS (20/ml of water and 5/ml of sediment) were reported in studies conducted in 1974-75 in Cochin backwater (Chandrika, 1983). In the present study, FC ranged between 100 and 1000 per ml of water and per gram of sediment respectively. FS fluctuated between 50 and 1000 per ml and 100 and 1000 per gram in water and sediment respectively in most of the months. Also TC, FC, FS and E. coli were detected in all the months. From this it is obvious that faecal pollution in Cochin backwater had increased considerably during these years.

During the investigation on faecal indicator organisms in Chesapeake Bay, Sayler et al (1975) observed significant proportions of faecal indicator bacteria and total viable bacteria associated with suspended sediments. Since utilization of Cochin backwater for various activities has considerably increased, a good amount of suspended sediments with heavy bacterial load (TC and FC) was noticed. Similarly Gerba et al (1977) reported higher level of TC and FC in sediment than overlying water in coastal canals of Texas.

Fluctuations of FS in sediments at Station 1 and 3 were of irregular nature. Sediment at station 2 maintained a rather steady FS population. Similar to water, population of FS exceeded that of TC in April at stations 2 and 3 whereas at station 1 it occurred in February. FC/FS ratio in sediment exceeded 4 from July to December which indicated human faecal pollution during these months. This also suggests that the source of faecal pollution in Cochin backwater is through the surface run off brought in through the six major rivers during monsoon rains.

2.3.3 Ecology of faecal indicator bacteria in zooplankton

Compared with water and sediment, variations of faecal indicator bacteria in zooplankton was unique. TC.

population at station 1 increased from March onwards till September, after which it showed a downward trend. Fluctuations of FC and FS were also very irregular at this station. An increase of E.coli was observed at station 1 coinciding with the zooplankton bloom in August. At station 2 E.coli showed a bimodal fluctuation. FC and FS were high during southwest monsoon period and they both recorded a trough in October at station 2. TC fluctuation was irregular. Except for a peak in August, E.coli population was low in the rest of the months at stations 1 and 3. TC, FC and FS did not show any definite pattern of fluctuation in zooplankton at station 3.

It may be assumed from the data that the variations in physico-chemical parameters may not play a crucial role in the seasonality of faecal bacteria associated with zooplankton. However, a coincidence of high E.coli population with zooplankton bloom in August at all stations suggest that there exists some interrelationship between E.coli and zooplankton which needs further investigation. Amino acids or other organic substances which attract and trigger multiplication of E.coli may be excreted by zooplankton during their bloom period. Such an interrelationship may be the reason for the higher occurrence of E.coli in zooplankton during their bloom period in August. The absence of a peak density of E.coli associated with zooplankton during their bloom period in October may be due to a different species composition of

the zooplankton in this bloom or some environmental factors might have adversely affected the attachment or multiplication of E.coli on zooplankton. Kaneko and Colwell (1975) reported that adsorption of V. parahaemolyticus onto chitin and copepods were decreased when p^H and concentratic of NaCl increased. In the present study when peak density of E.coli in zooplankton and zooplankton bloom coincided, salinity of water was low at all stations. From this it is suggested that the association of E.coli with zooplankton may be influenced by salinity.

As in sediment, faecal index, characteristic of human faecal pollution was recorded in zooplankton during months following monsoon rains. Significant positive correlation existed between TC and dissolved oxygen. Faust et al (1975) observed a direct relationship between survival of E.coli and dissolved oxygen of water. They reported high reduction in number of E.coli when dissolved oxygen content decreased below 4 mg/litre. In the present study peaks in dissolved oxygen content generally coincided with zooplankton blooms. This might have had an added advantage in association of faecal coliforms with zooplankton during their bloom period.

At stations 1 and 3 significant negative correlations were seen between E.coli and salinity. The detrimental affect of salinity on E.coli was reported earlier (Faust et al., 1975; Anderson et al., 1979). When compared with station 1 and

3 salinity at station 2 was higher due to direct influence of the sea. Therefore a significant negative relationship between E.coli and salinity was expected. However such relationship was not observed. Perhaps, in an association with zooplankton, there might have variations in sensitivity of E.coli to salinity. The extend of protection offered by zooplankton to E.coli during various environmental stresses needs detailed investigation. Nakayama and Ohno (1981) while experimenting on the effect of digested night soil, marine phyto and zooplankton and light on the viability of E.coli , Salmonella typhimurium and V. parahaemolyticus in sea water, found out that in the dark these three types of bacteria survived more than 6 days at 20^{o}C whereas in illuminated condition all the three types were dead within three days. Under illuminated conditions growth of phytoplankton might have occurred which acted against these bacteria. Phytoplankton playing a crucial role in the antibacterial property of seawater was reported earlier (Moebus, 1972 a,b). Occurrence of faecal indicator bacteria on zooplankton throughout the year and higher density of E.coli in zooplankton during their bloom period observed in the present study suggest that zooplankton may not have any antibacterial action on faecal indicator bacteria. Hirn et al (1980) did not find any significant relationship between faecal indicator bacteria and various groups in phytoplankton. However they observed significant positive correlations between TC and FC,

TC and FS, and FC and FS. In the present study TC and FC associated with zooplankton showed significant positive correlation at two stations.

2.3.4 Ecology of faecal indicator bacteria associated with fish

Studies on faecal indicator bacteria associated with finfish as well as shellfish pertain mostly to prepared and processed seafoods. Very few reports are available on the isolation of faecal bacteria from fresh finfishes. From June onwards, population of TC and FC in fish at station 1 and 2 followed closely. E.coli population in fish decreased more than 2 logs during summer months and recorded a lowest value (19/100g) in May. It increased to the highest values (29.5×10^4 and 11.22×10^5/g) in June at stations 1 and 2 respectively. At station 3, though an increase was noticed in June, the peak value was attained only in September. FS density was around 10^3 and 10^4/100g in most of the months.

FC in fish showed significant negative correlations with temperature and particulate organic carbon whereas FS showed significant positive correlation with particulate organic carbon of water. Among the bacterial parameters, significant positive correlations were observed between TC and FC and FC and E.coli. At station 3, FS showed significant negative

correlations with FC and E.coli. During summer season (March - May), when E.coli population in fish showed a decreasing trend, FS population showed an increasing trend. In May, when E.coli recorded lowest value in fish, FS marked its peak density. However the lowest value of FS was not coincided with a peak value in E.coli.

Among the coliform flora of migrating Sockeye Salmon,50% were Enterobacter species and the rest constituted by Citrobacter and Klebsiella (Strasdine and Dubetz, 1974) whereas in carp and white suckers Proteus dominated, followed by Enterobacter species (Souter et al., 1976). In fresh channel cat fish, Andrews et al (1977) observed a FC density of approximately 400/g. In the present study, density of FC was always higher than this value. In an investigation of bacterial flora of fishes like sardines, prawns, lobsters and seer fish caught off Cochin, Karthiyani and Iyer (1975a,b) found Pseudomonas , Achromobacter and Vibrio as dominant genera. They also recorded highest bacterial density in gills and intestine followed by body surface and then bottom mud. A higher density of bacterial indicators in fishes when compared with sediment was observed in this study also. Enteropathogenicity and serotyping were not conducted on E.coli strain. Therefore, presence of pathogenic E.coli

associated with these estuarine fishes could not be identified as reported by Rao and Gupta (1978).

Gibbons (1934 a) was of the opinion that bacterial flora of fish solely depends on the fish's recent intake of food and the degree of contamination in food and water. He also stated that coliforms are not associated with the normal intestinal flora of fish. Geldreich and Clarke (1966) reported that the composition of intestinal bacterial flora was related in varying degree to the level of contamination of water and food. Whether the coliforms are classified into autochthonous or allocthonous bacteria in fish intestine is of insignificance in assessing the sanitary quality of fish. Niemi and Taipalinen (1982) observed higher number of FC and FS in effluent water and fish faeces in fish ponds even when influent water and food contained very low FC and FS. They suggested that these bacteria multiply in fish intestine. The consistent number of faecal indicator bacteria present in fish shows that fish play as a major reservoir of these indicators in this ecosystem. Higher incidence of FC/FS ratio greater than 4 in most of the months shows the significance of finfish in retaining human faecal pollution in this estuary.

2.3.5 Ecology of faecal indicator bacteria in prawn

In the present study TC and FC recorded higher values in prawn from July till the end of the study period at stations 2 and 3. Population of FS and E.coli fluctuated closely at these stations during these months. At station 1, bimodal fluctuation in FC and E.coli were observed. TC and FS showed an irregular pattern of fluctuation at this station . TC and FC associated with prawn showed a significant negative correlation with water temperature. This is contrary to the finding of Rowse and Fleet (1984) who reported that at above 22°C elimination of bacteria by Sydney rock oyster was retarded and consequently a higher bacterial density was observed. They also reported an inverse relationship between population of S.charity and E.coli and salinity of water. No significant relationship between salinity and coliform bacteria could be observed in this study. At station 2 , coliform bacteria (TC,FC,EC) in prawn showed significant positive correlations with dissolved oxygen of water. In the light of the present result it needs thorough investigation. Among the bacterial indicators in prawn TC and FC showed significant positive correlation at all stations.

From June till the end of the study period faecal index (FC/FS ratio) was more than 4 indicating human faecal

pollution in prawn. Higher incidence of human faecal pollution may be attributed to the feeding behaviour of prawn. A number of environmental factors may also be in operation favouring retention of these faecal bacteria. Because of the "microorganism-concentrating" power of the shell fishes, greater sanitary control of harvest area were implemented in developed countries (Hunt, 1970, 77). He also pointed out that there was no constant pathogen/indicator ratio in sewage or treated effluents. The ratio changed according to the level of disease in the population, type or degree of sewage treatment and sewage dilution by storm drains. The high number of association of human faecal bacteria with prawn indicates its great public health significance.

2.3.6 Annual cycle of faecal indicator bacteria in Cochin backwater

All living organisms are inseparably interrelated with its non-living (abiotic) environment and they are at a state of constant interaction. In an ecosystem, this interaction further extends in some magnitude to other biotic factors such as an array of living organisms in that habitat. Microorganisms such as bacteria are very diverse in their nutritional habits, so that the same species may be found

occupying diverse ecological niches in an ecosystem. This kind of nutritional diversity is of great ecological significance when the annual cycle of the organism is concerned. The effect of conditioning factors on different niches may not be the same. Moreover the natural ecosystem may be considered as an open system and it is always changing due to interaction of various factors. In such an ever changing state, the characteristics of that ecosystem is governed mostly by the effects of the factors on the organisms and the organisms response to these factors. In a dynamic environment, such as estuaries, the situation is further complexed by continuous interaction of the sea and freshwater along with their native flora and fauna.

Faecal indicator bacteria are of crucial importance in sanitary evaluation of a water body. Interaction and survival of these allochthonous faecal bacteria in an estuarine environment is of a complex nature influenced by a number of physico - chemical and biological factors. The quantitative variations of faecal bacteria encountered in different samples in this study show the net success achieved by them in the complex interaction. The five types of samples analysed in this study offered an unique ecological niche for these bacteria. The seasonal variation of them

in these niches also reflects the extend of protection given by these niches from various environmental stresses.

TC, FC, E.coli and FS were present in all five types of samples throughout the study period. The physico-chemical parameters monitored did not show a definite significant correlation with faecal indicator bacteria in water column and sediment. Form this, it is obvious, that variations in faecal indicator bacteria in water and sediment in this estuary may be influenced by some other factors or combination of all factors. The bimodal fluctuation of E.coli in water, and FC and E.coli in sediment deserves great attention. Their peak values usually coincided with periods of zooplankton bloom. However, peak densities of E. coli coinciding with zooplankton bloom was observed only during August.

Seasonal variation of V. parahaemolyticus and allied organisms in estuarine waters were much influenced by zooplankton dynamics (Kaneko and Colwell, 1978; Abraham, 1981; Nair , 1981). This was attributed mainly to the chitinoclastic nature of these bacteria. Detailed investigation is warranted to suggest any valid reason for the relationship of zooplankton blooms with E.coli and FC in water.

Salinity showed significant negative correlation with coliform at different occasions whereas dissolved oxygen showed significant positive correlation. Detrimental effect of salinity on E.coli was already reported (Calucci and Pramer, 1960 a ; Faust et al ., 1975). When dissolved oxygen of water of numerous experiments were plotted against survival of E.coli in the Rhode river, a positive relationship was observed (Faust et al., 1975).

Grimes (1975) reported that dredging operation released large number of sediment-bound FC into water column, whereas Babinchak et al (1977) and Grimes (1982) reported that there was no appreciable increase in FC during clamshell dredging and dredge spoil deposition respectively. In a shallow water body like Cochin backwater where sediment is frequently disturbed by dredging, water transport and turbulance through flood water, a resuspension of sediment-bound coliforms may be taking place. Rain water contributing to faecal pollution through surface runoff was reported earlier (Dutka, 1977; Chandrika, 1983).

Temperature and particulate organic carbon of water showed significant negative correlations with FC associated with fish and prawn. The high incidence of faecal index, characteristic of human faecal pollution in

fish and prawn showed the ecological significance of these organisms in retaining faecal bacteria of human origin in Cochin backwater. Existence of viable but nonculturable E.coli and other enteric pathogens in estuary were reported (Xu et al. 1982; Roszak et al., 1984). Though validity of coliforms as a reliable indicator of water quality is questioned (Dutka, 1973) they were recommended as reliable indicators of sanitary quality of water (Hunt, 1977) until the discovery of a new more reliable indicator. Because of occasional pitfalls in sanitary evaluation by coliform criteria, it is advisable to rely on more than one type of indicator bacteria.

During this study, higher densities of faecal bacteria were observed at station 3. Due to the presence of fishing harbour at this station a number of fishing boats pass by this area every day. Moreover a large number of fish processing industries are situated near this station. Gates et al (1985) reported high organic load in effluents of seafood processing factories polluting estuaries of south-eastern USA. Similar organic pollution and enteric wastes containing faecal bacteria may account for higher faecal indicators at this station.

Table -1. Correlation coefficient matrix between environmental parameters monitored in water (Significance level *P < 0.05; **P < 0.01)

	Temperature	pH	Salinity	Oxygen	Particulate organic carbon
			Station - 1		
Temperature	1.0	0.2017	0.5344	-0.6440*	0.5378
pH		1.0	0.6941*	-0.3065	-0.3940
Salinity			1.0	-0.5592	-0.2527
Oxygen				1.0	-0.1657
Particulate organic carbon					1.0
			Station - 2		
Temperature	1.0	-0.1765	0.6456*	-0.6485*	0.3547
pH		1.0	0.4795	-0.2122	-0.2361
Salinity			1.0	-0.5973*	-0.0028
Oxygen				1.0	-0.0108
Particulate organic carbon					1.0
			Station - 3		
Temperature	1.0	-0.1099	0.3526	-0.4437	0.2569
pH		1.0	0.5146	-0.3761	-0.0005
Salinity			1.0	-0.7752**	-0.0734
Oxygen				1.0	-0.0560
Particulate organic carbon					1.0

Table -2. Correlation coefficient matrix between environmental parameters monitored in sediment (Significance level *P < 0.05; **p < 0.01)

	Temperature	pH	Organic Carbon	Total Nitrogen	Total Phosphorus
			Station - 1		
Temperature	1.0	0.0907	-0.3142	-0.6213*	-0.0652
pH		1.0	-0.5635	-0.4284	0.4346
Organic Carbon			1.0	0.5891*	-0.6190*
Nitrogen				1.0	0.0003
Phosphorus					1.0
			Station - 2		
Temperature	1.0	-0.0197	-0.6769*	-0.6434*	-0.5926*
pH		1.0	-0.3207	0.0330	-0.3494
Organic Carbon			1.0	0.3820	0.8304**
Nitrogen				1.0	0.5896*
Phosphorus					1.0
			Station - 3		
Temperature	1.0	0.2578	-0.6909*	-0.2882	0.2462
pH		1.0	-0.0674	0.0183	0.2576
Organic Carbon			1.0	0.7961**	0.2159
Nitrogen				1.0	0.6026*
Phosphorus					1.0

Table -3. FC/FS ratio in water collected from stations 1, 2 and 3.

Months	Station 1	Station 2	Station 3
March '82	>10.00	7.24	>10.00
April	0.06	0.04	0.03
May	>10.00	0.52	0.20
June	>10.00	>10.00	4.57
July	2.19	1.59	1.26
August	>10.00	>10.00	0.18
September	>10.00	< 0.01	0.56
October	>10.00	>10.00	>10.00
November	>10.00	>10.00	>10.00
December	0.66	7.08	>10.00
January '83	0.18	< 0.01	0.02
February	1.86	2.40	0.22

Table -4. Correlation coefficient between environmental parameters and TC, FC, FS, and EC in water (Significance level *P< 0.05, **P< 0.01)

	Tempe-rature	pH	Salinity	Oxygen	Particulate organic carbon
			Station - 1		
TC	-0.4589	0.0631	-0.3115	0.7018*	-0.1430
FC	-0.4766	-0.6138*	-0.8727**	0.4645	0.2619
EC	-0.3764	-0.5640	-0.5177	0.4408	0.1440
FS	-0.3329	-0.4110	-0.3708	0.1642	-0.0134
			Station - 2		
TC	-0.4326	-0.0507	-0.2369	0.6075*	-0.4366
FC	-0.3953	-0.3369	-0.4289	0.2374	-0.1778
EC	-0.5247	-0.4152	-0.6519*	0.5280	-0.1492
FS	-0.0622	0.1653	0.1252	-0.2873	-0.4016
			Station - 3		
TC	-0.2880	0.5369	-0.2748	0.1752	0.2324
FC	0.2551	0.4303	-0.1031	-0.0316	0.1703
EC	-0.0595	0.1841	-0.3649	0.5308	-0.2845
FS	-0.3266	0.4191	0.3253	-0.5145	0.1877

Table -5. Correlation coefficient among TC, FC, FS and EC in water (Significance level *P < 0.05; **P < 0.01)

	TC	FC	EC	FS
		Station - 1		
TC	1.0	0.3017	0.3015	-0.2797
FC		1.0	0.5515	0.2999
EC			1.0	0.1818
FS				1.0
		Station - 2		
TC	1.0	0.1883	0.1923	-0.1442
FC		1.0	0.4757	-0.0831
EC			1.0	-0.4475
FS				1.0
		Station - 3		
TC	1.0	0.7989**	0.2427	0.0358
FC		1.0	0.3150	-0.3083
EC			1.0	-0.4551
FS				1.0

TC : Total Coliforms; FC : Faecal coliforms;
EC : Eschcrichia coli; FS : Faecal streptococci.

Table -6. FC/FS ratio in sediment collected from stations 1, 2 and 3.

Months	Station 1	Station 2	Station 3
March '82	5.13	2.29	>10.00
April	0.11	0.04	0.04
May	1.59	0.04	0.17
June	0.26	0.55	0.23
July	4.37	3.72	0.58
August	>10.00	> 10.00	> 10.00
September	< 0.01	< 0.01	0.01
October	1.00	> 10.00	1.74
November	>10.00	>10.00	> 10.00
December	1.00	1.74	> 10.00
January '83	0.08	0.07	< 0.01
February	0.03	0.05	0.062

Table -7. Correlation coefficient between environmental parameters and TC, FC, FS and FC in sediment (Significance level at *P< 0.05; **P < 0.01)

	Tempe-rature	pH	Organic carbon	Total Nitrogen	Phos-phorous
			Station - 1		
TC	-0.6192*	-0.4906	-0.6071*	0.5705	-0.1060
FC	-0.6518*	-0.4304	0.1923	0.2403	-0.2770
EC	-0.4754	-0.4546	0.1427	0.0252	-0.3035
FS	-0.5993*	0.1821	0.2336	0.0853	-0.0887
			Station - 2		
TC	-0.4081	0.0549	0.3546	0.2467	0.4530
FC	-0.3398	-0.4062	0.3082	0.4408	0.2904
EC	-0.1646	-0.5140	0.1790	0.1966	0.1134
FS	-0.0328	0.5100	0.0382	-0.1138	-0.1580
			Station - 3		
TC	0.1024	0.2405	0.0950	0.0898	0.4235
FC	0.1440	0.2314	-0.2348	-0.1435	0.0523
EC	-0.1506	0.2821	0.2415	0.3249	0.1723
FS	-0.2864	-0.0102	0.0073	-0.4170	-0.3650

Table -8. Correlation coefficient among TC, FC, FS and and EC in sediment (Significance level *P < 0.05; **P < 0.01)

	TC	FC	EC	FS
			Station - 1	
TC	1.0	0.5305	0.3585	0.4360
FC		1.0	0.8840**	0.3173
EC			1.0	0.1647
FS				1.0
			Station - 2	
TC	1.0	-0.1617	-0.1375	-0.1475
FC		1.0	0.8646**	-0.4508
EC			1.0	-0.3129
FS				1.0
			Station - 3	
TC	1.0	0.2119	0.2136	0.0034
FC		1.0	0.7957**	-0.3589
EC			1.0	-0.4705
FS				1.0

Table -9. FC/FS ratio in zooplankton collected from Stations 1, 2 and 3.

Months	Station 1	Station 2	Station 3
March '82	3.80	6.17	>10.00
April	< 0.01	0.02	0.25
May	3.10	0.04	1.86
June	1.66	3.09	< 0.01
July	0.14	2.40	0.16
August	4.79	2.40	2.40
September	< 0.01	>10.00	3.98
October	>10.00	0.34	>10.00
November	>10.00	>10.00	>10.00
December	>10.00	>10.00	>10.00
January '83	< 0.01	< 0.01	0.13
February	0.16	0.42	2.63

Table -10. Correlation coefficient between environmental parameters and TC, FC, FS and EC in zooplankton (Significance level *P < 0.05; **P < 0.01)

	Temperature	pH	Salinity	Oxygen	Particulate organic carbon
			Station - 1		
TC	-0.7776**	-0.0240	-0.4870	0.6859*	-0.4574
FC	-0.1770	0.0691	-0.2024	0.1146	0.0253
EC	-0.5263	-0.7478**	-0.7453**	0.3950	0.1555
FS	-0.0712	0.2237	0.1867	-0.2148	-0.2572
			Station - 2		
TC	-0.5425	-0.0201	-0.4152	0.7458**	0.3589
FC	-0.2345	-0.2190	-0.4362	0.4779	-0.2015
EC	-0.4229	-0.0202	-0.4534	0.4234	-0.1747
FS	-0.4083	0.1773	-0.2069	-0.1028	0.0570
			Station - 3		
TC	-0.3069	-0.0543	-0.4692	0.7670**	-0.1130
FC	-0.1209	0.1521	-0.2015	0.4859	-0.4578
EC	-0.4399	-0.2791	-0.7305**	0.6401*	-0.0557
FS	-0.6690*	-0.1126	-0.4258	0.4762	-0.0292

Table -11. Correlation coefficient among TC, FC, EC and FS in zooplankton (Significance level *P < 0.05; **P < 0.01)

	TC	FC	EC	FS
		Station - 1		
TC	1.0	0.3212	0.2382	-0.0485
FC		1.0	0.1748	-0.4985
EC			1.0	-0.0194
FS				1.0
		Station - 2		
TC	1.0	0.8054**	0.5250	-0.0419
FC		1.0	0.5971*	0.2030
EC			1.0	0.2716
FS				1.0
		Station - 3		
TC	1.0	0.6460*	0.4228	0.2807
FC		1.0	0.4680	0.0481
EC			1.0	0.4803
FS				1.0

Table -12. FC/FS ratio in fish collected from stations 1, 2 and 3.

Months	Station 1	Station 2	Station 3
March '82	5.75	5.75	4.79
April	>10.00	>10.00	1.23
May	0.30	0.02	< 0.01
June	>10.00	>10.00	4.07
July	>10.00	>10.00	>10.00
August	>10.00	>10.00	>10.00
September	>10.00	>10.00	>10.00
October	>10.00	>10.00	>10.00
November	>10.00	>10.00	>10.00
December	>10.00	>10.00	>10.00
January '83	>10.00	>10.00	>10.00
February	>10.00	>10.00	>10.00

Table -13. Correlation coefficient among TC, FC, EC and FS in fish (Significance level *P $<$ 0.05; **P $<$ 0.01)

	Temperature	pH	Salinity	Oxygen	Particulate organic carbon
			Station - 1		
TC	-0.3123	0.2525	0.0020	0.1373	-0.2703
FC	-0.8379**	0.1176	-0.3556	0.6332*	-0.6680*
EC	-0.6093*	-0.0854	-0.4444	0.3975	-0.4223
FS	0.2271	-0.4318	-0.2526	-0.3510	0.5439
			Station - 2		
TC	-0.2316	0.3669	-0.0807	0.3736	0.0682
FC	-0.6063*	0.3230	-0.3005	0.4774	-0.6029*
EC	-0.4659	-0.0426	-0.3770	0.4204	-0.1917
FS	0.4268	-0.0736	0.1891	-0.4095	0.6958*
			Station - 3		
TC	-0.1661	0.2616	-0.0826	0.4724	-0.2078
FC	-0.4324	-0.1485	-0.1927	0.5177	-0.6185*
EC	-0.3902	-0.1687	-0.3906	0.6854*	-0.5942*
FS	0.0730	-0.1254	0.2481	-0.3969	0.6343*

Table -14. Correlation coefficient among TC, FC, EC and FS in fish (Significance level *P < 0.05; **P < 0.01)

	TC	FC	EC	FS
		Station - 1		
TC	1.0	0.6253*	-0.0024	0.0789
FC		1.0	0.5806*	-0.3531
EC			1.0	-0.3983
FS				1.0
		Station - 2		
TC	1.0	0.6212*	0.5769*	-0.1613
FC		1.0	0.5528	-0.5100
EC			1.0	-0.4487
FS				1.0
		Station - 3		
TC	1.0	0.5794*	0.2892	-0.5305
FC		1.0	0.6455*	-0.6456*
EC			1.0	-0.6852*
FS				1.0

Table -15. FC/FS ratio in prawn collected from stations 1, 2 and 3.

Months	Station 1	Station 2	Station 3
March '82	2.57	0.39	0.21
April	0.63	1.00	2.34
May	0.05	0.54	0.10
June	>10.00	>10.00	5.62
July	>10.00	>10.00	>10.00
August	>10.00	>10.00	>10.00
September	>10.00	>10.00	>10.00
October	>10.00	>10.00	>10.00
November	>10.00	>10.00	>10.00
December	>10.00	>10.00	>10.00
January '83	>10.00	>10.00	>10.00
February	>10.00	>10.00	>10.00

Table -16. Correlation coefficient between environmental parameters and TC, FC, FS and EC in prawn (Significance level *P < 0.05; **P < 0.01)

	Tempe-rature	pH	Salinity	Oxygen	Particulate organic carbon
			Station - 1		
TC	-0.4078	0.1798	-0.1314	0.5331	-0.2549
FC	-0.6513*	0.1093	-0.3007	0.7129**	-0.4439
EC	-0.3199	-0.2579	-0.4009	-0.4218	-0.0507
FS	0.3808	-0.2365	-0.0027	-0.4386	0.4166
			Station - 2		
TC	-0.6791*	0.1111	-0.4180	0.6970*	-0.3704
FC	-0.7625**	0.0391	-0.4940	0.6979*	-0.5363
EC	-0.3971	-0.3692	-0.5118	0.6049*	0.2724
FS	0.4138	-0.1387	0.2495	-0.2346	0.4633
			Station - 3		
TC	-0.6339*	0.1833	-0.2785	0.4412	-0.3751
FC	-0.7602**	-0.0393	-0.4726	0.5175	-0.5905*
EC	-0.5204	0.1380	-0.5089	0.4175	-0.1404
FS	0.2605	0.2606	0.3606	-0.6272*	0.5874*

Table -17. Correlation coefficient among TC, FC, EC and FS in prawn (Significance level *P < 0.05; **P < 0.01)

	TC	FC	EC	FS
		Station - 1		
TC	1.0	0.7549**	0.1430	-0.1955
FC		1.0	0.5307	-0.4801
EC			1.0	0.0286
FS				1.0
		Station - 2		
TC	1.0	0.8653**	0.1118	-0.3162
FC		1.0	0.3044	-0.3607
EC			1.0	0.2380
FS				1.0
		Station - 3		
TC	1.0	0.8307**	0.2417	-0.2891
FC		1.0	0.5660	-0.5603
EC			1.0	-0.3363
FS				1.0

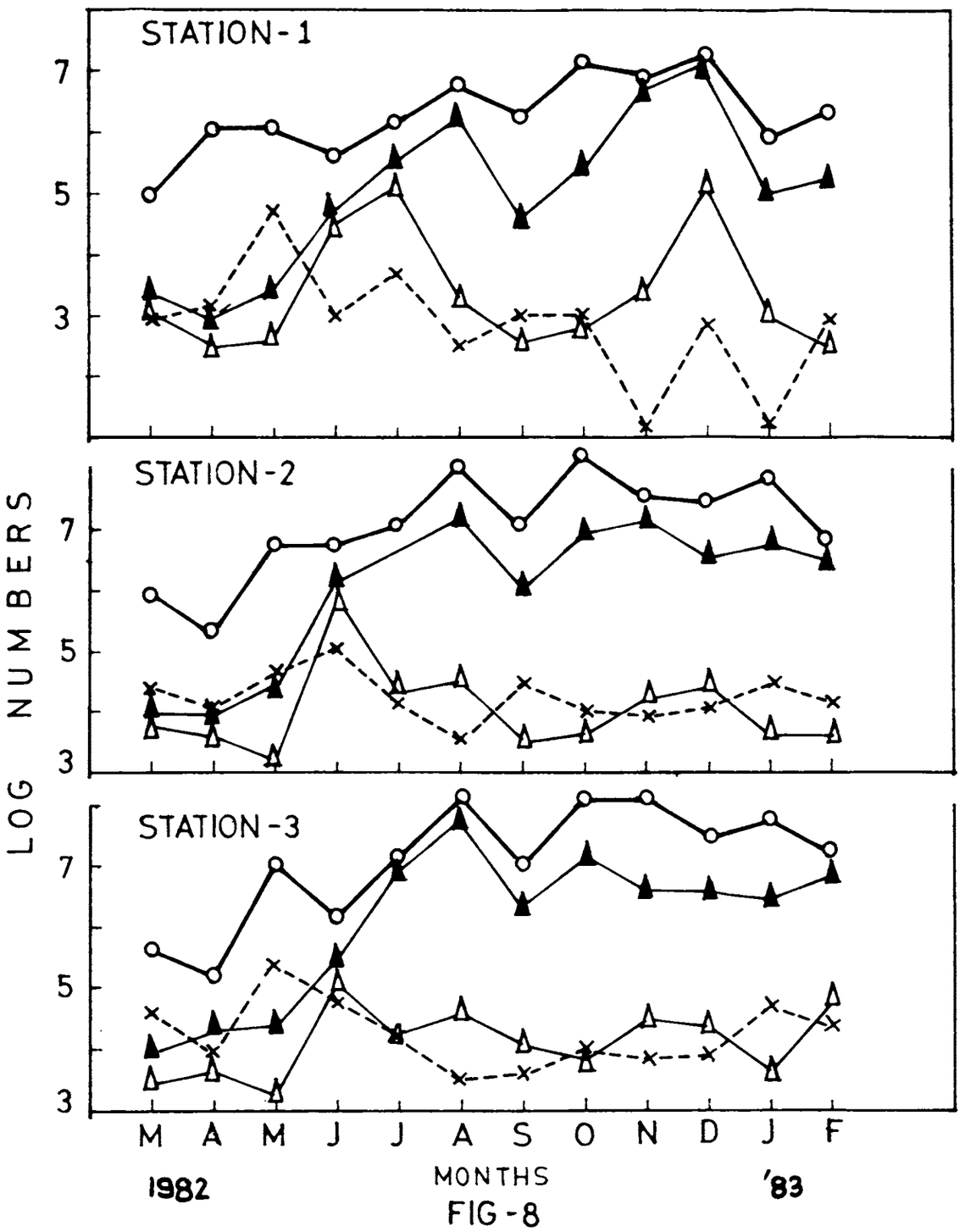

STATION - 1
STATION - 2
STATION - 3
LOG NUMBERS
7
5
3
M A M J J A S O N D J F
1982
MONTHS
'83

FIG - 8

Seasonal variation of TC, FC, FS and EC in prawn (100 g^{-1}) from March 1982 to February '83 at Stations 1, 2 and 3.

Total Coliform (TC) - (O — O)

Faecal Coliform (FC) - (▲ — ▲)

Faecal Streptococci (FS) - (X---X)

Escherichia coli (EC) - (Δ — Δ)

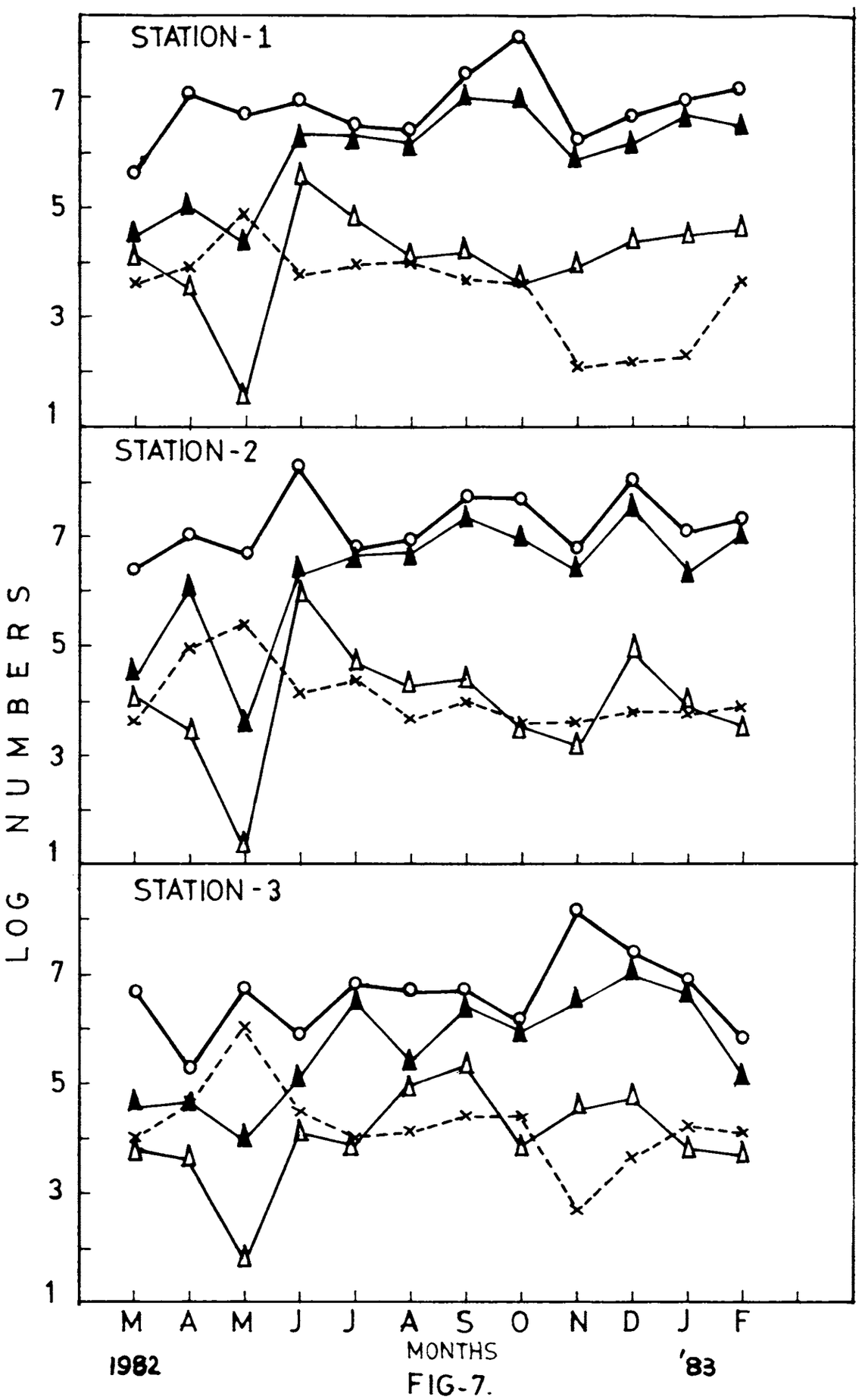

STATION - 1
STATION - 2
STATION - 3
LOG NUMBERS
7
5
3
1
M A M J J A S O N D J F
MONTHS
1982
'83

FIG-7.

Figure 7. Seasonal variation of TC, FC, FS and EC in fish (100 g^{-1}) from March 1982 to February '83 at Stations, 1, 2 and 3.

Total Coliform (TC) - (O — O)

Faecal Coliform (FC) - (▲ - ▲)

Faecal Streptococci (FS) - (X---X)

Escherichia coli (EC) - (Δ — Δ)

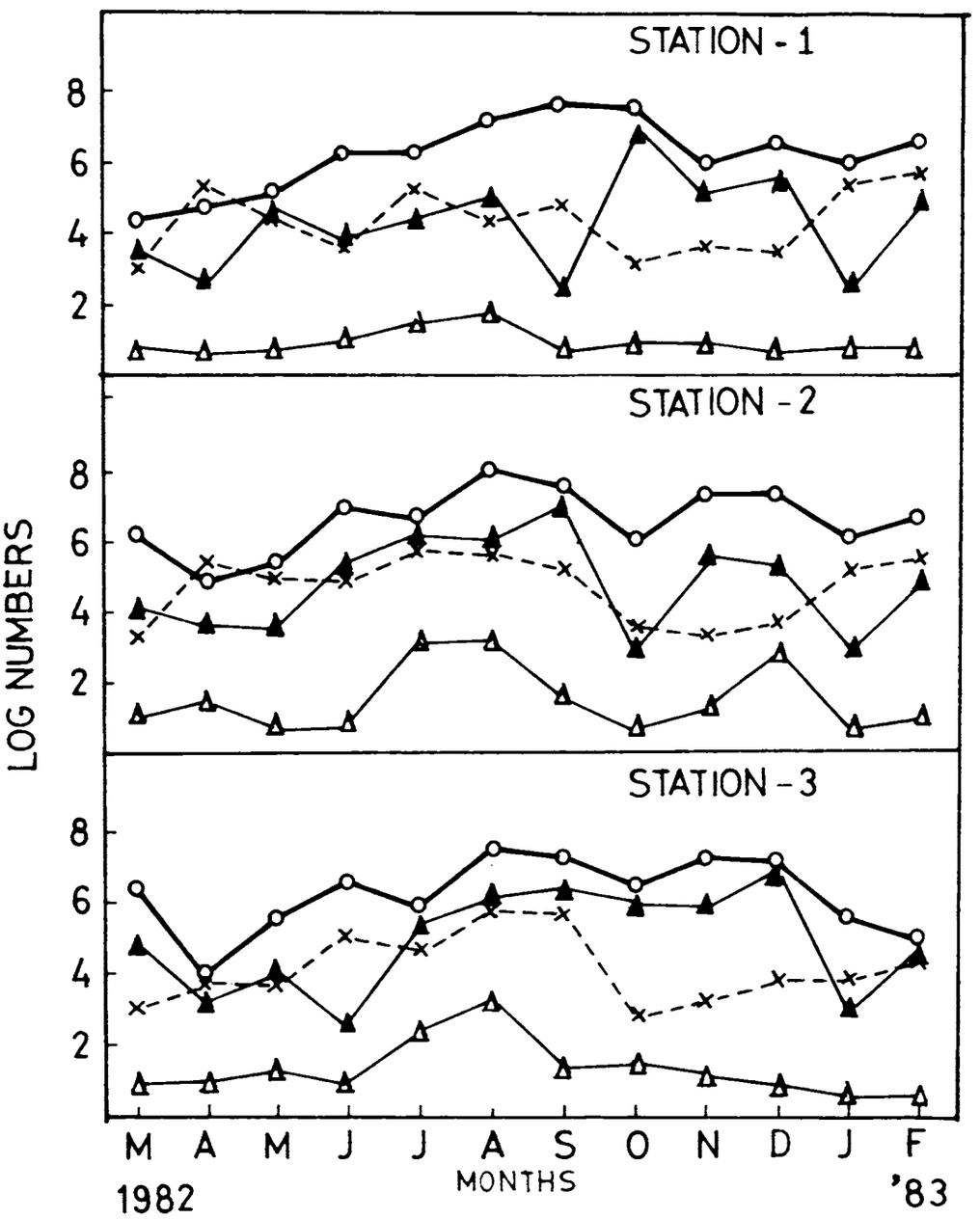

STATION - 1
STATION - 2
STATION - 3
LOG NUMBERS
8
6
4
2
M A M J J A S O N D J F
1982
MONTHS
'83

FIG. 6

Figure 6. Seasonal variation of TC, FC, FS and EC in Zooplankton (g^{-1}) from March 1982 to February '83 at Stations 1, 2 and 3.

Total Coliform (TC) - (O — O)

Faecal Coliform (FC) - (▲ — ▲)

Faecal Streptococci (FS) - (X---X)

Escherichia coli (EC) - (Δ — Δ)

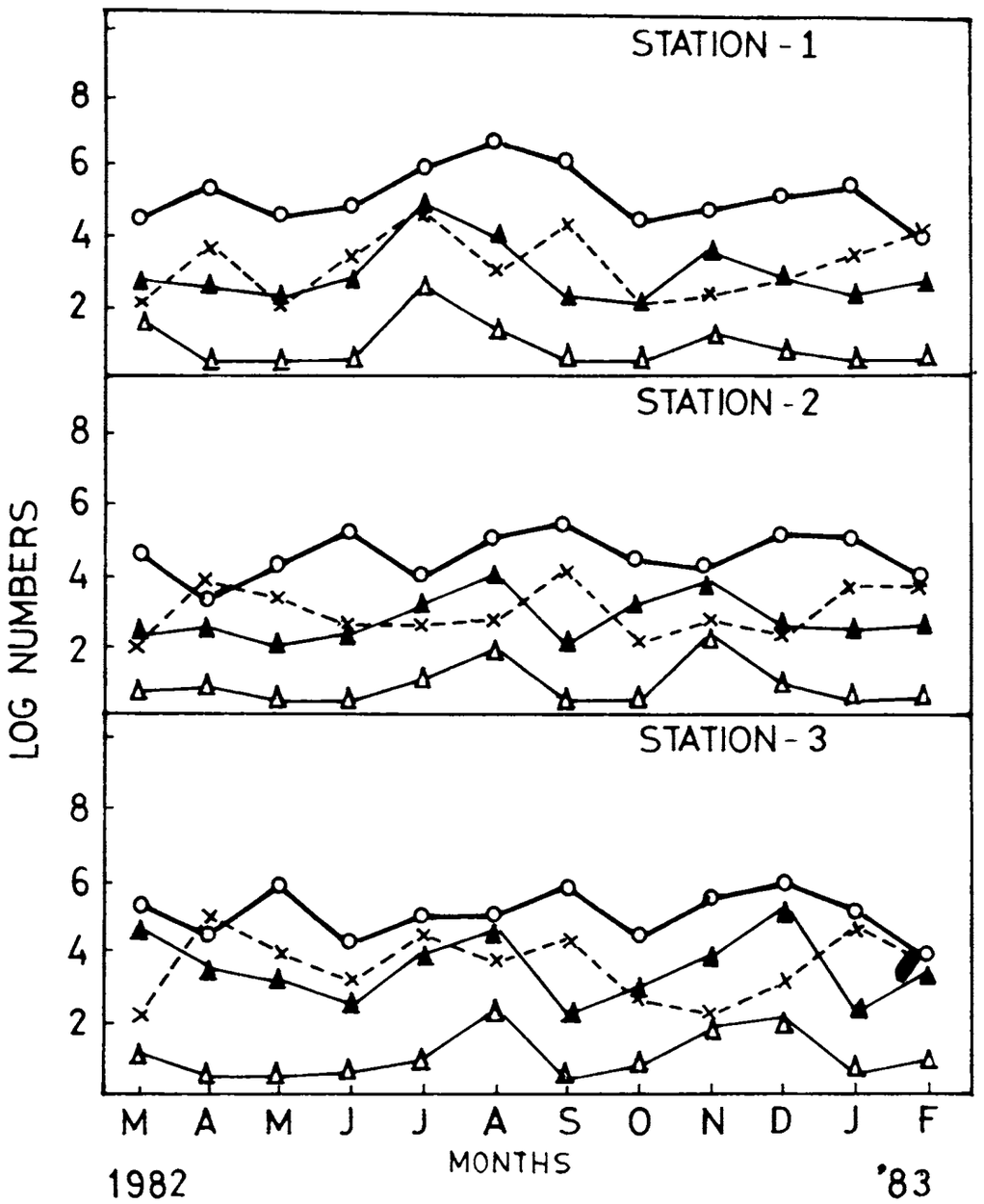

STATION - 1
STATION - 2
STATION - 3
LOG NUMBERS
8
6
4
2
M A M J J A S O N D J F
MONTHS
1982
'83

FIG. 5

Figure 5. Seasonal variation of TC, FC, FS and EC in sediment (g^{-1}) from March 1982 to February '83 at Stations 1, 2 and 3.

Total Coliform (TC) - (O — O)

Faecal Coliform (FC) - (▲ — ▲)

Faecal Streptococci (FS) - (X---X)

Escherichia coli (EC)- (Δ — Δ)

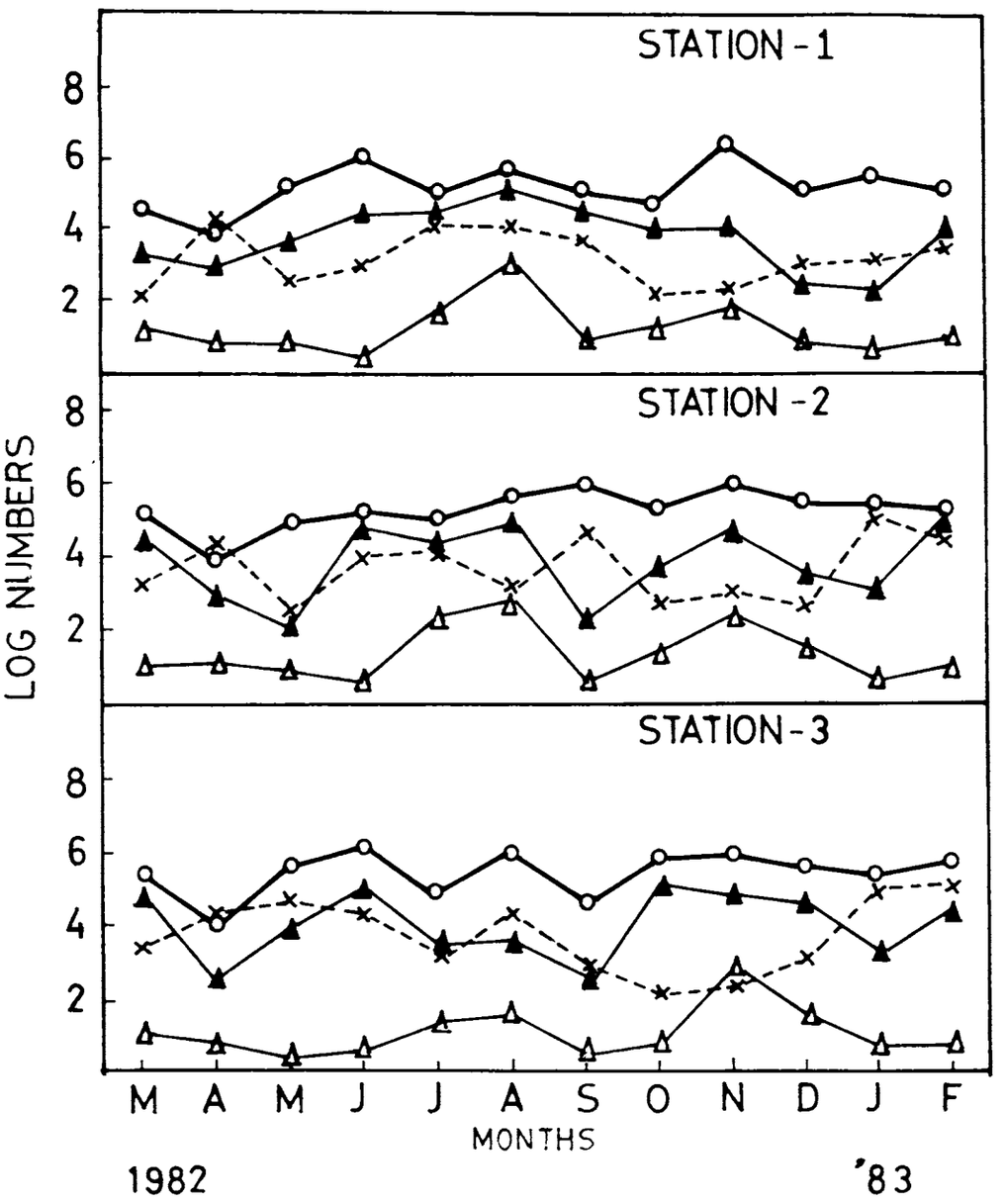

STATION - 1
STATION - 2
STATION - 3
LOG NUMBERS
8
6
4
2
M A M J J A S O N D J F
MONTHS
1982
'83

FIG. 4

Figure 4. Seasonal variation of TC, FC, FS, and EC in water column (100 ml^{-1}) from March 1982 to February '83 at Stations 1, 2 and 3.

Total Coliform (TC) - (O — O)

Faecal Coliform (FC) - (▲ — ▲)

Faecal Streptococci (FS) - (X---X)

Escherichia coli (EC)- (Δ—Δ)

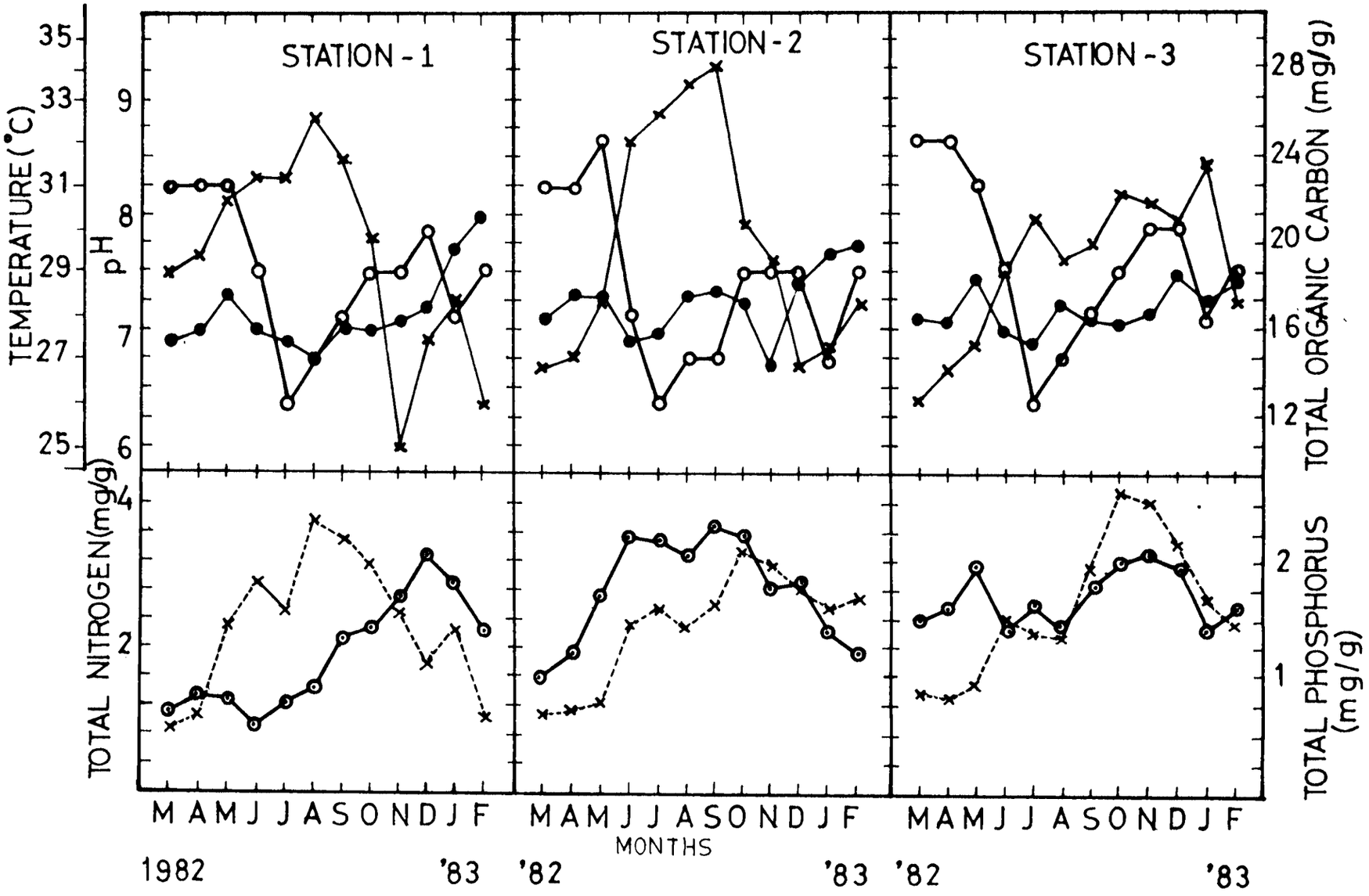

STATION - 1
STATION - 2
STATION - 3
TEMPERATURE (°C)
pH
TOTAL ORGANIC CARBON (mg/g)
TOTAL NITROGEN (mg/g)
TOTAL PHOSPHORUS (mg/g)
35
33
31
29
27
25
9
8
7
6
28
24
20
16
12
4
2
2
1
M A M J J A S O N D J F
MONTHS
1982
'83
'82
'83
'82
'83

Figure 3. Seasonal variation of physico-chemical parameters measured in sediment from March 1982 to February '83 at Stations 1, 2 and 3.

Temperature	-	(O— O)
p^H	-	(●— ●)
Total Organic Carbon	-	(X— X)
Total Nitrogen	-	(X---X)
Total Phosphorus	-	(⊙— ⊙)

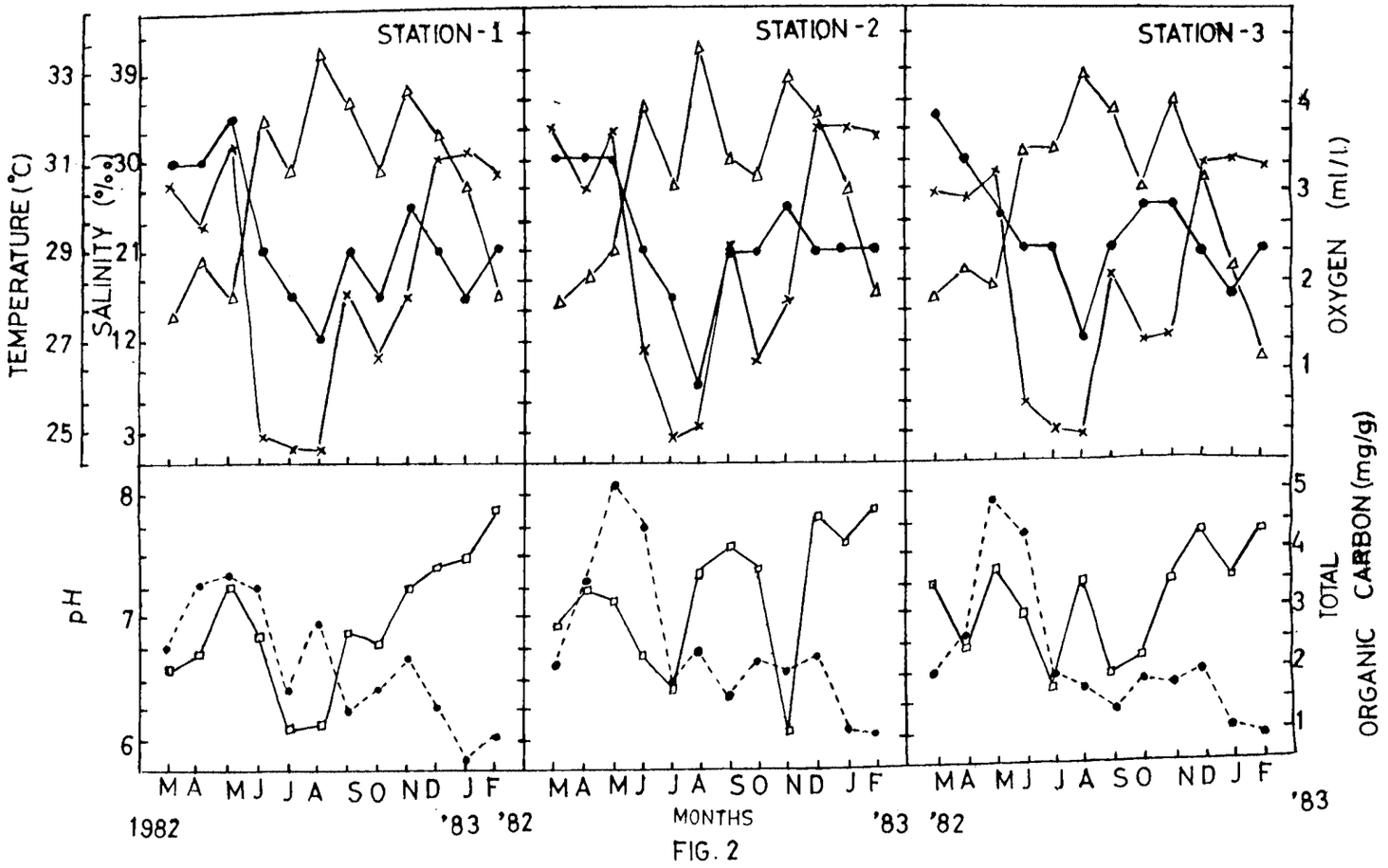

STATION - 1
STATION - 2
STATION - 3
TEMPERATURE (°C)
SALINITY (‰)
OXYGEN (ml/l)
pH
TOTAL ORGANIC CARBON (mg/g)
33
31
29
27
25
39
30
21
12
3
4
3
2
1
8
7
6
5
4
3
2
1
M A M J J A S O N D J F
1982
'83 '82
MONTHS
'83 '82
'83

FIG. 2

Figure 2. Seasonal variation of physico-chemical parameters monitored in water from March 1982 to February 83.

Temperature	-	(●— ●)
Salinity	-	(X— X)
Oxygen	-	(Δ— Δ)
p^H	-	(□—□)
Particulate organic carbon	-	(●---●)

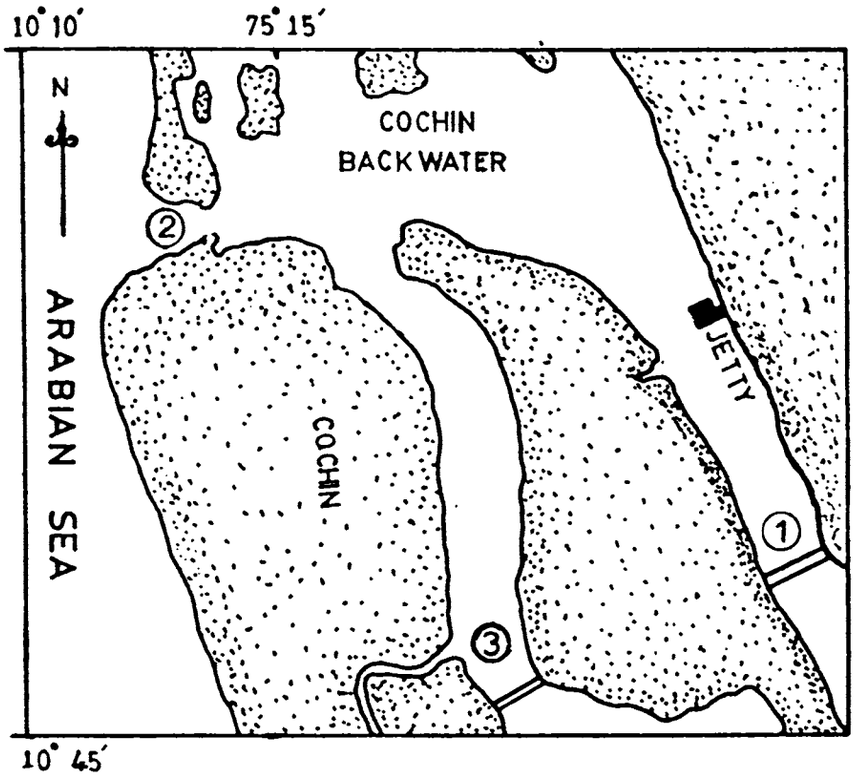

10° 10′
75° 15′
N
COCHIN
BACKWATER
ARABIAN SEA
COCHN
JETTY
1
2
3
10° 45′

Fig 1.

Figure 1. Map of Cochin backwater showing sampling stations.

3 ECOLOGY OF VIBRIO PARAHAEMOLYTICUS AND ALLIED ORGANISMS

3.1 MATERIAL AND METHODS

3.1.1 Preparation of samples for enumeration of total viable bacteria (TVB), V. parahaemolyticus (VP) and allied organisms (VLO and VPLO)

Preparation of samples were similar to those done for enumeration of indicator bacteria with a slight modification. Phosphate buffer solution was substituted by 50% aged sea water. After removing adhering sand and detritus from the body surface of fish and prawn 50g portions were aseptically comminuted as described earlier (2.1.5.1) and blended with 450 ml of sterile 3% NaCl solution. From the supernatent solution decimal dilutions were prepared with sterile 3% NaCl solution.

3.1.2 Enumeration methods

3.1.2.1 Enumeration of total viable bacteria (TVB)

Total viable bacteria (TVB) was enumerated by pour plate method using ZoBell's agar 2216 e(Schwan, 1980. Personnel communication).

ZoBell's Agar 2216 e

Peptone	5.0 g
Yeast extract	2.5 g
Fe PO_4	0.1 g
Agar (BDH)	20.0 g
Aged seawater	750 ml
Distilled water (Copper free)	250 ml
p^H	7 ± 0.1

Aliquots of the samples (1 ml) were transferred aseptically into sterile petri plates and sterile, molten medium (40-45°C) was added into the plates. Medium and inoculum were well mixed, allowed to solidify and incubated for 5-7 days at room temperature (28±2°C). Bacterial colonies developed were counted at the end of incubation period and the TVB were calculated.

3.1.2.2 Enumeration of *Vibrio*-like organisms (VLO)

V. Parahaemolyticus - like organisms (VPLO) and

V. parahaemolyticus (VP)

Three tube MPN method was used for the enumeration of VLO, VPLO and VP (Kaneko 1973, Kaneko and Colwell, 1973) 10 ml portions of decimal dilutions of the samples were inoculated in triplicate into 10 ml of sterile double strength sea water yeast extract (SWYE) broth.

Sea Water Yeast Extract (SWYE) broth

Peptone	:	10.0 g
Yeast extract	:	3.0 g
NaCl	:	24.0 g
$MgSO_4$ $7H_2O$	:	7.0 g
Mg Cl_2	:	5.5 g
KCl	:	0.7 g
Aged sea water	:	500 ml
Distilled water	:	500 ml
p^H	:	7.4 $\pm$ 0.2

Similarly 1 ml and 0.1 ml portions of the decimal dilutions were inoculated in triplicate into 10 ml of sterile single strength SWYE broth. Inoculated SWYE broth tubes were incubated at 37°C for 18 hr. A loopfull of culture from all turbid SWYE broth tubes were streaked into thiosulfate citrate bile salts sucrose (TCBS) agar plates.

TCBS Agar (HI - Media)

Yeast extract	:	5.0 g
Peptone	:	10.0 g
NaCl	:	10.0 g
$Na_2S_2O_3$	:	10.0 g

Oxgall	:	5.0 g
Sodium cholate	:	3.0 g
Sucrose	:	20.0 g
NaCl	:	10.0 g
Ferric citrate	:	1.0 g
Thymol blue	:	0.04 g
Bromothymol Blue	:	0.04 g
Agar	:	15.0 g
Distilled water	:	1000 ml
p^H	:	8.6 ± 0.2

Streaked TCBS plates were incubated at 37°C for 18 hr. Bacterial colonies developing on TCBS agar plates were presumptively identified following the scheme of Kaneko (1973) and Kaneko and Colwell (1973, 75). Colonies developing on TCBS agar were regarded as VLO while colonies appearing "typically" green were regarded as VPLO. Two such colonies appearing as VPLO were isolated from each plate, purified on TCBS agar and inoculated into VP medium (Kaper et al., 1980) for presumptive identification.

VP Medium (Kaper et al., 1980)

Gelysate peptone	:	5.0 g
Yeast extract	:	3.0 g

Tryptone	:	10.0 g
NaCl	:	30.0 g
Sucrose	:	20.0 g
Lactose	:	20.0 g
Mannitol	:	1.0 g
Arginine HCl	:	5.0 g
Ferric ammonium citrate	:	0.5 g
$Na_2S_2O_3$	:	0.3 g
Bromocresol purple	:	0.02 g
Agar	:	13.5 g
Distilled water	:	1000 ml
p^H	:	6.7

Isolates showing an alkaline reaction in the slant and acid reaction in the butt were further identified by tests described by Kaneko (1973) extended by Abraham(1981). The tests are given in Table 18. Populations of VLO, VPLO and VP were computed from the appropriate MPN tables.

3.2 RESULTS

3.2.1 Seasonal variation of TVB, VP and allied organisms

3.2.1.1 Water

Seasonal variations of TVB and vibrios in water are

given per 100 ml in Figure 9 and in Appendix Table 8 . Total viable bacteria (TVB) in water varied between $0.36x10^7$ and $14.8x10^8$. At all the stations the highest TVB was recorded in May and lowest in June. Apart from these, variations in TVB were independent of each station. At station 2 a bimodal trend could be observed, a major peak in May followed by another peak in October. On an average TVB fluctuated between 10^6 and 10^8 at all stations. Highest TVB was recorded at station 3 in May and lowest at station 1 in June.

Fluctuation of VLO was between 10^3 and 10^5. At stations 2 and 3 VLO densities were comparatively high during summer months (March-May). The highest VLO density was recorded at station 3 in May. A lowest value of $0.4x10^4$ was recorded at stations 1 and 2 in August and November respectively. During south-west monsoon period (June-August) VLO densities were low at station 1 and 2 . Except for a minor peak in July, this was true for station 3 also. After August VLO fluctuation was erratic at station 1. At station 2 it showed a peak in September, decreased through next two months, again increased and maintained around 10^4 in rest of the months. Variations of VLO after August at station 3 was more or less similar to that at station 2 except that a notable peak was formed in January instead of September.

Variations in VPLO were independent at each station. VPLO population was comparatively higher at station 3. At station 1 highest VPLO density was observed in November (10.97×10^4). Two minor peaks, one in June and another in February were also observed at this station. At station 2 highest VPLO density was observed in June (21.88×10^3) . At this station VPLO was fluctuating around 10^3 in rest of the months . From an initial value of 0.89×10^3 in March VPLO increased continuously at station 3 and attained a peak density (19.95×10^4) in May. It varied around 10^4 in next two months and recorded a trough in September (3×10^2). VPLO then increased and maintained around 10^3 in rest of the months.

Fluctuation of VP population at each station was independent. They were at below detectable level at station 1 and 2 in July and October respectively. Fluctuation of VP was more erratic at station 3. They were at below detectable levels in April, August, September and December. In the rest of the months VP was between 10^2 and 10^3 at this station. During summer months (March - May) VP population was around 10^3 at station 1. By the onset of south west monsoon it decreased sharply in June-July. By August they reattained a density 10^2 and 10^3 and maintained this level till January. In February it showed the highest peak of 36.31×10^2. At station 2 after a small trough in

April VP population increased and recorded a maximum density of 42.66×10^2 in July. It then started decreasing and went to below detectable level in October. By November VP reappeared and later maintained between 10^2 and 10^3 during rest of the months.

Correlation coefficient between environmental parameters and vibrios in water column showed a few significant relations (Table 19). TVB showed significant positive correlation ($P < 0.05$) with salinity at station 1 and p^H at station 2. It also showed a significant negative correlation with oxygen ($P < 0.01$). VLO showed significant positive correlation ($P < 0.05$) with temperature and salinity at station 2. VLO exhibited significant positive correlation with oxygen at station 3 ($P < 0.05$). VP did not show any significant correlation with environmental parameters at stations 2 and 3. At station 1 VP showed significant positive correlation ($P < 0.05$) with p^H and salinity.

Among TVC and vibrios only a single significant correlation was observed. This was a positive relationship between TVC and VLO ($P < 0.05$) at station 3 (Table 20).

3.2.1.2 Sediment

Seasonal variation of TVB and vibrios per g of dry sediment are given in Figure 10 and in Appendix Table 9.

TVB in sediment varied between $5x10^5$ and $79.43x10^8$. Highest and lowest TVB values were recorded at station 3 in May and January respectively. Maximum TVB was recorded in May at all stations. After this peak in May TVB fluctuated between 10^7 and 10^8 at station 1 till October. Then it was decreasing in the succeeding months and recorded a lowest value $27x10^5$ in February. At station 2 after the peak in May TVB decreased and recorded a trough in July - August. It then recorded two peaks, one in October and another in January and then decreased sharply. The lowest TVB values were recorded in February at stations 1 and 2. At station 3 TVB was fluctuating around 10^7 from June to September. It was then showing a downward trend and recorded a lowest value of $5x10^5$ in January.

VLO fluctuated between 10^3 and 10^6. Highest VLO densities were recorded in April at all stations. Apart from the peaks in April a second major peak was observed at stations 2 and 3. These peaks were in November and December at stations 2 and 3 respectively. At station 1 VLO decreased after the peak in April till July. At station 2 also a similar decrease was observed and it continued upto September. At station 1 after a small increase in August, VLO fluctuated around 10^4 in the rest of the months. At station 2 after the trough in September, VLO increased and recorded a major peak in November. It then dropped to a value

just below 10^4 and again increased slowly during the rest of the months. At station 3 VLO decreased after April and fluctuated around 10^4 from June to September. After a trough in October it increased and recorded a major peak in December.

Fluctuations of VPLO was between 10^1 and 10^5. The highest and lowest values were recorded at station 2 itself in November and February respectively. Fluctuations of VPLO were independent of each station. At station 1 from an initial value of $2x10^3$ in March VPLO increased continuously and recorded $29.51x10^3$ in June. It fluctuated between 10^2 and 10^4 till November and then decreased in the succeeding months. At station 2 though there was a slight increase in VPLO population in summer months (March-May), it decreased by the onset of south-west monsoon and maintained around 10^3 till October. In November VPLO increased to a peak value of $13.8x10^4$. It then decreased sharply and recorded a lowest value of 80/g in February. At station 3 fluctuations of VPLO closely followed that of VLO. The peaks and troughs of VPLO coincided with those of VLO.

VP population varied between below detectable level to 10^4. At station 1 VP population was at below detectable level in July. In rest of the months it fluctuated around 10^3. A notable peak was not observed at this station. Compared with station 1 fluctuation of VP was wider at

station 2. It showed an increasing trend in summer months. By the onset of south-west monsoon VP started decreasing and in July it was at below detectable level at this station. VP reappeared in August and maintained a population around 10^2 till January with a peak in November. In February it showed a sharp decrease. At station 3 still wider fluctuations in VP population were observed. There was a sharp increase in the summer months and recorded a peak density (13.5×10^3) in May. It then decreased and was at below detectable level in September. VP again increased in the succeeding months and recorded a highest density of 15.5×10^3 in December. Further it decreased and fluctuated around 10^2 in rest of the months.

TVB and vibrios in sediment showed a few significant correlations with physico-chemical parameters in sediment (Table 21). TVB and VP at station 1 showed significant positive correlation ($P < 0.05$) with total organic carbon and temperature respectively. At station 2 VLO showed significant negative correlations ($P < 0.05$) with total organic carbon and total phosphorus. It also showed a significant positive correlation with temperature at the same level. VPLO at this station also showed a significant positive correlation with temperature at the same level. VPLO at this station also showed a significant negative correlation ($P < 0.01$) with p^H. In sediment at station 3 no significant correlations could be observed.

Among TVB and vibrios in sediment a number of significant correlations were found at station 3 (Table 22). TVB correlated significantly ($P<0.01$) with VLO. VLO and VPLO also correlated at the same level. Similarly VP showed a significant positive correlation ($P<0.05$) with VPLO at stations 2 and 3. No significant correlations could be observed at station 1 among TVB and vibrios.

3.2.1.3 Zooplankton

Seasonal variations of TVB and vibrios associated with zooplankton per g wet wt are given in Figure 11 and in Appendix Table 10. TVB in zooplankton varied between 10^6 and 10^9. Highest and lowest TVB values were recorded at station 1 itself in February and July respectively. In summer months TVB was around 10^8 and 10^9 at station 1. It decreased sharply in June-July. In August following a bloom in zooplankton it shot up to a peak value. It further decreased and recorded another trough in November. TVB then started increasing in the succeeding months and recorded a peak density in February. TVB fluctuated in a narrow range in zooplankton at station 2 (between 10^7 and 10^9). Maximum density was observed in April (26.3×10^8) and minimum in September (9.55×10^7). At station 3 TVB increased from March onwards and recorded a highest density (16.22×10^8) in May. In June- July they were below 10^8. Further TVB fluctuated between 10^7 and 10^8 in rest of the months. Lowest value was recorded in December at this station.

VLO in zooplankton varied between 10^4 and 10^6. Fluctuations were independent at each station. Highest VLO density was recorded at station 3 in August and lowest at station 1 in February. From an initial density of 1.29×10^5 in March VLO increased and recorded a peak density in May (23.44×10^5) at station 1. It dropped in June and maintained around 10^5 and 10^6 till September. It further decreased from October onwards. After recording a minor peak in January VLO decreased sharply. At station 2 from an initial value of 1.58×10^5 VLO increased and fluctuated around 10^5 till July. It further showed a downward trend. In January VLO shot up to record a highest value (45.71×10^5) and then decreased sharply as in station 1. At station 3, VLO fluctuated around 10^5 and 10^6 from March-June. After a trough in July VLO increased and recorded a peak value (50.12×10^5) in August. VLO was showing a downward trend in rest of the months and recorded a lowest value in February (11×10^3) . A peak in VLO population as at stations 1 and 2 in January was not observed at station 3.

VPLO population in zooplankton ranged from 40 to 10.97×10^5. Highest density was recorded at station 3 in August and lowest at station 2 in October. VPLO population was comparatively higher at station 3. Fluctuation patterns were independent at each station. At station 1 after a

minor increase in summer months VPLO dropped to a low value of $3x10^2$ in June. In the succeeding months they increased sharply and recorded a highest value of $61.66x10^4$ in August. By September VPLO again decreased, After a minor peak in november VPLO showed a downward trend as in sediments in rest of the months at this station. At station 2 VPLO increased from April and recorded a peak value in July ($23.44x10^4$). In the succeeding months they showed a sharp decline and recorded a lowest=value 40/g in October. After showing another peak in November VPLO decreased as at station 1. At station 3 from an initial value of 180/g in March VPLO shot up to $39.8x10^4$ in April. After a small decrease in next 43 months VPLO again increased and recorded a highest density ($10.97x10^5$) in August. Afterwards it declined sharply and fluctuated between 10^3 and 10^4 in rest of the months.

VP in zooplankton fluctuated between 10^2 and 10^4 in most of the months. They were at below detectable level in July at station 1, in October at station 2 and in both these months at station 3. At station 1 and 3 VP fluctuations were similar. In summer months VP showed an increasing trend at both these stations. After recording a minor peak in May they decreased by onset of south-west monsoon and were at below detectable levels in July. But in August they shot up to record highest densities at these station

VP further decreased and recorded a trough in October. After recording a minor peak in December VP decreased in rest of the months. At station 2 VP occurred between 10^2 and 10^3 during summer months. In August they shot up to a hgihest value as at stations 1 and 3. In October a trough was observed. VP further increased and fluctuated around 10^3.

Correlation coefficient between environmental parameters and vibrios showed only two significant relations (Table 23) p^H showed a significant negative correlation with VLO at station 1 and a positive correlation with VP at station 3 ($P < 0.05$) . Among TVB and vibrios in zooplankton significant relations could be observed at two instances (Table 24). VP correlated positively with TVB at station 1 and with VPLO at station 2 ($P < 0.01$).

3.2.1.4 Fish

Seasonal variations of TVB and vibrios in fish per 100g wet wt are given in Figure 12 and in Appendix Table 11. TVB ranged between $3x10^6$ and $3.4x10^9$. Lowest TVB value was recorded at station 3 in February and highest at station 1 in April. From March to October TVB fluctuations at stations 2 and 3 were similar. From an initial density of 10^8 and 10^7. TVB at these stations increased and recorded peak values in May. These peaks $2.5x10^9$ and $1.5x10^9$ at stations 2 and 3

respectively) were the maximum values recorded at these stations. By the onset of south-west monsoon rains a sharp decrease was observed in TVB at stations 2 and 3. This went to form a trough in August after which they started increasing. At station 2 TVB varied around 10^7 and 10^8 after October while at station 3 the increase from August continued further to November where they recorded a major peak (10.2×10^8) Afterwards TVB decreased sharply at this station. At station 1 TVB fluctuated around 10^8 and 10^9 from March to June. It then decreased and formed a trough in August. TVB increased again in the succeeding months, formed a minor peak in October and afterwards varied around 10^7 in rest of the months.

VLO in fishes varied between 10^4 and 10^8. Lowest value was recorded at station 1 in March and highest at station 2 in May. From March to September VLO fluctuation was more erratic at stations 1 and 2. During September and October they maintained a density around 10^7. In December there was a sharp reduction after which VLO started increasing at both these stations. At station 3 fluctuation pattern of VLO was close to that of TVB. It exhibited rather a bimodal trend with a major peak in April (83.2×10^6) and another one in November (10.5×10^6) From June to September VLO density was around 10^5 . After the peak in November VLO decreased continuously in rest of the months at this station.

Fluctuation of VPLO was between 10^3 and 10^7. The lowest value was recorded at station 1 in May and highest at station 3 in April. At each station VPLO varied independentl: in fishes. At stations 1 and 2 VPLO was around 10^4 from March to May. In June it formed a peak at station 1 and then continuously decreased till September. It further increased in the succeeding months and recorded a highest value (3.89×10^6) in November at this station . After showing a trough in December it again started increasing till the end of the study period. At station 2 after the summer months VPLO increased and recorded a peak value in July(5.01×10^6) After recording a major trough in August it again increased and formed another peak in November (7.59×10^6). This was the highest VPLO population recorded in fish at station 2. Another trough was observed in December after which VPLO showed an increasing trend till February. At station 3 two major peaks were observed in VPLO population, one in April and another in November. Except for a minor peak in August VPLO was decreasing from April till October. After the second major peak in November it formed another trough in January.

Fluctuation of VP was very erratic at stations 1 and 2 in most of the months. At station 1 VP could be detected only for 8 months . When VP was detected it was between 10^3 and 10^4 in all except months November and February. Highest VP population at station 1 was recorded in November

($12.6x10^5$). At station 2 VP could be detected in 9 months when it was detected, it was around 10^3 and 10^4 as in station 1. Higher densities were observed during July ($50.12x10^4$) and November ($16.6x10^5$) at station 2. Except in July, VP could be detected throughout the study period in fishes at station 3. Here also VP was fluctuating around 10^3 and 10^4 with two notable peaks, one in August ($22.4x10^4$) and another in November ($12.6x10^5$).

Simple linear correlation of TVB and vibrios with physicochemical parameters showed a number of significant relations (Table 25) . TVB showed significant positive correlation with particularate organic carbon at all stations. Significance was at $P<0.01$ at stations 1 and 2 at $P<0.05$ at station 3. It also showed significant positive correlation with temperature at station 1 ($P<0.05$) and 2 ($P<0.01$) showed a significant positive correlation ($P<0.05$) with temperature at station 3. p^H of water showed a negative correlation with VPLO at station 2 and a positive correlation with VP at station 3($P<0.05$).

Among TVB and vibrios in fishes a few significant correlations were observed (Table 26) . VPLO and VP correlated positively at significant levels at station 1 ($P<0.05$) and station 2 ($p<0.01$). TVB showed significant positive correlation with VLO and VPLO ($P<0.01$) at station 3. VLO and VPLO also correlated positively at this station at the same level of significance.

3.2.1.5 Prawn

Seasonal variations of TVB and vibrios in prawn per 100g wet wt are given in Figure 13 and in Appendix Table 12. Fluctuation of TVB was between 10^7 and 10^9. The lowest value was recorded at station 3 in February and the highest at station 2 in May. Fluctuation of TVB was independent at each station . Except for a drop in April, TVB were around 10^9 during summer season at station 1. It then decreased and recorded a trough in September ($4x10^7$). After this TVB was increasing, reached another small peak in December and then started declining. At stations 2 and 3 TVB was increasing from March onwards. It recorded a peak value in May at station 2 while at station 3 the peak was recorded in June only. After these peaks at both these stations TVB decreased in the months followed and a trough was recorded in September at both these stations. At station 2 TVB then fluctuated around 10^8 in the rest of the months. But at station 3 TVB decreased sharply after June . Later it recorded a major peak in November ($3.9x10^9$) and then dropped to a lowest value (2.6×10^7) by February.

VLO population in prawn ranged from 10^5 to 10^8 The lowest and highest values were recorded at station 1 itself in April and May respectively. Fluctuation patterns were more or less similar at stations 1 and 3. After an

initial drop in April VLO increased at all stations and recorded a peak in May. VLO decreased in the succeeding months at stations 1 and 3 and marked a trough in September. Except for a minor peak in August this was true at station 2 also. VLO afterwards fluctuated around 10^6 at station 2. At stations 1 and 3 VLO increased till November and then showed a decreasing trend till the end of the study period. At station 1 fluctuation of VLO was similar to that of TVB. From September to February this was true at stations 2 and 3 also.

Population of VPLO varied between 10^3 and 10^7, the lowest being recorded at station 2 in september and higher at station 3 in November. At stations 2 and 3 an initial drop in VPLO was observed in April after which they started increasing. VPLO density which was around 10^4 in summer months increased in June-July at station 1 . In June -July VPLO population was around 10^6 at all stations. A drop in VPLO population after the retreat of south-west monsoon rain could be observed at all stations in September. Following this trough a peak in VPLO was observed at all stations in November. This was succeeded by another trough in December. A major increase in VPLO could be observed at station 2 by February.

VP population varied considerably from station to station. At station 1 it was around 10^3 and 10^4 in most of

the month. In November a peak density of 6.61×10^5 was observed at this station and in the succeeding month it went to below detectable level. At station 2 VP population attained above 10^6 in August, November and Februar hey were at below detectable level in April. In rest of the months VP density at station 2 varied around 10^3 and 10^4. Fluctuation of VP was close to that of VPLO . At station 3 wider oscillations were observed in VP population . They were at below detectable level in May and September, A VP density of around 10^5 was recorded in March, November and January at this station. In rest of the months they maintained a population between 10^3 and 10^4 .

TVB and vibrio showed very few significant correlations with physico-chemical parameters (Table 27) TVB showed significant positive correlation ($P<0.01$) with particulate organic carbon at stations 2 and 3. At station 1 VPLO and salinity showed a significant negative correlation ($P<0.05$)

Among TVB and vibrios in prawn a number of significant relations could be observed (Table 28). At stations 1 and 3 TVB correlated positively with VLO ($P<0.05$). VLO at station 2 positively correlated at significant level ($P<0.05$) with VPLO and VP. VPLO showed another significant positive correlation ($P<0.01$) with VP at station 2 and VLO at station 3.

3.2.2 Inter relationships between faecal indicator bacteria, TVB and vibrios

3.2.2.1 Water

Correlation coefficients between indicators and vibrios are given in Table 29. VLO showed significant positive correlations ($P < 0.05$) with FC at station 1 and FS at station 3. It also showed a significant negative correlation ($P < 0.05$) with EC at station 2. At the same significance level a positive correlation between TC and VPLO at station 1 and a negative correlation between FC and TVB at station 2 were also found. VP in water column did not show any significant relations with indicator bacteria.

3.2.2.2 Sediment

Significant relationships were observed between indicator bacteria and vibrios at stations 1 and 2 only (Table 30). At station 1 VP showed significant negative correlations with FC, EC ($P < 0.01$) and FS ($P < 0.05$). At station 2 TC correlated negatively with VLO ($P < 0.01$) and EC correlated positively with VPLO ($P < 0.05$). No significant relations could be observed at station 3.

3.2.2.3 Zooplankton

Significant correlations between indicators and vibrios in zooplankton were scattered (Table 31). EC showed significant positive correlations with VLO at station 3 ($P < 0.05$) and VPLO at station 1 ($P < 0.01$). TVB showed significant negative correlations ($P < 0.05$) with TC and FC at station 2. As in water VP did not showany significant relations with indicator bacteria.

3.2.2.4 Fish

In fish none of the vibrios monitored (VLO,VPLO and VP) showed significant relations with indicator bacteria (Table 32). However TVB showed significant negative relation with FC at station 1 ($P < 0.05$) and positive relation ($P < 0.01$) with FS at station 2. No significant correlations were observed at station 3.

3.2.2.5 Prawn

Unlike in fish VP in prawn showed significant positive correlations ($P < 0.05$) with TC and FC at station 2 (Table 33). At this station TVB also showed signigicant negative correlation ($P < 0.05$) with TC and FC. VLO and VPLO did not show any significant relations with indicator bacteria. Similarly stations 1 and 3 were devoid of any

significant relations between indicators and vibrios in prawn.

3.3 DISCUSSION

3.3.1 Ecology of *V. parahaemolyticus* and allied organisms in water

From the study it is evident that *V. parahaemolytic* and allied organisms were present in consistant numbers throughout the year in water. Occassional disappearance of *V. parahaemolyticus* was much pronounced at station 3 which receives more industrial and domestic waste. Oshiro (1964) Ose and Ikeda (1964) and Baross and Liston (1970) reported higher incidence of *V. parahaemolyticus* in natural waters which receive sewage and industrial waste. The nutrients and organic matter present in these effluents were found responsible for these high numbers. Unlike these reports a higher incidence of *V. parahaemolyticus* in water at station 3 receiving higher discharge of sewage and industrial effluents are not seen in this study. This may be due to the nature of effluent which contain more of detergents and disinfectants, used in the fish processing industry for maintaining hygeinic conditions.

V. parahaemolyticus being a moderate halophile, its distribution in water is dependent on the salinity. Extremes of salinity (high or low) are detrimental to this bacterium. Rheinheimer (1977) reported the occurrence of specific brackish water bacteria which was moderate halophiles (with optimum salinity 10-20‰). Abraham (1981) opined that V. parahaemolyticus may be put into this group because of its moderate halophilic nature. Yamamoto et al (1982) reported physical and chemical factors of sand assisting survival of V. parahaemolyticus in sand suspended seawater. However physico-chemical characters of suspended sediments were not mointored in this study. A significant relationship with salinity was seen in water at station 1 only. At stations 2 and 3 variations in salinities were not reflected in seasonality of V. parahaemolyticus. The detrimental effect of low salinity can be overcome by this bacterium if the water body is rich in organic matter of human or animal origin (Horie et al., 1967). In this study, particulate organic carbon showed considerable decrease from July onwards at all stations. In that case if the disappearance of V. parahaemolyticus from water column at station 1 is attributed to lowering of particulate organic matter, the survival of this bacterium at station 2 which is also low in organic carbon content needs further explanation. The direct influence of sea at station 2 may have a significant role in survival of this bacterium at thi station.

The role of phytoplankton on bacterial populations was studied by Simidu and coworkers (Simidu et al., 1977). They suggested that a depletion in bacterial population during phytoplankton blooms may be due to excretion of the metabolic products which are inhibitory to bacteria. During a bloom, there is a large amount of this antibacterial substance released into surrounding water which inhibits bacterial growth. They also suggested that during bloom period there is a high depletion of nutrient content in water column arising from their profuse multiplication. This also affects bacterial growth to a considerable extent. During monsoon period an increase in nutrients occurs through surface run off water. This can very well trigger a phytoplankton bloom which might have been the reason for depletion of V. parahaemolyticus in water at station 1.

Growth of V. parahaemolyticus is favoured by alkaline p^H (Beuchat, 1975). During July, water at all stations showed lower p^H. In few other months also lower p^H was recorded at different stations. Except at station 1 no significant relation was observed between V. parahaemolyticus density and p^H. Kaper (1979) reported an inverse relationship of V. parahaemolyticus and dissolved oxygen. Such a relationship was not observed in this study. In subtropical water bodies where temperature falls below 10°C, population of

V. parahaemolyticus is much influenced by temperature (Kaneko and Colwell, 1978). In Cochin backwater, being a tropical estuary, temperature fluctuation is between 25 and 35°C. This range of temperature is most favourable for growth of a mesophilic bacterium like V. parahaemolyticus. Therefore temperature is not a limiting factor for the distribution of V. parahaemolyticus in Cochin backwater.

Apart from the physical and chemical factors, there are a number of biological factors affecting the survival of V. parahaemolyticus. Miyamoto and Kuroda (1975) and Horie and Kobayashi (1981) reported the parasitic and lytic action of Bdellovibrio on V. parahaemolyticus. Yamamoto et al (1982b) in their experimental studies observed an increase in population of amoebae, bdellovibrio, myxobacteria and plaque forming units with the simultaneous reduction of V. parahaemolyticus. Similar biological factors may also be in operation in Cochin backwater affecting the dynamics of V. parahaemolyticus.

It is obvious that fluctuation of V. parahaemolyticus in water at station 3 is a complex phenonema. Apart from the physico-chemical parameters monitored in this study, some unknown factors which drastically affect the population dynamics of this bacterium at this station, are also involved. The erratic fluctuation of V. parahaemolyticus at this station

is a reflection of the unsteady ecological nature probably arising from the larger sewage and industrial effluents falling into this area.

Population of total viable bacteria (TVB) was around 10^7 and 10^8 in water at all stations. It showed a peak density in May probably due to increased temperature. During south-west monsoon rains TVB decreased and recorded lowest value at all stations. It showed a significant positive relationship with salinity and a negative relationship with dissolved oxygen at station 1. At station 2 a significant positive relationship was observed between TVB and p^H and no other physico-chemical parameters moitored in this study seemed to influence TVB.

VLO population at station 2 showed significant positive correlations with temperature and salinity and negative correlation with dissolved oxygen. It is probable that at station 2 which is much influenced by marine conditions, the VLO population is dominated by halophilc vibrios. Population of VPLO did not show a definite significant relationship with physico-chemical parameters. But at stations 1 and 2 fluctuations in VP was close to VPLO in a number of months. From the fluctuation patterns of VPLO and VP at station 2, it appears that a large

population of VPLO at this station is constituted by VP.

3.3.2 Ecology of *V. parahaemolyticus* and allied organism in sediment

Factors controlling the population dynamics of bacteria in sediment are complex. When compared with water, sediment offers a more stable ecological niche with more nutrients for bacterial proliferation. During most of the months population of *V. parahaemolyticus* were between 10^3 and 10^4 in sediment at station 1. Variations increased at station 2 and further at station 3. *V. parahaemolyticus* was reported to survive adverse seasons through association with sediment (Kaneko and Colwell, 1978). Such a survival is much pronounced at station 3 because in water at this station fluctuation of *V. parahaemolyticus* was very erratic while in sediment it was more stable. The south-west monsoon rains had an effect on distribution of *V. parahaemolyticus* in sediments at stations 1 and 2. A definite significant relationship was not observed with any of the physico-chemical parameters monitored in this study. But at station 3, a number of significant positive relationship existed between *V. parahaemolyticus* and allied organisms and TVB . From this it may be assumed that *V. parahaemolyticus* and allied organisms play a major role

in the population dynamics of TVB in sediment at this statio Since this station receives large amount of sewage and industrial effluents the sediments might be rich in mineral and organic nutrients. Higher densities of V. parahaemolytic and VPLO were observed at station 3 than in other stations. A number of fish processing industries are situated nearby this area and their effluents are rich in chitin and other materials of fish and prawn origin. They gradually settle in the bottom sediment at this station. It is natural that chitinoclastic bacteria like V. parahaemolyticus and allied organisms are seen in greater numbers in these sediments.

Anderson and Meadows (1978) suggested that in sediments of shallow water bodies great heterogeneity exists over minute distances and a number of diverse species find suitable ecological niche in close proximity. Such microenviornments play a major role in survival and growth of a number of diverse microorganisms. Yamamoto and Lopez (1985) demonstrated a positive relationship between bacterial abundance and surface area and organic content of marine sediments. The sediments encountered in this study were always of clayey nature. These fine sediments contain high organic content and this probably might have supported good bacterial growth.

3.3.3 Ecology of *V. parahaemolyticus* and allied vibrios in zooplankton

An organism flurish in an ecological niche only if the niche can provide adequate food and protection to the organism. This natural law bids to all living organism irrespective of their size and evolutionary status. *V. parahaemolyticus* was reported to absorb onto chitin of copepods with highest efficiency than other types of bacteria (Kaneko and Colwell, 1975). The primary reason for this was attributed to the chitinoclastic nature of *V. parahaemolyticus* and abundance of chitin in exoskeleton of copepods. The association of *V. parahaemolyticus* with copepod and chitinous materials has an important ecological role in nutrient regeneration. The dead and decaying zooplankton is acted upon by *V. parahaemolyticus* and other chitinoclastic bacteria and the chitin is degraded into other materials.

In a comparative study of bacterial flora in phytoplankton, zooplankton and in seawater, Simidu *et al* (1971) observed dominance of *Vibrio* and *Aeromonas* . *V. parahaemolyticus* was found to be present in higher numbers in zooplankton than in phytoplankton (Thompson *et al*., 1976). Keneko and Colwell (1973) reported that in summer

season 100% of the heterotrophic bacterial population isolated from plankton were Vibrio sp. In the present study, VLO was fluctuating around 10^5 and 10^6 in zooplankton. V. parahaemolyticus showed a significant positive relationsh with p^H at station 3. A contradictory observation was reported by Kaneko and Colwell (1975). They observed that adsorption of V. parahaemolyticus onto copepods was decreased when p^H was increased. However in general, Vibrio sp are favoured by alkaline p^H.

Population of TVB and VLO were higher in zooplankton than in water and sediment. At station 1, fluctuations in V. parahaemolyticus followed close to VPLO suggesting that major protion of VPLO in most of the months were constituted by V. parahaemolyticus. Their fluctuations were further apart at stations 2 and 3 . Only a small portion of VPLO was constituted by V. parahaemolyticus at station 3 from April to August. Occurence of zooplankton blooms at the retreat of monsoon rains in Cochin backwater were reported (Silas and Pillai, 1975). These blooms occur in August and November. Peaks in VPLO and V. parahaemolyticus densities in zooplankton were observed either at this bloom period or soon after the bloom. Abraham (1981) also observed higher counts of V. parahaemolyticus in plankton during zooplankton blooms in Vellar estuary.

Apart from p^H, salinity and concentration of divalent ions also influence adsorption of V. parahaemolyticus onto copepods and chitin (Kaneko and Colwell, 1975). They are of the opinion that electrostatic forces may exist between bacterial cell wall and chitinous materials favouring bacterial attachment. Also the induction of peritrichous flagellation in bacteria by substrata (Boer et al., 1975) is of great ecological significance because lateral flagellation can result in firmer attachment with the substratum. Kogure et al (1980) assessed the effect of phytoplankton and zooplankton on growth of heterotrophic bacteria. The overall results showed that phytoplankton specifically suppressed the growth of Vibrio sp while zooplankton enhanced it. In the present study also higher counts of V. parahaemolyticus and VPLO were observed in zooplankton especially during their bloom period. Sarkar et al (1983) reported the role of plankton in survival of V. parahaemolyticus in fresh waters. Through association with plankton this bacterium was able to survive in fresh water. Huq et al (1984) observed higher temperature, alkaline p^H and higher salinity favouring attachment and multiplication of V. cholerae to live planktonic crustanceans such as copepods. From the observation in Cochin backwater it is suggested that similar environmental conditions prevailing in this backwater might have favoured attachment

of V. parahaemolyticus to zooplankton. However the occassional disappearance of V. parahaemolyticus from zooplankton at different stations cannot be attributed to any cause with the parameters monitored in this study.

3.3.4 Ecology of V.parahaemolyticus and allied organisms in fish

Predominance of Vibrio sp in intestines of marine and estuarine fishes were reported earlier (Liston, 1957; Colwell, 1962). Higher number of V. parahaemolyticus was isolated from intenstine than from gills and skin in fishes caught from Vellar estuary (Abraham, 1981). A number of reasons were attributed for the dominance of Vibrio sp in intestine of fishes. Sera et al (1974) is of opinion that among heterotrophic bacterial group entering the intestine through food and water, Vibrio sp are able to survive the peculiar conditions (high acidity, presence of gastric juice and bile salts) encountered in the stomach. Therefore in due course of time they emerge as the major component of the gut microflora.

O'Brien and Sizemore (1979) observed a correlation between Beneckea harveyi and availability of chitin in intestine

of fishes. The food of the fishes include copepods and other zooplankton which are rich in chitin. It is then probable that chitinoclastic bacteria such as vibrio proliferate in the intestine of fishes where they get plenty of chitin. Then apart from finding a suitable ecological niche in the intestine, vibrios perform the ecological role of regeneration of nutrients through chitin degradation.

In the present study an organ-wise analyses of V. parahaemolyticus and allied organisms were not conducted. A portion of intestine, gills and skin with flesh were homogenated and the bacterial parameters were estimated. V. parahaemolyticus population was more erratic at station 1 whereas a stable population was monitored at station 3. It did not show a definite correlating pattern with the physico-chemical parameters monitored in this study. However in fishes caught from stations 1 and 2 population of V. parahaemolyticus significantly correlated positively with population of VPLO.

TVB in fishes caught from all stations recorded a significant positive correlation with particulate organic carbon. Goulder (1977) observed that most of the bacteria were attached to suspended solids in Humber estuary. The glucose mineralization potential of attached bacteria were

higher than free bacteria. It is natural that during feeding process large quantity of particulate matter are ingested by fishes. Apart from this an increase in particulate matter in water can result in adherence of these particles to gills and body surface . Since gill, skin and intestine of fishes were used in estimation of bacterial parameters it is obvious that an increase in particulate matter which is rich in bacterial load resulted in an increase of bacteria associated with fishes.

At station 3, TVB positively correlated with VLO and VPLO at significant levels. Similar significant correlation was also observed between VLO and VPLO at this station. From these it is obvious that in fishes at station 3, VLO and VPLO influence the dynamics of the TVB population. The close fluctuation of VPLO and V. parahaemolyticus at stations 1 and 2 reflect that the major fraction of VPLO at these stations are usually constituted by V. parahaemolyticus. The fluctuations of TVB were between 10^7 and 10^9 at all stations. During the summer months when temperature was higher TVB population in fishes were also higher.

Ruby and Morin (1979) in their experimental studies demonstrated progressive mevement of labelled bacteria

through the gastro intestinal tract of fishes. These bacteria proliferated during the movement and ultimately discharged into the surrounding water along with faeces. Intestine of fish act as enrichment tubes where multiplication of the bacteria takes place and latter they are shed into water . Such an enrichment in the intestine of fishes have a greater ecological significance to bacteria especially during adverse environmental conditions (Abraham, 1981).

3.3.5 Ecology of *V. parahaemolyticus* and allied organisms in prawn

When compared with fish, population of *V. parahaemolyticus* in prawn was more stable. Generally prawn harboured more number of *V. parahaemolyticus* and allied organisms. The higher number of TVB present in prawn can be attributed to the food and feeding mechanism. Prawn is a botton dwelling organisms and can be considered as omnivorous, scavenger or detritus feeder. Detritus are high in organic content and bacteria. Moreover the filter-feeding nature of prawn concentrate large number of bacteria including pathogen and particulate matter during feeding process of shellfish. (Cabelli and Hefferman, 1970; Plusquellec *et al.*, 1983; Timoney and Abston, 1984; Kelly and Dinuzzo, 1985).

Chitin constitute 60-80% of total organic constituents of prawn exoskeleton (Richards, 1951). It is obvious that chitnoclastic bacteria like vibrios find a suitable ecological niche in prawn than in fish. Since vibrios are able to produce chitinolytic enzyme they have a major ecological role in regeneration of nutrients as in their association with zooplankton. Hood and Meyers (1973) observed predominance of Vibrio and Beneckea in the intestine of penaeid shrimps and their role was suggested as production of chitinolytic enzymes. Though an organ-wise analysis of various bacterial parameters associated with prawn was not conducted it may be assumed that intestine of prawn also acts as a kind of enrichment tube for bacteria as in the case of fish (Abraham, 1981).

TVB associated with prawn showed significant positive correlations with particulate organic matter as in the case of fishes. Since higher number of bacteria are seen attached to suspended particles and prawn being a bottom feeding organism it is obvious that an increase in particulate matter in water results in an increase of bacterial load in prawn. Other environmental parameters monitored in this study did not show a definite significant correlation with V. parahaemolyticus and allied

organisms associated with prawn. Among the bacterial parameters, TVB showed significant positive correlation with VLO and VLO with VPLO. These correlations suggest that population dynamics of TVB associated with prawn are influenced by VLO and the close fluctuation of VLO and VPLO in prawn suggest that major portion of VLO population are constituted by VPLO. V. parahaemolyticus at station 2 alone showed significant positive correlations with VLO and VPLO.

A marked seasonality in fluctuation of V. parahaemolyticus and allied organisms in prawn was not observed in this study. However in May when temperature in water was maximum, population of TVB and VLO were higher. In November, VPLO and V. parahaemolyticus were higher in prawn at all stations. A similar peak in this month was observed in fish also. This may be considered as the after effect of zooplankton blooms. After the retreat of north-east monsoon in October a zooplankton bloom is expected. During the late phase of this bloom the zooplankton die and disintegrate. These suspended zooplankton particles are actively colonized by bacteria, especially by chitinoclastic vibrios. The filter feeding crustaceans concentrate these bacteria-rich suspended particles actively, whereas they may adhere to surface and

gills of fin fishes or ingested along with food and water. This can effect an increased density of VPLO and V. parahaemolyticus in fish and prawn in November.

3.3.6 Interrelationship between faecal indicator bacteria, TVB and vibrios

The concept that microorganisms indigenous to human faeces to index water-borne disease outbreak is a centuary old. Significant relationships between faecal indicator bacteria and enteric pathogens like Salmonellae were reported (Grunnet et al., 1970; Geldreich, 1974; Miskimin et al., 1976). In marine recreational waters swimming associated illness rate increased with bacterial indicator density (Cabelli, 1983). Until 1979 no significant relations between faecal indicators and V. parahaemolyticus were reported (Sayler et al 1976, Thompson and Vanderzant, 1976) Probable reason for this was that V. parahaemolyticus, though an enteric pathogen, was considered to be an autochthonous bacterial flora of the estuarine environment (Colwell et al., 1973; Sayler et al., 1976) whereas the primary source of faecal indicator bacteria was the intestinal tract of man and other higher animals. Therefore

in an estuarine environment allochthonous bacteria such as faecal indicators have a different, probably a diminisial, survival pattern when compared to the autochthonous bacteria, V. parahaemolyticus . Hence occurrence of any significant relationship between V. parahaemolyticus and faecal indicators in an estuarine or coastal environment was not seriously considered. However in 1979 Kaper et al (1979) reported a positive relationship of V. parahaemolyticus with total coliforms and faecal coliforms in chesapeake Bay. Subsequently Robertson and Tobin (1983) reported that Candida albicans, Pseudomonas aeruginosa and V. parahaemolyticus can complement faecal indicator such as faecal coliforms and faecal streptococ as indicators of human faecal pollution in Nova Scotian coastal waters. Watkin and Cabelli (1985) also found out that V. parahaemolyticus densities were significantly positively correlated with levels of faecal streptococci, E.coli and Clostridium perfringens in the Narragansett Bay.

In the present study V. parahaemolyticus did not show any significant correlation with bacterial indicators in water column, zooplankton or fish. However in sediment at station 1 significant negative correlations were observed between V. parahaemolyticus and bacterial indicators (FC, EC and FS). Similarly in prawn at station 2 significant

positive correlations between V. parahaemolyticus and coliforms (TC and FC) were observed . Except at single instance in water, sediment and zooplankton, VPLO also did not show a definite significant correlation pattern with indicator bacteria. The same is true in the case of VLO also. In fish and prawn VPLO and VLO did not exhibit any significant correlation with faecal indicators . Except in the case of sediment, TVB always correlated significalntly with one or more bacterial indicators. The correlations were positive with coliforms (TC,FC and EC) and negative with faecal streptococci. From this it is obvious that in Cochin backwater there do not exist a definite correlation between V. parahaemolyticus and faecal indicators. The few correlations which occurred at few instances were only a random phenomenon.

Colwell (1984) observed little relationship between V. parahaemolyticus , V. cholerae, V. alginolyticus, V. vulnificus and faecal coliforms in water and oyster meats. However the seawater and improperly . cooked oyster meat can serve as vehicles for transmission of vibrio infections. She also suggested that because of lack of correlation between faecal califorms and vibrios, a new combination of criteria should be adopted in evaluating shellfish quality. In the report of Watkin and Cabelli (1985) waste water had

an effect on V. parahaemolyticus densities through biostimulation of the food chain. Such an amount of waste water to produce a bio-stimulation does not occur in Cochin backwater because of two reasons. First of all such large amounts of sewage are not discharged into this backwater system as reported in Rhode Island and secondly water coming through the six major rivers into Vembanad lake dilute the sewage and the regular tidal cycles flush the estuary thoroughly.

3.3.7 Annual cycle of V. parahaemolytifus and allied organisms in Cochin Backwater

Seasonal variation of V. parahaemolyticus and allied organisms in water on sediment alone will not give any relevant information on the population dynamics of these bacteria in the estuary. Cochin backwater is a dynamic habitat influenced by six major rivers. The condition is further complexed by water transports and sewage of domestic and industrial orgin. The population dynamics of these bacteria associated with organisms occupying different trophic levels apparently give a more clear picture about their annual cycle and survival patterns.

In water, sediment and zooplankton V. parahaemolyticus was not detected at station 1 in July. It was also absent in sediment at station 2 and zooplankton at station 3 in this month. Large amount of fresh water with onset of south-west monsoon rains, lowering of salinity, low p^H and probable proliferation of phytoplankton during this season had an adverse effect on the population of V. parahaemolyticus. Kaneko and Colwell (1978) reported the survival of V. parahaemolyticus in association with sediment during winter season when the temperature of water is not congenial for growth of this bacterium. They suggested that during winter season when temperature falls below 10°C V. parahaemolyticus survive in sediments in a dormant state with hardly any multiplication. The adverse environmental conditions encountered in Cochin backwater are low salinity during monsoon period, occasional lower p^H and rather high salinity and temperature during summer season. Temperature never exceeded 33°C and receded below 25°C . At this range temperature will not be a limiting factor on V. parahaemolyticus Salinity in Cochin backwater ranged from 1.5 to 33.3% . During the higher salinity periods V. parahaemolyticus was detected in water, sediment and zooplankton.

The association of V. parahaemolyticus with fish and prawn plays an important role in annual cycle of this

bacterium in Cochin backwater. In July when V. parahaemolyticus was not detected in water, sediment and zooplankton at station 1 , they were isolated in consistent numbers from fish and prawn collected from this station. Mukhamedov et al (1971) reported, lake frogs, fish and water crayfish acting as reservoirs of vibrios in nature.

The highly erratic fluctuation of V. parahaemolyticus observed in water at station 3 and fish station 1 cannot be explained in the light of physico-chemical parameters monitored in this study. However the sewage and industrial effluents falling into station 3 may be a reason for such a type of fluctuation of V. parahaemolyticus in water at this station. In an earlier study (James, 1983) population of V. parahaemolyticus in freshly caught fish ranged from 1.1 to 5.4 x 10^2/g and in prawn it ranged from 2.7 to 5.4 x10^3/g. He also reportedhigher incidence of Vibrio counts in prawn than in fish. Less than 50% of the samples in his study were positive for V. parahaemolyticus. Results of the present study show higher level of V. parahaemolyticus associated with fish and prawn than reported by James (1983).

Among water, mud, crabs, shrimps, mussels and fishes examined in Mangalore, high incidence of V. parahaemolyticus

(33.3%) was found in shrimps (Karunasagar and Mohankumar, 1980). Out of sixteen strains isolated by them four were kanagawa positive. They also reported lower incidence of this bacterium in molluscs (8.6%) and crabs (8.33%) compared with estuarine water (18.75%) and mud (16.66%). Abraham (1981) observed that 40.6% of finfish, 51.7% of crustaceans and 63.3% of oysters were positive for V. parahaemolyticus in freshly harvested condition. Population of this bacterium varied from 1.5 to 460/g in finfish, 1.5 to 930/g in crustaceans and 1.5 to 9300/g in oysters. Nair (1981) reported that mean counts of V. parahaemolyticus in estuarine fishes and crustaceans at Porto Novo were 450/g and 530/g respectively. In the present study V. parahaemolyticus population ranged from below detectable level to 16.6×10^3/g in fish and from below detectable level to 28.84×10^3/g in prawn.

Except for an occassional disappearance from few samples V. parahaemolyticus was consistently found in all samples throughout the study period. There seems to be a high degree of interaction between trophic levels (water, sediment, zooplankton, fish and prawn) in the annual cycle of V. parahaemolyticus in Cochin backwater.

Table -18. Diagnostic criteria employed for identification of *V. parahaemolyticus*.

Test	Response
Gram stain	-
Motility	+
Growth in 1% peptone water containing	
0% NaCl	-
3% NaCl	+
7% NaCl	+
10% NaCl	-
Cytochrome Oxidase	+
Gas from glucose	-
Fermentation of	
Glucose	+
Sucrose	-
Cellobiose	-
Rhamnose	-
Dulcitol	-
Inositol	-
Sorbitol	-

Contd.....2.

Table -18. (Contd.....2)

Test	Response
Lactose	-
Mannitol	+
Maltose	+
Trehalose	+
Voges Proskauer	-
Methyl red	+
Nitrate reduction	+
Indole	+
Lysine decarboxylase	+
Arginine dihydrolase	-
Ornithine decarboxylase	+
H_2S production (TSI agar)	-
Gelatin liquefaction	+
Growth in Koser's citrate	+
Starch hydrolysis (aerobic)	+
Starch hydrolysis (anoerobic)	+
Urease	-
Catalase	+
Pleomorphism	+
Luminescence	-
Growth at 42^{o}C	+

Table -19. Correlation coefficient matrix between physico - chemical parameters and TVB, VLO VPLO and VP in water of stations 1, 2 and 3 (significance level *P < 0.05; **P < 0.01)

	Tempe-rature	pH	Salinity	Oxygen	Particulate organic carbon
			Station - 1		
TVB	0.2952	0.4409	0.6739*	-0.7204**	-0.2441
VLO	0.3290	0.5474	0.5226	-0.1503	-0.1568
VPLO	0.1324	0.1557	-0.2473	0.1877	0.1852
VP	0.4513	0.5916*	0.6349*	-0.3490	0.1091
			Station - 2		
TVB	0.5441	0.2086	0.3306	-0.4114	0.3704
VLO	0.5787*	0.3062	0.6379*	-0.6992*	0.4307
VPLO	-0.1495	-0.5179	-0.4495	0.1814	0.3454
VP	-0.0866	0.2404	0.0924	0.0881	0.0676
			Station -3		
TVB	0.0647	0.6031*	0.5698	-0.5369	0.2913
VLO	0.2946	0.1714	0.4527	0.7325**	0.4069
VPLO	0.0782	0.0020	-0.1342	-0.1438	0.7009*
VP	0.2445	0.2069	0.0844	-0.4375	0.0389

Table -20. Correlation coefficient matrix among TVB, VLO, VPLO and VP in water column of stations 1, 2 and 3 (Significance level *P < 0.05; **P < 0.01)

	TVB	VLO	VPLO	VP
		Station - 1		
TVB	1.0	0.2608	-0.1521	0.3774
VLO		1.0	0.3068	0.2133
VPLO			1.0	-0.0547
VP				1.0
		Station - 2		
TVB	1.0	0.5181	-0.5651	-0.4761
VLO		1.0	-0.1271	0.2073
VPLO			1.0	0.4317
VP				1.0
		Station - 3		
TVB	1.0	0.6453*	0.3418	0.2580
VLO		1.0	0.5340	0.4075
VPLO			1.0	0.0743
VP				1.0

Table -21. Correlation coefficient matrix between physico-chemical parameters and TVB, VLO, VPLO and VP in sediment of stations 1, 2 and 3 (Significance level *P < 0.05; **P < 0.01)

	Tempe-rature	pH	Total organic carbon	Total nitrogen	Total phosphorous
			Station - 1		
TVB	0.1537	-0.5229	0.6363*	0.4198	-0.5619
VLO	0.2094	0.1265	0.1705	0.0331	-0.2447
VPLO	-0.0107	0.4485	0.1802	0.4895	-0.0834
VP	0.6162*	0.1380	-0.1604	-0.0687	0.3175
			Station - 2		
TVB	0.1464	0.0896	-0.1250	-0.3003	0.1020
VLO	0.6351*	-0.2081	-0.6250*	-0.5014	-0.6616*
VPLO	0.2986	-0.7344**	0.0217	-0.1857	0.0620
VP	0.5094	-0.1838	-0.2622	-0.2005	-0.1264
			Station - 3		
TVB	0.2226	0.0751	-0.4531	-0.4424	0.2448
VLO	0.1886	0.1866	-0.1033	-0.0937	0.4229
VPLO	0.3260	0.1678	-0.2271	-0.2006	0.3366
VP	0.2221	0.3840	-0.0903	-0.1550	0.1535

Table -22. Correlation coefficient matrix between physico-chemical parameters and TVB, VLO, VPLO and VP in sediment of stations 1, 2 and 3 (Significance level *P < 0.05; **P < 0.01)

	TVB	VLO	VPLO	VP
		Station - 1		
TVB	1.0	0.3549	0.4411	0.0192
VLO		1.0	0.2979	0.2299
VPLO			1.0	0.3354
VP				1.0
		Station - 2		
TVB	1.0	-0.0798	0.0896	0.2445
VLO		1.0	0.0682	0.3098
VPLO			1.0	0.7077*
VP				1.0
		Station - 3		
TVB	1.0	0.7575**	0.7554**	0.2949
VLO		1.0	0.9089**	0.4484
VPLO			1.0	0.6900*
VP				1.0

Table -23. Correlation coefficient matrix between physico-chemical parameters and TVB, VLO, VPLO and VP in zooplankton at stations 1, 2 and 3 (Significance level *P < 0.05; **P < 0.01)

	Tempe-rature	pH	Salinity	Oxygen	Particulate organic carbon
			Station - 1		
TVB	0.1594	0.2901	0.3716	-0.3684	0.1901
VLO	0.0264	-0.5795*	-0.2338	0.2693	0.3751
VPLO	-0.3537	-0.5330	-0.4360	0.4488	0.0500
VP	0.1182	0.3042	0.4089	0.0824	0.1403
			Station - 2		
TVB	0.3442	-0.1864	0.2965	-0.2907	0.3325
VLO	-0.1753	-0.2574	-0.0396	-0.0034	0.2083
VPLO	-0.2728	-0.5246	-0.3607	-0.3939	0.2328
VP	-0.2220	-0.2697	-0.0570	0.2007	0.0278
			Station - 3		
TVB	0.2179	0.1940	0.1107	-0.3050	0.3123
VLO	0.0803	-0.4464	-0.5552	0.4288	0.5058
VPLO	-0.3700	-0.0271	-0.3772	0.2332	0.4392
VP	-0.3544	0.5853*	0.2634	0.1320	0.1050

Table -24. Correlation coefficient matrix among TVB, VLO, VPLO and VP in zooplankton of stations 1, 2 and 3 (Significance level *P < 0.05; **P < 0.01)

	TVB	VLO	VPLO	VP
		Station - 1		
TVB	1.0	-0.0904	-0.3128	0.7747**
VLO		1.0	0.4221	0.0823
VPLO			1.0	-0.0848
VP				1.0
		Station - 2		
TVB	1.0	0.3807	0.0213	0.1610
VLO		1.0	0.5188	0.5075
VPLO			1.0	0.8773**
VP				1.0
		Station - 3		
TVB	1.0	0.4549	0.2848	0.0411
VLO		1.0	0.4052	-0.0253
VPLO			1.0	0.3219
VP				1.0

Table -25. Correlation coefficient matrix between physico-chemical parameters and TVB, VLO, VPLO and VP in fish at stations 1, 2 and 3 (Significance level *P < 0.05; **P < 0.01)

	Temperature	pH	Salinity	Oxygen	Particulate organic carbon
			Station - 1		
TVB	0.6270*	-0.2331	0.0023	-0.4577	0.7474**
VLO	-0.1930	-0.1958	-0.4296	0.3409	-0.0341
VPLO	-0.2888	0.2071	-0.3204	0.3549	-0.1839
VP	-0.2409	-0.0327	-0.0626	-0.1076	-0.2869
			Station - 2		
TVB	0.7191**	-0.1545	0.2893	-0.4027	0.8274**
VLO	0.2446	-0.3604	-0.0915	-0.1931	0.1229
VPLO	0.0127	-0.6747*	-0.2906	0.0993	-0.3051
VP	0.1780	-0.4478	0.0233	-0.0306	-0.1897
			Station - 3		
TVB	0.4575	0.0291	0.1277	0.0518	0.6571*
VLO	0.6629*	-0.1425	0.1335	-0.1146	0.4410
VPLO	0.3220	-0.0379	-0.0874	0.0921	0.5253
VP	0.0557	0.5946*	0.2231	0.0305	-0.0348

Table -26. Correlation coefficient matrix among TVB, VLO, VPLO and VP in fish at stations 1, 2 and 3 (Significance level *P < 0.05; **P < 0.01)

	TVB	VLO	VPLO	VP
		Station - 1		
TVB	1.0	-0.1809	-0.3752	-0.3046
VLO		1.0	0.1542	-0.0183
VPLO			1.0	0.6253*
VP				1.0
		Station - 2		
TVB	1.0	0.1429	-0.1810	-0.0438
VLO		1.0	0.2822	0.3875
VPLO			1.0	0.8047**
VP				1.0
		Station - 3		
TVB	1.0	0.8431**	0.7403**	0.2998
VLO		1.0	0.7534**	0.2996
VPLO			1.0	0.2977
VP				1.0

Table -27. Correlation coefficient matrix between physico-chemical parameters and TVB, VLO, VPLO and VP in prawn at stations 1, 2 and 3 (Significance level *P < 0.05; **P < 0.01)

	Tempe-rature	pH	Salinity	Oxygen	Particulate organic carbon
			Station - 1		
TVB	0.2956	-0.0375	-0.3263	-0.2189	0.3299
VLO	-0.0164	-0.0113	-0.2591	0.1279	0.3502
VPLO	-0.4142	-0.0904	-0.6298*	0.3581	0.0310
VP	-0.0588	-0.2399	-0.3494	-0.0086	0.0961
			Station - 2		
TVB	0.5470	-0.4004	0.1366	-0.3978	0.8171**
VLO	-0.2468	-0.1957	-0.1798	0.2223	0.3456
VPLO	-0.4921	-0.3408	-0.4224	0.3860	-0.0525
VP	-0.4605	-0.1819	-0.2413	0.3818	-0.3692
			Station - 3		
TVB	0.2722	0.0803	-0.1638	0.1882	0.7187**
VLO	0.0162	0.2043	-0.3324	0.3496	0.3764
VPLO	0.1626	0.1198	-0.2339	0.0516	0.2611
VP	-0.0442	0.2360	-0.1867	-0.0169	-0.4402

Table -28. Correlation coefficient matrix among TVB, VLO, VPLO and VP in prawn at stations 1, 2 and 3 (Significance level *P < 0.05; **P < 0.01)

	TVB	VLO	VPLO	VP
		Station - 1		
TVB	1.0	0.6848*	0.2752	-0.1400
VLO		1.0	0.3609	-0.0092
VPLO			1.0	0.4184
VP				1.0
		Station - 2		
TVB	1.0	0.3543	0.0221	-0.3275
VLO		1.0	0.6809*	0.6506*
VPLO			1.0	0.7297**
VP				1.0
		Station - 3		
TVB	1.0	0.6620*	0.5234	0.0663
VLO		1.0	0.8142**	0.1631
VPLO			1.0	0.2778
VP				1.0

Table -29. Correlation coefficient matrix of TC, FC, FS, EC, TVB, VLO, VPLO and VP in water (Significance level *P < 0.05; **P < 0.01)

	TVB	VLO	VPLO	VP
		Station - 1		
TC	-0.2490	0.1065	0.6305*	-0.1125
FC	-0.4489	0.5817*	0.3492	-0.3968
EC	-0.1070	-0.3533	0.2285	-0.2260
FS	-0.2458	-0.0219	-0.0531	-0.3966
		Station -2		
TC	-0.2209	-0.5759	-0.1107	0.0147
FC	-0.6793*	-0.5608	0.5634	0.2133
EC	-0.2830	-0.6205*	0.1775	0.1703
FS	-0.3410	0.2129	-0.0688	0.1920
		Station - 3		
TC	0.1280	-0.1794	-0.0080	0.4026
FC	-0.0129	-0.1368	-0.1458	0.5265
EC	-0.2174	-0.4418	-0.0408	-0.0071
FS	0.4893	0.5874*	0.4214	0.1135

Table -30. Correlation coefficient matrix of TC, FC, FS, EC, TVB, VLO, VPLO and VP in sediment (Significance level *P < 0.05; **P < 0.01)

	TVB	VLO	VPLO	VP
		Station - 1		
TC	0.1561	0.1726	0.2160	-0.2382
FC	0.0808	-0.1615	0.1662	-0.7475**
EC	0.0237	-0.4874	0.1000	-0.7474**
FS	-0.3727	0.1360	-0.4183	-0.5831*
		Station - 2		
TC	0.3434	-0.7391**	-0.1490	0.1167
FC	-0.3733	-0.0983	0.3379	0.0650
EC	-0.4325	0.1162	0.5859*	0.2959
FS	-0.0207	0.1775	-0.1062	0.0948
		Station - 3		
TC	0.2553	0.3822	0.2111	-0.0225
FC	-0.0059	0.1984	0.2437	0.5172
EC	-0.2621	0.1054	0.1275	0.3983
FS	0.3722	0.3572	0.1690	-0.1023

Table -31. Correlation coefficient matrix of TC, FC, FS, EC, TVB, VLO, VPLO and VP in zooplankton (Significance level *P < 0.05; **P < 0.01)

	TVB	VLO	VPLO	VP
		Station - 1		
TC	-0.0792	-0.0129	0.1758	0.0431
FC	-0.0204	-0.3235	0.2822	0.0961
EC	-0.1899	0.3396	0.8047**	-0.2051
FS	0.1070	0.1244	0.1006	0.1592
		Station - 2		
TC	-0.6488*	-0.2126	0.3365	0.2937
FC	-0.6620*	-0.0480	0.4472	0.3932
EC	-0.3485	-0.0647	0.5433	0.5160
FS	-0.0318	0.5175	0.3791	0.4291
		Station - 3		
TC	-0.2985	0.2869	-0.2236	0.1766
FC	-0.1467	-0.0159	-0.2192	-0.0585
EC	0.2317	0.5885*	0.5180	-0.0746
FS	-0.2971	0.2946	0.5047	0.3647

Table -32. Correlation coefficient matrix of TC, FC, FS, EC, TVB, VLO, VPLO and VP in fish (Significance level *P < 0.05; **P < 0.01)

	TVB	VLO	VPLO	VP
		Station - 1		
TC	0.0103	0.4210	-0.1179	-0.1414
FC	-0.6022*	0.3734	0.3424	0.0855
EC	-0.2459	-0.2268	0.4396	0.1352
FS	0.5180	0.2995	-0.4764	-0.2733
		Station - 2		
TC	0.0975	-0.3939	-0.1843	-0.1250
FC	-0.5587	-0.4829	0.1286	-0.1116
EC	-0.2676	-0.5258	0.0429	-0.0544
FS	0.7762**	0.2474	-0.1847	-0.2343
		Station - 3		
TC	0.1866	-0.1286	-0.1006	0.2230
FC	-0.2750	-0.4317	-0.4867	-0.1797
EC	-0.4110	-0.4040	-0.2428	0.1574
FS	0.2130	0.2051	0.1333	-0.2280

Table -33. Correlation coefficient matrix of TC, FC, FS, EC, TVB, VLO, VPLO and VP in prawn (Significance level *P < 0.05; **P < 0.01)

	TVB	VLO	VPLO	VP
		Station - 1		
TC	-0.3233	0.1137	0.1837	-0.2796
FC	-0.0614	0.1818	0.4735	-0.1821
EC	0.5585	0.3490	0.4538	-0.3757
FS	0.3066	0.3320	-0.2756	-0.3245
		Station - 2		
TC	-0.6421*	0.3166	0.2865	0.5998*
FC	-0.6899*	0.2118	0.5188	0.6309*
EC	0.1412	0.3726	0.5642	0.1976
FS	0.4621	0.1300	-0.1085	-0.1422
		Station - 3		
TC	-0.1805	0.4397	0.2208	0.1460
FC	-0.4300	0.0450	-0.1159	0.2922
EC	-0.0695	0.0116	-0.0820	0.3389
FS	0.2731	0.2568	0.4043	-0.1568

Figure 9. Seasonal variation of TVB, VLO, VPLO and VP in water (100 ml^{-1}) of stations 1, 2 and 3.

Total viable bacteria (TVB) - (●---●)

Vibrio-like organisms (VLO) - (O — O)

Vibrio parahaemolyticus like organisms (VPLO) - (Δ — Δ)

Vibrio parahaemolyticus (VP) - (X---X)

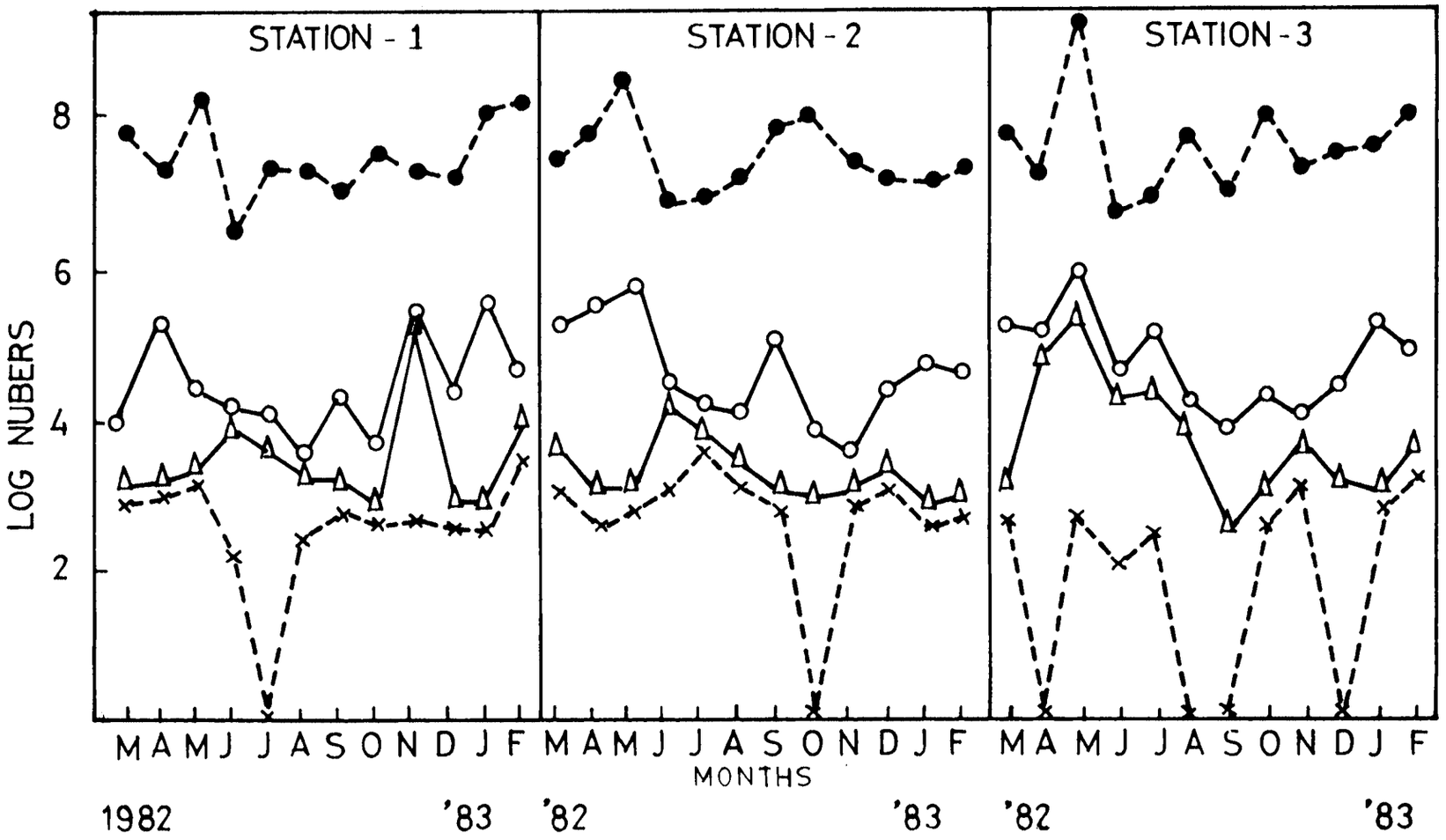

STATION - 1
STATION - 2
STATION - 3
LOG NUBERS
8
6
4
2
M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
MONTHS
1982
'83
'82
'83
'82
'83

FIG. 9

Figure 10. Seasonal variation of TVB, VLO, VPLO and VP in sediment (g^{-1}) of stations 1, 2 and 3.

Total viable bacteria (TVB) - (●---●)

Vibrio-like organisms (VLO) - (O — O)

Vibrio parahaemolyticus like organisms (VPLO) - (Δ — Δ)

Vibrio parahaemolyticus (VP) - (X---X)

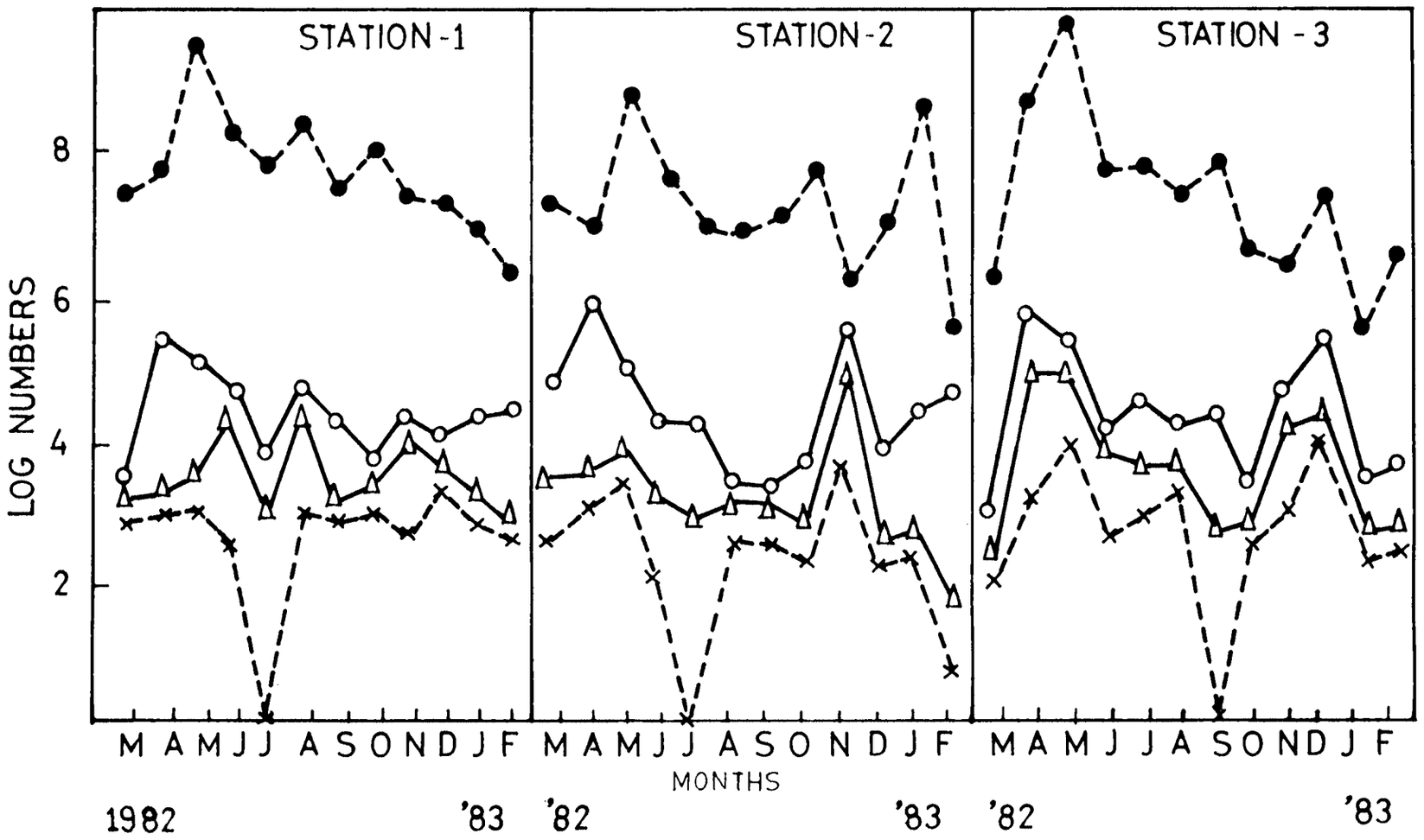

STATION-1
STATION-2
STATION - 3
LOG NUMBERS
8
6
4
2
M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
MONTHS
1982
'83
'82
'83
'82
'83

FIG.10

Figure 11. Seasonal variation of TVB, VLO, VPLO and VP in zooplankton (g^{-1}) of stations 1, 2 and 3.

Total viable bacteria (TVB) - (●---●)

Vibrio-like organisms (VLO) - (O — O)

Vibrio parahaemolyticus like organisms (VPLO) - (Δ — Δ)

Vibrio parahaemolyticus (VP) - (X---X)

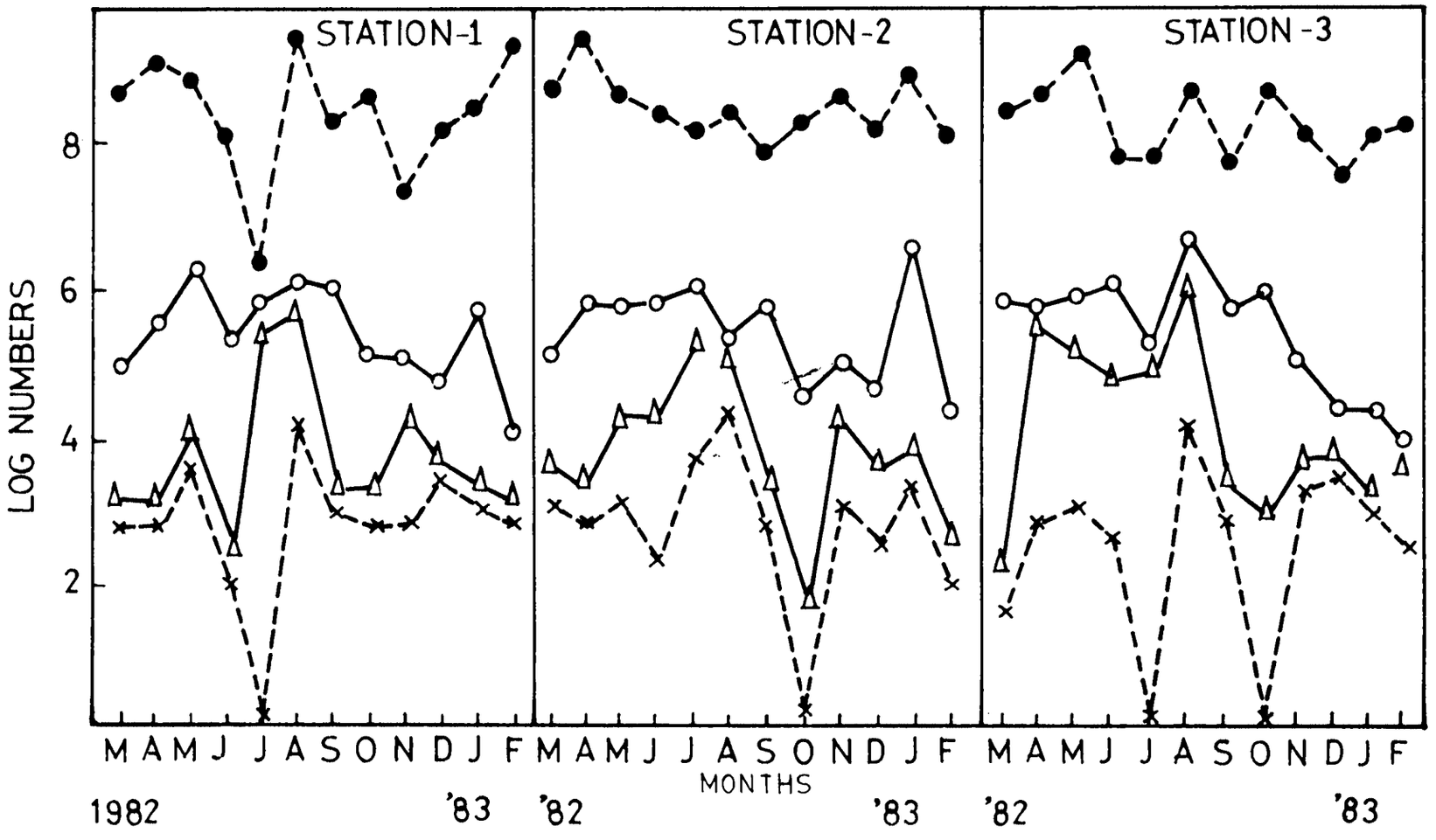

STATION-1
STATION-2
STATION-3
LOG NUMBERS
8
6
4
2
M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
MONTHS
1982
'83
'82
'83
'82
'83

FIG-11

Figure 12. Seasonal variation of TVB, VLO, VPLO and VP in fish (100 g^{-1}) of stations 1,2 and 3.

Total viable bacteria (TVB) - (●---●)

Vibrio-like organisms (VLO) - (O — O)

Vibrio parahaemolyticus like organisms (VPLO) - (▲ — ▲)

Vibrio parahaemolyticus (VP) - (X——X)

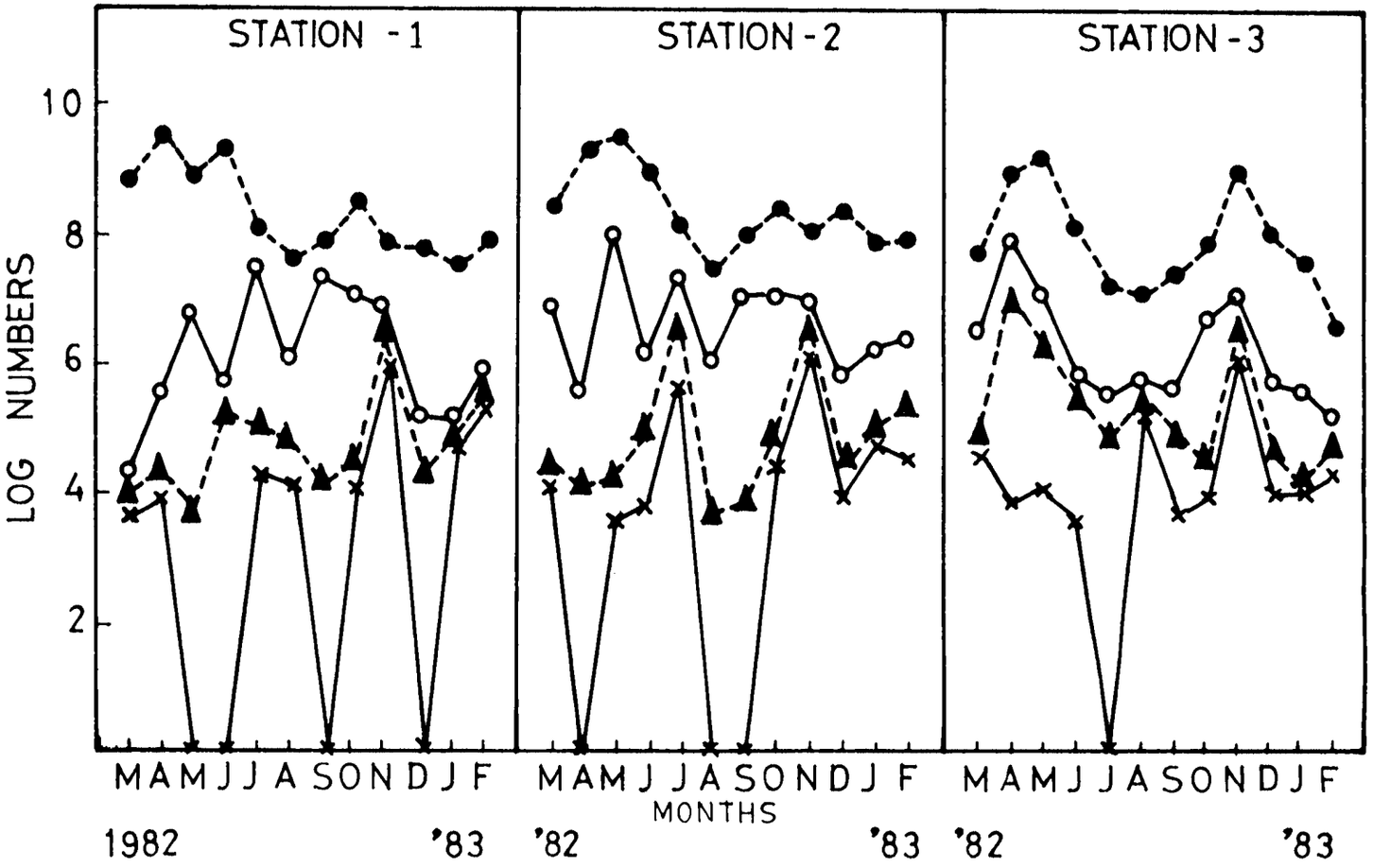

STATION - 1
STATION - 2
STATION - 3
LOG NUMBERS
10
8
6
4
2
M A M J J A S O N D J F
M A M J J A S O N D J F
M A M J J A S O N D J F
MONTHS
1982
'83
'82
'83
'82
'83

FIG .12

Figure 13. Seasonal variations of TVB, VLO, VPLO and VP in prawn (100 g^{-1}) of stations 1, 2 and 3.

Total viable bacteria (TVB) - (●- - -●)

Vibrio-like organisms (VLO) - (O — O)

Vibrio parahaemolyticus like organisms (VPLO) - (▲- - -▲)

Vibrio parahaemolyticus (VP) - (X——X)

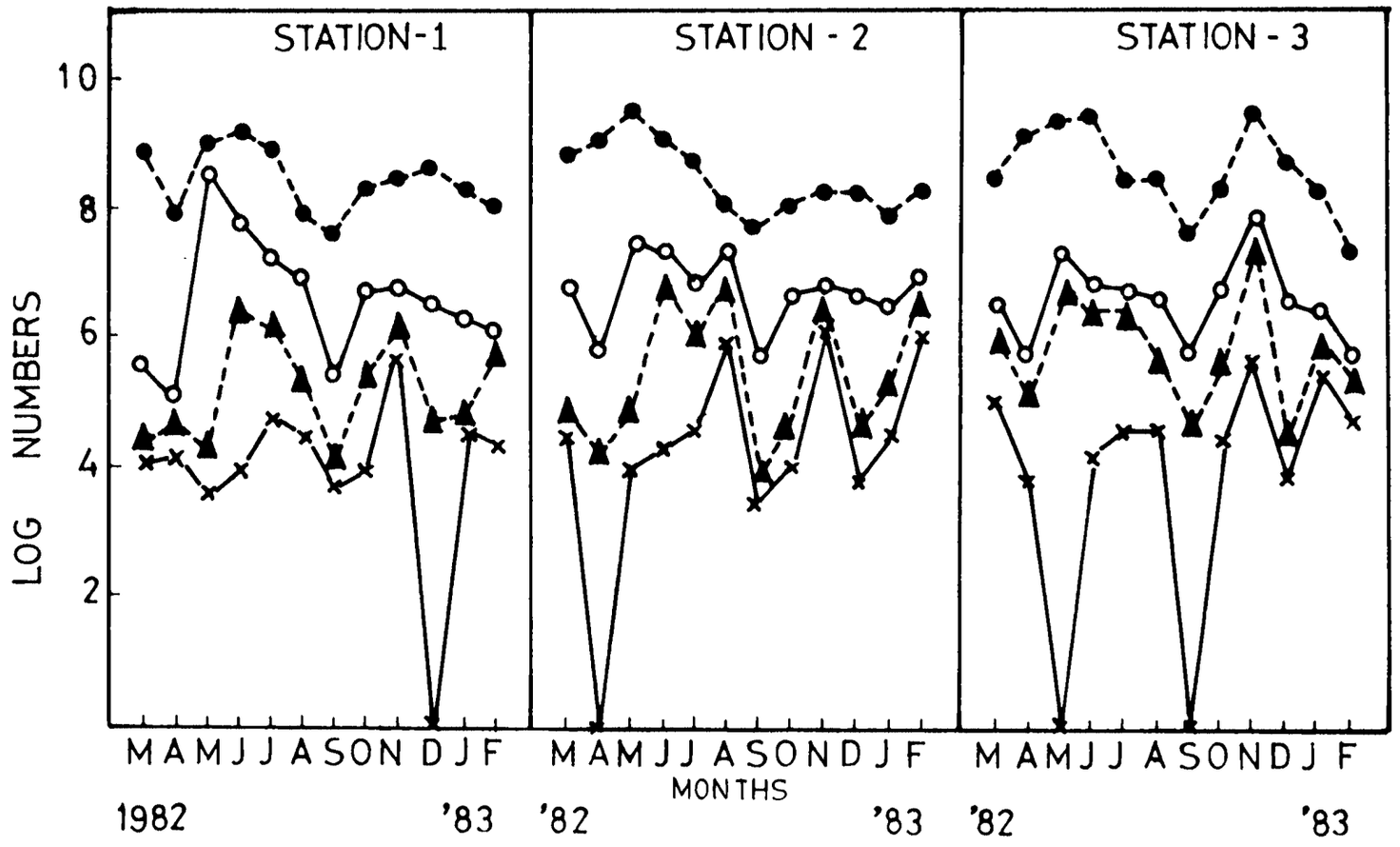

STATION-1
STATION - 2
STATION - 3
LOG NUMBERS
10
8
6
4
2
M A M J J A S O N D J F
MONTHS
1982
'83
'82
'83
'82
'83

FIG. 13

4 CHARACTERIZATION AND EXPERIMENTAL STUDIES ON SELECTED *V. PARAHAEMOLYTICUS* STRAINS

4.1 CHARACTERIZATION

4.1.1 MATERIAL AND METHODS

4.1.1.1 Source of strains

The strains used in this study (120) were those isolated from water, sediment, zooplankton, fish and prawn collected from three stations during March 1982 to Feb. 1983. Ten isolates each from a month were selected at random for characterization experiments. Sources and isolation details of the strains are given in Table 34 and they were previously identified as *Vibrio parahaemolyticus* using minimal tests.

4.1.1.2 Reference strains

Apart from the 120 strains *V. parahaemolyticus*, 7 strains received from other geographical regions were also included to serve as reference strains. Their numbers and names of donors are given in Table 35. Cultures were maintained on ZoBell's agar slants.

4.1.1.3 Characterization of strains

All the strains including reference strains were examined for 84 unit characters (Table 36). Tests were

repeated only in the event of inconclusive results.

a) Morphological and cultural characters

The tests included were gram staining, pigment production, swarming, motility and luminescence. Heat-fixed smears were prepared from fresh cultures (12-16 hrs.) for gram staining. Hucker's modification of Gram stain was adopted (Hucker and Conn, 1923). Pigment production was tested by growing the cultures on ZoBell's agar and seawater yeast extract (SWYE) agar. For testing swarming, cultures were inoculated on ZoBell's agar along with a known positive organism (V. alginolyticus) and observed after overnight incubation at room temperature (28± 2°C). Motility was tested using mannitol motility agar and doubtful cases alone were confirmed by hanging drop method. Cultures were grown on ZoBell's agar and photobacterium agar. Observations were made in a dark room after overnight incubation.

b) Physiological characters

This included tests to determine the ability of the isolates to grow at various temperatures and sodium chloride concentrations. For testing growth at different temperatures enriched isolates were inoculated into nutrient broth supplemented with 2% NaCl and incubated at respective temperatures (4, 15, 25, 30, 37 and 42°C). Observations were made after 14 days on cultures incubated at 4°C, after 96 hrs on those kept at 15°C and after

48 hrs in rest of the temperatures. Growth at various NaCl. concentrations were tested by inoculating enriched cultures into nutrient broth containing 0, 3, 5, 7, 8 and 10% (w /v) NaCl. Observations were made after 48 hours incubation at room temperature (28 ± 2°C).

c) Biochemical characters

These included tests for indole production, Kovac's oxidase, catalase, methyl red, voges-Proskauer, citrate utilization, nitrate reduction, hydrogen sulphide prodiction, gas production, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase. The results were usually read after five days incubation period. Production of indole was tested in 1% tryptone broth using Kovac's reagent (Kovacs, 1928). Kovac's oxidase test was done adopting method of Kovacs (1956) with 18 hr. cultures from ZoBell's agar. Methyl red and Voges-Prokauer tests were performed by growing the cultures in MR-VP medium supplemented with 2% NaCl. Catalase test was performed as per the method of Cowan(1974). Nitrate reduction was tested with 48 hr. cultures grown in 1% peptone water with 2% NaCl and 0.1% KNO_3. Citrate utilization was tested on Simmon's citrate agar. Hydrogen sulphide production was tested on Triple Sugar Iron (TSI) agar after 7 days incubation period. The mode of glucose metabolism and gas producti were tested using modified oxidation - fermentation medium of Leifson (1963). Production of arginine dihydrolase, lysine and ornithine decarboxylase were tested in the medium of Moeller(1955).

d) Production of extracellular enzymes

ZoBell's agar was used as the basal medium for the demonstration of extracellular enzymes and incubations were done at room temperature (28± 2°C). Amylase production was determined by growing the strains on ZoBell's agar supplemented with 2% starch. After 48 hr. incubation, plates were flooded with Lugol's iodine solution (Mannual of Microbiological methods, 1957). Basal medium was supplemented with 2%(w/v) collodial chitin (Berger and Reynolds, 1958) and chitinase activity of the strains were tested. Similarly gelatinase, lipase and urease activities were tested by incorporating 5% (w/v) gelatin (Skerman, 1967) 1% (v/v) Tween - 80 (Sierra, 1957) and 1% (w/v) urea respectively. Strains were recorded as positive when the zones of hydrolysis extended beyond the limits of growth.

e) Fermentation of carbohydrates

Modified marine oxidation - fermentation medium (Leifson, 1963) was used as the basal medium for these tests. Filter sterilized carbohydrates were incorporated at a concerntr of 1% (w/v) in sterile basal medium. Various carbohydrates tested are given in Table 36.

f) Utilization of various substrates

Various substrates were tested as single source of carbon and energy for the strains. Substrates included were

sugars and amino acids (Table 36). They were filter sterilized and incorporated at a concentration of 0.1% in sterile basal medium (Baumann et al., 1971). The medium with substrate was dispensed into petriplates and allowed to dry for 4-6 hr. Plates were then inoculated and incubated at room temperature (28± 2°C) for 2 weeks. Comparisons were made with a positive control (glucose as carbon source) and a negative control (medium lacking the carbon source) for confirmation of growth.

g) Sensitivity to 0/129

Sensitivity to 2,4 - diamino - 6,7 - diisopropyl pteridine phosphate (0/129) was determined on Brain Heart Infusion agar (HI-media). The agar plates were seeded with enriched cultures and sensitivity discs of 10 µg and 100µg concentrations were placed over it. Incubations were done at 37° for 18-24 hrs and observed for zones of inhibition.

4.1.2 RESULTS

Results of characterization tests of reference strains (7 numbers) and 120 strains of parahaemolyticus collected from various samples throughout the study period are presented below. Reactions of type strains, percentage of strains showing similar reactions and similarity index are given in Table 37. A few strains showed swarming in SWYE medium. Some failed to grow at 15°C and 42°C. Tolerance to different sodium chloride

concentrations were same for all strains. Few strains showed deviations in biochemical characters such as methyl red, utilization of citrate and lysine decarboxylase. One was catalase negative. In extra-cellular enzyme production, 5% of them failed to produce lipase. Lower similarities were observed in a few fermentation experiements such as fermentation of arabinose, cellobiose and glycerol. Lesser level of similarities were seen in utilization of arabinose, cellobiose and glycine as sole source of carbon and energy. All strains were sensitive to 10 µg and 100 µg of 0/129.

On the basis of percentage deviations in reactions of present strains from reference strain reactions, tests were classified into different classes (Table 38). 41 tests were put in one class since 100% of them showed reactions similar to those of reference strains. The test numbers are given in Table 38 and the corresponding reactions are presented in Table 36. These tests are very much useful in identification and confirmation of V. parahaemolyticus. A deviation of 0.1 to 5% were recorded in 29 tests. 7 tests showed deviation of 0.1 to 10% . They were fermentation of maltose, rhamnose, sorbital, and utilization of adonitol, lucine, proline and sorbitol. More than 10% deviations were observed in the case of methyl red test, fermentation of arabinose, cellobiose, glycerol, utilization of arabinose, cellobiose, and glycine. These tests may not have a direct application in confirming the identity of V. parahaemolyticus.

Swarming was observed in 3.3% of V. parahaemolyticus strains. Four strains did not grow at 15°C and two of them failed to grow at 42°C. One was catalase negative. This strain was negative for methyl red test, did not ferment mannose, and fermented and utilized rhamnose. It showed 92.86% similarity with type strain reactions. 88.3% of strains were positive for methyl red test. Four strains were not able to utilize citrate as sole source of carbon and among these there were negative in methyl red test also. Negative reactions for lysine decarboxylase and ornithine decarboxylase were shown by 0.8% and 2.5% of strains respectively.

In production of extracellular enzymes one strain did not produce chitinase and six of them failed to produce lipase. Arabinose, cellobiose and glycerol were fermented by less than 80% of the strains. Two strains were able to ferment dulcitol. Galactose was not fermented by four strains and among these two strains did not ferment fructose also. These two strains were negative in fermentation of arabinose, glycerol, maltose, mannitol, mannose and trehalose. Their overall similarities with reference strains were 88.1 and 89.20%. Inositol was fermented by 3 strains. Less than 6% of the strains were not able to ferment maltose, mannitol, mannose and trehalose. 10% of them were able to ferment and utilize sorbitol. 80.8 and 82.5% of strains showed similariries in utilization of arabinose and cellobiose respectively (Table 37). A few strains showed deviations in utilization of duicitol, fructose and galactose. Glycine was not utilized by any of the reference

strains of V. parachaemolyticus, but 48.3% of V.parahaemolyticus strains from Cochin backwater were capable of utilizing it. This was the test in which maximum deviation between strains isolated from Cochin backwater and type strain was observed. Less than 3% of strains were negative in utilization of maltose, mannitol and mannose. Three strains were able to ferment and utilize melezitose.

Percentage similarity of individual strains with type strains are given in Table 39. Three isolates VP 42, VP 44 and VP 72 showed 100% similarity with type strains in characterization experiments. Similarly two strains, VP 58 and VP 61, showed below 90% similarity with type strains. Consolidated data showing frequency of strains falling at different percentage similarily levels are given in Figure 14. Among the 120 strains 95 strains showed 95% and above similarity (cumulative frequency) with reference strains. Maximum frequencies of strains (28 strains) were observed at similarities of 97% and 98%. 90,89 and 88% of similarities were shown by one strain each respectively.

4.1.3 DISCUSSION

Characterization experiment was conducted to find out whether there exists any marked variations in morphological, cultural or biochemical characters between V. parahaemolyticus strains isolated from Cochin backwater and those reported from other regions of the world. All the strains in the present study formed a single group along with reference strains at 88% and

above similarity level. Only 3 of them showed 100% similarity with reference strains while maximum number of strains (28 each) clustered at 98% and 97% similarity levels. 95% and above similarity was shown by 95 strains. Variations in characters at different levels were shown by a number of strains. Though such variations in strains of a species are common in nature, variations in cardinal characters which form the basis of diagnostic criteria deserves great attention.

According to Hugh and Sakazaki (1972) growth at 42°C and presence of lysine decarboxylase enzyme are important diagnostic characters in the identification of V. parahaemolyticus. Two strains did not grow at 42°C and three strains did not produce lysine decarboxylase in the present study. Sucrose fermentation is a cardinal character in differentiating V. parahaemolyticus from other halphilic Vibrio spp such as V. alginolyticus and V. harveyi. None of the strains included in this study fermented sucrose. Similarly lactose was also not fermented by any of the strains . In a numerical taxonomic study on Vibrio spp (Kaper et al., 1983), V. parahaemolyticus clustered into three phena among which two were sucrose positive. In characterization studies of marine Vibrio spp, Gilmour (1977) observed a large number of V. parahaemolyticus fermenting lactose. In the present study sucrose or

lactose fermenting V. parahaemolyticus were not encountered. This can be attributed to the incorporation of VP medium which screens off sucrose and lactose positive forms during initial isolation itself.

Though urease positive and indole negative strains of V. parahaemolyticus were reported earlier (Colwell, 1970; Kaper et al., 1983), none of the strains included in this study showed these variations. Spreading on solid media, though not an important identifying character, was very uncommon in V. parahaemolyticus. From strains in the present study exhibited swarming. A number of reports exist on the production of acetyl methyl carbinol and negative to methyl red test by V. parahaemolyticus (Twedt et al., 1969; Abraham, 1981; Nair, 1981). 11.7% of the strains in this study showed negative to methyl red test but none were positive to Voges-Proskauer test. These two tests are important in differentiating V. parahaemolyticus from V. alginolyticus. Variabilities in these tests raises problems in the differentiation of these two species. Golten and Scheffers (1975), Nair (1980a) opine that a number of intermediate forms between V. parahaemolyticus and V. alginolyticus exist in nature.

It can be assumed that the diagnostic criteria employed in the present study with the incorporation of

VP medium eliminated a number of intermediate strains of V. parahaemolyticus. This is evidenced by high degree of homogenity (95 strains agreed $\geq$ 95% of characters) encountered in the strains. Complete absence of growth at 10% NaCl can be attributed to the moderate halophilic nature of this bacterium. Minor variations in other characters such as fermentation and utilization of sugars can be regarded as the result of ecological variability existing in Cochin backwater.

4.2 EFFECT OF PHYSICO-CHEMICAL PARAMETERS ON THE GROWTH OF SELECTED V. PARAHAEMOLYTICUS STRAINS

4.2.1 OBJECTIVES

Growth of V. parahaemolyticus is influenced by various physico-chemical parameters such as temperature, p^H, salt concentrations etc. Except for a few sporadic reports (Covert and Woodburn, 1972; Vanderzant and Nickelson, 1972; Rottini et al; 1973; Beuchat, 1974, 75) no precise systematic studies on the effect of the above parameters on the growth of V. parahaemolyticus have been conducted so far. Hence this experiment was carried out to determine the effect of temperature, p^H and NaCl concentrations individually and collectively on the growth of V. parahaemolyticus.

4.2.2 MATERIAL AND METHODS

Tryptic soy broth was used as the basal medium for determining the effect of temperature, P^H and NaCl concentration on growth of V. parahaemolyticus strains K23 and K132.

Tryptic Soy Broth (Hi-media)

Pancreatic digest of casein	:	17.0 g
Papaic digest of soya - meal	:	3.0 g
Sodium chloride	:	5.0 g
Potassium phosphate (dibasic)	:	2.5 g
Dextrose	:	2.5 g
Distilled water	:	1000 ml
P^H	:	7.3 $\pm$ 0.2

Sodium chloride concentration and P^H of the basal medium were modified appropriately and incubated after inoculation at different temperatures. Two cultures (K 23 and K 132) were selected for these experiments.

Six hour old cultures enriched in tryptic soy broth supplemented with 2% NaCl was used as the inoculum. A standard inoculation loop of 3mm diameter was used for inoculation throughout the experiments. Inoculations were

done into 10 ml of sterile appropriately modified (NaCl and p^H) medium and incubated in water bath at different temperatures. After appropriate incubation periods the optical density (OD) of the broth was measured in a spectrophotometer (Hitachi Model - 200) at 600 nm. All experiments were carried out in duplicate.

4.2.2.1 Effect of temperature on growth

Various temperatures tested in the experiment were 10, 15, 20, 25, 30, 35, 40 and 45°C. A water bath with $\pm$ 0.2°C accuracy was used for this purpose. NaCl concentration of the basal medium was raised to 3% and inoculations were done as mentioned in 4.2.2 and incubated at appropriate temperatures. OD values were taken after 18 hrs. except for those incubated at 10°C. Growth of the cultures incubated at 10°C was measured after 5 days only.

4.2.2.2 Effect of p^H on growth

Different p^H values tested were 5, 6, 7, 8 and 9. NaCl concentration of the basal medium was raised to 2% and p^H was adjusted using 0.1 N NaOH or 0.1 N HCl. After completing inoculations, broths were incubated at 35°C for 18 hrs. and turbidity were measured.

4.2.2.3 Effect of NaCl concentration on growth

NaCl concentrations of the basal medium was adjusted to 0.5, 2, 4, 6 and 8 %. p^H was maintained at 7.3 ± 0.2 and incubation temperature at 35°C. After inoculation, broths were incubated for 18 hrs. and OD values were taken.

4.2.2.4 Combined effect of temperature, p^H and NaCl concentrations on growth

Effect of individual parameters on growth of V. parahaemolyticus was determined, normally keeping the other parameters constant. In this experiment, the three parameters were changed simultaneously and their effect on growth was determined. Basal medium with different combination of NaCl concentration and p^H were prepared, inoculated and incubated at different temperatures. OD values were taken after 18 hrs. (except for those incubated at 10°C which was taken after 5 days).

4.2.3 RESULTS

4.2.3.1 Effect of temperature on growth

The effect of temperature on growth of V. parahaemolyticus at p^H 7.5 and 3% NaCl concentration is recorded in Figure 15. The optimum temperature, NaCl

concentration and p^H for maximum growth and pattern of growth were similar for both the strains . Hence the value of optical density exhibited by one of the strain (K 132) was taken and ploted against temperature, p^H and NaCl concentration. There was a sharp increase in growth from 10 to 20°C , whereas from 20 to 25°C no appreciable increase in growth could be observed. Again,from 25 to 30°C remarkable increase in growth was observed. 30°C was found to be the optimum temperature for maximum growth at the specified p^H and NaCl concentrarion. Later,a sharp decline in growth was observed. Growth rate at 45°C was below that at 10°C.

4.2.3.2 Effect of p^H on growth

Effect of varying p^H on growth of V.parahaemolyticus (K 132) at 30°C and 3% NaCl concentration is given in Figure 16. Optimum growth was at p^H 8. At p^H 5 growth was very limited. On increasing p^H, there was a sharp increase in growth. This rapid increase was continued upto p^H 7. From p^H 7 increase in growth was slow and this continued upto p^H 8. Maximum growth was recorded at this p^H and on further increase in p^H growth decreased.

4.2.3.3 Effect of NaCl concentration on growth

Being a halophilic bacterium, growth of *V. parahaemolyticus* is influenced by changes in sodium chloride co concentration. Effect of varying NaCl concentrarion on growth of *V. parahaemolyticus* (K 132) is given in Figure 17. p^H and temperature of incubation during this experiment were maintained at 7.5 and 30°C respectively. Good growth was observed at 0.5% NaCl concentration itself. Growth increased substantially on increasing NaCl concentration. Maximum growth was observed at 4% NaCl concentration. On further increase of NaCl concentration, growth showed a sharp decline and at 8% NaCl concentration very poor growth was observed.

4.2.3.4 Combined effect of temperature, p^H and NaCl concentration on growth

Effect of simultaneous variations in temperature, p^H and NaCl concentrations on growth of *V. parahaemolyticus* (K 132) are given in Figure 18 a-h. At 10°C, p^H and salinity ranges for growth of *V. parahaemolyticus* (K 132) were narrow (Figure 18a) No growth was observed at p^H 5. Similarly at 6 % and 8% NaCl concentrations no growth was recorded. At 2% NaCl concentration growth could be observed from pH 6 to 9. Likewise at pH 7 good growth was seen from 0.5 to 6% NaCl concentration. This was followed by pH 7

in 4% NaCl concentration and p^H 8 in 2% NaCl concentration. At p^H 9 faint growth could be observed at 2% NaCl concentration at this temperature.

At 15°C also no growth was observed at p^H 5 as well as at 8% NaCl concentration (Figure 18b). Ranges in p^H and NaCl concentration for growth of V. parahaemolyticus (K 132) were wider at 15°C than those at 10°C. Maximum growth at 15°C was observed at p^H 6 in 2% NaCl concentration. At 6% NaCl concentration growth was observed only at p^H 7, whereas at p^H 9 growth could be observed from 0.5 to 4% NaCl concentrations.

Growth was much accelerated at 20°C and it extended further to p^H 5 and 8% NaCl concentration (Figure 18c) which never occurred at previous temperature (19 and 15°C). At p^H 5 growth was seen at 0.5 and 2% NaCl concentration at this temperature. Similarly at 8% NaCl concentration faint growth observed at p^H 7 and 8. From p^H 6 to 9 and 0.5 to 6% NaCl concentrations, good growth was observed at 20°C. Maximum growth was observed at p^H 7 in 4% NaCl concentration.

Growth pattern of V. parahaemolyticus (K 132) at different p^H and NaCl concentrations at 25°C is given in Figure 18d. Growth extended further at p^H 9 to 8% NaCl concentration. Apart from this, growth ranges of V. parahaemolyticus in respect of NaCl concentration and p^H were similar to those at 20°C. At p^H 8 and 9 maximum growth was observed at

6% NaCl concentration at 25°C while it was at 4% NaCl concentration at 20°C. At 20 and 25°C maximum growth was seen at pH 7 and 4% NaCl concentration.

Growth range at pH 5 has extended upto 4% NaCl concentration at 30°C (Figure 18 e) Maximum growth was observed at pH 7 in 6% NaCl concentration. At 35°C (Figure 18 f) and pH 5 growth of V. parahaemolyticus (K 132) occurred at 2% NaCl concentration only. The optimum pH and NaCl concentration for maximum growth at 35°C were 8 and 6% respectively. At 40°C (Figure 18 g) ranges of pH and NaCl concentration for growth of V. parahaemolyticus (K 132) had still narrowed. There was no growth at pH 5 as well as 8% NaCl concentration. Further growth was not observed at pH 6 in 6% NaCl concentration at 40°C. However, optimum pH and Nacl concentration for growth of V. parahaemolyticus (K 132) occurred at a very low level at different pH and NaCl concentrations. There was no growth at pH 5 and 6. Similarly growth was not observed in 8% Nacl concentration. It was found that growth of V. parahaemolyticus (K 132) at this temperature was mostly confined to pH 7 and 8, and NaCl concentrations of 2-4%. The optimum pH and NaCl concentration for growth of V. parahaemolyticus (K 132) at 45°C were found to be 7 and 2% respectively (Figure 18 h).

Overall assessment of growth pattern of *V. parahaemolyticus* (K 132) showed remarkable shifts in optimum pH and NaCl concentrations when temperature was increased from 10°C to 45°C (Table 40). At 10 and 15°C optimum pH NaCl concentration were 7 and 2% respectively. At 20 and 25°C optimum NaCl concentration had shifted to 4% while optimum pH remained at 7 itself. At 30°C optimum NaCl concentration had further increased to 6% while there was no change for optimum pH. At 35 and 40°C, there was no change for optimum NaCl concentration and it remained at 6% itself. But optimum pH had shifted from 7 to 8. at 45°C all these were reversed and optimum pH and NaCl concentration had come back to 7 and 2% respectively as at 10 and 15°C.

V. parahaemolyticus can grow at wider range of pH and NaCl concentrations at 30°C. Deviations to either side of this temperature resulted in shortening of these ranges.

Growth was always more in the alkaline pH (pH 8 and 9) than in the acidic pH (pH 5 and 6) at all temperatures On deviation on either side of optimum temperature (30°C) retardation of growth was faster in the acidic pH than in the alkaline pH.

4.2.4 DISCUSSION

4.2.4.1 Effect of varying temperature, pH and NaCl concentration on the growth of V. parahaemolyticus

Growth of V. parahaemolyticus is influenced by temperature, pH and NaCl concentration. These parameters have got a maximum and minimum values, beyond which growth of the bacterium is lethally affected and an optimum value at which it proliferate rapidly.

The effects of physico-chemical parameters on growth and survival of V. parahaemolyticus have been carried out in tissue homogenates of fish and prawn and in semisynthetic media (Tryptic soy broth and Nutrient broth) (cited in 1.2.23) Being a seafood brone pathogen, studies on the effect of physico-chemical parameters on V. paramaemolyticus were centered round their inactivation or survival at higher temperatures and refrigeration and frozen temperatures. The halophilic nature of V. parahaemolyticus prompted the workers to study the effect of sodium chloride and other cations influencing their survival at the above metnioned temperature ranges. There exists a wide gap of knowledge

on the effect of temperature, pH and NaCl concentration individually and collectively on the growth of V.parahaemolyticus between these inactivation temperatures. The main objective of the present work was to systematicaly analyse the effect of temperature, pH and NaCl concentration individually and collectively on the growth of V. parahaemolyticus (K 23 and K 132).

In temperatures above 10°C growth of V. parahaemolyticus (K 23 and K 132) was very much accelerated. From 20 to 25°C much increase in growth was not observed . However,there was rapid proliferation of bacteria from 25 to 30°C. Amount of growth was similar at 20, 25 and 35°C. Optimum temperature for growth of V. parahaemolyticus was reported to be 35 to 37°C (Beuchat, 1975). But in the present investigation maximum growth was obtained at 30°C. A difference in survival ability between kanagawa positive and negative strains of V. parahaemolyticus was reported by Barrow and Miller (1974). Kanagawa positive strains were usually isolated from clinical case while Kanagawa negative strains were isol ed from marine sources. Kanagawa positive strains grew better and quickly in tissue homogenates and survived longer under extremes of acid and alkaline conditions than Kanagawa negative strains. Whereas in

autoclaved seawater, Kanagawa negative strains survived longer than Kanagawa positive strains. In the present study, the strains of V. parahaemolyticus (K23 and K132) tested were isolated from estuarine environment. Hence, optimum growth temperature might be at 30°C which is very close to the ambient temperature rather than 35 and 37°C which is the body temperature of the vertebrates. High retardation of growth at 35, 40 and 45°C also reflected the inability of the isolates to aclimatise and rapidly proliferate at these temperatures.

The influence of pH on growth showed that pH 8 was most favourable for V. parahaemolyticus (K 23 and K 132). pH 7.6 to 8.6 was reported to be the optimum for growth of V. parahaemolyticus (Beuchat, 1975). Marine bacteria such as vibrios have an optimum pH in slightly alkaline conditions and V. parahaemolyticus is found to be no exception for this. Growth of V. parahaemolyticus in pH ranging from 5 to 11 (Twedt et al., 1969), 5 to 8 (Beuchat, 1973) and 5 to 10 (Ermolina and Shikalov, 1975) were already reported.

Being a halophilic bacterium, growth of V. parahaemolyticus is much influenced by sodium

chloride concentration. Palasuntheram (1981) reported that there is no specific requirement for sodium and chloride ions and they can be substituted by sodium sulfate, sodium nitrate, potassium sulphate and magnesium chloride. He classified V. parahaemolyticus to group 3 of the halophiles put forth by Larsen (1962). Optimum NaCl concentration for V. parahaemolyticus (K 23 and K 132) were found to be 4%, whereas approximately 3% NaCl concentration was reported earlier to be optimum (Beuchat 1974, 75). NaCl was found to have a protective effect on growth and survival of V. parahaemolyticus at lower temperatures (Temmyo et al., 1966; Covert and Woodburn, 1972) and at higher temperatures (Covert and Woodburn, 1972; Beuchat, 1975). An increase in the NaCl concentration reduces the water activity of the medium and thereby alter the osmotic sensitivity of the cells (Beuchat, 1974). Except in two months in the year (June and July), salinity in Cochin backwater was higher and pH was generally alkaline. Therefore, in the experimental condition in which temperature and pH were maintained at 30°C and 7.5 respectively (which were close to the ambient temperature and pH) the strains naturally showed an optimum preference to higher salinity. A difference in tolerance levels to higher salinities between clinical and environmental isolates were already reported (Sakazaki and Balows, 1981). Clinical isolates

cannot survive at salinities more than 7% whereas environmental isolates survive in 8 or 10% salinity also. The strains of V. parahaemolyticus (K 23 and K 132) have been isolated from Cochin backwater. Probably, this may be the reason for affinity towards slightly higher salinity for maximum growth.

Combined effect of temperature, pH and NaCl concentration on growth of V. parahaemolyticus (K 23 and K 132) also revealed some interesting patterns. Earlier reports (Beuchat, 1973; Goldmintz, 1974) showed that highest resistance of V. Parahaemolyticus to heat was at pH 7. A change in both ways from pH 7 increased sensitivity to heat. Sodium chloride has a protective effect against inactivation by heating (Covert and Woodburn, 1972) and freezing (Temmyo, 1966; Covert and Woodburn, 1972). At lower temperatures, growth was observed only in the alkaline pH (Beuchat, 1973). In V. parahaemolyticus (K 23 and K 132) also similar observations were made at higher temperature. More growth at near inactivation temperatures was in alkaline pH. From the findings it is obvious that V. parahaemolyticus (K 23 and K 132) have maximum growth at pH 7 and 2% NaCl concentration at extremes of temperature range tested in the study. Near optimum temperature it preferred an alkaline pH and higher NaCl concentration.

At 35 and 40°C good growth was obtained at 6% NaCl concentration. This may be because of the protective effect of NaCl at higher temperatures as reported earlier (Covert and Woodburn, 1972; Beuchat, 1975; James, 1983). At 45°C the protective effect of NaCl seems to be lost because maximum growth at this temperature was observed at 2% NaCl concentration. Moreover the pH preferendum for maximum growth also have come back from 8 to 7.

4.3 ANTIBIOTIC AND METAL RESISTANCE/SESNITIVITY OF SELECTED V.PARAHAEMOLYTICUS STRAINS

4.3.1 METRIAL AND METHODS

4.3.1.1 Antibiotic sensitivity test

Antibiotic sensitivity tests were carried out by disc diffusion method. Nutrient agar supplemented with 2% sodium chloride was used as the basal medium. Cultures enriched in nutrient broth (6 hrs) containing 2% sodium chloride were seeded over nutrient agar with sterile cotton swabs. Using flamed forceps antibiotic discs were placed on the agar surface sufficiently separated from each other to avoid overlapping of inhibition zones.

The discs were lightly pressed with the forceps to make complete contact with the medium. After 30 minutes (pre-diffusion time), the plates were incubated at 37°C for 18-24 hours. The antibiotics used, their concentrations and interpretation of inhibition zones are given in Table 41. Diameter of inhibition zones were measured at the end of incubation period, compared with the interpretive chart of Kirby - Bauer sensitivity test method modified in July 1969 (Schering Corporation, U.S.A. Bloomfield, N.J.) and classified accordingly. For convenience of discussion intermediate strains were also included under resistant forms. Thus strains were classified into two types namely sensitive and resistant.

4.3.1.2 Metal sensitivity test

Metal sensitivity was also tested on nutrient agar medium supplemented with 2% NaCl. Filter sterilized salts of 10 metals were incorporated in the sterile molten basal medium at different concentrations (Table 42) before dispensing into petriplates. Cultures enriched as described in section 4.3.1.1. were spot inoculated on the metal incorporated medium. For comparision of growth, basal medium without addition of metals were also inoculated with the cultures. Plates were incubated at 37°C for 18-24 hours.

If no growth was seen after 24 hours, plates were reincubated for an additional 24 hours. If growth was observed within 48 hours the isolate was treated as resistant to that concentration of the metal. The criteria used by Austin et al (1977) was further extended to, other metals in this study and the critical concentration was fixed as 10 ppm for mercury and 100 ppm for other metals for differentiation of the strains into sensitive and resistant forms. Minimal inhibitory concentrations (MIC) of the resistant strains were found out and the maximum tolerance levels were also worked out.

4.3.2 RESULTS

4.3.2.1 Antibiotic resistance/sensitivity of *V. parahaemolyticus* strains

Result of antibiotic sensitivity tests of 120 strains of *V. parahaemolyticus* are given in Table 43. All strains were resistant to penicillin. This was followed by kanamycin (95%), sulphadiazine (88.3%) and ampicillin (85%). Resistance to streptomycin was shown by 82.5% of the strains. The least resistance was observed against chloramphenicol (6.7%) and gentamycin (3.3%).

V. parahaemolyticus strains showed highest sensitivity towards gentamycin (96.7%) followed by chloramphenicol (93.3%). Only 66.3% of the strains were sensitive to oxytetracycline. Sensitivity to neomycin and polymyxin-B were less than 50%. Only 5% were sensitive to kanaymycin.

Variations could be observed in percentage resistance/sensitivity of strains from different samples (Figure 19). Highest resistance to ampicillin (95.6%) was shown by strains from prawn. Strains from water, sediment and associated with fish showed resistance around 86%. Lowest resistance to ampicillin was recorded among strains isolated from zooplankton (77.3%). Resistance to chloramphenicol was around 8% among strains from water, sediment and prawn while it was around 4.5% from zooplankton and fish. Strains from sediment samples did not show resistance to gentamycin, while in other samples around 4% of the strains were found resistant to the above antibiotic.

All strains isolated from fish and prawn exhibited resistance to kanamycin while 96.3% of strain from sediment and around 90% of strains from water and zooplankton showed resistance to the same antibiotic. There was wide variations in resistance of strains to neomycin. Highest resistance was observed among strains

from sediment (92.6%) followed by strains from prawns (86.9%). Lowest resistance to neomycin was observed among V. parahaemolyticus strains isolated from zooplankton. Resistance to oxytetracycline varied from 25% in prawn to 44.4% in zooplankton. All strains irrespective of the samples, were resistant to pencillin. Resistance to polymyxin-B varied from 59.1% in zooplankton to 74.1% in sediment. Strains associated with fish and prawn showed a resistance of 73.9% to that antibiotic. Lowest resistance to streptomycin was shown by strains from water samples. 90% of strains from sediment and prawn showed resistance to streptomycin while among those from zooplankton and fish it was around 82%. High resistance to sulphadiazine was observed. It varied from 95.7% in fish to 80% in water. Among the strains isolated from zooplankton and prawn the resistance to sulphadiazine was around 91% while from sediment it was only 85.2%.

Strains from water samples showed highest resistance to penicillin followed by ampicillin and kanamycin (Figure 19) whereas high resistance was observed towards penicillin,(100%) kanamycin (96%) and neomycin (93%) among the strains from sediments. The strains isolated from zooplankton exhibited resistance (100%) towards

penicillin followed by kanamycin and sulphadiazine (91% each). All strains from fish showed resistance to penicillin as well as kanamycin. This was followed by resistance to sulphadiazine (95.7%). 100% of the strains from prawn samples also showed resistance to penicillin and kanamycin. 95.6% of strains were resistant to ampicillin and was followed by streptomycin and sulphadiazine (91.3% each).

The most common antibiotic resistance patterns are given in Table 44. Among the 120 isolates, 36 numbers showed a resistance pattern of IKNPXSZ. This was followed by two other patterns IKNOPXSZ and IKNPSZ represented by 13 and 12 strains respectively. Seven strains showed a resistance pattern of IKNOPSZ and 5 strains exhibited a pattern of IKPXSZ. 28 strains of V. parahaemolyticus showed unique patterns represented by only one, and six other patterns were represented by 2 strains each (Table 44). All strains showed multiple resistance. Of the 10 antitiotics tested, multiple resistance patterns to 9 antibiotics were (IJKNOPXSZ) represented by 3 strains and ICKNOPXSZ was represented by a single strain. Multiple resistance patterns to minimum number of antibiotics were OP and PZ represented by one strain each.

Among the 120 strains of V. parahaemolyticus, antibiotic resistance decreased from pneicillin to gentamycin

The strains isolated from water exhibited the following trend:

P > I = K > Z > S > N > X > O > C > J

The strains isolated from sediment showed the following antibiotic resistance pattern :

P > K > N > S > I = Z > X > O > C > J

The pattern among strains associated with zooplankton was as below:

P > K = Z > S > I > X > N > C = J

Decreasing drug resistance pattern in fish and prawn were similar to some extend. The strains associated with fish showed the resistance pattern as follows :

K = P > Z > I > S > N > X > O > C = J and

V. parahaemolyticus strains associated with prawn exhibited

K = P > I > S = Z > N > X > O > C > J pattern.

4.3.2.2 Metal resistance/sensitivity of *V. parahaemolyticus* strains

Results of metal sensitivity tests carried out on *V. parahaemolyticus* strains are given in Table 45. Among 10 metals tested all the strains were resistant to 5 metals namely cadmium, iron, zinc, molybdenum and lead. None of the strains were resistant to silver and cobalt. Resistance to mercury, copper and nickel varied among strains. Sensitivity to 10 ppm of mercury was shown by 1.67% of strains. Similarly sensitivity to 100 ppm of copper and nickel were exhibited by 0.83% and 38.33% of strains respectively.

As in the case of antibiotic sensitivity pattern, some difference could be noticed in metal sensitivity patterns from sample to sample also. Sensitivity to mercury was shown by 4% of strains isolated from water and 4.54% of strains isolated from zooplankton. None of the *V. parahaemolyticus* strains from sediment, fish and prawn exhibited sensitivity to mercury. Another difference was in the sensitivity towards copper. Sensitivity to 100 ppm of copper was shown by 4.54% of strains from zooplankton only. Lowest resistance to nickel was elaborated by strains from water (48%). Resistance to nickel from other samples varied between 60 - 70%.

The most common metal resistance patterns are given in Table 46. 60.84% of strains showed resistance pattern of mercury copper and nickel. This was followed by a pattern of mercury and copper (36.67%). Other patterns were at 0.83% each.

Minimal inhibitory concentration (MIC) of all the metals to 120 V. parahaemolyticus strains were worked out. Concentrations of each metal tested and the cumulative inhibition at each concentrations are given in Table 47. The critical concentration for classifying a strain into resistant or sensitive was taken as 10 ppm for mercury and 100 ppm for other metals (Austin et al., 1977). None of the strains tested showed inhibition towards the cadmium, iron, zinc, molybdenum and lead. MIC for these metals were found to be 400 ppm for cadmium and zinc, 450 ppm for iron and 1000 ppm for molybdenum and lead. All the strains were inhibited at 25 ppm of mercury, 75 ppm of silver and 50 ppm of cobalt. Highest inhibitions at MIC levels were shown by cadmium (88.33%) followed by lead (87.5%) and zinc (81.67%).

4.3.2.3 Comparison of antibiotic and metal resistance/ sensitivity patterns of V. parahaemolyticus strains

Results of antibiotic and metal resistance tests were compared to find out whether there exist any qualitative or quantitative relationship between antibiotic and metal resistance patterns of V. parahaemolyticus. All strains of V. parahaemolyticus tested were found to be multiple resistant to antibiotic and metals. The most common antibiotic resistant pattern was IKNPXSZ exhibited by 36 strains. Among them 27 strains showed a metal resistance pattern of Cd, Fe, Zn, Mo, Pb, Hg, Cu, Ni (Table 46). There was only a single strain each showing a metal resistance pattern of Cd, Fe, Zn, Mo, Pb, Hg. Antibiotic resistance pattern of the above strain was also unique - PSZ, which was not found in any of the strains tested.

4.3.3 DISCUSSION

There are a number of reports available on the antibiotic sensitivity of V. parahaemolyticus whereas no information is available on their metal sensitivity. Because of differences in the concentration of antibiotics used, methods adopted and types of antibiotics used (Sakazaki et al., 1963; Sanyal et al., 1973), an overall comparison of antibiotic sensitivity studies conducted by various workers is impractical.

In the present study, V. parahaemolyticus strains were sensitive to chloramphenicol (93.3%) and gentamycin (96.7%). Similar observations were reported by Sanyal et al (1973), Joseph (1974), Sircar et al (1976) and Sen et al (1977). Kaneko and Colwell (1978) observed that all strains of V. parahaemolyticus were sensitive to chloramphenicol and they did not include gentamycin in their experiment. 66.3% of the strains showed sensitivity to oxytetracycline. Lower sensitivity to oxytetracycline was reported by Sanyal et al (1973) and James (1983). Contradicting reports are available on the kanamycin and streptomycin sensitivity of V. parahaemolyticus. Higher resistance to kanamycin was reported by Joseph (1974), Sircar et al (1976) and Sen et al (1977) whereas Chatterjee et al (1970), Sanyal et al (1973) and James (1983) reported higher sensitivity of V. parahaemolyticus to this antibiotic. Sensitivity to kanamycin among strains isolated from Cochin backwaters was very low (5%). Similarly, higher sensitivity to streptomycin by V. parahaemolyticus was reported by Chatterjee et al (1970), Sanyal et al (1973), Kaneko and Colwell (1978), Abraham (1981) and James (1983) whereas higher resistance to streptomycin was reported by Joseph (1974), Sircar et al (1976) and Sen et al (1977). Sensitivity to streptomycin was found to be only 17.5% among V. parahaemolyticus strains.

Higher resistance to ampicillin (Sanyal et al., 1973; Joseph, 1974; Sircar et al., 1976; Sen et al., 1977; James 1983), sulphadiazine and penicillin (James, 1983) were reported earlier and the present study also corroborate with their findings. Only one-fourth of the strains tested here were sensitive to neomycin, whereas in earlier studies (Sanyal et al.,1973; Kaneko and Colwell, 1978; James,1983) majority of V. parahaemolyticus were found to be sensitive to the above drug. Joseph (1974) reported that all isolates of V. parahaemolyticus tested were found to be intermediate in their sensitivity to neoymycin. The classification of strains into intermediate group raises problems in their clinical and epidemiological significance. They should also be considered with equal seriousness as resistant groups.

Colwell and Sizemore (1974) reported that frequencies in antibiotic resistance among bacteria depend on the amount and kinds of antibiotics used in that area. Grabow and Prozesky (1973) observed higher number of antibiotic resistant bacteria in hospital sewages. Application of sulphonamide and tetracycline in fish ponds resulted in an increase of bacteria resistant to these antibiotics (Aoki, 1974). Penicillin,

ampicillin, streptomycin and sulphadiazine are frequently used in large amounts in this area in chemotherapy. This may be the reason for the occurrence of higher resistance in *V. parahaemolyticus* to these drugs.

Godwin and Slater (1979) reported that resistance to ampicillin, streptomycin and sulphonamide were lost in *E. coli* on phosphate limited growth while resistance to ampicillin was lost on phosphate as well as carbon limited growth. In the present study, *V. parahaemolyticus* was isolated from samples occupying various trophic levels in Cochin backwater. It could be speculated that bacteria associated with organisms at higher trophic level (fish and prawn) are rather in a nutrient rich environment than those associated with organisms of lower trophic level (zooplankton) and abiotic factors (water and sediment). Moreover in an association with organisms at higher trophic level, bacteria have an easier access to various growth factors and seldom face any nutrient limited conditions. In such a stable environment they are well protected against stress from environment. Therefore, it is obvious that drug resistance factors acquired in

bacteria associated with organisms at higher trophic levels are seldom lost due to stress or nutrient limitation when compared with bacteria associated with lower trophic level organisms. Multiple antibiotic resistant V. parahaemolyticus were higher in prawn than in other samples. Also, the antibiotic resistance to individual antibiotics, in general, was more in fish and prawn.

As there are no reports available on the metal sensitivity pattern of V. parahaemolyticus, a comparative study of the present work cannot be made. However, resistance to antibiotics are usually associated with resistance to metals (Allen et al., 1977; Timoney et al., 1978; Devanas et al., 1980., Sjogren and Port, 1981; Michaels et al., 1984). Resistance to antibiotics, metals and other toxic substances were associated with the possession of an extra-chromosomal genetic element commonly known as R-plasmid (Colwell and Sizemore, 1974). Resistance, if once acquired is a stable characteristic and it exhibits a heritable continuity. Since information for resistance to antibiotics as well as metals are present in R-plasmid, it is natural that bacteria resistant to antibiotics show resistance to metals also.

The question of to which antibiotics or to which metals that one organism shows resistance, depends on the information coded in the R-plasmid. Moreover R-plasmid transfer between bacteria are of frequent occurrence in nature (Koditschek and Guyre, 1974; Goyal and Hoadlay, 1979)

Reports on the resistance of bacteria to metals include resistance to nickel (Kurata et al., 1977), arsenic (Smith, 1978), mercury (Timoney et al., 1978), cadmium (Devanas et al., 1980), chromium (Luli et al., 1983), cadmium, nickel and iron (Kadri and Salem, 1985), copper (Rho, 1984) and zinc and cadmium (Gauthier et al., 1985). In the present study all V. parahaemolyticus strains were resistant to cadmium, iron, zinc, molybdenum and lead. Around 99% of the strains showed resistance to mercury and copper whereas resistance to nickel was approximately 62%. None of the strains showed resistance to silver and cobalt. These two metals may not be present in sufficient levels in Cochin backwater to initiate resistance in bacterial communities.

Minimal inhibitory concentrations of metals like molybdenum and lead have gone upto 1000 ppm. These

metals may be present in Cochin backwater in higher concentrations and bacteria might have developed resistance. Or the basal medium (nutrient agar) used in this study which contain coagulizing substances like peptone might have interfered in the sensitivity of V. parahaemolyticus to these metals. But such general media were used with reasonable success by Austin et al (1977), Timoney et al (1978) and Devanas et al (1980).

Association of drug resistance with metal resistance was reported earlier (Devanas et al., 1980; Michaels et al., 1984; Kadri and Salem, 1985). In the present study, though a statistically significant relationship was not observed between antibiotic resistance and metal resistance, higher resistance to antibiotics as well as metals could be observed among V. parahaemolyticus. Similar to antibiotic resistance, higher resistance to metals were also shown by V. parahaemolyticus isolated from fish and prawn. These findings corroborate with the report of Cooke (1976 a) who reported higher incidence of antibiotic resistant coliform and faecal coliform bacteria in shellfish than in seawater. Dowding and Davis (1975)

and Cooke (1976 b) stated that bacteria with antibiotic resistance have a selective advantage over antibiotic sensitive forms in natural environment. This can also imply to metal resistant bacteria. In such case the exhaustive use of antibiotics in chemotherapy and release of untreated or primarily treated industrial or domestic sewage containing metals into natural environment has a definite impact on the ecosystem.

Table - 34. Details of strains isolated in the study employed for characterization experiments

Serial No.	Isolate No.	Sample	Location	Month
VP 1	K 8	Fish	Station 2	March 1982
VP 2	K 10	Prawn	Station 1	,,
VP 3	K 23	Prawn	Station 3	,,
Vp 4	K 35	Fish	Station 3	,,
VP 5	K 45	Sediment	Station 2	,,
VP 6	K 49	Zooplankton	Station 2	,,
VP 7	K 50	Water	Station 1	,,
VP 8	K 54	Sediment	Station 1	,,
VP 9	K 55	Water	Station 1	,,
VP 10	K 57	Zooplankton	Station 1	,,
VP 11	K 60	Prawn	Station 1	April 1982
VP 12	K 63	Sediment	Station 3	,,
VP 13	K 66	Water	Station 2	,,
VP 14	K 71	Zooplankton	Station 2	,,
VP 15	K 72	Fish	Station 3	,,
VP 16	K 73	Prawn	Station 3	,,
VP 17	K 75	Fish	Station 1	,,
VP 18	K 78	Zooplankton	Station 3	,,
VP 19	K 82	Water	Station 1	,,
VP 20	K 84	Sediment	Station 1	,,

Contd.....2.

Table -34. (Contd.....2.)

Serial No.	Isolate No.	Sample	Location	Month
VP 21	K 88	Prawn	Station 1	May 1982
VP 22	K 95	Fish	Station 3	,,
VP 23	K 97	Fish	Station 2	,,
VP 24	K 103	Prawn	Station 2	,,
VP 25	K 104	Water	Station 3	,,
VP 26	K 109	Zooplankton	Station 3	,,
VP 27	K 111	Zooplankton	Station 2	,,
VP 28	K 116	Sediment	Station 1	,,
VP 29	K 121	Sediment	Station 2	,,
Vp 30	K 124	Water	Station 1	,,
VP 31	K 129	Sediment	Station 3	June 1982
VP 32	K 137	Water	Station 2	,,
VP 33	K 151	Zooplankton	Station 2	,,
VP 34	K 159	Sediment	Station 1	,,
VP 35	K 164	Water	Station 3	,,
VP 36	K 169	Zooplankton	Station 1	,,
VP 37	K 173	Fish	Station 2	,,
VP 38	K 187	Prawn	Station 2	,,
VP 39	K 191	Prawn	Station 3	,,
VP 40	K 192	Fish	Station 3	,,

Contd.....3.

Table -34. (Contd.....3.)

Serial No.	Isolate No.	Sample	Location	Month
VP 41	K 204	Fish	Station 2	July 1982
VP 42	K 218	Prawn	Station 1	,,
VP 43	K 230	Fish	Station 1	,,
VP 44	K 238	Prawn	Station 3	,,
VP 45	K 247	Water	Station 2	,,
VP 46	K 254	Zooplankton	Station 2	,,
VP 47	K 255	Zooplankton	Station 2	,,
VP 48	K 256	Sediment	Station 3	,,
VP 49	K 257	Sediment	Station 3	,,
VP 50	K 262	Water	Station 3	,,
VP 51	K 265	Prawn	Station 2	August 1982
VP 52	K 278	Prawn	Station 1	,,
VP 53	K 289	Fish	Station 3	,,
VP 54	K 292	Fish	Station 3	,,
VP 55	K 310	Sediment	Station 3	,,
VP 56	K 314	Zooplankton	Station 3	,,
VP 57	K 316	Water	Station 2	,,
VP 58	K 329	Water	Station 2	,,
VP 59	K 338	Water	Station 2	,,
VP 60	K 345	Sediment	Station 1	,,

Contd.....4.

Table -34. (Contd.....4.)

Serial No.	Isolate No.	Sample	Location	Month
VP 61	K 346	Prawn	Station 1	September 1982
VP 62	K 350	Sediment	Station 1	,,
VP 63	K 351	Sediment	Station 1	,,
VP 64	K 352	Water	Station 2	,,
VP 65	K 353	Sediment	Station 2	,,
VP 66	K 355	Sediment	Station 2	,,
VP 67	K 357	Zooplankton	Station 2	,,
VP 68	K 359	Water	Station 1	,,
VP 69	K 361	Zooplankton	Station 3	,,
VP 70	K 362	Zooplankton	Station 3	,,
VP 71	K 369	Prawn	Station 3	October 1982
VP 72	K 381	Fish	Station 3	,,
VP 73	K 388	Prawn	Station 1	,,
VP 74	K 390	Fish	Station 2	,,
VP 75	K 399	Water	Station 1	,,
VP 76	K 400	Water	Station 1	,,
VP 77	K 409	Sediment	Station 2	,,
VP 78	K 411	Sediment	Station 3	,,
VP 79	K 413	Sediment	Station 1	,,
VP 80	K 415	Zooplankton	Station 1	,,

Contd.....5.

Table -34. (Contd.....5.)

Serial No.	Isolate No.	Sample	Location	Month
VP 81	K 418	Fish	Station 1	November 1982
VP 82	K 429	Fish	Station 2	,,
VP 83	K 451	Prawn	Station 2	,,
VP 84	K 457	Prawn	Station 3	,,
VP 85	K 464	Water	Station 2	,,
VP 86	K 468	Water	Station 3	,,
VP 87	K 472	Sediment	Station 3	,,
VP 88	K 475	Zooplankton	Station 3	,,
VP 89	K 478	Sediment	Station 2	,,
VP 90	K 482	Zooplankton	Station 2	,,
VP 91	K 495	Water	Station 1	December 1982
VP 92	K 497	Sediment	Station 1	,,
VP 93	K 515	Water	Station 2	,,
VP 94	K 522	Zooplankton	Station 2	,,
VP 95	K 523	Sediment	Station 3	,,
VP 96	K 543	Zooplankton	Station 3	,,
VP 97	K 546	Fish	Station 3	,,
VP 98	K 553	Prawn	Station 3	,,
VP 99	K 555	Prawn	Station 2	,,
VP 100	K 556	Fish	Station 2	,,

Contd.....6.

Table -34. (Contd.....6.)

Serial No.	Isolate No.	Sample	Location	Month
VP 101	K 573	Prawn	Station 2	January 1983
VP 102	K 586	Fish	Station 2	,,
VP 103	K 595	Prawn	Station 1	,,
VP 104	K 608	Prawn	Station 3	,,
VP 105	K 624	Fish	Station 3	,,
VP 106	K 630	Sediment	Station 1	,,
VP 107	K 635	Water	Station 3	,,
VP 108	K 636	Water	Station 3	,,
VP 109	K 637	Sediment	Station 3	,,
VP 110	K 638	Water	Station 3	,,
VP 111	K 641	Water	Station 1	February 1983
VP 112	K 663	Sediment	Station 2	,,
VP 113	K 665	Zooplankton	Station 2	,,
VP 114	K 675	Sediment	Station 3	,,
VP 115	K 684	Zooplankton	Station 3	,,
VP 116	K 701	Water	Station 2	,,
VP 117	K 718	Fish	Station 1	,,
VP 118	K 733	Prawn	Station 2	,,
VP 119	K 749	Fish	Station 2	,,
VP 120	K 754	Prawn	Station 3	,,

Table -35. Details of reference strains tested for characterization experiments.

Serial No.	Name	Strain	Donar
1.	Vibrio parahaemolyticus	ATCC 17802	Dr.R.R.Colwell, U.S.A.
2.	,,	TY 17	Dr.I.Karunasagar, Mangalore.
3.	,,	TY 20	,,
4.	,,	TY 36	,,
5.	,,	TY 81	,,
6.	,,	RIMD 2210087 (WP-28)	Dr.T.Mori, Japan.
7.	,,	RIMD 2210100 (TNK-11)	,,

Table -36. Details of tests employed for characterization experiments.

No.	Test	Reaction
	Morphological and cultural characters	
1	Gram stain	-
2	Pigment production	-
3	Swarming	-
4	Motility	+
5	Luminescence	-
	Physiological characters	
6	Growth at 4°C	-
7	,, 15°C	+
8	,, 25°C	+
9	,, 30°C	+
10	,, 37°C	+
11	,, 42°C	+
	Growth in peptone water	
12	with 0% NaCl	-
13	,, 3% NaCl	+
14	,, 5% NaCl	+
15	,, 7% NaCl	+
16	,, 8% NaCl	+
17	,, 10% NaCl	-
	Biochemical characters	
18	Indole	+
19	Oxidase	+

Contd....2.

Table -36. (Contd....2.)

No	Test	Reaction
20	Catalase	+
21	Methyl red	+
22	Voges - Proskauer	-
23	Citrate	+
24	Nitrate reduction	+
25	Hydrogen sulphide	-
26	Gas from glucose	-
27	Arginine dihydrolase	-
28	Lysine decarboxylase	+
29	Ornithine decarboxylase	+
	Production of extracellular enzymes	
30	Amylase	+
31	Chitinase	+
32	Gelatinase	+
33	Lipase	+
34	Urease	+
	Fermentation of	
35	Arabinose	+
36	Adonitol	-
37	Cellobiose	-
38	Dulcitol	-
39	Fructose	+
40	Galactose	+

Table -36. (Contd.....3.)

No.	Test	Reaction
41	Glucose	+
42	Glycerol	+
43	Inositol	-
44	Lactose	-
45	Maltose	+
46	Mannitol	+
47	Mannose	+
48	Melobiose	-
49	Melezitose	-
50	Raffinose	-
51	Rhamnose	-
52	Salicin	-
53	Sorbitol	-
54	Sucrose	-
55	Trehalose	+
56	Xylose	-
	Utilization of	
57	Alanine	-
58	Adonitol	-
59	Arabinose	+
60	Cellobiose	-
61	Dulcitol	-
62	Fructose	+

Table -36. (Contd.....4.)

No.	Test	Reaction
63	Galactose	+
64	Glucose	+
65	Glycerol	+
66	Glycine	-
67	Inositol	-
68	Lactose	-
69	Leucine	+
70	Maltose	+
71	Mannitol	+
72	Mannose	+
73	Melobiose	-
74	Melezitose	-
75	Proline	+
76	Raffinose	-
77	Rhamnose	-
78	Salicin	-
79	Sorbitol	-
80	Sucrose	-
81	Trehalose	+
82	Xylose	-
	Sensitivity to	
83	0/129 (10 µg)	+
84	0/129 (100 µg)	+

Table - 37. Response of type strains, percentage of VP strains isolated in the present study showing similar reactions and similarity index.

Sl. No.	Tests	Reaction of type strains*	% of similar response of isolates **	Similarity index
1	Gram stain	-	100	1
2	Pigment production	-	100	1
3	Swarming	-	96.7	0.97
4	Motility	+	100	1
5	Luminescence	-	100	1
6	Growth at 4°C	-	100	1
7	,, 15°C	+	96.7	0.97
8	,, 25°C	+	100	1
9	,, 30°C	+	100	1
10	,, 37°C	+	100	1
11	,, 42°C	+	98.3	0.98
12	Growth in 0% NaCl conc	-	100	1
13	,, 3% ,,	+	100	1
14	,, 5% ,,	+	100	1
15	,, 7% ,,	+	100	1
16	,, 8% ,,	+	100	1
17	,, 10% ,,	-	100	1
18	Indole	+	100	1
19	Oxidase	+	100	1
20	Catalase	+	99.2	0.99
21	Methyl red	+	88.3	0.88

Contd.....2.

Table -37. (Contd.....2.)

Sl. No.	Tests	Reaction of type strains*	% of similar response of isolates **	Similarity index
22	Voges - Proskauer	-	100	1
23	Citrate	+	96.7	0.97
24	Nitrate reduction	+	100	1
25	Hydrogen sulphide	-	98.3	0.98
26	Gas fromglucose	-	100	1
27	Arginine dihydrolase	-	100	1
28	Lysine decarboxylase	+	97.5	0.98
29	Ornithine decarboxylase	+	100	1
30	Amylase	+	100	1
31	Chitinase	+	99.2	0.99
32	Gelatinase	+	100	1
33	Lipase	+	95	0.95
34	Urease	-	100	1
35	Fermentation of Arabinose	+	70.8	0.71
36	Adonitol	-	100	1
37	Cellobiose	-	74.17	0.74
38	Dulcitol	-	98.3	0.98
39	Fructose	+	98.3	0.98
40	Galactose	+	96.7	0.97
41	Glucose	+	100	1
42	Glycerol	+	76.67	0.77

Contd.....3.

Table -37. (Contd.....3.)

Sl. No.	Tests		Reaction of type strains*	% of similar response of isolates **	Similarity index
43		Inositol	-	97.5	0.98
44		Lactose	-	100	1
45		Maltose	+	94.2	0.94
46		Mannitol	+	95.8	0.96
47		Mannose	+	95	0.95
48		Melobiose	-	100	1
49		Melizitose	-	97.5	0.98
50		Raffinose	-	100	1
51		Rhamnose	-	94.2	0.94
52		Salicin	-	100	1
53		Sorbitol	-	90	0.90
54		Sucrose	-	100	1
55		Trehalose	+	95.8	0.96
56		Xylose	-	100	1
57	Utilization of	Alanine	-	100	1
58		Adonitol	-	94.2	0.94
59		Arabinose	+	80.8	0.81
60		Cellobiose	-	82.5	0.83
61		Dulcitol	-	99.2	0.99
62		Fructose	+	98.3	0.98
63		Galactose	+	98.3	0.98

Contd.....4.

Table -37. (Contd.....4.)

Sl. No.	Tests		Reaction of type strains*	% of similar response of isolates **	Similarity index
64		Glucose	+	100	1
65		Glycerol	+	95	0.75
66		Glycine	-	51.7	0.52
67		Inositol	-	98.3	0.98
68		Lactose	-	100	1
69		Lucine	+	93.3	0.93
70		Maltose	+	97.5	0.98
71		Mannitol	+	97.5	0.98
72		Mannose	+	98.3	0.98
73		Melobiose	-	100	1
74		Melezitose	-	97.5	0.98
75		Proline	+	94.2	0.94
76		Raffinose	-	100	1
77		Rhamnose	-	97.5	0.98
78		Salicin	-	100	1
79		Sorbitol	-	90	0.9
80		Sucrose	-	100	1
81		Trehalose	+	95	0.95
82		Xylose	-	100	1
83	Sensitivity to 0/129	(10 µg)	+	100	1
84	Sensitivity to 0/129	(100 µg)	+	100	1

* total 7 strains

** total 120 isolates

Table -38. Classification of tests on the basis of deviation in reactions from the response of reference strains

Deviation (%)	Test numbers *	Total number of tests	per centage
0	1, 2, 4, 5, 6, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 22, 24, 26, 27, 30, 32, 34, 36, 41, 44, 48, 50, 52, 54, 56, 57, 64, 68, 73, 76, 78, 80, 82, 83, 84.	41	48.81
0.1 to 5.0	3, 7, 11, 20, 23, 25, 28, 29, 31, 33, 38, 39, 40, 43, 46, 47, 49, 55, 61, 62, 63, 65, 67, 70, 71, 72, 74, 77, 81.	29	34.52
5.1 to 10.0	45, 51, 53, 58, 69, 75, 79.	7	8.33
10.1 to 15.0	21	1	1.19
15.1 to 20.0	59, 60.	2	2.38
More than 20	35, 37, 42, 66.	4	4.76

* Details of the tests are given in table 36

Table -39. Percentage similarity of individual *V. parahaemolyticus* strains with tube strains.

Isolate No.	Similarity (%)	Isolate No.	Similarity (%)
VP 1	97.62	VP 21	97.62
VP 2	97.62	VP 22	98.81
VP 3	97.62	VP 23	96.43
VP 4	97.62	VP 24	97.62
VP 5	97.62	VP 25	97.62
VP 6	97.62	VP 26	98.81
VP 7	97.62	VP 27	97.62
VP 8	95.24	VP 28	90.48
VP 9	97.62	VP 29	91.67
VP 10	98.81	VP 30	96.43
VP 11	98.81	VP 31	97.62
VP 12	96.43	VP 32	97.62
VP 13	98.81	VP 33	96.43
VP 14	98.81	VP 34	96.43
VP 15	98.81	VP 35	96.43
VP 16	98.81	VP 36	95.24
VP 17	95.24	VP 37	97.62
VP 18	98.81	VP 38	95.24
VP 19	91.67	VP 39	95.24
VP 20	98.81	VP 40	98.81

Contd.....2.

Table -39. (Contd.....2.)

Isolate No.	Similarity (%)	Isolate No.	Similarity (%)
VP 41	98.81	VP 61	89.29
VP 42	100	VP 62	96.43
VP 43	98.81	VP 63	95.24
VP 44	100	VP 64	92.86
VP 45	96.43	VP 65	95.24
VP 46	91.67	VP 66	95.24
VP 47	92.86	VP 67	96.43
VP 48	95.24	VP 68	92.86
VP 49	94.05	VP 69	97.62
VP 50	92.86	VP 70	97.62
VP 51	97.62	VP 71	98.81
VP 52	95.24	VP 72	100
VP 53	92.86	VP 73	94.05
VP 54	95.24	VP 74	96.43
VP 55	97.62	VP 75	98.81
VP 56	97.62	VP 76	91.67
VP 57	96.43	VP 77	98.81
VP 58	88.10	VP 78	97.62
VP 59	94.05	VP 79	95.24
VP 60	94.05	VP 80	98.81

Contd.....3.

Table -39. (Contd.....3)

Isolate No.	Similarity (%)	Isolate No.	Similarity (%)
VP 81	97.62	VP 101	98.81
VP 82	94.05	VP 102	98.81
VP 83	94.05	VP 103	98.81
VP 84	97.62	VP 104	96.43
VP 85	94.05	VP 105	98.81
VP 86	96.43	VP 106	96.43
VP 87	98.81	VP 107	94.05
VP 88	96.43	VP 108	92.06
VP 89	95.24	VP 109	97.62
VP 90	96.43	VP 110	97.62
VP 91	96.43	VP 111	98.81
VP 92	97.62	VP 112	98.81
VP 93	92.86	VP 113	96.43
VP 94	92.86	VP 114	92.86
VP 95	94.05	VP 115	96.43
VP 96	96.43	VP 116	98.81
VP 97	95.24	VP 117	97.62
VP 98	98.81	VP 118	95.24
VP 99	96.43	VP 119	98.81
VP 100	98.81	VP 120	97.62

Table -40. Effect of temperature on the requirements of pH and sodium chloride concentrations for maximum growth of *V. parahaemolyticus* strain K 132.

Temperature	Optimum pH	Optimum NaCl Con (%)
10°C	7	2
15°C	7	2
20°C	7	4
25°C	7	4
30°C	7	6
35°C	8	6
40°C	8	6
45°C	7	2

Table -41. Concentration of different antibiotic discs and classification of inhibition zone.

	Antibiotic	Symbol	Strength per disc*	Resistant mm or less	Intermediate mm	Sensitive mm or more
1	Ampicillin	I	10 mcg	11	12-13	14
2	Chloramphenicol	C	30 mcg	12	13-17	18
3	Gentamycin	J	10 mcg	12	-	13
4	Kanamycin	K	30 mcg	13	14-17	18
5	Neomycin	N	30 mcg	12	13-16	17
6	Oxytetracycline	O	30 mcg	14	15-18	19
7	Penicillin	P	10 µ	11	12-21	22
8	Polymyxin - B	X	300 µ	8	9-11	12
9	Streptomycin	S	10 mcg	11	12-14	15
10	Sulphadiazine	Z	300 mcg	12	13-16	17

* Span Diagnostics, Udhna, India.

Table -42. Metallic salts and their different concentrations used in this study.

	Metals	Salts used	Conc. tested (ppm)
1	Mercury	$HgCl_2$	5, 10, 15, 20, 25.
2	Silver	$AgNO_3$	50, 60, 70, 75, 100.
3	Copper	$CuSO_4\ 5H_2O$	50, 100, 200, 300, 400.
4	Cadmium	$CdCl_2H_2O$	350, 400, 450, 500, 600.
5	Cobalt	$CoCl_2\ 6H_2O$	20, 30, 40, 50, 60.
6	Iron	$FeCl_3$	400, 450, 500, 550, 600.
7	Zinc	$ZnCl_2$	350, 400, 450, 500, 600.
8	Molybdenum	$(NH_4)_2\ Mo\ O_4$	900, 1000, 1100, 1200, 1500.
9	Lead	$Pb(NO_3)_2$	900, 1000, 1100, 1200, 1500.
10	Nickel	$NiCl_26H_2O$	50, 100, 150, 200, 250.

Table -43. Percentage distribution of antibiotic resistance and sensitivity of *V. parahaemolyticus* strains (n = 120).

	I	C	J	K	N	O	P	X	S	Z
Resistance	85	6.7	3.3	95	75.8	33.7	100	68.3	82.5	88.3
Sensitivity	15	93.3	96.7	5	24.2	66.3	0	31.7	17.5	11.7

I - Ampicillin; C - Chloramphenicol; J - Gentamycin; K - Kanamycin;
N - Neomycin; O - Oxytetracycline; P - Penicillin; X - Polymyxin -B;
S - Streptomycin; Z - Sulphadiazine.

Table -44. Occurrence of antibiotic resistance patterns of V. parahaemolyticus strains (n = 120).

Sl. No.	Pattern	No. of isolates
1	IKNPXSZ	36
2	IKNOPXSZ	13
3	IKNPSZ	12
4	IKNOPSZ	7
5	IKPXSZ	5
6	IKPX	4
7	IJKNOPXSZ	3
8	KNOPXSZ	2
9	KNPXSZ	2
10	KPXSZ	2
11	IKNPX	2
12	IKPXZ	2
13	IKP	2
14	OP	1
15	PZ	1
16	KPX	1
17	PSZ	1
18	IKNP	1
19	IKPZ	1
20	KOPX	1

Contd.....2.

Table -44. (Contd.....2.)

Sl. No.	Pattern	No. pf isolates
21	KPSZ	1
22	OPXZ	1
23	ICKPZ	1
24	IKPSZ	1
25	IKNPS	1
26	KNPSZ	1
27	OPXSZ	1
28	IKNOPZ	1
29	IKNPXZ	1
30	INOPXS	1
31	CKNPSZ	1
32	KNOPSZ	1
33	ICKNPSZ	1
34	ICKOPSZ	1
35	IKOPXSZ	1
36	CKNOPSZ	1
37	IKNOPXZ	1
38	ICKNPXSZ	1
39	ICKOPXSZ	1
40	IJKNOPSZ	1
41	ICKNOPXSZ	1

Table -45. Percentage frequency of sensitivity and resistance of *V. parahaemolyticus* strains to various metals (n = 120)

		Hg*	Ag	Cu	Cd	Co	Fe	Zn	Mo	Pb	Ni
Water	R	96	0	100	100	0	100	100	100	100	48
	S	4	100	0	0	100	0	0	0	0	52
Sediment	R	100	0	100	100	0	100	100	100	100	66.67
	S	0	100	0	0	100	0	0	0	0	33.33
Zooplankton	R	95.46	0	95.46	100	0	100	100	100	100	63.64
	S	4.54	100	4.56	0	100	0	0	0	0	36.36
Fish	R	100	0	100	100	0	100	100	100	100	69.57
	S	0	100	0	0	100	0	0	0	0	30.43
Prawn	R	100	0	100	100	0	100	100	100	100	60.87
	S	0	100	0	0	100	100	0	0	0	39.13
Total	R	98.33	0	99.17	100	0	100	100	100	100	61.67
	S	1.67	100	0.83	0	100	0	0	0	0	38.33

*Hg - Mercury; Ag - Silver; Cu - Copper; Cd - Cadmium; Co - Cobalt; Fe - Iron; Zn - Zinc; Mo - Molybdenum; Pb - Lead; Ni - Nickel.

R - resistant; S - Sensitive.

Table -46. Percentage occurrence of common metal resistance patterns of *V. parahaemolyticus* strains (n = 120).

Pattern*	No. of isolates	Percentage
Cd, Fe, Zn, Mo, Pb, Hg, Cu, Ni.	73	60.84
Cd, Fe, Zn, Mo, Pb, Hg, Cu.	44	36.67
Cd, Fe, Zn, Mo, Pb, Cu, Ni.	1	0.83
Cd, Fe, Zn, Mo, Pb, Hg.	1	0.83
Cd, Fe, Zn, Mo, Pb, Cu.	1	0.83

* Cd - Cadmium; Fe - Iron; Zn - Zinc;
Mo - Molybdenum; Pb - Lead; Hg - Mercury;
Cu - Copper; Ni - Nickel.

Table -47. Minimum inhibition concentrations (MIC) and level of tolerance to various concentrations of metals by *V. parahaemolyticus* strains (n = 120).

Mercury (10 ppm)[a]		Silver (60 ppm)		Copper (100 ppm)		Cadmium (400 ppm)		Cobalt (30 ppm)		Iron (450 ppm)		Zinc (400 ppm)		Molybdenum (1000 ppm)		Lead (1000 ppm)		Nickel (100 ppm)	
C*	I**	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I
5	0	50	0	50	0	350	0	20	0	400	0	350	0	900	0	900	0	50	0
10	2 (1.67)[b]	60	20 (16.67)	100	1 (0.83)	400	106 (88.33)	30	6 (5)	450	25 (20.83)	400	98 (81.67)	1000	39 (32.5)	1000	105 (87.5)	100	46 (38.33)
15	35 (29.17)	70	108 (90)	200	16 (13.33)	450	120 (100)	40	62 (51.67)	500	57 (47.5)	450	120 (100)	1100	103 (85.83)	1100	120 (100)	150	112 (93.33)
20	67 (55.83)	75	120 (100)	300	120 (100)	500	120 (100)	50	120 (100)	550	120 (100)	500	120 (100)	1200	120 (100)	1200	120 (100)	200	120 (100)
25	120 (100)	100	120 (100)	400	120 (100)	600	120 (100)	60	120 (100)	600	120 (100)	600	120 (100)	1500	120 (100)	1500	120 (100)	250	120 (100)

a - minimal inhibitory concentration.

b - percentages given in brackets.

* - concentration in ppm tested

** - cumulative inhibition at each concentration

igure 14. Frequency distribution of V. parahaemolyticus strains falling at different percentage similarity levels.

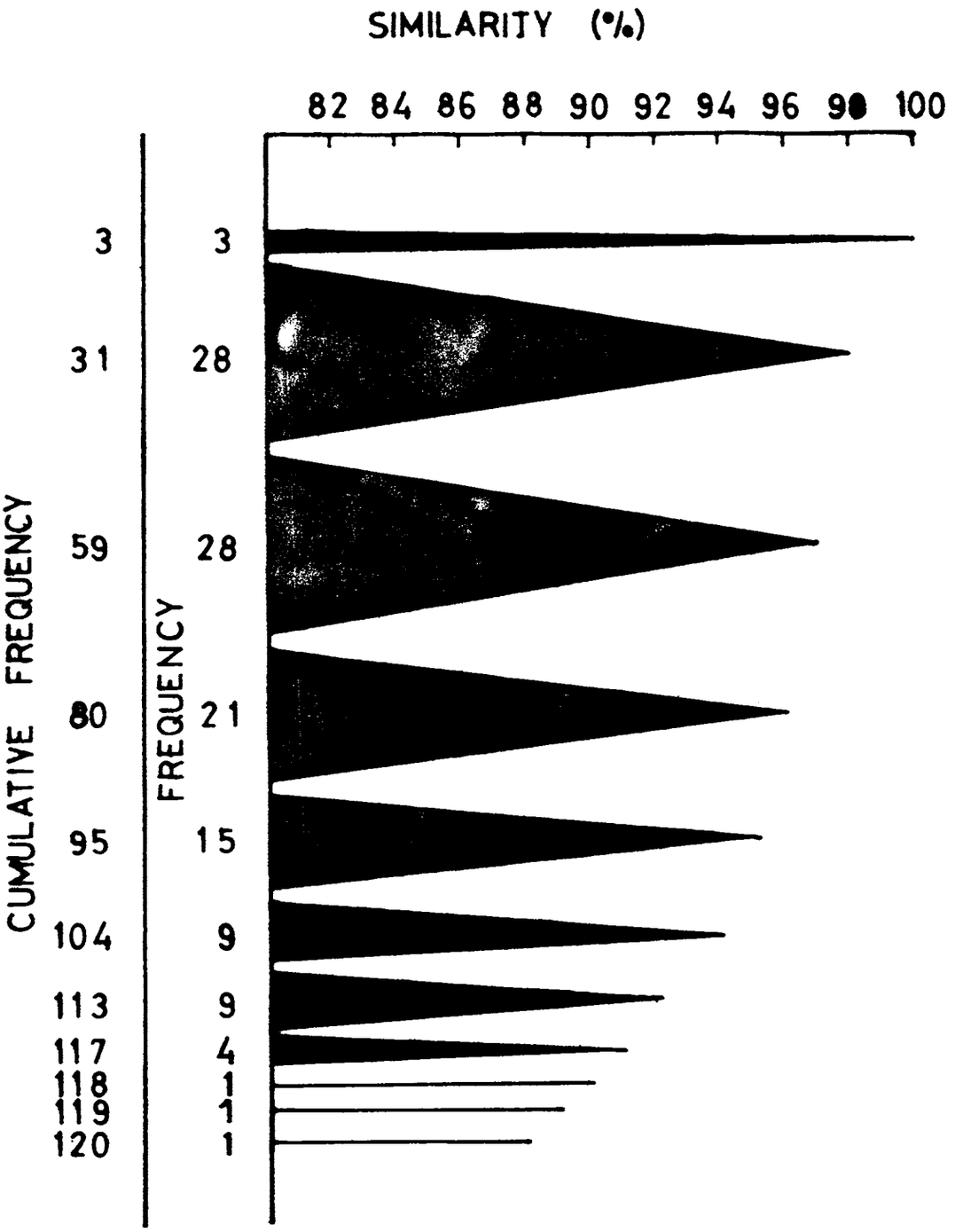
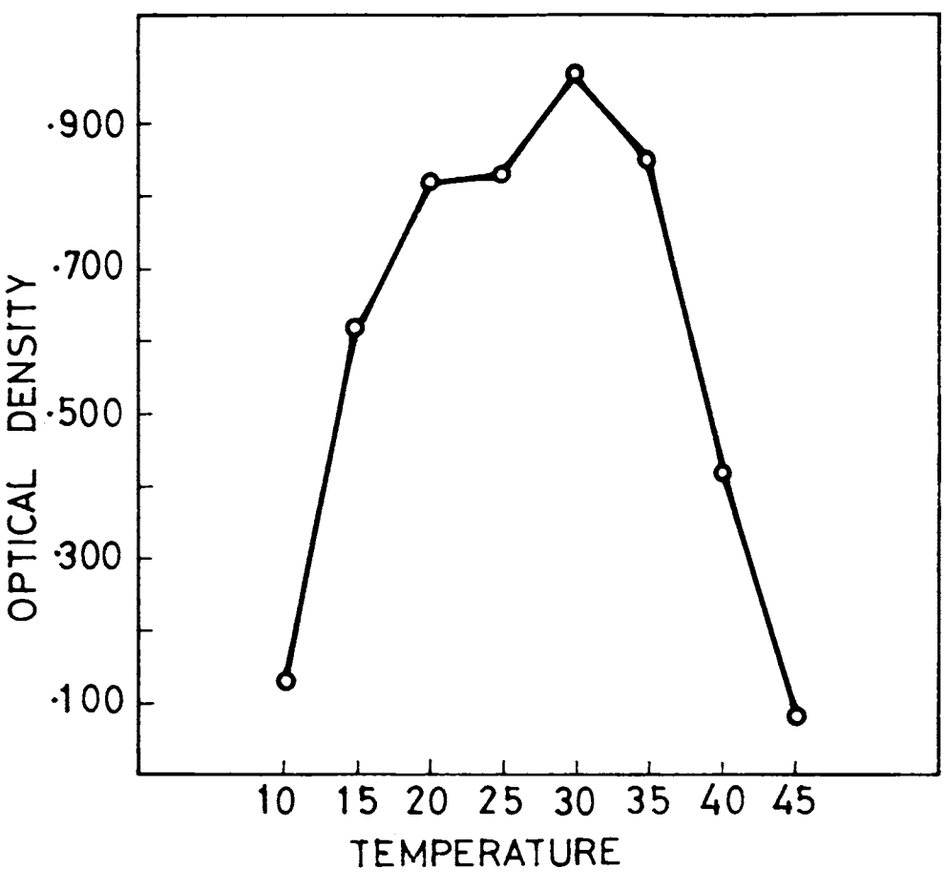

SIMILARITY (%)
82 84 86 88 90 92 94 96 98 100
CUMULATIVE FREQUENCY
FREQUENCY
3
31
59
80
95
104
113
117
118
119
120
3
28
28
21
15
9
9
4
1
1
1

FIG.14

Effect of different temperatures on the growth of *V. parahaemolyticus* strain (K 132)


·900
·700
·500
·300
·100
OPTICAL DENSITY
10 15 20 25 30 35 40 45
TEMPERATURE

FIG·15

Figure 16. Effect of varying p^H on the growth of V. parahaemolyticus strain (K 132)

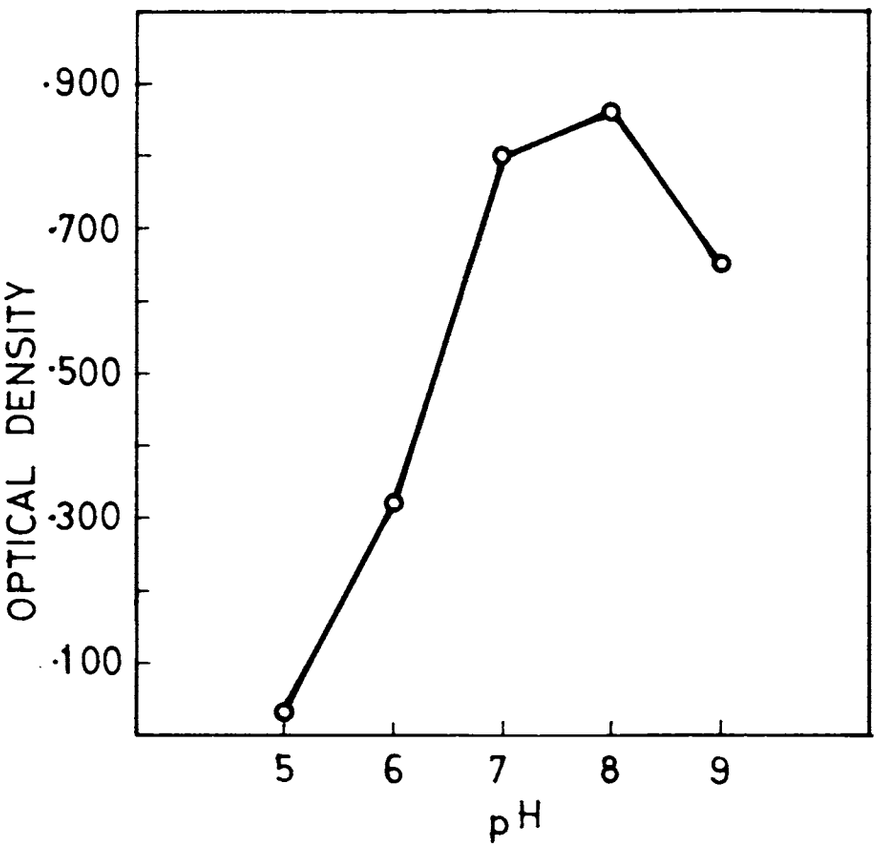


FIG.16

Effect of Sodium Chloride concentrations on the growth of V. parahaemolyticus strains (K 132)

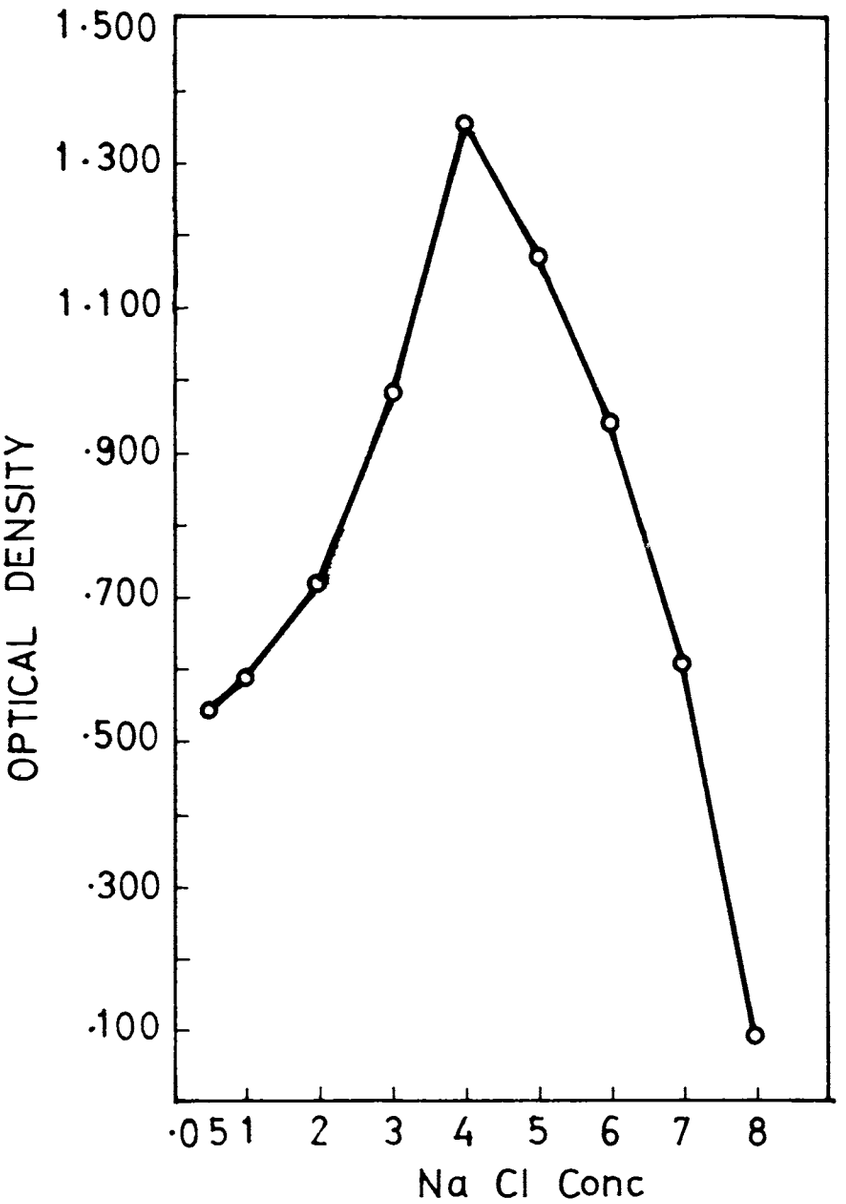

1·500
1·300
1·100
·900
·700
·500
·300
·100
OPTICAL DENSITY
·05 1 2 3 4 5 6 7 8
Na Cl Conc

FIG. 17

Figure 18 a. Combined effect of varying temperatures, p^H and sodium chloride concentration on the growth of *V. parahaemolyticus* strain (K 132) at 10°C.

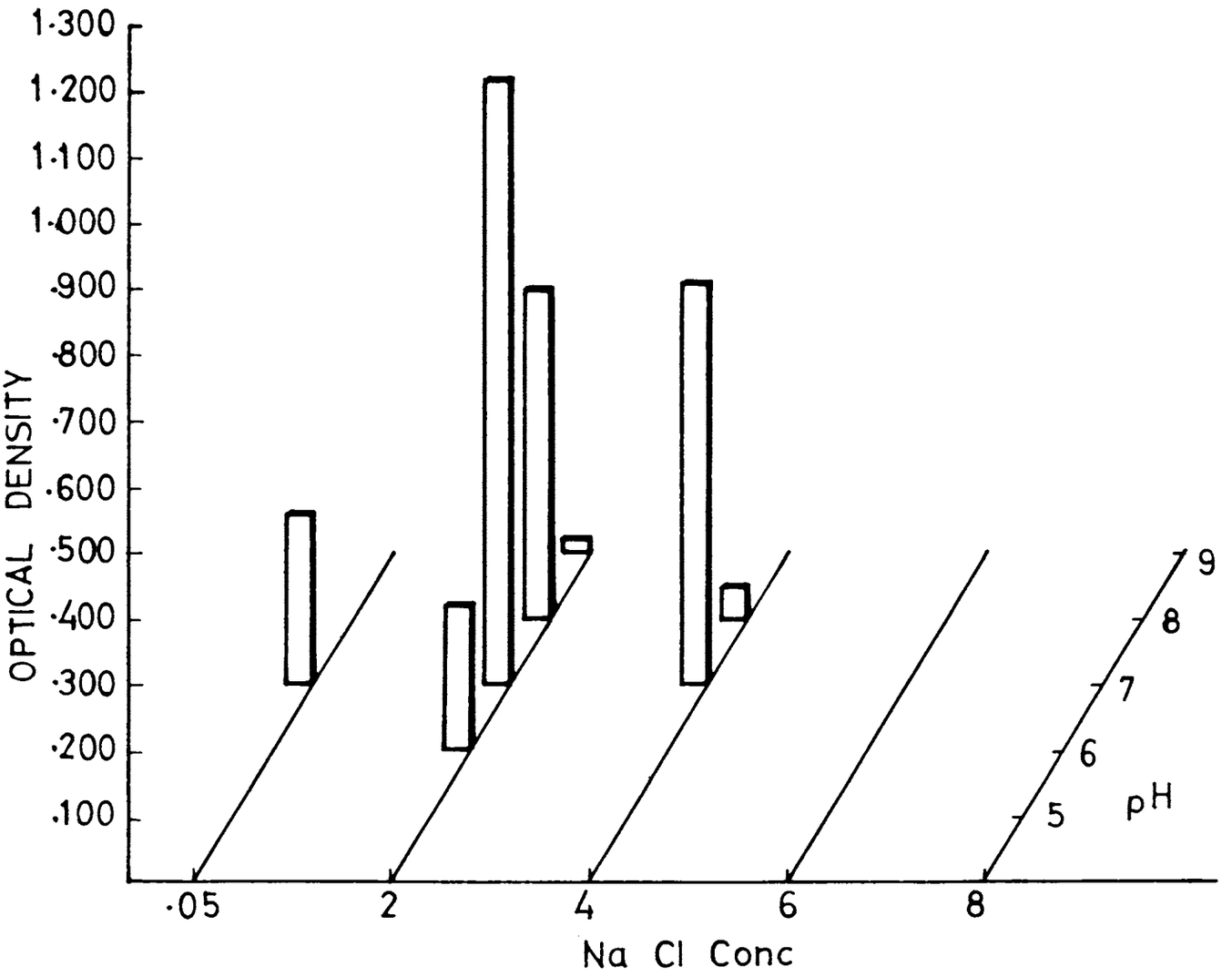

1·300
1·200
1·100
1·000
·900
·800
·700
·600
·500
·400
·300
·200
·100
OPTICAL DENSITY
·05
2
4
6
8
Na Cl Conc
9
8
7
6
5
pH

FIG. 18-a

Figure 18 b. Combined effect of varying temperatures, p^H and sodium chloride concentration on the growth of *V. parahaemolyticus* strain (K 132) at 15°C.

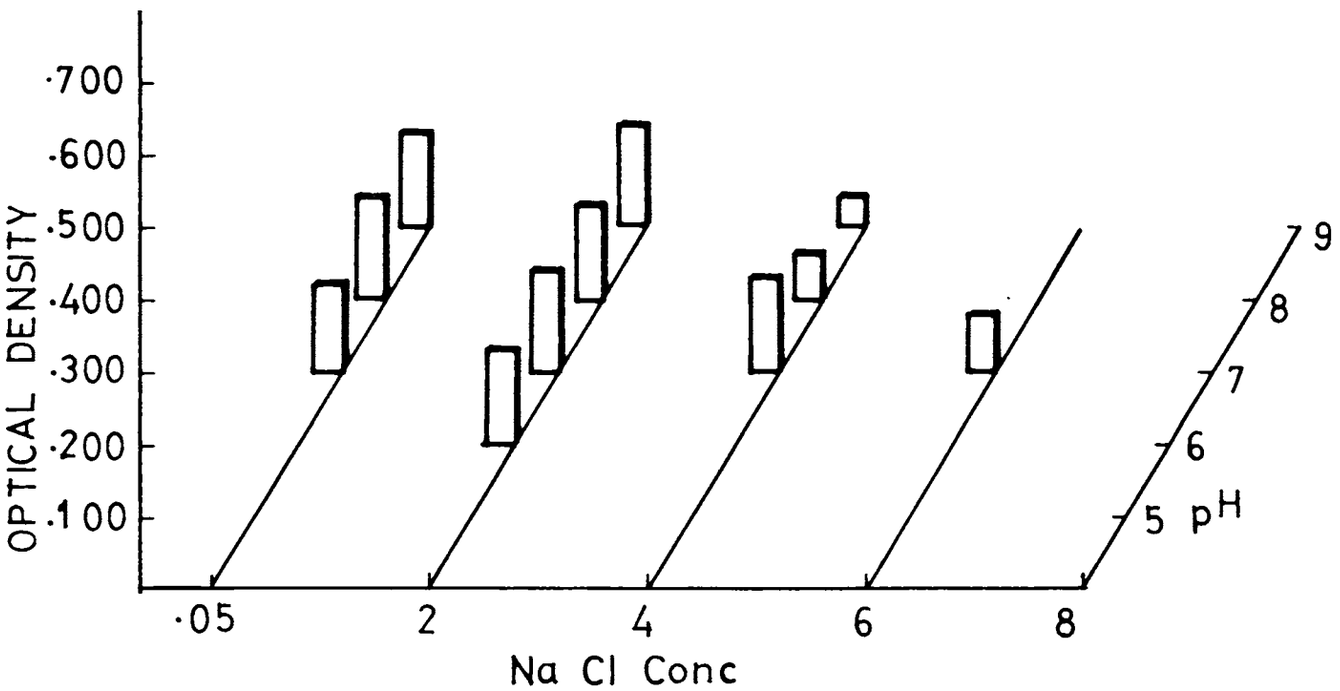

·700
·600
·500
·400
·300
·200
·100
OPTICAL DENSITY
·05
2
4
6
8
Na Cl Conc
9
8
7
6
5 pH

FIG. 18-b

Figure 18 c. Combined effect of varying temperatures, p^H and sodium chloride concentration on the growth of V. parahaemolyticus strain (K 132) at 20°C.

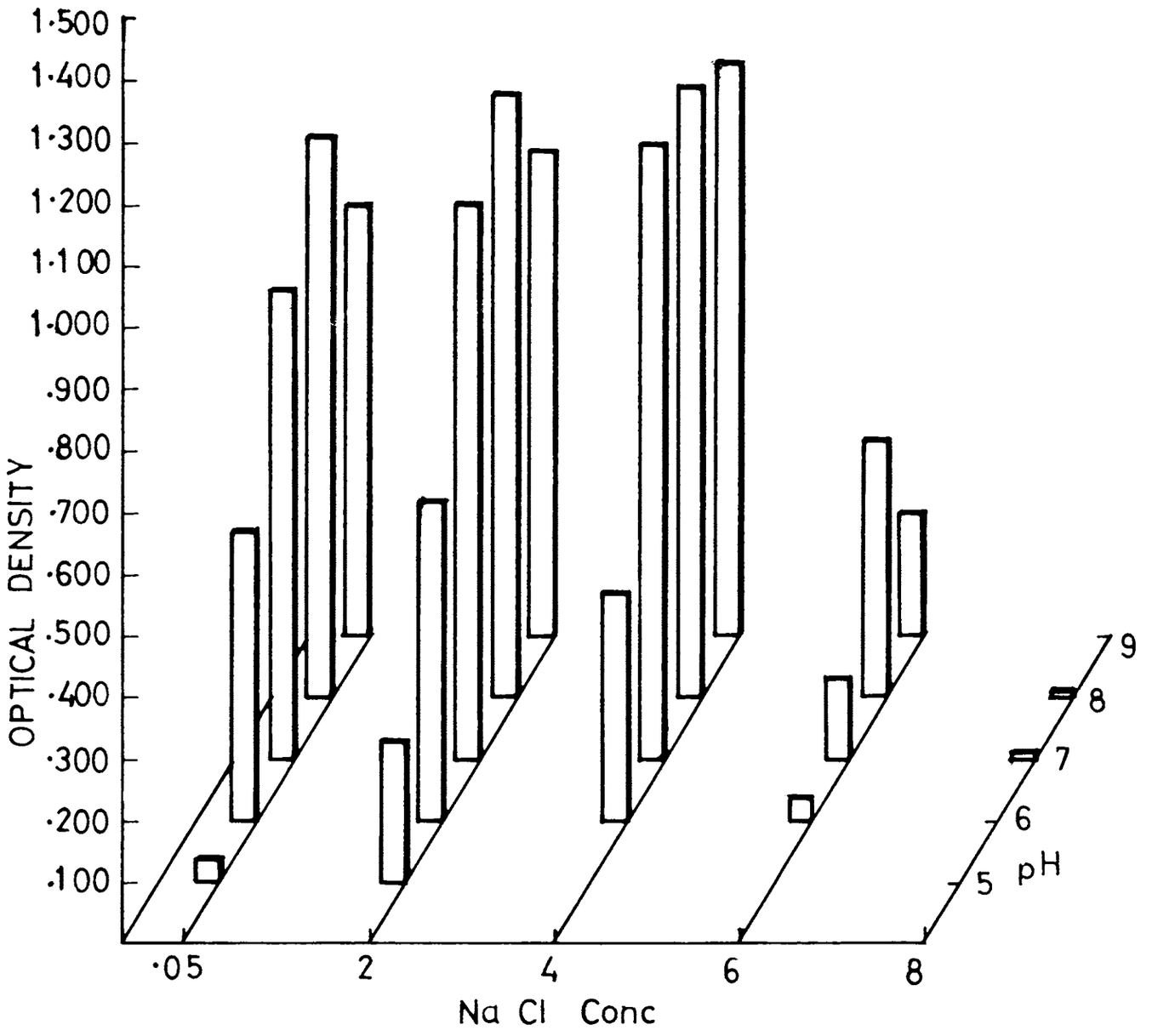

1·500
1·400
1·300
1·200
1·100
1·000
·900
·800
·700
·600
·500
·400
·300
·200
·100
OPTICAL DENSITY
·05
2
4
6
8
Na Cl Conc
9
8
7
6
5
pH

FIG. 18-c

Figure 18 d. Combined effect of varying temperatures, p^H and sodium chloride concentration on the growth of V. parahaemolyticus strain (K 132) at 25°C.

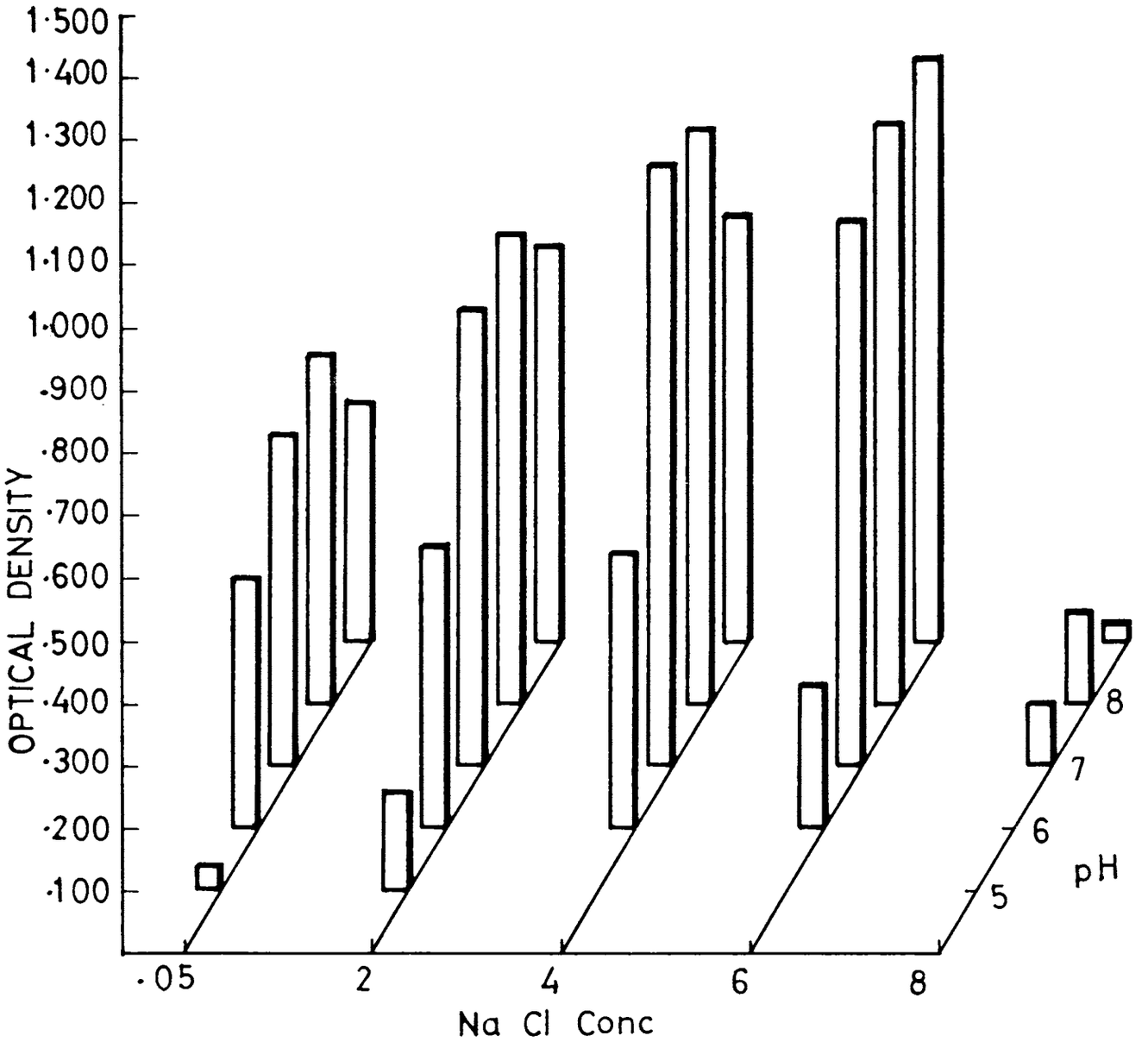

1·500
1·400
1·300
1·200
1·100
1·000
·900
·800
·700
·600
·500
·400
·300
·200
·100
OPTICAL DENSITY
·05
2
4
6
8
Na Cl Conc
5
6
7
8
pH

FIG. 18-d

Figure 18 e. Combined effect of varying temperatures, p^H and sodium chloride concentration on the growth of *V. parahaemolyticus* strain (K 132) at 30°C.

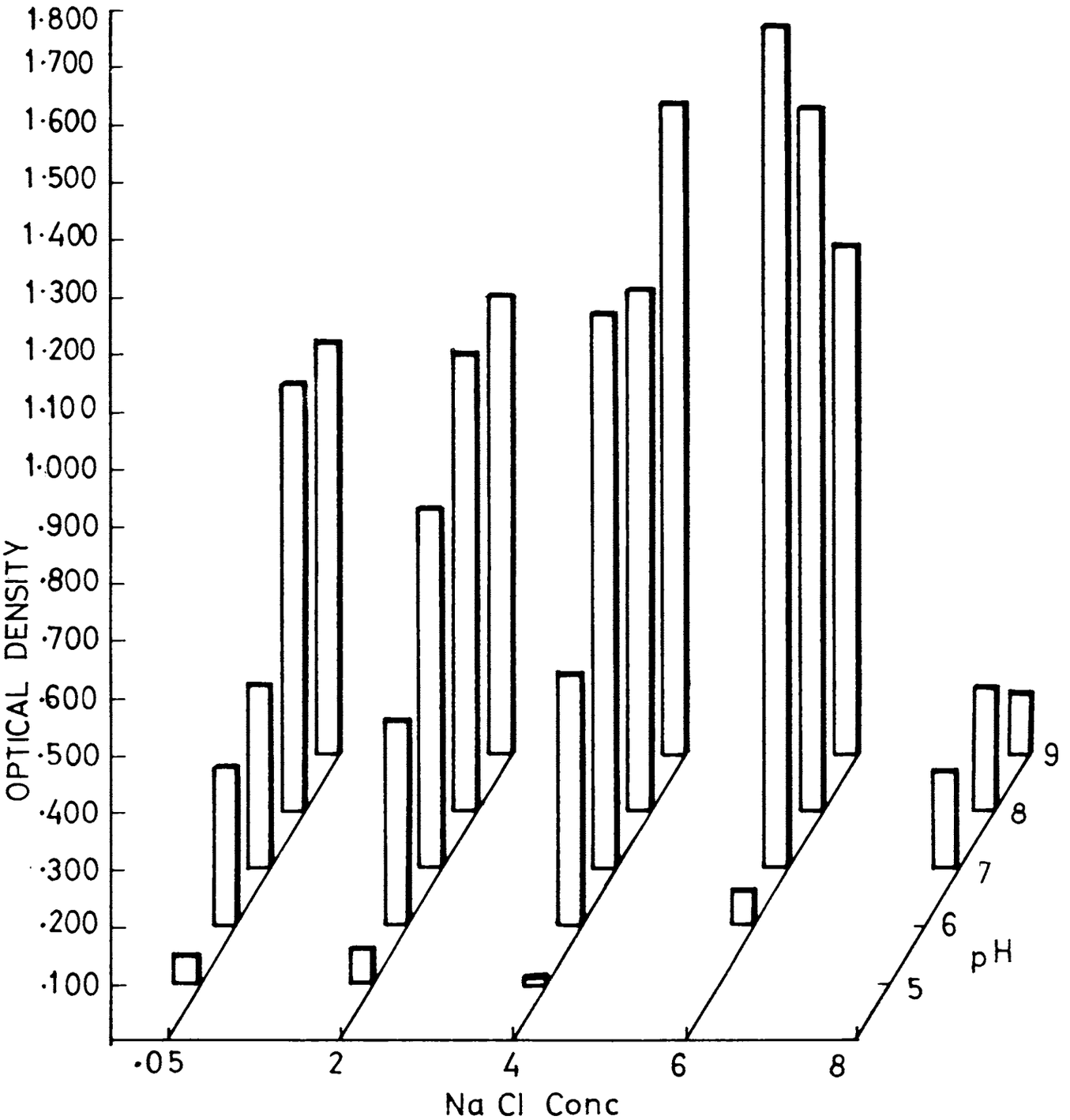

1·800
1·700
1·600
1·500
1·400
1·300
1·200
1·100
1·000
·900
·800
·700
·600
·500
·400
·300
·200
·100
OPTICAL DENSITY
·05
2
4
6
8
Na Cl Conc
5
6
7
8
9
pH

FIG. 18 - e

Figure 18 f. Combined effect of varying temperatures, p^H and sodium chloride concentration on the growth of V. parahaemolyticus strain (K 132) at 35°C.

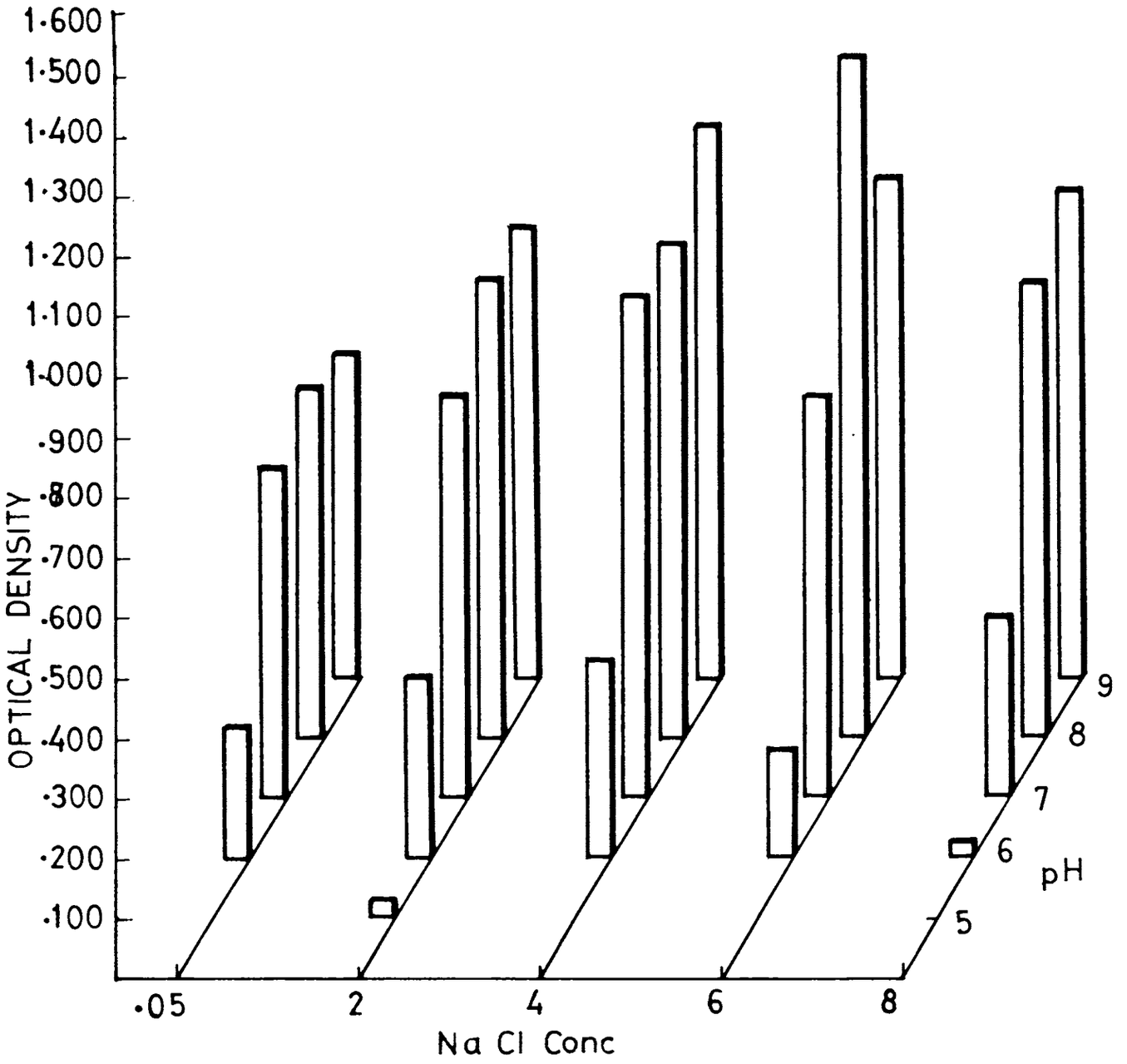

1·600
1·500
1·400
1·300
1·200
1·100
1·000
·900
·800
·700
·600
·500
·400
·300
·200
·100
OPTICAL DENSITY
·05
2
4
6
8
Na Cl Conc
9
8
7
6
5
pH

FIG.18-f

Figure 18 g. Combined effect of varying temperatures, p^H and sodium chloride concentration on the growth of *V. parahaemolyticus* strain (K 132) at 40°C.

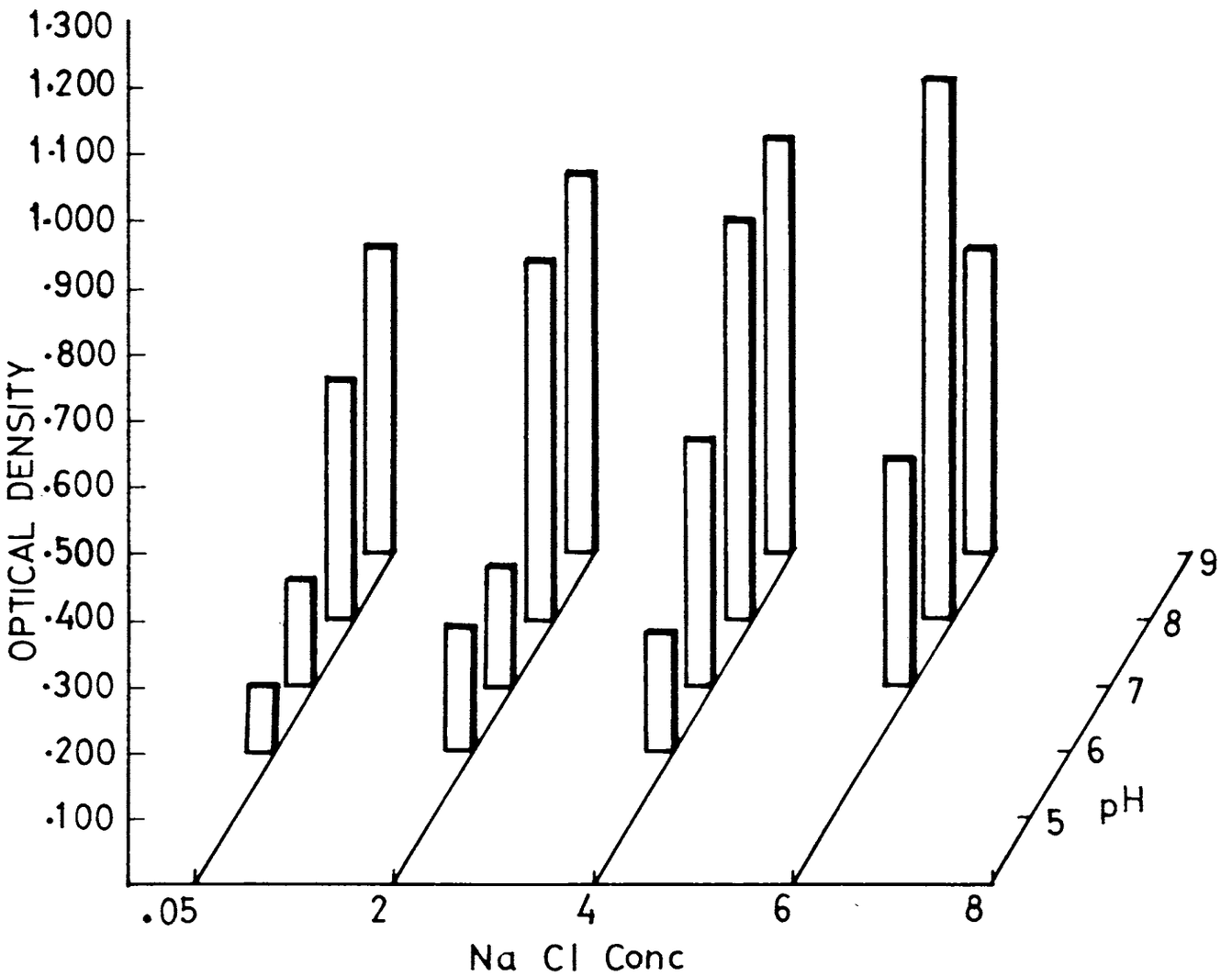

1·300
1·200
1·100
1·000
·900
·800
·700
·600
·500
·400
·300
·200
·100
OPTICAL DENSITY
.05
2
4
6
8
Na Cl Conc
9
8
7
6
5
pH

FIG 18-g

Figure 18 h. Combined effect of varying temperatures, p^H and sodium chloride concentration on the growth of V. parahaemolyticus strain (K 132) at 45°C.

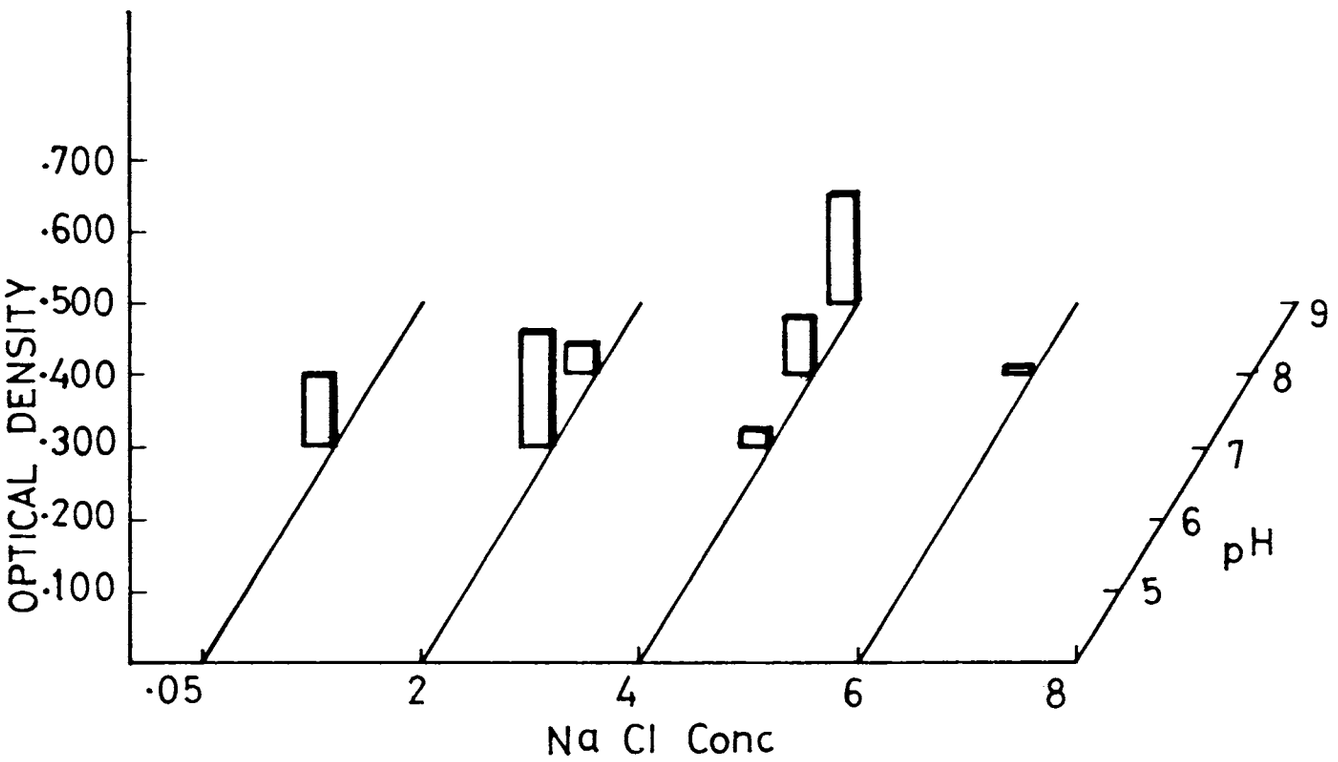

OPTICAL DENSITY
·700
·600
·500
·400
·300
·200
·100
·05
2
4
6
8
Na Cl Conc
9
8
7
6
5
pH

FIG.18-h

Figure 19. Percentage distribution of antibiotic resistant *V. parahaemolyticus* strains isolated from water, sediment, zooplankton, fish and prawn.

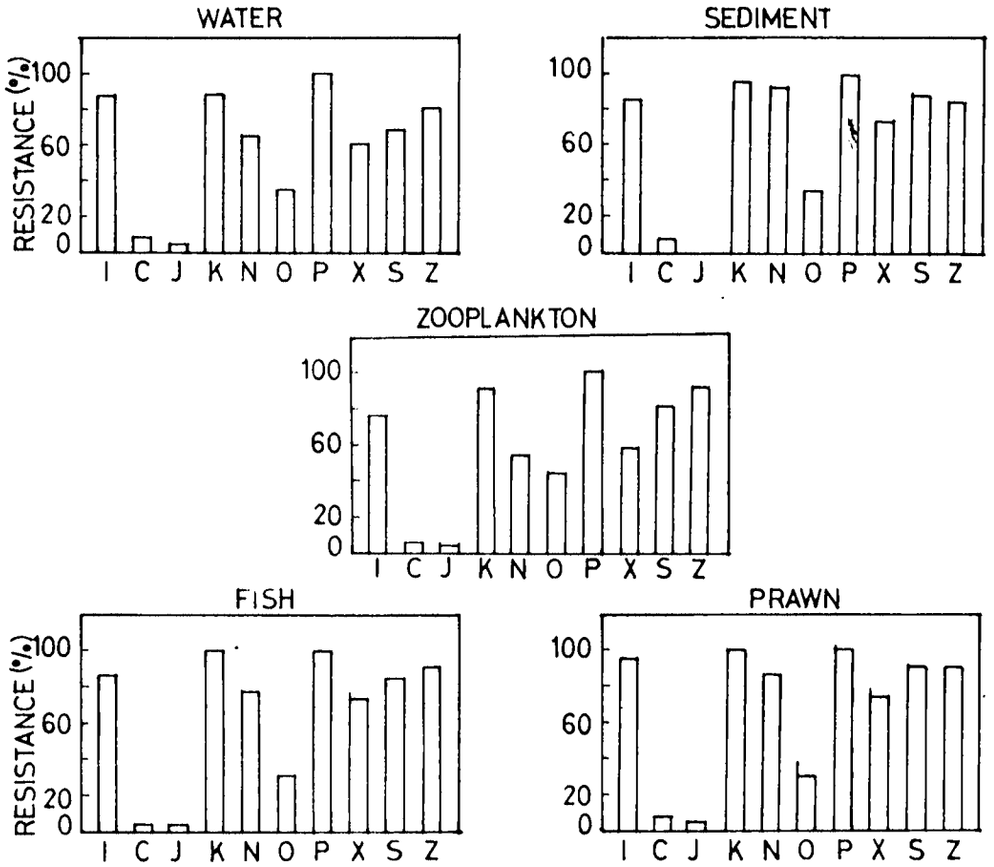

WATER
SEDIMENT
ZOOPLANKTON
FISH
PRAWN
RESISTANCE (%)
100
60
20
0
I C J K N O P X S Z

FIG-19.

Studies on the distribution and seasonal variations of faecal indicator bacteria have been reported from a number of estuarine and coastal waters of the world. Similarly, information on Vibrionaceae also are voluminous which resulted in recognition of the pathogenic potential of members of Vibrionaceae. The halophilic nature of a number of Vibio Spp and their pathogenic potential triggered a series of investigations on halophilic vibrios in estuarine and coastal environments. Because of the cosmopolitan distribution of Vibrio spp in estuarine, coastal and ocean waters of the world and pathogenic potential inherent in them, Colwell and Kaper (1977) suggested the usefulness of Vibrio spp as an indicator of water quality in estuarine and marine enviornment. Hence, a detailed comparative study on vibrios and faecal indicators has to be conducted to understand their interrelationship in an ecosystem. Till recently, Vibrio spp subjected for this kind of comparative studies are V. parahaemalyticus and V. alginolyticus. The present work reveals, apart from the ecology of faecal indicator bacteria and V. parahaemolyticus, the interrelationship existing between them in Cochin backwater.

Regulations on estuarine and coastal water

bodies for fishing and recreational activities on bacteriological standards are not practiced in India. Greater emphasis is given to potable water only. Compared with the existing criteria of 200 FC/100ml of water in USA, and 100 *E. coli*/100ml in European and Mediterranean countries (Cabelli *et al* ., 1982), Cochin backwater may be considered as highly polluted (Appendix, Table 3-7).

Organisms at different trophic levels play significant role in annual cycle of faecal indicator bacteria and *V. parahaemolyticus* in Cochin backwater. Also fish and prawn are found to be good reservoirs of faecal bacteria and *V. parahaemolyticus*. This also shows the high risk of disease outbreaks with enteric pathogens and importance of strict hygienic measures during processing and consumption of these edible items collected from this region. Brayton *et al* (1984) while highlighting the public health significance of human pathogens in the ocean, stated that microbiological cultural techniques alone will not portray the safety of coastal waters and shell fishes. The major reason attributed for this was that human pathogens entering sea water become non-culturable but they remain viable and capable of producing patholigical changes when

introduced into experimental animals. In modern times, even after the widespread recognition of human pathogens existing in freshly harvested seafoods, the practice of eating raw sea food is popularising in developed countries (McGinnis, 1983). It is fortunate that such habits are not spreading in India. High incidence of ear, eye, skin, throat and gastro-intestinal complaints in swimmers were reported due to unsatisfactory microbiological quality of recreational waters (Mujeriego et al., 1982 ; Foulon et al., 1983; Powis and Hazzard, 1984). Peters (1982) reported high prevalence of several diseases and pathological changes occurring in coastal and estuarine fishes due to pollution. It may be summerized at this point that estuarine and coastal pollution apart from giving a direct impact on man's health, also deteriorate his primary source of food and recreation. In the present study, a definite significant relationship was not observed between faecal indicator bacteria and V. parahaemolyticus. It further implies that microbiological safety of this backwater cannot be judged solely on faecal indicator density. Simultaneous monitoring of other pathogens in this region may give a more reliable sanitary quality as suggested by Colwell and Kaper (1977), Cabelli (1977) and Scarpino (1978).

Experimental studies conducted on selected *V. parahaemolyticus* strains revealed that they are very similar to those isolated in other parts of the world. Minor differences in some of their cultural and biochemical characters can be attributed to the ecological variability of the environment. Existence of high resistance to antibiotics and metals is of major ecological and epidemiological significance. High resistance to antibiotics and metals in coliform bacteria are extensively reported from estuarine and coastal water bodies and the major cause for this was attributed to the extensive use of antibiotics and discharge of industrial effluents containing high concentrations of metals into estuarine and coastal waters. A large number of major industries are situated in and around Cochin. Their effluents are discharged into the nearby rivers which empty into Cochin backwater. A number of hospitals situated in this city and neighbouring areas may be contributing a significant role in the development of bacterial populations with drug resistance. From all these findings it appears that a strict microbiological monitoring of this ecosystem is essential from sanitary and epedemiological points of view.

SUMMARY

The thesis furnishes information on the following aspects of faecal indicator bacteria and the seafood borne pathogen *Vibrio parahaemolyticus* at three selected stations in Cochin backwater (Lat.9°28' and 10° 10' N; Long.76°13' and 76° 30' E)

a) Ecology and annual cycle of indicator bacteria such as total coliforms(TC), faecal coliforms(FC), *Escherichia coil* (EC) and faecal streptococci(FS) in three selected stations in Cochin backwater stemming from an year round study (March 1982 to February 1983) of their population in water, sediment, associated with zooplankton, fish(*Etroplus suratensis* Bloch) and prawn (*Metapenaeus dobsoni* Miers).

b) Ecology and annual cycle of total viable bacteria (TVB), *Vibrio*-like organisms (VLO), *Vibrio parahaemolyticus*-like organism(VPLO) and *Vibrio parahaemolyticus*(VP) in the above mentioned samples during the same period.

c) Interrelationship and hydrobiological parameters influencing the annual cycle of these indicators and pathogen (*V parahaemolyticus*) in the Cochin backwater region.

d) Similarity arising in characterization experiments performed on *V. parahaemolyticus* strains isolated from Cochin backwater with those isolated from other parts of the world.

e) Antibiotic and metal resistance/sensitivity pattern of selected V. parahaemolyticus strains.

f) Effect of varying p^H, temperature, and NaCl concentration (individually and in combination) on growth of V. parahaemolyticus strains.

Higlights of the present investigation are summarized below:

1) Indicator bacteria such as total coliforms, faecal coliforms, faecal streptococci and Escherichia coli were consistently present in water, sediment, zooplankton, fish and prawn throughout the study period, March 1982 to February, 1983.

2) The influx of solar radiation and higher salinity during summer months, and large inflow of freshwater with abundant nutrients during monsoon period play a significant role in seasonal variation of indicator bacteria in Cochin backwater.

3) Indicator bacteria associated with sediment(g^{-1}) was higher than those in water (ml^{-1}). Disturbances to sediments caused by water transports, dredging and silt-laden flood water influence the distribution of these indicator bacteria.

4) High numbers of indicator bacteria were present in fish and prawn than water, sediment and plankton throughout

the study period. A marked seasonal variation was not observed in their distribution.

Incidence of faecal index, characteristic of human faecal contamination (more than 4), was higher in association with fish and prawn than in other samples. This reflects the high risk of disease outbreak by consumption of these edible items harvested from the ecosystem.

6) Through bacterial indicator density and faecal index it can be concluded that human faecal contamination has increased considerably in Cochin backwater during the last few years.

7) Except in few months, V.parahaemolyticus was isolated in consistent numbers from all the samples analysed during the investigation. Organisms at different trophic levels play a significant role in annual cycle of V. parahaemolyticus in Cochin backwater.

8) As in the case of indicator bacteria, monson rains influenced the distribution of V. parahaemolyticus in Cochin backwater.

9) Sediments playing a major role in the survival of V. parahaemolyticus, is much pronounced at station 3.

10) Zooplankton, playing a significant role in annual cycle of V. parahaemolyticus, as reported earlier from various estuaries, was observed in the Cochin backwater also.

11) Environmental parameters monitored did not show a definite significant relationship with distribution of V. parahaemolyticus associated with fish and prawn. However, total viable bacteria associated with prawn showed significant positive correlation with particulate organic carbon of water.

12) In general various bacterial parameters such as, TC, FC, EC, FS, TVB, VLO, VPLO and VP were higher in prawn than in fish.

13) Except in a few instances, faecal indicator bacteria did not show a definite significant correlation with V. parahaemolyticus.

14) Higher density of vibrios in fish and prawn also warrant strict hygienic measures to be taken during preparation and processing of these edible items.

15) Association of V. parahaemolyticus with organisms at different trophic levels is of great significance in completing the annual cycle of the seafood borne pathogen in the Cochin backwater.

16) Through characterization experiments, it is found that V. parahaemolyticus isolated from Cochin backwater were similar to those isolated from other parts of the world.

17) The systematic experimental study conducted on slected strains of V. parahaemolyticus showed the influence of temperature, p^H and salinity, individually and collectively, on the growth of V. parahaemolyticus strains. Optimum temperature, p^H and salinity for the growth of V. parahaemolyticus was found to be 30°C, 8 and 4% respectively. Simultaneous variation of these factors showed an increase in optimum p^H and NaCl concentration with increase in temperature. However, the increase was continued only upto 40°C beyond which a sharp decrease in optimum p^H and NaCl concentration values occured with increase of temperature.

18) Higher sensitivity to antibiotics such as gentamycin and chloramphenicol and higher resistance towards penicillin, kanamycin, sulphadiazine ampicillin and streptomycin was exhibited by V. parahaemolyticus strains.

19) All strains of V. parahaemolyticus were sensitive to 100 ppm of silver and cobalt, whereas none of the isolates showed sensitivity to cadmium, iron, zinc, molybdenum and lead at the same concentration. Similarly 98.33% of the strains were resistant to 10 ppm of mercury.

20. Generally resistance to large number of antibiotics as well as metals was observed in the V. parahaemolyticus strains isolated from fish and prawn. Resistance to antibiotics in V. parahaemolyticus strains was usually associated with resistance to metals. The minimum inhibitory concentration (MIC) of metals to V. parahaemolyticus strains varied widely and V. parahaemolyticus strains were able to tolerate higher concentrations of metals.

21. The results clearly point out that the trophic levels of Cochin backwater ecosystem is ingested not only with heavy loads of faecal bacteria and food borne pathogen like V. parahaemolyticus, but also with lot of antibiotic and metal resistant V. parahaemolyticus which may pose severe threat to aquacultural practices in and around Cochin backwater environment and to the health of animals and man.

REFERENCES

Abdelnoor, A.M. and B.M. Roumani, 1980. Characterization of some Vibrio parahaemolyticus strains isolated from seafoods in Lebanon. Zentralbl. Bakteriol. Mikrobiol. Hyg., I Abt. B 170: 502-507.

Abraham, M., 1981. Studies on Vibrio parahaemolyticus and allied vibrios from the Pitchavaram mangrove-Kille backwater complex interconnecting the Vellar and Coleroon estuarine systems. Ph.D. Thesis, Annamalai University, India.322 P.

Abraham, M., G.B Nair and R. Natarajan, 1980. Ecology of Vibrio parahaemolyticus and allied vibrios in estuarine and mangrove environments at Porto Novo, S. India. Paper presented at the second International Symposium on Microbial Ecology, 7th - 12th September 1980, Univ. Warwick, England. Abstract No.7.6.

Ahn, C.W. and Y.H.Hwang, 1975. Studies on salinity quality of Jarhidom, Chromis notatus. Bull. Korean Fish. Soc.,8 : 217-221.

Al-Jebouri, M.M. and D.R. Trollope, 1981. The Escherichia coli content of Mytilus edulis from analysis of whole tissue or digestive tract. J. Appl. Bacteriol., 51: 135-142.

Allen, D.A., B. Austin and R.R. Colwell, 1977. Antibiotic resistance patterns of metal - tolerant bacteria isolated from an estuary. Antimicrob. Agents Chemother. 12 : 545-547.

Allen, L.A., M.A. Pierce and H.M. Smith, 1953. Enumeration of Streptococcus faecalis with particular reference to polluted waters. J. Hyg., 51: 458-461.

Alton, L.V. and P.Kh. Rakhno, 1979. Effect of temperature on the viability of Escherichia coli strains in sea water. Mikrobiologiya, 48 : 149-152.

Amyot, J., 1921, Is the Colon bacillus a normal habitant of intestines of fish ? Am. J. Public Health., 27: 400-401.

Anderson, J.S. and P.S. Meadows, 1978. Microenvironments in marine sediments. Proc. Roy. Soc. Edinb., 76 B: 1-16.

Anderson, I.C., M. Rhodes and H. Kator, 1979. Sublethal stress in Escherichia coli: a function of salinity. Appl. Enviorn. Microbiol., 38:1147-1152.

Andrews, W.H., C.R. Wilson, P.C. Poelma and A. Romero, 1977. Bacteriological survey of the Channel catfish (Ictalurus punctatus) at the retail level. J. Fd. Sci., 42: 359-363.

Aoki, T., 1974. Studies on drug-resistant bacteria isolated from water of carp ponds and intestinal tracts of carp. Bull. Jap. Soc. Sci. Fish., 40: 247-254.

APHA., 1980. Standard methods for examination of water, waste water and sediment. American Public Health Association, New York.

Asakawa, S., 1967. A laboratory study on the resistivity of Vibrio parahaemolyticus against low temperature. J. Fac. Fish. Anim. Husb. Hiroshima Univ., 7: 113-118.

Austin, B., D.A. Allen, A.L.Mills and R.R. Colwell, 1977. Numerical taxonomy of heavy-metal tolerant bacteria isolated from an estuary. Can. J. Microbiol., 23:1433-1447.

Ayres, P.A., 1975. The quantitative bacteriology of some commercial bivalve shellfish entering British markets. J. Hyg., 74: 431-440.

Azariah, J. and M. Subramaniam. 1982. Faecal coliform pollution in Madras inshore water (India), March-April 1980. Atlantica, 5:10.

Babinchak, J.A., J.T. Graikoski, S. Dudley, and M.F. Nitkowski, 1977. Distribution of faecal coliforms in bottom sediment from the New York Bight. Mar. Pollut. Bull., 8: 150-153.

Bandekar, J.R., R. Chander, D.P. Nerkar and N.F. Lewis, 1982. Occurence of Kanagawa positive Vibrio parahaemolyticus strains inshrimp. (Penaeus indicus) Indian J.Microbio 22:247-248.

Barnes, H., 1959. Apparatus and methods in oceanography (Chemical). Academic Press. London.341p.

Barros, G.C., P.G. Robbs and L.C. Soares, 1977. Microbilogical study of peeled, frozen shrimp for consumption in Rio de Janeiro Rev. Latinoam. Microbiol., 19 : 203-208.

Baross, J. and J. Liston, 1970. Occurrence of Vibrio parahaemolyticus and related hemolytic vibrios in marine environments of Washington State. Appl. Microbiol., 20: 179-186.

Baross, J.A., F.J. Hanus and Morita, R.Y., 1975. Survival of human enteric and other sewage microorganisms under simulated deep sea conditions. Appl. Microbiol., 30 : 309-318.

Barrow, G. I. and D.C. Miller, 1974. Growth Studies on Vibrio parahaemolyticus in relation to pathogenicity. In: Int. Symp.on Vibrio parahaemolyticus (T. Fujino, G. Sakaguchi, R. Sakazaki and Y. Takeda. Eds) Saikon Publishing Co.Ltd., Tokyo. pp:205-210.

Baumann, P., L. Baumann and M. Mandel, 1971. Taxonomy of Marine bacteria. The Genus Beneckea. J. Bacteriol., 107:268-294.

Berger, L.R. and D.M. Reynolds, 1958. The chitinase system of a strain of Streptomyces griseus. Biochem. Biophys. Acta., 29:522-534.

Bernad, F.R., 1973. Bacterial flora of positive coliform tests of Pacific oysters from polluted and clean regions of Vancouver Island. Tech. Rep. Fish.Res. Bd. Can., 421:6.

Bethoux, N. and G.C. Montegut, 1976. Study on the relationship between nutrient salt and test mocroorganisms of faecal contamination during summer, along the Mediterranean coast. In: Protection of the Mediterranean coast, Part 1. 3rd study session on marine pollution (Split, 22-23 Oct ., 1976) Publ. by CIESM, Monaco. pp: 143-146.

Beuchat, L.R. 1973. Interacting effects of p^H, temperature and salt concentration on growth and survival of Vibrio parahaemolyticus. Appl. Microbiol., 25: 844-846.

Beuchat, L.R., 1974. Combined effects of water activity, solute and temperature on the growth of Vibrio parahaemolyticus. Appl. Microbiol., 27: 1075 - 1080.

Beuchat, L.R., 1975. Environmental factors affecting survival and growth of Vibrio parahaemolyticus : A review. J. Milk Fd. Technol., 38: 476 - 480.

Beuchat, L.R., 1976. Sensitivity of Vibrio parahaemolyticus to spices and organic acids. J. Fd. Sci., 41:899-902.

Beuchat, L.R., and W.K.Jones, 1979. Growth of Vibrio parahaemolyticus in seafoods extended with soy protein concentrate, structural protein fibre, and textured vegetable protein. J. Fd. Sci., 44: 1114 - 1117.

Beuchat, L.R. and R.E. Worthington, 1976. Relationships between heat resistance and phospholipid fatty acid composition of Vibrio parahaemolyticus. Appl. Environ. Microbiol., 31: 389 - 394.

Bhattacharya, S., A.K. Bose and A.K. Ghosh, 1971. permeability and enterotoxic factors of nonagglutinable vibrios, Vibrio alcaligenes and Vibrio parahaemolyticus Appl. Microbiol., 22: 1159 - 1161.

Bianchi, A.,V. Mircea, and R.P.V. Reun, 1976. The disapperance of Escherichia coli in sea water: Experiments in dialyzing chambers. Comm. Int. Explor. Sci. Mer. Mediterr. Monaco., 23: 95- 96.

Binta, M.G., P.N. Nyaga and T.B. Tjaberg, 1982. Vibrio parahaemolyticus: occurence in marine seafish, shellfish, water and sediment in Kenya. East Afr. Med. J., 59: 442 - 445.

Bireley, L.E. and J.D. Buck, 1975. Microbilogy of a former dredge spoil disposal area. Mar. Pollut. Bull., 6:107-110.

Bitton, G. and R. Michell, 1974. Protection of Escherichia coli by montmorillonite in seawater. J. Environ. Engg. Div. Am. Soc. Civ. Engg., 100(EE6):1310-1313.

Boeddu, M. ,L.Villa and L. Volterra, 1977. State of pollution on Italian coast (Summer 1976). Ann. SelavoRiv. Microbiol. Immunol., 19: 451 - 454.

Boer, W.E.,C. Golten and W.A. Scheffers, 1975. Effects of some physical factors on flagellation and swarming of Vibrio alginolyticus. Neth. J. Sea. Res., 9: 197 - 213.

Bonang, G., M.Lintong and U.S. Santoso, 1974. The isolation and susceptibility to various antibacterial agents of Vibrio parahaemolyticus from acute gastroenteritis cases and from seafood in Jakarta. Int. Symp. Vibrio parahaemolyticus., 27-33.

Bradshaw, J.G., D.W. Francis and R.M. Twedt, 1974. Survival of Vibrio parahaemolyticus in cooked seafood at refrigeration temperatures. Appl. Microbiol., 27: 657 - 661.

Brayton, P.,D. Roszak, S.A. Hug, L.M. Palmer, D.J. Grimes and R.R. Colwell, 1984. Public health significance of human pathogens in the ocean. In: Oceans '84 conference Record : Industry, Govt., Education. Mar. Technol. Soc. Wash. DC. IEEE, NY., USA. pp:263-267.

Breittmayer, J.P. and M.J. Gauthier, 1978. Bacterial contamination of a bathing area related to the frequency with which it is used. Water Res., 12: 193 - 197.

Brown, R.K., T.A. McMeekin and C.Balis, 1977. Effect of some unicellular algae on Escherichia coli populations in sea water and oysters. J,Appl. Bacteriol., 43: 129 - 136.

Buck, J.D., 1976. Pollution microbiology of Biscayne Bay beaches. Florida Sci., 39: 111 - 120.

Burgess, J.S., 1974. Measurement of bacterial water pollution and the log - normal distribution. NewZealand J. Mar. Freshwater Res., 8: 551 - 553.

Burke, V. and L.A. Baird, 1931. Fate of freshwater bacteria in the sea. J. Bacteriol., 21: 287-298.

Cabelli, V.J., 1977. Indicators of recreational water quality. In: Bacterial indicators/health hazard associated with water. (A.W. Hoadley and B.J.Dutka, Eds), ASTM, Philadelphia, p.222.

Cabelli, V.J., 1983. Health effects criteria for marine recreational waters. US. EPA. Research and development. North Carolina, USA. 98.

Cabelli, V.J. and W.P. Hefferman, 1970. Accumulation of Escherichia coli by the Northern Quahang. Appl. Microbiol., 19: 239-244.

Cabelli, V.J., M.A. Levin, and A.P. Dufour, 1982. Public health consequences of coastal and estuarine pollution : Infectious diseases. In: Ocean disposal of municipal wastewater: Impact on the coastal environment. (E.P. Myers, Ed.) MIT Sea Grant Programme, Mass., pp: 520-575.

Calomiris, J.J., J.L. Armstrong and R.J. Seidler, 1984. Association of metal tolerance with multiple antibiotic resistance of bacteria. Appl. Environ. Microbiol., 47: 1238 - 1242.

Cann, D.C., L.Y. Taylor and Z.Merican, 1981. A study of the incidence of Vibrio parahaemolyticus in Malaysian Shrimp undergoing processing for export. J.Hyg., 87: 485 - 491.

Carlucci, A.F. and D.Pramer, 1960a. An evaluation of factors affecting survival of Escherichia coli in seawater. II. Salinity, p and nutrients. Appl. Microbiol., 8: 247 - 250.

Carlucci, A.F. and D.Pramer, 1960b. An evaluation of factors affecting survival of Escherichia coli in seawater IV. Bacteriophages. Appl. Microbiol., 8: 254 - 256.

Carney, J.F., C.E. Carty and R.R. Colwell, 1975. Seasonal occurrence and distribution of microbial indicators and pathogens in the Rhode River of the Chesapeake Bay. Appl. Microbiol., 30: 771 - 780.

Chamberlin, C.E. and R. Mitchell, 1978. A decay model for enteric bacteria in natural waters. In: Water pollution microbilogy Vol.2. (R.Mitchell, Ed.) John Wiley and Sons, New York.pp.325-348.

Chan, K.Y., S.H. Wong and C.Y.Mak, 1979. Effect of bottom sediments on the survival of Enterobacter aerogenes in Seawater. Mar.Pollut. Bull., 10: 205 - 210.

Chandrabose, S.V. and F. Chandrasekaran, 1976. Occurance of Vibrio parahaemolyticus in marine prawns and environments. Fish. Technol., Cochin, 13: 36-40.

Chandramohan, D., P. Lakshmanaperumalsamy and K. Dhevendaran, 1980. Iodophor: An effective disinfectant in aquaculture. Bull. Dept. Mar. Sci., Univ. Cochin, XI: 71-75.

Chandrika,V., 1983. Studies on the ecophysiology of some heterotrophic and indicator bacteria in the marine environment of Kerala. Ph.D Thesis, Cochin University of Science and Technology, Cochin, India 294p.

Chatterjee, B.D. and K.N.Neogy, 1971 b. Significance of some characteristics of Vibrio parahaemolyticus in relation to its taxonomic position. J. Pathol. Bacteriol., 14: 158-169.

Chatterjee, B.D. and K.N. Neogy, 1972 a. On the etiology of choleraic diarrhoea. Indian J. Med. Res., 60: 531 - 541.

Chatterjee, B.D. and K.N.Neogy, 1972 b. Vibrio alginolyticus, Vibrio parahaemolyticus and the related biotypes isolated from non-marine fishes in Calcutta. Bull. Cal. Sch. Trop. Med., 20: 29 - 30.

Chatterjee, B.D. and T. Sen, 1974. Vibrio parahaemolyticus serotypes in Calcutta, India. Bull. WHO., 50: 559 - 561.

Chatterjee, B.D., K.N.Neogy and S.L.Gorbach, 1970. Study of Vibrio parahaemolyticus from cases of diarrhoea in Calcutta. Indian J. Med. Res., 58: 234 - 238.

Chatterjee, B.D. and K.N.Neogy, 1971 a. Isolation of Vibrio parahaemolyticus from some cold blooded animals of Calcutta. Bull. Cal.Sch.Trop. Med., 19: 84 - 85.

Chatterjee, B.D., P.K.De, A.K. Hati and N.Tandon, 1978. Fly borne Vibrio parahaemolyticus in Calcutta. Trop. Georg. Med., 30: 499 - 503.

Chun, D., S.Y. Seol, R. Tak and C.K. Park, 1972. Inhibitory effect of glycerine on Vibrio parahaemolyticus and Salmonella. Appl. Microbiol., 24: 675 - 678.

Colwell, R.R., 1962. The bacterial flora of Puget Sound fish. J. Appl. Bacteriol., 25: 147 - 158.

Colwell, R.R., 1970. Polyphasic taxonomy of the genus Vibrio: Numerical taxonomy of Vibrio cholerae, Vibrio parahaemolyticus, and related Vibrio species. J. Bacteriol., 104: 410 - 433.

Colwell, R.R., 1979. Toxic effects of pollutants on microorganisms. In: Ecotoxicology. SCOPE Project, 4: 275-294.

Colwell, R.R.,(Ed.)1984. Vibrios in the environment. John Wiley & Sons, New york.

Colwell, R.R. and R.K. Sizemore, 1974. Drug resistant bacteria in the marine environment. In: Proc. of Marine Technology Society Tenth Annual Conference, p 427 - 430.

Colwell, R.R. and J. Kaper, 1977. Vibrio species as bacterial indicators of potential health hazards associated with water. In: Bacterial indicators/ health hazards associated with water ASTM STP 645 (A.W. Hoadley and B.J. Dutaka, Eds.) American Society for Testing and Materials. pp: 115-125.

Colwell, R.R. T. Kaneko and T. Staley, 1973. Vibrio parahaemolyticus, an estuarine bacterium resident in Chesapeake Bay. In: proc. 3rd conf. Food-Drugs from the sea., 20th August 1972 (L.R. Worthen, Ed.) MTS, Washington, D.C., USA. 396 P.

Colwell, R.R.J.B.Kaper, E.E.Remmers and H.Lockman, 1981. Distribution of Vibrio parahaemolyticus in Chesapeake Bay during the summer season. Estuaries, 4: 321 - 327.

Cooke, M.D., 1976a. Antibiotic resistance among coliform and faecal coliform bacteria isolated from sewage, seawater and marine shellfish. Antimicrob. Agents and Chemother., 9: 879 - 884.

Cooke, M.D., 1976b. Antibiotic resistance in coliform and faecal coliform bacteria from natural waters and effluents. N.Z.J. Mar. Freshwater Res., 10: 391 - 397.

Covert, D. and M.Woodburn, 1972. Relationship of temperature and Nacl concentration to the survival of Vibrio parahaemolyticus in broth and fish homogenate. Appl. Microbiol., 23: 321 - 325.

Davis, J.W. and R.K.Sizemore, 1982. Incidence of Vibrio species associated with blue crab(Callinectes sapidus)collected from Galveston Bay, Texas. Appl. Environ. Microbiol., 43: 1092 - 1097.

De, S.P., M.Banerjee, B.C.Deb, P.G.Sengupta, J. Sil, B.K.Sircar, D.Sen, A.Ghosh and S.C.Pal, 1977. Distribution of vibrios in Calcutta environment with particular reference to Vibrio parahaemolyticus. Indian J.Med. Res., 65:21 - 28.

Deb, B.C., R. Sinha, S.P.De, P.G.Sengupta, S.N.Sikdar and A. Mondal, 1975. Studies on Vibrio parahaemolyticus infection in Calcutta as compared to cholera infection. Prog. Drug Res. Trop. Dis. II, 19:490 - 502

Devanas, M.A., C.D.Litchfield, C.McClean and J.Gianni, 1980. Coincidence of cadmium and antibiotic resistance in New York Bight apex benthic microorganisms. Mar. Pollut. Bull., 11: 264 - 269.

Dillion, E.J. and B.H. Sellers, 1984. A bacteriological assessment of the Mersey estuary and north wirral coast, U.K. Water Air Soil Pollut. 22: 241 - 255.

Domek, M.J., M.W. Lechavallier, S.C. Cameron and G.A.McFeters, 1984. Evidence for the role of copper in the injury process of coliform bacteria in drinking water. Appl. Environ. Microbiol., 48: 289 - 293.

Dowding, J. and J. Davies, 1975. Mechanisms and origins of plasmid - determined antibiotic resistance. In Microbiology - 1974, (D.Schlessinger Ed.), ASM, Washington DC, 314pp.

Dawe, L.L. and W.R. Penrose, 1978. 'Bactericidal' property of seawater: death or debilitation ? Appl. Environ. Microbiol., 35: 829 - 833.

Ducklow, H.W., H.M. Tarraza and R. Jr. Mitchell, 1980. Experimental pathogenicity of Vibrio parahaemolyticus for the schistome - bearing snail Biomphalaria globata. Can. J. Microbiol.,26: 503 - 506.

Dudley, S., J.A.Babinchak and J.T.Graikoski, 1977. Enumeration and distribution of bacterial populations of Long Island sound. Mar. Pollut. Bull., 8: 271 - 273.

Durairaj, S., G. Chinnaswamy and M.S. Mohamed, 1983. A bacteriological study of the natural flora of edible oyster Crassostria madrasensis. Fish. Technol. 20:111-114.

Dutka, B.J., 1977. Microbiological study of storm runoff waters from a 100% residential area in Canada. Canadian Res.,10: 51-53.

Dwivedi, A.N. and S.A.H. Abidi, 1977. Studies on the occurrence of coliforms and streptococci in coastal waters of Bombay. J. Indian Fish. Assoc., 7: 77 - 81.

Edenborn, H.M. and W.H. Renwick, 1981. Pollutant levels in New Jersey dredge spoil disposal. Estuaries., 4: 303.

El - Sahn, M.A., A.A. El - Banna and A.M. El - Tabey Shehata, 1982. Occurence of Vibrio parahaemolyticus in selected marine invertebrates, sediment, and seawater around Alexandria, Egypt. Can.J.Microbiol., 28: 1261 - 1264.

el Wakeel, S.K. and J.P.Riley, 1956. The determination of organic carbon in marine muds. J. du. Coun., 22: 180 - 183.

Enzinger, R.M. and R.C. Cooper, 1976. Role of bacteria and Protozoa in the removal of Escherichia coli from estuarine waters. Appl. Environ. Microbiol., 31: 758 - 763.

Ermolina, E.P. and G.M. Shikalov, 1975. An experimental study of the effect of some physical and chemical factors on the survival and propagation of Vibrio parahaemolyticus in food products. Vopr. Pitaniya., 5: 74 - 79.

Eyles, M.J. G.R. Davey and G. Arnold, 1985. Behavior and incidence of Vibrio parahaemolyticus in Sidney rock oysters (Crassostrea commercialis). Int. J. Fd. Microbiol., 1: 327 - 334.

Faust, M.A., A.E.Aotaky and M.T. Hargadon, 1975. Effect of physical prameters on the in situ survival of Escherichia coli MC - 6 in an estuarine environment. Appl. Microbiol., 30: 800 - 806.

Fernandez, E.A., 1973. Observations on the pollution of the coastal area near Cumana. Biol. Inst. Oceanogr., Univ. Oriente., 12: 23 - 32.

Figueiredo, S.F., L.C.M. Hagler and A.N. Hagler, 1978. Survival of Escherichia coli, Salmonella and Shigella in polluted estuary water. In:Brazilian Congress on Microbiology. Sociedade Brasileire de Microbiologia (Brazil).p.49.

Flestcher, M. and K.C. Marshall, 1982. Solid surface of ecological significance to aquatic bacteria. Adv. Microbiol. Ecol., 6: 199 - 236.

Foulon, G., J.Maurin, N.Q.Nguen and G.M. Bouyer, 1983. Relationships between the microbiological quality of bathing water and health effects: A preliminary survey. Rev. Sci. Eau. Fr. J. Water Sci., 2: 127-143.

Fujino, T., Y.Okuno, D. Nakada, A.Aoyama, K.Fukai, T.Mukai and T. Ueho, 1951. On the bacteriological examination of Shirasu food poisoning. J. Jap. Assoc. infect. Dis., 35:11.

Fujino, T., Y. Okuno, D. Nakada, A. Aoyama, K. Fukai, T. Mukai and T. Ueho, 1953. On the bacteriological examination of Shirasu food poisoning. Med.J. Osaka Univ., 4: 299 - 304.

Fujioka, R.S. and O.T. Narikawa, 1982. Effect of sunlight on enumeration of indicator bacteria under field conditions. Appl. Environ. Microbiol.,44:395 - 401.

Fujioka, R.S., H.H. Hashimoto, E.B. Siwak and H.F. Reginald, 1981. Effect of sunlight on survival of indicator bacteria in seawater. Appl. Environ. Microbiol., 41: 690 - 696.

Fujiwara, K., K. Ashino and M.Kono, 1972. Bacteriological survey of preserved foods. II. Bacteriological examination of frozen ready-to-serve seafoods and enterotoxigenicity of staphylococcal strains isolated from shellfish. J. Fd. Hyg. Soc. Jap., 13: 392 - 397.

Gameson, A.L.H. and J.R. Saxon, 1967. Field Studies on effect ofdaylight on mortality of coliform bacteria Water Res., 1: 279 - 295.

Gameson, A.L.H. and D.J. Gould, 1975. Effect of solar radiation on the mortality of some terrestrial bacteria in seawater. In: Discharge of sewage from sea outfalls. (A.L.H. Gameson, Ed.) Pergamon, UK. pp: 209-219.

Gameson, A.L.H., E.B. Pike and K.W. Davey, 1971. Mortality of coliform bacteria in sea water in daylight. In: Proceedings of the 5th International colloquim of Medical Oceanography, Messina, October 4-7, 1971. (S. Genovese, Ed.) ELLEBI, Editrice Libraria Bonanzinga, Messina (Italy).pp:353-362.

Gameson, A.L.H., E.B. Pike and K.W. Davey, 1973. Mortality of coliform bacteria in sea water samples in daylight. Colli.nt. oceanogr, med. Messina. A tit., 5: 353-362.

Gates, K.W., B.E. Perkins, J.G. EuDaly, A.S. Harrison and W.A. Bough, 1985. The impact of discharges from seafood processing on south eastern estuaries. Estuaries, 8: 244-251.

Gauthier, M.J., F. Cauvin and J.P.Breittmayer, 1985 a. Influence of salts and temperature on the transfer of mercury resistance from a marine pseudomonad to Escherichia coli. Appl. Environ. Microbiol., 50: 38 - 40.

Gauthier, M.J., V.Breittmayer, R. Clement, G.N.Flatan and J.C. Amiard, 1985. Tolerance au zinc at au cadmium et accumulation du zinc par les bacteries marines a Gram negatif: Relations avec leur type respiratoire. Can. J. Microbiol., 31: 793 - 798.

Geldreich, E.E. 1974. Microbiological criteria concepts for coastal bathing waters. Ocean Management, 3: 225 - 248.

Geldreich, E.E. and N.A. Clarke, 1966. B cterial pollution indicators in the intestinal tract of freshwater fish. Appl. Microbiol., 14: 429 - 437.

Geldreich, E.E. and B.A. Kenner, 1969. Concepts of fecal streptococci in stream pollution. J. Water Pollut. Contr. Fed., 41: 336 - 352.

Gerasimenko, D.D., 1977. On survival rate of colon bacillus in oil-polluted sea water. Mikrobiol. zh., 39: 726 - 728.

Gerba, C.P. and J.S. McLeod, 1976. Effect of sediments on the survival of Escherichia coli in marine water. Appl. Environ. Microbiol. 32: 114 - 120.

Gerba, C.P., S.M. Goyal, E.M. Smith and J.L. Melnick, 1977. Distribution of viral and bacterial pathogens in a coastal canal community. Mar. Pollut. Bull. 8: 279 - 282.

Gibbons, N.E., 1934 a. The slime and intestinal flora of some marine fishes. Contrib. Can. Biol. Fisheries., 8: 275 - 290.

Gibbons, N.E., 1934 b. Lactose fermenting bacteria from the intestinal contents of marine fish. Contrib. Can. Biol. Fisheries., 8: 291 - 300.

Gilmour, A., 1977. Characteristics of marine vibrios isolated from fish farm tanks. Aquaculture., 11: 51 - 62.

Glantz, P.J. and G.E. Krantz, 1965. Escherichia coli serotypes isolated from fish and their environment. Health Lab. Sci., 2: 59 - 63.

Goatcher, I.J. and D.C. Westhoff, 1975. Repression of Vibrio parahaemolyticus by Pseudomonas species isolated from processed oysters. J. Fd. Sci., 40: 533 - 536.

Goatcher, L.J., S.E.Engler, D.C. Wagner and D.C.Westhoff, 1974. Effect of storage at 5°C on survival of Vibrio parahaemolyticus in processed Maryland oysters (Crassostrea virginica). J. Milk. Fd. Technol., 37:74.

Godwin, D. and J.H. Slater, 1979. The influence of the growth environment on the stability of a drug resistance plasmid in Escherichia coli K12. J. Gen. Microbiol., 111: 201 - 210.

Goldmintz, D., 1974. Food technological aspects of Vibrio parahaemolyticus in market seafoods. In: Int. Symp. on Vibrio parahaemolyticus (T. Fujino, G. Sakaguchi, R. Sakazaki and Y. Takeda, Eds.) Saikon publishing Co., Ltd., Tokyo. pp: 147 - 152.

Goldmintz, D., R.C. Simpson and D.L. Dubrow, 1973. Effect of temperature on Vibrio parahaemolyticus in artifically contaminated seafood. Dev. Ind. Microbiol.,15: 288 - 293.

Golten, C. and W.A. Scheffers, 1975. Marine vibrios isolated from water along the Dutch coast. Neth. J. Sea Res., 9: 351 - 364.

Gore, P.S., O.Raveendran and R.V. Unnithan, 1979a. Pollution in Cochinbackwater with reference to indicator bacteria. Indian. J. Mar. Sci. 8: 43 - 46.

Gore, P.S., O.Raveendran and R.V. Unnithan, 1979b. Pollution status of the Cochin beach. Indian J. Mar. Sci., 8: 187 - 190.

Gore, P.S., T.S.G. Iyer, O.Raveendran and R.V. Unnithan, 1980. Isolation and significance of Salmonella Sp. from some beaches of Kerala. Mahasagar , 13: 147-152.

Goulder, R., 1977. Attached and free bacteria in an estuary with abundant suspended solids. J. Appl. Bacteriol., 43: 399 - 405.

Goyal, S.M. and A.W.Hodley, 1979. Salmonellae and their associated R-plasmids in poultry processing wastes. Rev. Microbiol., 10: 50 - 58.

Goyal, S.M., C.P. Gerba and J.L. Melnick, 1977. Occurrence and distribution of bacterial indicators and pathogens in canal communities along the Texas coast. Appl. Environ, Microbiol., 34: 139 - 149.

Grabow, W.O.K. and O.W. Prozesky, 1973. Drug resistance of coliform bacteria in hospital and city sewage. Antimicrob. Agents and Chemother., 3: 175 - 180.

Gray, R.J.H. and D.H. L. Hsu, 1979. Effectiveness of iodophor in the destruction of Vibrio parahaemolyticus. J. Fd. Sci., 44: 1097 -1100.

Greenberg, E.P., M.Duboise and B.Palhof, 1982. The survival of marine vibrios in Mercenaria mercenaria, the hardshell clam. J. Fd. Saf.,4: 113 - 123.

Greenwood, M.H., E.F.C. Coetzee, B.M. Ford, P.Gill, W.L. Hooper, S.C.W.Matthews, S.Patrick, J.V.S.Pether and R.J.D. Scott, 1985. The bacteriological quality of selected retail, ready - to - eat food products. Environ. Health., 93: 236 - 239.

Grigsby, P. and J. Calkins, 1980. The inactivation of a natural population of coliform bacteria by sunlight. Photochem. Photobiol., 31: 291 - 294.

Grimes, D.J. 1975. Release of sediment - bound faecal coliforms by dredging. Appl. Microbiol., 29: 109 - 111.

Grimes, D.J., 1982. Bacteriological water quality effects of clamshell dredging. J. Freshwater Ecol., 1: 407 - 419.

Grimes, D.J., F.L. Singleton, J. Stemmler, L.M. Palmer, P. Brayton and R.R. Colwell, 1984. Microbiological effects of waste water effluent discharge into coastal waters of puerto Rico. Water Res.,18: 613 - 619.

Grunnet, K., A. Gundstrep and G.J. Bonde, 1970. Isolation of Salmonella from a polluted marine environment, technique and enrichment media. Rev. Int. Oceanogr. Med., 17: 165 - 177.

Guhathakurta, B., G.C.Datta and D.Sasmal, 1978. Skin permeability and enterotoxic factors of Vibrio parahaemolyticus isolated from human diarrheal cases and river water. Indian J. Microbiol,18: 44-48.

Hajna, A.A., 1951. A buffered azide glucose - glycerol broth for the presumptive and confirmative tests for faecal streptococci. Public Health Lab., 9: 80.

Hanes, N.B., W.B. Sarles, and G.A. Rohlich, 1964. Dissolved oxygen and survival of coliform organisms and enterococci. J. Am. Water Works Assoc., 56: 441 - 446.

Harvey, H.W., (Ed.) 1955. The chemistry and fertility of sea waters. cambridge University Press. 224 P.

Hashimoto, H., H. Kawakami, M.Murata, H.Ushijina, M. Nakao, M. Yanagi and K.Kono, 1976. On the contamination of sea water with Salmonella and faecal indicator organisms. 1. Occurance and distribution of Salmonella and facal indicator organisms in coastal sea water of Fukuyama. J. Fac. Fish. Anim, Husb. Hiroshima Univ.. 15: 207 - 218.

Heinis, J.J., L.R. Beuchat and W.K. Jones, 1977. Growth of heat injured Vibrio parahaemolyticus in media supplemented with various cations. Appl. Environ. Microbiol., 33: 1079 - 1084.

Hirn, J., H. Viljamaa and M. Raevuori, 1980. The effect of physico-chemical, phyloplankton and seasonal factors on faecal indicator bacteria in northern brackish water. Water Res., 14: 279 - 285.

Hood, M.A. and S.P. Meyers, 1973. Microbial aspects of penaeid shrimp digestion. Proc. Gulf and Carribbean Fish. Inst., 26th Annual Session, October 1973. pp: 81-92.

Hood, M.A. and G.E. Ness, 1982. Survival of Vibrio cholerae and Escherichia coli in estuarine waters and sediments. Appl. Environ. Microbiol., 43: 578 - 584.

Hood, M.A., G.E.Ness and N.J.Blake, 1983. Relationship among faecal coliforms, Escherichia coli, and Salmonella spp. in shellfish. Appl. Environ. Microbiol., 45: 122 - 126.

Horie, S. and T. Kobayashi, 1981. Biological and ecological studies on Bdellovibrio parasitic and lytic to Vibrio parahaemolyticus. Bull. Jap. Soc. Sci. Fish., 47: 1209 - 1222.

Horie, S., S. Saheki and M. Okuzumi, 1967. Quantitative enumeration of Vibrio parahaemolyticus in sea and estuarine waters. Bull. Jap. Soc. Sci. Fish., 33: 126 - 130.

Houston A.C. 1900. On the value of examination of water for streptococci and staphylococci with a view to detection of its recent contamination with animal organic matter. In: Sup. 29th Ann. Rep.Loc. Gov. Bd. containing Rep. of Med. Off.London City Council England. P.458.

Hucker, G.J. and H.J. Conn, 1923. Methods of Gram staining. Tech. Bull. N.Y. State Agricul. Exper. Sta., No. 93.

Hugh, R. and R. Sakazaki, 1972. Minimal number of characters for the identification of Vibrio species, Vibrio cholerae and Vibrio parahaemolyticus. Public Health Laboratory. 30: 133 - 137.

Hunt, D.A., 1970. Sanitary control of shellfish and marine pollution. FAO Technical conference on marine pollution and its effects on living resources and fishes, 9-18 Dec. 1970, Rome, Italy.

Hunt, D.A. 1977. Indicators of quality for shellfish waters. In: Bacterial indicators/health hazards associated with water (A.W. Hoadley, and B.J. Dutka, Eds.) ASTM, Philadelphia, pp. 337 - 345.

Huq, M.I,D.Huber and G. Kibryia, 1979. Isolation of urease producing Vibrio parahaemolyticus strains from cases of gastroenteritis. Indian J. Med. Res., 70:540-553.

Huq, A., P.A. West, E.B.Small, M.I. Huq and R.R. Colwell, 1984. Influence of water temperature, salinity, and pH on survival and growth of toxigenic Vibrio cholerae serovar 01 associated with live copepodes in laboratory microcosms. Appl. Environ. Microbiol., 48: 420 - 424.

Iyer, T.S.G., 1979. Pathogens and faecal indicator organisms in fish and fishery products. In: Quality control in fish processing (M.K. Kandoran and M.Thomas, Comps.) Central Institute of Fisheries Technology, Cochin. pp: 45-59.

Iyer, T.S.G. and V.K. Pillai, 1971. Distribution of coliforms in fresh fishery products and environments. J. Fd. Sci. Technol., 8: 146 - 147.

Izzo, G., E.Tosti and L. Volterra, 1983. Faecal contamination of marine sediments in a stretch of the Gulf of Naples. Water Air Soil Pollut., 20: 191 - 198.

Jackson, H., 1974. Temperature relationships of Vibrio parahaemolyticus.In: Int. Symp. Vibrio parahaemolyticus (T. Fujino, G. Sakaguchi, R. Sakazaki and Y. Takeda, Ed.), Saikon Publishing Co.Ltd., Tokyo pp:139 - 144)

James, M.A., 1983. Studies on the microbial pathogens Salmonella and Vibrio parahaemolyticus in selected marine products. Ph.D. Thesis, Cochin, Univ. Sci. Technol.

Johnson, G.A., 1904. Isolation of Bacillus coli communis from the alimentary tract of fish and the significance thereof. J. Infect. Diseases, 1: 348 - 354.

Johnson, H.C. and J. Liston, 1973. Sensitivity of Vibrio parahaemolyticus to cold in oysters, fish fillets and crabmeat. J. Fd. Sci., 38: 437 - 441.

Johnson, Jr. W.G. , A.C. Salinger, and W.C.King, 1973. Survival of Vibrio parahaemolyticus in oyster shellstock at two different storage temperatures. Appl. Microbiol. 26: 122 - 123.

Johnson, D.E., L. Weinberg, J. Ciarkowski, P. West. and R.R.Colwell, 1984. Wound infection caused by Kanagawa - negative Vibrio parahaemolyticus. J. Clin. Microbiol. 20: 811 - 812.

Joseph, S.W., 1974. Observations on Vibrio parahaemolyticus in Indonesia.In:Int. Symp. on Vibrio parahaemolyticus (T. Fujino, G. Sakaguchi, R. Sakazaki and Y. Takeda.Eds), Saikon publishing Co.Ltd., Tokyo.pp. 35 - 40

Joshi, L., C.K. Desaprade and D. Rajagopalan, 1985. Isolation of Vibrio parahaemolyticus from Indian shrimps. In: Absts. XXVI Ann, Conf. Assoc. Microbiol. India Abst. Ve. Ma 14.

Kadri, M.H. and A.R. Salem, 1985. Incidence of heavy metal and antibiotic resistant coliforms in coastal waters and sediments of Kuwait city. Indian J. Mar. Sci., 14 : 48 - 49.

Kakubo, Y.S. Matsushita, A. Kai, M. Yamada and H. Konuma, 1978. Incidence of Escherichia coli in raw oyster and enterotoxin producibility of the isolates. J. Fd. Hyg. Soc. Jap., 19: 117 -121.

Kaneko, T., 1973. Ecology of Vibrio parahaemolyticus and related organisms in Chesapeake Bay. Ph.D. Thesis, Georgetown University, Washington, D.C., U.S.A.315P.

Kaneko, T. and R.R. Colwell, 1973. Ecology of Vibrio parahaemolyticus in chesapeake Bay. J. Bacteriol., 113: 24 - 32.

Kaneko, T. and R.R. Colwell, 1975. Adsorption of Vibrio parahaemolyticus on to chitin and copepods. Appl. Microbiol. 29: 269 - 274.

Kaneko, T. and R.R. Colwell, 1978. The annual cycle of Vibrio parahaemolyticus in Chesapeake Bay. Microb. Ecol., 4: 135 - 155.

Kaper, J., 1979. Isolation, ecology and taxonomy of human pathogens in an estuary. Ph.D. Thesis, University of Maryland, Maryland, U.S.A. 309.p.

Kaper, J.B., E.F. Remmers and R.R. Colwell, 1980. A medium for presumptive identification of Vibrio parahaemolyticus. J. Fd. Protect.,43: 936-938.

Kaper, J.B., H. Lockman, E.E. Remmers, K. Kristenser and R.R. Colwell, 1983. Numerical taxonomy of vibrios isolated from estuarine environments. Int. J. Syst. Bacteriol., 33: 229 - 255.

Kaper J., H. Lockman, R.R. Colwell, and S.W. Joseph, 1979. Ecology, Serology and enterotoxin production of Vibrio chlorerae in Chesapeake Bay. Appl. Environ. Microbiol., 37: 91 - 103.

Kapuscinski, R.B. and R. Mitchell, 1981. Solar radiation induces sublethal injury in Escherichia coli in seawater. Appl. Environ. Microbiol., 41: 670 - 674.

Kapuscinski, R.B. and R. Mitchell, 1983. Sunlight- induced mortality of viruses and Escherichia coli in coastal seawater. Environ. Sci. Technol., 17: 1-6.

Karthiayani, T.C. and K.M. Iyer, 1967. Qualitative and quantitative studies on the bacterial flora of fresh sardines. Fish. Technol., 4: 89 - 97.

Karthiayani, T.C. and K.M. Iyer, 1975. The bacterial flora of certain marine fishes and prawns in Cochin waters in relation to their environs. J. Mar. Biol. Assoc. India., 17: 96 - 100

Karunasagar, I., 1981. Production of hemolysin by Vibrio parahaemolyticus in a chemically defined medium, Appl. Environ, Microbiol, 41: 1274 - 1275.

Karunasagar, I. and K.C. Mohankumar, 1980. Occurence of Kanagawa positive Vibrio parahaemolyticus strains around Mangalore (South India). Indian J. Med. Res., 72: 619 - 621.

Karunasagar, I., M.N. Venugopal and I. Karunasagar, 1984. Levels of Vibrio parahaemolyticus in Indian shrimp undergoing processing for export. Can. J. Microbiol., 30: 713 - 715.

Karunasagar, I., S.W.Joseph, R.M. Twedt, H. Hada and R.R. Colwell, 1984. Enhancement of Vibrio parahaemolyticus virulence by lysed erythrocyte factor and iron. Infect. Immun., 46: 141 - 144.

Karunasagar, I and I. Karunasagar, 1985. Isolation of filamento phages acting on Vibrio parahemolyticus In: Abts.XXVI Ann. Conf. Assoc. Micro. India Abst. Med 12.

Kelly, M.T. and A. Dinuzzo, 1985. Uptake and clearence of Vibrio vulnificus from Gulf Coast oysters (Cassostrea virginica). Appl. Environ. Microbiol., 50: 1548 - 1549.

Kenner, B.A. 1978. Fecal streptococcal indicators. In: Indicators of Viruses in Water and Food (G. Berg, Ed) Ann Arbor, Michigan.pp: 147 - 169.

Kenner., B.A., H.F. Clarke and P.W. Kabler, 1961.Fecal Streptococci; I. Cultivation and enumeration of Streptococci insurface waters. Appl. Microbiol., 9: 15 - 20.

Kibbey, H.J., C. Hagedorn and E.L. McCoy, 1978. Use of fecal streptococci as indicators of pollution in soil. Appl. Environ. Microbiol., 35: 711 - 717.

Kim, J. 1975. Indicative, pathogenic, and marine bacteria in the sea. In: Coastal Water Research Project. Ann Rep. for the year ended 30th June 1975.p.15-22.

Kim J.H., 1983. Microbiological water quality of major swimming beaches in Jelu Island. Bull. Mar. Resour. Res. Inst. Jelu Natl Univ., 7: 53 - 58.

Kim., Y.K. and D.S. Chang, 1981. Bacteriological quality of seawater at swimming beaches in Busan area. Bull. Korean fish. Soc., 14: 148 - 157.

Kim., J.H. and D.C. Oh, 1982. A study on distribution of Vibrio parahaemolyticus in Jelu harbor. Bull. Mar. Resour. Res. Inst. Jelu Natl. Univ.,6: 27 - 32.

Kimura, K., S. Tateiri and H. Iida, 1979. Effects of p^H of the medium on flagellation of Vibrio parahaemolyticus Appl. Environ. Microbiol., 37: 1248 - 1249.

Koditschek, L.R. and P. Guyre, 1974. Antimicrobial - resistant coliforms in New York Bight. Mar. Pollut. Bull., 5: 71 - 74.

Kogure, K., U.Simidu and N. Taga, 1980. Effect of phyto and zooplankton on the growth of marine bacteria in filtered seawater. Bull. Jap. Soc. Sci. Fish., 46: 323 - 326.

Kovacs, N., 1928. Eine vereinfachte Method zum Nachweis der indolbildung durch Bakterien. Z. Immunetacts. Exp. Iher. , 55: 311 - 315.

Kovacs, N. 1956. Identification of Pseudomonas pyocyanea by the oxidase reaction. Nature, 178: 703.

Kuch, C.S.W., 1974. An investigation on the nutrients, coliform bacteria and other indicators of marine pollution in Tolo Harbour, Hong Kong. Hongkong Fish. Bull., 4A: 115 - 124.

Kurata, A., Y.Yoshida, H. Kadota, and F.Taguchi, 1977. Distribution of Nickel tolerant bacteria in water and sediment of the sea of Aso. Bull. Jap. Soc. Sci. Fish., 43: 1203 - 1208.

Lakshmanaperumalsamy, P., S. Premjith and A.Purushothaman, 1986. Incidence of faecal coliform and Escherichia coli in fresh fishery products. Bull. Dept. Mar. Sci., Univ. Cochin, 14: (In press)

Lakshmanaperumalsamy.P. M. Chandrasekaran, I.S. Bright Singh and D. Chandramohan, 1981. Microbial indicators and pathogens near the mouth region of Vembanad lake. Bull. Dept. Mar. Sci., Univ. Cochin, 12 : 103 - 119.

Lall, R.,D.Sen, M.R.Saha, A.K.Bose, S.P.De, N.C. Palchowdhury, and S.C.Pal, 1979. Prevalence of Vibrio parahaemolyti in Port Blair. Indian J. Med. Res., 69: 217 - 221.

Larkin, E.P., W.Litsky, and J.E. Fuller, 1956. Incidence of faecal streptococci and coliform bacteria in frozen food products. Am. J. Publ. Health., 46 : 464 - 468.

Larsen, H., 1962. Halophilism. In: The bacteria: A treatise on structure and function. Vol.4. (A.C.Gunsalus and R.Y.Stamier, Eds.) Academic Press, New York. pp. 297 - 342.

Larson, J.L.,A.F.Farid and I.Dalsgaard, 1981. Occurance of Vibrio parahaemolyticus and Vibrio alginolyticus in marine and estuarine bathing areas in Danish coast. Zentralbl. Bakteriol. Mikrobiol. Hyg. Abt. B., 173: 338 - 345.

Leclerc, J., 1971. Marine and freshwater pollution. Rev. Int. Oceanogr. Med. CERBOM., 24: 155 - 170.

Lee, J.S., 1972. Inactivation of Vibrio parahaemolyticus in distilled water. Appl. Microbiol., 23: 166 - 167.

Leifson, E., 1963. Determination of carbohydrate metabolism of marine bacteria. J. Bacteriol., 85: 1183 - 1184.

Leung, C.,K.F. Shortridge, B. Mortor and P.S. Wong, 1975. The seasonal incidence of faecal bacteria in the tissues of the commercial oyster, Crassostrea gigas correlated with the hydrology of Deep Bay, Hong Kong. In: Symposium papers of the Pacific Science association special symposium on marine science (B. Morton, Ed.) PSA, Hong Kong.pp:114 - 127.

Lin, A.C. and L.R. Beuchat, 1980. Recovery of chill-stressed Vibrio parahaemolyticus from oysters with enrichment broths supplemented with magnesium and iron salts. Appl. Environ, Microbiol., 39: 179 - 185.

Liston, J., 1957. The occurrence and distribution of bacterial types on flatfish. J. Gen. Microbiol., 16: 205 - 216.

Litsky, W, W.L. Mallamann and C.W.. Fifield, 1955. Comparison of the most probable numbers of Escherichia coli and Enterococci in river waters. Amer. J. Publ.Health, 45: 1049 - 1053.

Lombardo, A., 1973. Microbiological studies in the Venezia lagoon. 1. Presence and distribution of faecal coliforms as index of pollution of urban origin. Atti. Ist. Ver. Sci. Lett. Arte, Sci. Mat. Nat., 132: 57 - 74.

Luli, G.W., J.W.Talnagi, W.R. Strohl and R.M. Pfister, 1983. Hexavalent chromium - resistant bacteria isolated from river sediments. Appl. Environ. Microbiol., 46: 846 - 854.

MacLean, J.R., 1978. Bacteriological results of monitoring program of oyster shellstock harvesting areas Maritimes region 1977. Data Rep. Fish. Mar. Serv. (Canada) (No.86) 12P.

Manavalan, S., K. Dhevendaran, Vanajakumar and N. Selvakumar, 1977. Vibrio parahaemolyticus in the marine environment at Porto Novo. Curr. Sci., 46: 821 - 824.

Manual of Microbiological methods, 1957. (Soc.Am.Bacterioligists McGraw-Hill Book Co., New York. 315 p.

Marano, G., N. Casavola and R. Vaccarella, 1974. Survey on the Bacteria coli titre in the Teleostei fished along the coast of Bari. Mem. Biol.Mar.Oceanogr., Messira., 4: 445 - 457.

Marques, A.M. F.Congregado and D.M.S. Pujol., 1979. Antibiotic and heavy metal resistance of Pseudomonas aeruginosa isolated from soils. J. Appl. Bacteriol., 47: 347 - 350.

Matches, J.R. and J. Liston, 1971. Radiation destruction of Vibrio parahaemolyticus. J. Fd. Sci., 36: 339 - 340.

Matches, J.R. J. Liston and L.P. Daneault, 1971. Survival of Vibrio parahaemolyticus in fish homogenate during storage at low temperatures. Appl. Microbiol., 21: 951 - 952.

McCambridge, J. and T.A. McMeekin, 1979. Protozoan predation of Escherichia coli in estuarine waters. Water Res., 13: 659 - 663.

McCambridge, J. and T.A. McMeekin, 1980a. Relative effects of bacterial and protozoan predators on survival of Escherichia coli in estuarine water samples. Appl. Environ. Microbiol., 40: 907 - 911.

McCambridge, J. and T.A. McMeekin, 1980 b. Effect of temperature on activity of predators of Salmonella typhimurium and Escherichia coli in estuarine water. Aust. J. Mar. Freshwater Res., 31: 851 - 855.

McCambridge, J. and T.A. McMeekin, 1981. Effect of solar radiation and predacious microorganisms on survival of fecal and other bacteria. Appl. Environ. Microbiol., 41: 1083 - 1087.

McFeters, G.A. and D.G. Stuart, 1972. Survival of coliform bacteria in natural waters: Field and laboratory sutdies with membrane filter chambers. Appl. Microbiol., 24: 805 - 811.

McGinnis, J.A., 1983. Raw seafood can be a harzardous food. J. Environ. Health., 45: 182 - 185

Michaels, G.B., J.J. Hill, D.J. Schneck and P.Delannoy, 1984. Influence of heavy metals on the distribution of antibiotic resistant bacteria in a high altitude watershed. Abst. of the Ann. Meet. of the Am. Soc. for Microbiology, 1984. Publ. by ASM, washington DC, USA,, P. 206.

Miskimin, D.K., K.A. Berkowitz, M.Solberg, W.E.Jr. Riha, W.C. Franke, R.L. Buchanan and V.Oleary, 1976. Relationships between indicator organisms and specific pathogens in potentially hazardous foods. J. Fd. Sci., 41: 1001 - 1006.

Mitchell, R. 1971. Role of predators in the reversal of imbalances in microbial ecosystems. Nature (Lond)., 230: 257 - 258.

Miyamoto, S. and K. Kuroda, 1975. Lethal effect of fresh seawater on Vibrio parahaemolyticus and isolation of Bdellovibrio prasitic against the organism. Jap. J. Microbiol., 19: 309 - 317.

Moebus, K. 1972 a. Bactericidal properties of natural and synthetic seawater as influenced by addition of low amounts of organic matter. Mar. Biol., 15: 81 - 88.

Moebus, K. 1972 b. Seasonal changes in antibacterial activity of North Seawater. Mar. Biol., 13: 1-13

Moebus, K., 1972. Factors affecting survival of test bacteria in sea water: marine bacteria, test bacteria and solid surfaces. Helgolander wiss. Meeresunters. 23: 271 - 285.

Moeller, V. 1955. Simplified tests for some amino acids decarboxylases and for the arginine dihydrolase system. Acta. Pathol. Microbiol. Scand., 36: 158 - 172.

Molitoris, E., S.W. Joseph, M.I. Krichevsky, W. Sindhuhardja and R.R. Colwell, 1985. Characterization and distribution of Vibrio alginolyticus and Vibrio parahaemolyticus isolated in Indonesia. Appl. Environ. Microbiol., 50: 1388 - 1394.

Moore, B., 1954 a. A survey of beach pollution at a seaside resort. J. Hyg (Lond)., 52: 71 - 86.

Moore, B., 1954 b. Sewage contamination of coastal bathing waters. Bull. Hyg., 29: 689.

Moore, B., 1959. Sewage contamination of coastal bathing waters in England and Wales: A bacteriological and epidemiological study. J. Hyg., (Lond) 57:435 - 472.

Moreau, J.P., G. Feval and R. Bagnis, 1971. Study of the pollution of the lagoon at Tahiti by fecal germs. Rev. Epidemiol. Med. Soc. Sante Publique, 19: 613 - 626.

Morton, B. and K.F. Shortridge, 1976. Coliform bacteria levels correlated with the tidal cycle of feeding and digestion in the Pacific oyster(Crassostrea gigas) cultured in Deep Bay, Hong Kong. Proc. Natl. Shellfish. Assoc. Med., 65: 78 - 83.

Mujeriego, R., J.M.Bravo and M.T. Feliu, 1982, Recreation in coastal waters: Public health implications. Paper presented in workshop on pollution of the Mediterranean, 2nd - 4th December 1982. Cannes.

Mukhmedoe, S.M., M.V. Inzheatova, A.F. Aleinikova, N.M. Ostrovskaya, R.T. Radzhapova and M.A Alimov, 1971. Lake frogs, fish and water crayfish as potential reservoirs of vibrios in nature. Zh. Mikrobiol. Epidemiol. Immunobiol., 48: 56 - 59.

Murphy, J. and J.P. Riley, 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chem. Act., 12: 162 - 176.

Nair., G.B., 1981. Studies on Ecology, Serology and taxonomy of Vibrio parahaemolyticus and allied vibrios from estuarine and neritic enviorns of Porto Novo. Ph.D. Thesis, Annamalai University India, 322p.

Nair, G.B., M. Abraham and R. Natarajan, 1980a. Marine vibrios and related genera from the Vellar estuary. Mahasagar, 13: 285 - 290.

Nair, G.B., M. Abraham and R. Natarajan, 1980b. Distribution of Vibrio parahaemolyticus in finfish harvested from Porto Novo (South India) environs: a seasonal study. Can. J. Microbiol, 26: 1264 - 1269.

Nair, G.B., B.L. Sarkar, M. Abraham and S.C. Pal, 1985 a. Serotypes of Vibrio parahaemolyticus isolates from hydrobiologically dissimilar aquatic environments. Appl. Environ. Microbiol., 50: 724 - 726.

Nakayama, O. and M. Ohno, 1981. Bactericidal effect of seawater containing digested night soil and marine plankton against intestinal bacteria. Bull. Jap. Soc. Sci. Fish., 47: 165 - 169.

Natarajan, R., M. Abraham and G.B. Nair, 1979. Distribution of Vibrio parahaemolyticus and allied vibrios in backwater and mangrove biotopes at Porto Novo. Indian J. Mar. Sci., 8: 286 - 289.

Natarajan, R., M. Abraham and G.B. Nair, 1980 a. Distribution of Vibrio parahaemolyticus in Porto Novo environment. Indian J. Med. Res., 71: 679 - 687.

Natarajan, R., M. Abraham and G.B. Nair, 1980b. Monitoring food borne pathogenic bacteria in estuarine fish and shellfish. Paper presented in the Asian Symposium on mangrov Environment-Research and Management, 25th - 29th August, 1980., Kuala Lumpur, Malaysia.

Natarajan, R., M. Abraham and G.B.Nair, 1980c. Vibrio parahaemolyticus and the seafood industry. Fish, Technol., 17: 1 - 6.

Natarajan, R., G.B. Nair, and M. Abraham, 1979a. Incidence of Vibrio parahaemolyticus in relation to feeding habit of fishes. Curr. Sci., 48: 875 - 877.

Natarajan, R., G.B. Nair and M. Abraham, 1979b. characterization of marine food borne pathogen, Vibrio parahaemolyticus isolated from freshwater fishes. Indian J. Mar. Sci., 8: 166 - 169.

Natarajan, R., M. Abraham, R. Gautam and G.B. Nair, 1978. Vibrio parahaemolyticus marine food borne pathogen - a perspective. In: Proceedings of the Internat. Symp. on Environmental Agents and Their Biological Effects, Hyderabad, India. pp: Int 5.2.59 - 5.2.67.

Nelson.K.J. and N.N. Potter, 1976. Growth of Vibrio parahaemolyticus at low salt levels and in nonmarine foods. J. Fd. Sci., 41: 1413 - 1417.

Neogy, K.N., B.D. Chatterjee, M.K. Mukherjee and P. Manji, 1970. Isolation of vibrios and allied organisms from cases of acute diarrhoea in Calcutta during 1969. Bull. Cal. Sch. Trop. Med., 18:9.

Nickerson, J.T.R. and G.A. Pollak, 1972. Bacteriological examination of raw breaded frozen shrimp. J. Milk Fd.Technol.,35: 167 - 169.

Niemi, M., and I. Taipalinen, 1982. Faecal indicator bacteria in fish farms. Hydrobiologiya, 86: 171 - 175.

Nolan, C.M., J. Ballard, C.A. Kaysner, J.L. Lilja, L.P. Williams Jr. and F.C. Tenover, 1984. Vibrio parahaemolyticus. An outbreak associated with raw oysters in the pacific north west. Diagn. Microbiol. Infect. Dis., 2: 119 - 128.

Nuhi, A. and Y. Khorasani, 1981. Bacterial pollution indicators in the intestinal tract of various fish species living in Amir - Kolayeh Lagoon. Zentralbl. Bakteriol. Parasitenkd Infektionskr. Hyg. II., 136: 566-571.

Nusbaum, J. and R.M. Garver, 1955. Survival of coliform organisms in Pacific ocean coastal waters. Sewage Ind. Wastes. 27: 12.

O'Brien, C.H. and R.K. Sizemore, 1979. Distribution of the luminous bacterium Beneckea harveyi in a semitropical estuarine environment. Appl. Environ. Microbiol., 38: 928 - 933.

Ogawa, K., 1973. Distribution of coliform bacteria in the coastal water. J. Oceanogr. Soc. Jap., 29: 203 - 208.

Ogawa, K., 1977a. Primary participation of fecal bacteria in the formation of suspended organic matter in the sea. 1. Ion adsorption and floc formation by Escherichia coli. Bull. Jap. Soc. Sci. Fish., 43: 1081 - 1088.

Ogawa, K., 1977b. Primary participation of fecal bacteria in the formation of suspended organic matter in the sea. 2. Floc formation by fecal bacteria isolated from marine animals. Bull. Jap. Soc. Sci. Fish., 43: 1089 - 1096.

Oger, C., A. Philippo and H. Leclerc, 1974. The micorbiological pollution of North Sea and English Channel shores. Ann. Microbiol. (Inst. Pasteur). 125 B,: 513 - 527.

Olson, B.H. and R.C. Cooper, 1974. In situ methylation of mercury in estuarine sediment. Nature, 252: 682-683.

Olson, B.H., T. Barkay and R.R. Colwell, 1979. Role of plasmids in mercury transformation by bacteria isolated from the aquatic environment. Appl. Environ. Microbiol., 38:478-485.

Oragui, J.I. and D.D Mara, 1983. Investigation of the survival characteristics of Rhodococcus coprophilus and certain fecal indicator bacteria. Appl. Environ. Microbiol., 46: 356 - 360.

Orlob, G.T., 1956. Viability of sewage bacteria in sea water Sewage Ind. Wastes, 28: 1147 - 1167.

Ose, Y. and T. Ikeda, 1964. Studies on Vibrio parahaemolyticus from night soil. Isolation of V. parahaemolyticus from night soil. Shukuhin Eiseigaku Zasshi, 5: 206 - 210.

Oshiro, T., 1964, The distribution of Vibro parahaemolyticus in nature. In: Vibrio parahaemolyticus (T. Fujino and H. Fukumi. Eds). Isseido, Tokyo, Japan. pp. 263 - 277.

Ottunola, E.T. and C.O. Ogunsanya and J.A. Ekundayo, 1983. Microbial quality of Gryphaca gasar Adanson from the coastal waters of Nigeria and Ghana. Microbios Lett., 24: 91-98.

Owens, J.D., 1978. Coliform and Escherichia coli bacteria in sea water around Penang Island, Malaysia. Water Res., 12: 365 - 370.

Palasuntheram, C. 1981. The halophilic properties of Vibrio parahaemolyticus. J.Gen. Microbiol.,127: 427-428.

Papadakis, J. 1972. Coliforms and Escherichia coli as indicators of sewage pollution of seawater. Proc. 5th Natl. Symp. for Microbiol. pp: 61 - 66.

Parker, J.G., A.P. Damoglov and D.A. Stewart, 1979. The distribution of faecal bacteria in the estuary of the river Lagan. Rec. Agric. Res. Dep. Agric.,(North Irel.), 27 : 43 - 51.

Parvis, D., D. Reali, G. Caroli, and E. Levre, 1975. Research of Salmonella as a pollution index of the sea water near the coast. Ig. Mod., 67: 807 - 818.

Perry, C.A. and M. Baylise, 1936, Escherichia cali as an indicator of fecal pollution in oysters and oyster waters. Am. J. Public Health, 26: 406.

Peters, N., 1982. Fish diseases and water pollution in the coastal area. Decheniana Beih,26: 120 - 127.

Pike,E.B., A.L.H. Gameson and D.J. Gould, 1970. Mortality of coliform bacteria in sea water samples in dark. Rev. Intern. Oceanogr. Med. Tome, XVIII-XIX: 97-107.

Pillai,C.T. 1980. Microbial flora of mussels in the natural beds and farms. C.M.F.R.I. Bull., 29: 41-43.

Pinon, J. and Pijck, 1972. Microbiological seawater contamination along the Belgian Coast. II. Techniques, norms, preliminary results. Rev. Int. Oceanogr. Med. CERBOM, 27: 17-40.

Plusquellec, A., M. Beucher and Y. Legal, 1983. Enumeration of the bacterial contamination of bivalves in monitoring the marine bacterial pollution. Mar. Pollut. Bull., 14: 260-263.

Powis, B. and A. Hazzard, 1984. Effect of swimming on health. Med. J. Aust., 141: 235-236.

Pujol, S.M.D., M.J.E. Thomas, F. Congregado and A.M. Marques, 1980. Heavy metal tolerance of antibiotic resistant gram-negative bacteria isolated from seawater. Microbios Lett., 15: 151-157.

Raghavachari, T.N.S. and P.V.S. Iyer, 1939. The Coli-aerogenous index of pollution used in the bacteriological analysis of water. Ind. J. Med. Res., 26: 867-877.

Rao, C.C.P. and S.S. Gupta, 1978. Enteropathogenic Escherichia coli and other coliforms in marine Fish. Fish. Technol., 15: 45-47.

Rao, K.S. and R. Boopathy, 1985. Studies on the survival of coliforms and faecal streptococci in sewage and polluted waters. Asian Environ., 7: 20-24.

Raveendran, O., P.S. Gore, and R.V. Unnithan, 1978. Observations on faecal contamination of cherai beach in Kerala, Indian J. Mar. Sci., 7: 128-129.

Rheinheimer, G., 1977. Introduction. In: Ecological studies, Vol. 25. (G. Rheinheimer. Ed.), Springer- Verlag, Berlin Heidelberg. pp 1-4.

Rho, J., 1984. Characterization of copper tolerant bacteria from urban lake sediments. Paper presented in the Ann. Meet of the ASM 4th-9th March 1984 St. Louis, USA .

Rhodes, M.W. and H.I. Kator, 1984. In situ survival of Escherichia coli in estuarine water: Effects of sunlight and autochthonous microbiota. Paper presented in Ann. Meet of the ASM., 4th-9th March 1984. St. Louis, USA.

Richards, A.G., 1951. The integuments of arthropods. University of Minnesota press, Minneapolis, U.S.A.

Ro, S. and M. Woodburn, 1976. Survival of Vibrio parahaemolyticus in Korean style salted oysters. J. Fd. Sci., 41:1033-1035.

Robach, M.C. and C.S. Hickey, 1978. Inhibition of Vibrio parahaemolyticus by sorbic acid in crab meat and flounder homogenates. J. Fd. Protect., 41: 699-702.

Robertson, W.J., 1984. Pollution indicators and potential pathogenic microorganisms in estuarine recreational waters. Can.J. Public Health, 75: 19-24.

Robertson, W.J., and R.S.Tobin, 1983. The relationship between three potential pathogens and pollution indicator organisms in Nova scotian coastal waters. Can. J. Microbiol., 29: 1261-1269.

Rochford, D.J., 1951. Studies in Australian estuarine hydrography. I. Introductory and comparative features. Aust. J. Mar. Freshwater Res., 2: 1-116.

Roper, M.M. and K.C. Marshall, 1977. Lysis of Escherichia coli by a marine myxobacter. Microb. Ecol., 3: 167-171.

Roper, M.M. and K.C. Marshall, 1978. Biological control agents of sewage bacteria in marine habitats Aust. J. Mar. Freshwater Res., 29: 335-343.

Roszak, D.D., D.J. Grimes, and R.R. Colwell, 1984. Viable but non-recoverable stage of Salmonella enteritidis in aquatic systems. Can. J. Microbiol., 30: 334-338.

Rottini, G.D., M. Tamaro, M. Cinco, and C. Monti- Bragadin, 1974. Effects of Na^+, K^+ and Li^+ on the growth of Vibrio parahaemolyticus and related strains isolated from the Adriatic sea. In Int. Symp. Vibrio parahaemolyticus. (T. Fujino, G. Sakaguchi, R. Sakazaki and Y. Takeda. Eds.), Saikon Publishing Co. Ltd., Tokyo. pp 153-157.

Row, A., 1981. Microbiological studies in the Mandovi - Zuari river system. Indian J. Mar. Sci., 10: 189-191.

Rowse, A.J. and G.H. Fleet, 1984. Effects of water temperature and salinity on elimination of Salmonella charity and Escherichia coli from Sydney rock oysters. Appl. Environ, Microbiol., 48:,1061 - 1063.

Ruby, E.G. and J.G. Morin, 1979. Luminous enteric bacteria of marine fishes: A study of their distribution, densities and dispersion. Appl. Environ. Microbiol.,37: 1230-1238.

Sakazaki, R. and A. Ballows, 1981. The genera Vibrio Plesiomonas and Aeromonas. In: The Prokaryotes. A handbook on habitats, isolation, and identification of bacteria (M.P. Starr, H. Stolp, H.G. Trupar, A. Balows and H.G. Schlegel, Eds.) springer-Verlag, Berlin. pp: 1272-1302.

Sakazaki, R., S. Iwanami and H.Fukumi, 1963. Studies on the enteropathogenic facultatively halophilic bacteria, Vibrio parahaemolyticus: Morphological, cultural and biochemical properties and its taxonomic position. Jap. J. Med. Sci. Biol., 16: 161-188.

Sakazaki, R., K. Tamura, L.M. Prescott, Z. Bencic, S.C. Sanyal and R. Sinha 1971. Bacteriological examination of diarrheal stools in Calcutta. Indian J. Med. Res., 59: 1025-1034.

Salas, E.P. 1976. Pollution of faecal origin in the Herradura Bay, Coquimbo, Chile. Rev. Com. perm. Pae. Sur., 5: 147-160.

Saldanha, F.L., A.K. patil and M.V. Sant, 1975. Studies on Vibrio parahaemolyticus in Bombay. Progress in Drug Res. Trop. Dis., 19: 586.

Sanyal, S.C. and P.C. Sen. 1974. Human volunteer study on the pathogenicity of Vibrio parahemolyticus. Int. symp. Vibrio parahaemolyticus. (T.Fujino, G. Sakaguchi, R.Sakazaki and Y. Takeda, Eds.) Saikon Publishing Co.,Ltd., Tokyo. P 227.

Sanyal, S.C., M.G. Chowdhary, P.C. Sen and H. Singh, 1973. Sensitivity of Vibrio parahaemolyticus to antibacterial agents. Indian J. Med. Res., 61: 324-329.

Sarkar, B.L., G.B. Nair, B.K. Sircar and S.C. pal, 1983. Incidence and level of Vibrio parahaemolyticus associated with freshwater plankton. Appl. Environ. Microbiol., 46: 288-290.

Sarkar, B.L., G.B. Nair, A.K. Banarjee and S.C. Pal, 1985 Seasonal distribution of Vibrio parahaemolyticus in freshwater environments and in association with freshwater fishes in Calcutta. Appl. Environ. Microbiol., 49: 132-136.

Sasmal, D., B. Guhathakurta, and G.C. Dutta, 1973. Comparative studies on some biochemical aspects of different strains of vibrios . Indian J. Med. Res., 61: 321-323.

Sastry, C.A., K.M. Aboo, and M.N. Rao, 1969. Incidence of coliforms and enterococci in natural waters. Environ. Health. 11: 32-40.

Savage, H.P. and N.B. Hanes, 1971. Toxicity of seawater to coliform bacteria. J. Water Pollut. Contr. Fed., 43: 854-861.

Sayler, G.S., J.D. Nelson, Jr., A Justice and R.R. Colwell, 1975. Distribution and significance of faecal indicator organisms in the upper Chesapeake Bay. Appl. Microbiol., 30: 625-638.

Sayler, G.S., J.D., Nelson, A.Justice and R.R. Colwell, 1976. Incidence of Salmonella sp., Clostridium botulinum and Vibrio parahaemolyticus in an estuary. Appl. Environ. Microbiol., 31: 723-730.

Scarpino, P.V., 1978. Bacteriophage indicators, In: Indicators of viruses in water and food. (G.Berg, Ed.) Ann Arbor, Mich. USA pp 201-227.

Scarpino, P.V. and D. Pramer, 1962. Evaluation of factors affecting the survival of Escherichia coli in seawater. Appl. Microbiol., 10: 436-440.

Schawrz, J.R. and R.R. Colwell, 1974. Effect of hydrostatic pressure on growth and viability of Vibrio parahaemolyticus. Appl. Microbiol., 28: 977-981.

Schreiber, D.R., A.S. Gordon and F.J. Millero, 1985. The toxicity of copper to the marine bacterium Vibrio alginolyticus. Can J. Microbiol. 31: 83-87.

Sen, D. and S.N. Ghosh, 1970. A comparison of the enterococci index with coliform index as indication of faecal pollution of water. Indian J Med. Res. 58: 1164-1168.

Sen, D. S.P.De, S.N. Ghosh P.K.Chandra, A. Ghosh and S.C. Pal, 1977. Antibiotic sensitivity of Vibrio parahaemolyticus from cases of gastroenteritis. Indian J. Med., 65,628-631.

Sera, H., Y. Ishida and H. Kadota, 1974. Baterial flora in the digestive tracts of marine fish. In: Effect of the ocean environment on microbial activities (R.R. Colwell and R.Y. Morita, Eds.) Univ. Park, Press, Baltimore, pp: 467-490.

Shinoda, S., K. Itoh, Y. Hayashi, S. I. Miyoshi, Y. Yamosaki, M. Ikeda, T. Itoh and T. Tsuchile, 1985. Ecology of vibrios in estuarine region of Seto Inland sea: Distribution of some diarrhenogenic species. J. Hyg. Chem., 31: 220-226.

Sieburth, J. McN. and D.M. Pratt, 1962. Anticoliform activity of sea water associated with the termination of Skeletonema costatum blooms. Trans. N.Y. Acad. Sci. (Ser.2), 24: 498-501.

Sierra, G., 1957. A simple method for the detection of of lipolytic activity of microorganisms and some observations on the influence of contact between cells and fatty substances. Antonie Van Leewenhook (J.Microbiol. Serol), 23: 15-22.

Silas, E.G. and P.P. Pillai, 1975. Dynamics of zooplankton in a tropical estuary (Cochin backwater), with a view on the plankton fauna of the environment. Bull Dept. Mar. Sci., Univ. Cochin., 7 : 329-335.

Simidu, U, K. Ashino, and E. Kaneko, 1971. Bacterial flora of phyto and zooplankton in the inshore waters of Japan. Can. J. Microbiol, 17: 1157-1160

Simidu, U., E. Kaneko and N. Taga, 1977. Microbiological studies of Tokyo Bay. Microbial. Ecol., 3: 173-191.

Simmann, J. and G. Rheinheimer, 1975. Investigation on the migration and multiplication of Escherichia coli in mud sediments of coastal waters. Kiel. Meeresforsch., 31 : 95-106.

Sinha, S. and T.R. Doctor, 1983, Survival study of Vibrio parahaemolyticus in sterile and nonsterile coastal and seawater. Paper presented in 24th Ann. Conf. of Associ. Microbiol of India, 12th—14th November 1983, Osmania University, Hyderabad, India.

Sircar, B.K., B.C. Deb, S.P. De, A. Ghosh and S.C. Pal, 1976. Clinical and epidemiological studies on Vibrio parahaemolyticus infection in Calcutta. Indian J. Med. Res. 64 : 1576 - 1580.

Sivakumar, V., G.S. Thangaraj, R. Chandran, P. Lakshmanaperumalsamy and K. Ramamoorthi, 1980 . Studies on faecal coliform in an estuarine environment of Porto Novo. Paper presented in Symposium on coastal aquaculture, 12-18 Jan. 1980. Cochin, India.

Sjogren, R.E. and J. Port, 1981. Heavy metal and antibiotic resistant bacteria in a lake recreational area. Water Air Soil Pollut., 15: 29-44.

Skerman, V.B.D., 1967. A guide to the identification of the genera of bacteria. The Williams and Wilkins Co., Baltimore.

Slanetz, C.W. and C.H. Bartley, 1957. Numbers of enterococci in water, sewage, and feces determind by the membrane filter technique with an improved medium . J.Bacteriol., 74 : 591.

Smith, H.W., 1978. Arsenic resistance in Entrobacteriaceae: Its transmission by conjugation and by phage. J. Gen. Microbiol., 109: 49-56.

Souter, B.W., R.A. Sonstegard and L.A. McDermott, 1976. Enteric Bacteria in carp (Cyprinus carpio) and with suckers (Catostomus commersoni) J. Fish. Res. Bd. Can., 33: 1401 - 1403.

Sreenivasan,A., 1964. Bacterial pollution of inshore waters of Kerala and Madras states, India. Symp. de Monaco, Comm. Int . Explor. Sci.Mer.Medit, 55.

Stevenson, A.J. 1953. Studies of bathing water quality and health. Am. J. Public Health, 43 : 529-538.

Strasdine, G.A. and L. Dubetz, 1974. Coliform flora of migrating sockeye Salmon (Oncorhynchus nerka). J. Fish. Res. Bd. Can. 31: 1559 - 1560.

Strickland, J.D.H. and T.R. Parsons, 1968. A practical handbook of sea water analysis. Fish. Res. Bd. Canada, Bull., 167: 311.

Tello, G.P., R. Iturriaga and A. Vera, 1970. Note on the coliform bacteria in the shrimp Heterocarpus reedi Bahamonde. Santiago., 4: 33-40.

Temmyo, R, 1966. Studies on the prevention of outbreaks of food poisoning caused by Vibrio parahaemolyticus . Bull. Tokyo Med. Dent. Uni., 13 : 489-510.

Tepedino.A.A., 1982. Vibrio parahaemolyticus in Lond Island oysters. J. Fd. Port., 45: 150-151.

Thayib, S.S. and H. Suhadi, 1979. The distribution and occurrance of microbi al indicators in the strait of Bangkong and Jakarta Bay waters. Paper presented at 5th Internat. Symp. of Tropical Ecol., 16th April 1979. Kula Lumpur, Malaysia.

Thompson,C.A., Jr. C.Vanderzant, and S.M. Ray, 1976. Zooplankton and phytoplankton from Galveston Bay: Taxonomic distribution and coexistence with Vibrio parahaemolyticus. J. Fd. Sci., 41 : 725-727.

Thompson, W.K. and C.L. Thacker, 1973. Effect of temperature on Vibrio parahaemolyticus in oysters at refrigerator and deep freezer temperatures. Can. Inst. Fd. Sci. Technol. J., 6 : 156-158.

Thompson, C.A. and C.Vanderzant, 1976. Relationship of Vibrio parahaemolyticus in oysters, water and sediment, and bacteriological and environmental indices. J. Fd. Sci. 41 : 117-122.

Tilton,R.C. and B. Roseberg, 1978. Reversal of silver inhibition of microorganism by agar . Appl. Environ. Microbiol. 35: 1116-1120.

Timoney, J.F. and A. Abston, 1984. Accumulation of Escherichia coli and Salmonella typhimurium by hard clam in an invitro system. Appl. Environ. Microbiol., 47 : 980-988 .

Timoney, J.F., Port , J.Giles and J. Spanier, 1978. Heavy metal and antibiotic resistance in the bacterial flora of sediments of New York Bight. Appl. Environ. Microbiol, 36: 465-472.

Twedt, R.M., P.L. Spoulding and H.E. Hall, 1969, Morphological cultural, biochemical and serological comparison of Japanese strains of Vibrio parahaemolyticus with related cultures isolated in the United States. J. Bacteriol., 98 : 511-518.

Vaccaro, R.F., M.P. Briggs, C.L. Carey and B.H. Ketchum, 1950. Viability of Echerichia coli in sea water AM. J. publ. Health., 40: 1257-1266

van der Broek,M.J.M. and D.A.A. Mossel, 1977. Sublethal cold shock in Vibrio parahaemolyticus. Appl. Enviorn. Microbiol., 34: 97-98.

Vanderzant, C, and R. Nickelson, 1972. Survival of Vibrio parahaemolyticus in shrimp tissue under various environmental conditions. Appl. Microbiol, 23: 34-37 .

Velescu, S., 1982. Indicators of fecal pollution in the marine sediments under anthropogenic effects. Paper presented at workshop on pollution of the mediterranean, 2-4 December 1982 Cannes.

Venkataraman,R. and A Sreenivasan, 1953. The bacteriology of freshwater fish. Indian J. Med. Res., 41: 385-392.

Venugopal, M.N., K.Segar, I.Karunagar and I. Karunasagar, 1985. Comparison of methods for enumeration of Vibrio parahaemolyticus from seafoods: In Absts. XXVI Ann Conf. Assoc. Microbiol. India. Abst No. VE MA 9.

Volterra, L., G. Piccininno, E.Palliola, F.A. Anticino and M. Gianfranceschi, 1984. Environmental faecal pollution and concentration power of the clam Chamelea gallina. Water Air Soil Pollut., 21 : 425-424.

Volterra, L., E. Tosti, A. Vero, and G. Izzo, 1985. Microbiological pollution of marine sediments in the southern stretch of the Gulf of Naples. Water Air Soil Pollut., 26: 175-184.

Walkar,J.D. and R.R. Colwell, 1974. Mercury- resistant bacteria and petroleum degradation. Appl. Microbiol., 27 : 285-287.

Ware, G.C., A.E. Anson , and Y.F. Arianayagam, 1972. Bacterial pollution of the Bristol channel. Mar. pollut. Bull. 3: 88-90

Watkins, W.D. and V.J. Cabelli, 1985. Effects of fecal pollution on Vibrio parahaemolyticus in an estuarine environment. Appl. Environ, Microbiol, 49: 1307-1313.

Weagant, S.D. and C.A. Kaysner, 1982. The incidence and seasonal distribution of Yersinia enterocolitica and Vibrio parahaemolyticus in a puget sound commercial oyster bed.. J. Shellfish Res., 2: 122-123.

Williams, H.N., W.A. Falkler, Jr., and D.E. Shay, 1980. Incidence of marine Bdellovibrios lytic against Vibrio parayaemolyticus in Chesapeake Bay. Appl. Environ. Microbiol., 40: 970-972.

Wood.L.V., L.E. Ferguson, P. Hogan, D. Thurmon, D R.Morgan, H.L. Dupont and G.D. Erricsson, 1983. Incidence of bacterial enteropathogens in foods from Mexico. Appl. Environ. Microbiol., 46: 328- 332.

Xu, H,S., N. Roberts. F.L. Singleton, R.W. Altwell, D.J. Grimes and R.R. Colwell, 1982. Survival and viability of non-culturable Escherichia coli and Vibrio Cholerae in he estuarine environment. Microbiol, Ecol., 8: 313-323.

Yamane,T. (Ed.) 1967. Statistics: An introductory analysis. Harper and Row, New York.

Yamamoto, H., Y. Ezura, and T.Kimura, 1982 b. Evaluation of biological agents affecting the survival of Vibrio parahaemolyticus in sea water. Bull. Jap. Soc. Sci. Fish. 48. 1433-1439.

Yde, M., M. Cleempoel and S.De, 1980.Faecal pollution of Belgian coastal water. Mar. Pollut Bull., 11: 108-110.

Yoshikura,T., K. Oda and S. Iida, 1980. The distribution and seasonal fluctuations of heterotrophic bacteria and coliform bacteria in the river and estuaries in Osaka city , Japan. Bull.Jap.Soc.Sci. Fish., 46: 231-236.

Yoshpe-Purer, Y., 1981.Bacterial quality of coastal water on certain recreational areas in Israel. In: Developments in Arid Zone, Ecology and Environmental Quality. (H.I. Shuwal, Ed.) Balaben Intal. quality.'H.I Shuwal, Ed.) Balaban Intal. Sci.Serv. Rehovot (Israel). pp:241-248.

Yoshpe-Purer, Y., 1982. Bacterial quality of coastal waters in central and southern of Israel. Paper presented at workshop on pollution of the Mediteranean, 2nd - 4th December 1982. Cannes.

Yoshpe-Purer, Y. and H.I. Shuval, 1972 Salmonella and bacterial indicator organisms in pollutied coastal water and their hygeinic significance . In: Marine Pollution and sea life. (M.Ruivo, Ed.), Fishing News (Books) Ltd., London.; pp 574-580.

Zapatka, F.A. and B. Bartolomeo, 1973. Microbiological evaluation of cold-water shrimp (Pandalus borealis). Appl. Microbiol., 25: 858-861.

Yamamoto, N. and G. Lopez, 1985. Bacterial abundance in relation to surface area and organic content of marine sediments. J. Exp. Mar. Biol. Ecol., 90: 209-220.

APPENDIX I

Table 1. Seasonal Variation of Physico-chemical parameters monitored in water.

Months	Station 1					Station2					Station 3				
	Tempe-rature (°C)	Salinity (‰)	pH	Oxygen (ml/l)	POC (mg/l)	Tempe-rature (°C)	Salinity (‰)	pH	Oxygen (ml/l)	POC (mg/l)	Tempe-rature (°C)	Salinity (‰)	pH	Oxygen (ml/l)	POC (mg/l)
March '82	31	28.0	6.80	1.60	2.51	31	32	7.20	1.70	2.27	32	26.8	7.50	1.8	2.06
April	31	23.4	6.95	2.20	3.51	31	27	7.50	2.00	3.58	31	26.0	6.90	2.1	2.67
May	32	32.1	7.50	1.80	3.69	31	32.6	7.40	2.30	5.13	30	28.8	7.60	1.9	4.82
June	29	2.5	7.10	3.80	3.50	29	11.1	6.94	3.90	4.51	29	5.6	7.20	3.4	4.30
July	28	1.6	6.30	3.20	1.70	28	2.4	6.65	3.00	1.81	29	3.0	6.55	3.4	1.85
August	27	1.5	6.35	4.40	2.89	26	3.5	7.70	4.55	2.53	27	2.7	7.50	4.3	1.77
September	29	17.6	7.15	4.00	1.43	29	21.2	7.85	3.30	1.63	29	18.4	6.65	3.9	1.41
October	28	10.5	7.05	3.20	1.84	29	10.3	7.70	3.10	2.17	30	11.8	6.85	3.0	1.92
November	30	16.5	7.50	4.10	2.46	30	15.8	6.30	4.20	2.00	30	12.1	7.50	4.0	1.83
December	29	30.4	7.70	3.62	1.53	29	33.3	8.10	3.82	2.39	29	29.4	7.90	3.1	1.98
January '83	28	30.9	7.75	2.98	0.72	29	33.2	7.85	2.96	1.18	28	30.0	7.50	2.2	1.05
February 83	29.	28.5	8.15	1.80	1.09	29	32.5	8.15	1.85	1.13	29	29.6	7.90	1.1	0.96

Table 2. Seasonal Variation of Physico-Chemical Parameters Monitored in Sediment.

Months	Station 1					Station 2					Station 3.				
	Temperature (^oC)	pH	Total Organic Carbon (mg/g)	Total Nitrogen (mg/g)	Total Phosphorus (mg/g)	Temperature (^oC)	pH	Total Organic Carbon (mg/g)	Total Nitrogen (mg/g)	Total Phosphorus (mg/g)	Temperature (^oC)	pH	Total Organic Carbon (mg/g)	Total Nitrogen (mg/g)	Total Phosphorus (mg/g)
March 1982	31	6.91	18.7	0.91	0.71	31	7.07	14.2	1.08	1.01	32	7.09	12.8	1.42	1.52
April	31	7.01	19.4	1.09	0.83	31	7.27	14.9	1.13	1.21	32	7.07	14.1	1.37	1.63
May	31	7.30	22.1	2.31	0.79	32	7.27	17.3	1.22	1.70	31	7.42	15.3	1.52	1.95
June	29	7.02	23.2	2.87	0.62	28	6.89	24.7	2.31	2.21	29	6.98	18.7	2.41	1.48
July	26	6.91	23.0	2.53	0.77	26	6.97	25.9	2.57	2.17	26	6.88	21.2	2.23	1.63
August	27	6.79	25.7	3.76	0.91	27	7.27	27.2	2.35	2.05	27	7.22	19.3	2.17	1.47
September	28	7.03	24.1	3.52	1.37	27	7.33	28.1	2.87	2.30	28	7.10	20.1	3.18	1.82
October	29	6.98	20.3	3.18	1.42	29	7.26	20.9	3.41	2.22	29	7.06	22.3	4.21	1.98
November	29	7.08	10.7	2.47	1.70	29	6.70	19.2	3.17	1.81	30	7.13	21.8	4.05	2.07
December	30	7.17	15.8	1.82	2.10	29	7.42	14.3	2.80	1.78	30	7.48	21.1	3.52	1.96
January 1983	28	7.70	17.3	2.23	1.83	27	7.66	15.1	2.56	1.43	28	7.24	23.7	2.71	1.43
February	29	8.02	12.6	1.03	1.42	29	7.73	17.2	2.74	1.23	29	7.42	17.4	2.40	1.63

Table 3. Seasonal Distribution of TC, FC, FS and EC in water (100 ml^{-1}) from March 1982 to February 1983.

Months / Stations	TC (X 10^4)			FC (X 10^3)			FS (X 10^3)			EC (X 10^{-1})		
	1	2	3	1	2	3	1	2	3	1	2	3
March 1982	4.27	15.85	23.99	2.09	45.71	109.65	0.13	1.51	2.82	18.20	10.00	14.13
April	0.71	1.00	1.38	0.89	0.89	0.40	15.14	23.99	15.14	7.08	12.02	7.08
May	12.02	9.33	45.71	4.27	0.16	9.33	0.30	0.30	45.71	7.08	7.08	3.02
June	91.20	18.62	218.78	23.99	109.60	109.65	0.89	9.33	23.99	3.03	3.98	5.01
July	7.08	10.97	7.08	45.71	23.99	2.88	20.90	15.14	2.29	38.91	245.47	22.36
August	38.91	42.66	93.33	100.00	109.70	4.27	9.33	0.89	23.99	1122.00	691.33	45.71
September	8.91	93.33	3.98	39.81	0.16	0.50	3.89	45.71	0.89	7.08	3.02	3.98
October	3.98	22.91	75.86	9.33	4.27	138.04	0.13	0.40	0.15	18.20	26.30	7.08
November	239.88	93.33	93.33	9.33	45.71	109.65	0.16	0.89	0.40	95.50	245.47	467.74
December	8.91	22.91	42.66	0.40	2.82	45.71	0.60	0.40	1.29	7.08	30.90	46.77
January 1983	22.91	22.91	22.91	0.13	0.89	0.29	0.89	109.65	109.65	3.98	3.02	5.01
February	8.51	18.62	47.86	4.27	109.65	23.99	2.29	45.71	109.65	7.08	7.08	6.03

Table 4. Seasonal distribution of TC, FC, FS, and EC in sediment (g^{-1}) from March 1982 to February 1983.

Months / Stations	TC (X 10^5)			FC (X 10^2)			FS (X 10^2)			EC (X 10^{-1})		
	1	2	3	1	2	3	1	2	3	1	2	3
March 1982	0.39	0.55	2.40	6.46	2.57	575.44	1.26	1.12	1.59	44.67	6.03	14.13
April	2.75	0.03	0.44	3.98	3.47	35.48	37.15	79.43	955.00	3.02	7.08	3.02
May	0.39	0.25	11.48	2.00	1.26	15.85	1.26	30.20	91.2	3.02	3.02	3.02
June	0.72	2.14	0.19	7.94	2.51	3.16	30.90	4.57	13.8	3.02	3.02	3.98
July	8.91	0.11	1.23	1023.29	16.59	13.18	537.03	4.47	229.09	467.74	12.02	10.00
August	50.12	1.20	1.32	144.54	123.03	794.33	10.00	5.37	50.12	26.30	95.50	251.19
September	12.30	3.02	9.55	2.51	1.26	1.78	263.03	194.99	229.09	3.02	3.02	3.02
October	0.29	0.32	0.29	1.59	18.62	6.61	1.59	1.41	3.8	3.02	3.02	7.08
November	0.68	0.14	4.17	41.69	112.20	83.18	2.00	5.25	1.78	18.20	245.47	95.50
December	1.41	1.45	10.00	6.03	4.37	1819.70	6.03	2.51	11.75	6.03	10.00	154.88
January 1983	2.40	1.18	2.14	2.00	3.16	2.00	23.99	46.77	549.54	3.02	3.02	3.98
February	0.11	0.07	0.06	3.98	3.39	28.18	120.23	74.13	45.71	3.02	3.02	10.00

Table 5. Seasonal Distribution of TC, FC, FS and EC in zooplankton (g^{-1}) from March 1982 to February 1983.

Months / Stations	TC ($\times 10^5$)			FC ($\times 10^3$)			FS ($\times 10^3$)			EC ($\times 10^{-1}$)		
	1	2	3	1	2	3	1	2	3	1	2	3
March 1982	0.22	15.14	26.30	3.09	12.88	69.18	0.81	2.09	0.96	6.92	10.00	7.08
April	0.36	0.76	0.08	0.32	3.31	1.20	147.90	158.49	4.79	3.98	26.30	7.08
May	0.76	1.48	2.63	66.07	2.69	6.61	21.38	61.66	3.55	3.98	3.98	14.13
June	12.88	74.13	28.84	4.17	154.88	0.20	2.51	50.12	75.86	6.03	5.01	6.03
July	13.49	30.90	5.37	14.13	891.25	200.00	100.00	371.54	30.90	23.99	1096.50	154.88
August	100.00	741.31	239.88	57.54	741.30	1096.50	12.02	309.03	457.09	45.71	1112.00	1148.20
September	295.12	295.12	154.88	0.16	6456.5	1445.40	39.81	112.20	363.08	3.98	32.36	18.20
October	245.47	6.03	21.38	370.30	0.79	776.25	0.89	2.34	0.40	6.05	3.98	26.30
November	5.50	141.25	123.03	70.80	275.40	616.60	2.63	1.23	1.07	6.05	12.02	12.02
December	19.50	162.18	95.50	190.55	134.90	6025.60	1.91	2.24	5.37	3.02	467.74	7.08
January 1983	5.01	8.32	2.57	0.25	0.63	0.63	128.82	87.10	4.79	3.98	3.02	3.02
February	245.47	28.18	0.60	47.86	58.88	31.62	295.12	141.25	12.02	3.98	7.08	3.02

Table 6. Seasonal Distribution of TC, FC, FS and EC in fish (100 g^{-1}) from March 1982 to February '83.

Months / Stations	TC ($X10^6$)			FC ($X10^5$)			ES ($X10^3$)			EC ($X10^2$)		
	1	2	3	1	2	3	1	2	3	1	2	3
March 1982	0.43	2.4	4.57	0.23	0.23	0.43	3.98	3.98	8.91	125.89	125.89	60.26
April	10.96	10.96	0.19	0.93	10.97	0.53	7.08	93.33	42.66	31.62	28.18	39.81
May	4.57	4.57	4.57	0.23	0.04	0.09	75.86	239.88	1096.48	0.30	0.19	0.63
June	8.71	181.97	0.76	18.2	18.20	1.20	6.61	14.79	29.51	2951.20	11220.00	112.20
July	3.09	5.89	6.17	19.95	48.98	30.90	8.91	25.12	9.55	562.34	467.74	70.79
August	2.46	7.59	5.13	15.85	50.12	2.75	12.59	4.57	11.75	120.23	181.97	954.99
September	27.54	48.98	5.01	107.15	251.19	22.91	4.57	9.55	25.12	158.49	263.03	2138.00
October	120.23	50.12	1.32	83.18	102.30	10.23	4.37	4.37	25.12	39.81	30.20	70.80
November	1.86	5.89	120.23	7.94	25.12	31.62	0.13	3.55	0.50	70.80	15.14	323.60
December	5.01	102.33	26.30	16.60	426.58	102.33	0.16	6.61	4.37	263.03	776.25	467.74
January 1983	9.12	12.02	7.76	45.71	26.30	48.98	0.18	6.61	16.98	302.00	70.80	60.26
February	13.80	20.42	0.56	30.20	128.83	1.18	5.01	8.13	11.75	398.00	30.2	50.12

Table 7. Seasonal Distribution of TC, FC, FS and EC in prawn (100 g^{-1}) from March 1982 to February 1983.

Months / Stations	TC (X 10^6)			FC (X 10^5)			FS (X 10^3)			EC (X 10^3)		
	1	2	3	1	2	3	1	2	3	1	2	3
March 1982	0.93	0.76	0.43	0.23	0.09	0.09	8.91	22.91	42.66	12.02	6.03	3.02
April	10.96	0.19	0.16	0.09	0.09	0.21	14.13	8.91	8.91	3.02	3.98	5.01
May	10.96	4.57	10.97	0.23	0.23	0.23	457.01	42.66	239.88	3.98	1.51	2.00
June	3.89	4.79	1.48	5.01	12.02	3.24	9.77	102.33	57.54	316.23	794.33	112.20
July	13.18	10.47	15.49	31.62	37.15	91.2	47.86	12.88	20.42	1122.00	18.20	18.20
August	50.12	95.50	120.23	165.96	131.83	501.19	3.31	3.31	3.31	18.20	31.62	38.91
September	17.78	10.23	10.23	4.37	10.00	25.12	9.77	25.12	4.37	3.98	3.16	12.02
October	120.23	120.23	120.23	25.12	83.18	165.96	9.77	9.77	9.77	6.31	3.98	7.08
November	77.63	31.62	120.23	501.19	120.23	39.81	0.14	7.76	7.76	26.30	14.13	30.90
December	162.18	22.91	31.62	1202.3	31.62	38.91	7.76	9.77	7.76	1096.50	21.88	22.91
January 1983	8.32	47.86	52.48	10.00	44.67	33.11	0.16	23.99	48.98	8.91	3.98	5.02
February	19.50	4.79	14.79	14.45	25.12	79.43	9.12	12.02	26.92	3.02	3.55	63.10

Table 8. Seasonal Distribution of TVB, VLO, VPLO and VP in water column (100 ml^{-1}) from March 1982 to February '83

Months / Stations	TVB ($X10^7$)			VLO ($X10^4$)			VPLO ($X10^3$)			VP ($X10^2$)		
	1	2	3	1	2	3	1	2	3	1	2	3
March 1982	6.61	3.09	4.79	1.26	19.95	15.85	1.26	6.31	0.89	10.00	14.13	6.03
April	2.34	6.92	1.35	19.95	38.02	10.97	1.78	1.58	70.80	10.97	3.98	0.00
May	19.95	28.18	147.91	3.16	63.10	79.40	2.09	1.26	199.53	15.14	7.08	6.03
June	0.36	0.71	0.43	1.51	3.02	3.31	9.12	21.88	21.88	1.41	10.72	1.20
July	2.14	0.78	0.79	1.41	1.74	12.88	4.27	9.33	23.99	0.00	42.66	3.02
August	2.04	1.55	3.98	0.40	1.66	1.78	2.00	2.82	9.33	3.02	15.14	0.00
September	1.20	6.31	0.93	2.63	11.22	0.71	1.51	1.10	0.30	8.91	7.08	0.00
October	3.55	10.00	3.51	0.50	0.79	2.00	0.71	1.10	1.10	3.98	0.00	3.98
November	2.24	2.19	1.58	30.20	0.40	1.00	109.65	1.51	6.46	4.47	8.91	14.13
December	1.55	1.55	2.88	2.63	2.88	2.19	0.71	2.40	1.51	3.98	20.89	0.00
January '83	12.88	1.41	3.98	44.67	6.46	16.98	0.76	0.76	1.10	3.02	3.02	6.03
February	16.60	1.86	8.91	4.37	5.01	7.24	11.48	1.10	4.79	36.31	7.08	23.99

Table 9. Seasonal distribution of TVB, VLO, VPLO and VP in sediment (g^{-1}) from March 1982 to February '83.

Months / Stations	TVB (X 10^7)			VLO (X 10^4)			VPLO (X 10^3)			VP (X 10^2)		
	1	2	3	1	2	3	1	2	3	1	2	3
March 1982	3.24	1.91	0.26	0.32	7.94	0.10	2.00	3.16	0.37	11.22	3.31	0.16
April	6.31	0.87	60.26	31.62	104.71	63.10	2.63	5.01	131.83	10.97	13.18	23.99
May	323.60	61.66	794.33	12.60	13.80	27.54	3.55	7.94	120.23	12.02	30.20	134.90
June	22.91	3.80	5.37	7.41	2.40	1.78	29.51	1.74	14.13	4.27	1.62	0.65
July	7.76	1.15	6.46	0.89	2.14	5.25	0.89	1.00	6.46	0.00	0.00	12.30
August	25.12	0.87	3.31	6.46	0.27	2.34	30.90	1.86	7.08	13.18	5.25	30.20
September	3.09	1.15	7.24	2.57	0.26	3.31	1.70	2.09	0.74	8.51	5.25	0.00
October	12.02	5.01	0.58	0.79	0.63	0.32	3.72	0.89	1.05	14.45	2.46	5.75
November	2.82	0.21	0.41	2.46	42.66	7.41	14.45	138.04	19.06	3.89	91.20	13.49
December	2.69	1.05	3.16	1.41	0.98	33.10	5.50	0.44	39.81	28.18	2.51	154.88
January 1983	1.05	42.66	0.05	2.69	3.72	0.32	2.09	0.76	0.55	9.33	3.55	3.47
February	0.27	0.06	0.42	3.47	7.08	0.48	0.78	0.08	0.89	4.90	0.13	5.62

Table 10. Seasonal Distribution of TVB, VLO, VPLO and VP in Zooplankton (g^{-1}) from March 1982 to February 1983.

Months / Stations	TVB ($X10^7$)			VLO ($X10^5$)			VPLO ($X10^3$)			VP ($X10^2$)		
	1	2	3	1	2	3	1	2	3	1	2	3
March 1982	69.18	54.95	28.84	1.29	1.58	6.31	1.41	5.01	0.18	6.17	5.59	0.40
April	158.49	263.03	47.86	3.98	6.17	6.03	1.59	2.00	398.11	8.12	8.51	7.94
May	102.33	48.98	162.18	23.44	6.31	8.51	12.59	22.39	165.96	38.91	14.79	16.60
June	18.20	32.36	6.31	2.40	7.94	13.49	0.30	19.50	69.18	1.12	2.00	5.62
July	0.23	15.85	6.61	6.02	10.97	1.74	309.03	234.42	100.00	0.00	56.23	0.00
August	302.00	28.18	70.80	13.80	1.82	50.12	616.60	141.25	1096.48	199.50	234.42	151.36
September	24.55	9.55	5.89	12.88	6.76	5.37	1.86	2.40	2.34	10.72	7.94	10.60
October	47.86	25.70	58.88	1.62	0.34	8.32	2.46	0.04	1.00	6.76	0.00	0.00
November	2.51	51.29	16.60	1.48	1.15	1.12	21.88	19.95	5.35	7.08	18.62	23.99
December	19.05	17.38	3.98	0.62	0.42	0.28	5.01	3.98	5.75	25.12	4.37	39.81
January 1983	38.02	102.33	13.80	6.03	45.71	0.24	2.19	6.92	1.66	13.80	28.88	10.00
February	316.23	14.45	20.42	0.11	0.22	0.11	1.09	0.39	7.59	7.94	1.02	4.68

Table 11. Seasonal distribution of TVB, VLO, VPLO and VP in fish (100 g^{-1}) from March 1982 to February '83.

Months / Stations	TVB (X 10^7)			VLO (X 10^5)			VPLO (X 10^4)			VP (X 10^4)		
	1	2	3	1	2	3	1	2	3	1	2	3
March 1982	77.62	22.91	5.25	0.16	79.43	25.12	1.26	3.16	7.24	0.40	2.00	4.37
April	338.80	169.82	75.86	2.51	3.16	831.76	2.00	1.26	1202.30	0.71	0.00	0.71
March	74.13	251.19	154.88	60.26	1174.90	102.33	0.50	1.58	190.55	0.00	0.40	1.10
June	181.97	91.20	12.59	4.37	12.02	5.75	15.14	9.77	39.81	0.00	0.66	0.51
July	10.47	12.59	1.51	323.60	229.10	2.51	9.55	501.20	7.08	1.78	50.12	0.00
August	3.72	2.40	1.91	8.51	9.12	4.79	6.31	0.46	35.48	1.23	0.00	22.39
September	7.24	10.23	2.24	154.88	109.65	3.16	1.26	0.63	8.32	0.00	0.00	0.44
October	30.20	22.39	5.75	104.71	112.20	44.67	2.95	7.41	2.63	1.00	3.72	0.78
November	5.37	9.77	102.33	57.54	95.50	104.71	389.00	758.60	389.00	125.90	165.96	125.89
December	5.25	23.99	9.55	1.29	5.75	4.17	1.78	2.40	4.37	0.00	0.76	0.89
January 1983	2.95	6.76	3.72	1.02	16.22	3.02	8.13	8.51	1.26	4.90	5.89	1.02
February	6.03	7.76	0.30	7.94	21.38	1.17	36.31	23.44	6.46	25.12	1.05	1.95

Table 12. Seasonal Distribution of TVB, VLO, VPLO and VP in prawn (100 g^{-1}) from March 1982 to February 1983.

Months / Stations	TVB ($\times 10^8$)			VLO ($\times 10^6$)			VPLO ($\times 10^5$)			VP ($\times 10^4$)		
	1	2	3	1	2	3	1	2	3	1	2	3
March 1982	7.76	7.94	3.02	0.32	6.31	3.16	0.20	0.79	12.02	1.10	4.37	10.96
April	0.81	12.59	16.22	0.10	0.45	0.36	0.50	0.16	1.29	1.51	0.00	0.60
May	12.59	39.81	25.70	354.80	29.51	23.44	0.16	0.79	52.48	0.30	1.10	0.00
June	15.85	14.45	29.51	53.70	19.50	6.03	26.30	72.44	9.55	0.78	2.19	1.62
July	8.51	6.46	2.88	15.14	5.62	5.62	12.02	9.55	10.72	5.89	3.80	3.39
August	0.91	1.32	3.02	8.13	21.88	4.47	2.29	100.00	5.01	3.09	120.23	3.89
September	0.40	0.58	0.40	0.21	0.39	0.47	0.10	0.07	0.48	0.44	0.33	0.00
October	2.24	1.41	2.19	5.13	5.01	4.47	2.29	0.47	5.01	0.78	1.20	2.19
November	3.24	2.24	38.90	5.50	6.31	93.32	16.60	46.77	501.20	66.10	288.40	66.07
December	4.90	2.29	6.03	3.09	5.01	3.24	0.49	40.44	0.24	0.00	0.60	0.71
January 1983	2.24	1.05	2.19	2.04	3.24	2.69	0.63	2.19	12.59	3.89	3.63	33.11
February	1.29	2.24	0.26	1.29	9.55	0.48	5.89	75.86	2.69	2.51	120.20	5.89

APPENDIX II

APPENDIX II

LIST OF PUBLICATIONS

1. R. Pradeep and P. Lakshmanaperumals amy. 1984, Seasonal variations of Vibrio parahaemolyticus in Cochin backwater. Indian.J.Mar.Sci., 13: 113-115.

2. R. Pradeep and P. Lakshmanaperumals amy. 1985. Antibiotic sensitivity of Vibrio parahaemolyticus. Fish Technol., 22: 135-139.

3. R. Pradeep and P. Lakshmanaperumals amy. 1985. Health indicator bacteria associated with zooplankton of Cochin backwater. Presented in Second National Seminar on Marine Intertidal Ecology, Andhra University, Waltair. Feb. 14-16, 1985.

4. P. Lakshmanaperumals amy and R. Pradeep. 1985. Metal tolerance of Vibrio parahaemolyticus isolated from Cochin backwater. Abst. 26th Annual Conf. Assoc. Microbiologists India, Madras, Octo., 85.

5. R. Pradeep and P. Lakshmanaperumals amy. 1986. Quantitative study of Vibrio parahaemolyticus in fish and prawn of Cochin backwater. Fish Technol., 23: 66-69.

6. R. Pradeep and P. Lakshmanaperumals amy. 1986. Distribution of faecal indicator bacteria in Cochin backwater. Indian J. Mar. Sci., 15: 99-101.

7. R. Pradeep and P. Lakshmanaperumals amy. 1986. Drug resistant coliforms in Cochin backwater. Indian J. Mar. Sci. (in press)