MICROBIAL FLORA OF SELECTED SPECIES OF CRUSTACEANS

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C E R T I F I C A T E

This is to certify that this thesis is a bonafide record of research work done by Shri. V. Krishnamurthy, M.Sc., during the period of his study under my supervision, at the Inspection Laboratory, Marine Products Export Development Authority, Cochin and that no part thereof has been presented for any other degree in any University.

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D E C L A R A T I O N

I hereby declare that this thesis entitled "Microbial flora of selected species of Crustaceans" has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar title or recognition.

Cochin,
16, July, 1985.

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C O N T E N T S

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CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Food and Agricultural Organization (1981) has recorded India as one of the top ten fish-producing countries of the world and ranks first in Shrimp production. Among many marine products exported from India, Shrimp plays a significant role and earn nearly 310.37 crores (1983) of rupees of foreign exchange to our country. Statistical records of the Marine products Export Development Authority for the year 1983 also reveal that nearly 62.27% by quantity and 85.40% by value of the total marine exports comes from the single item i.e., Prawns; indicating its trade importance in the marine industry. Nearly one-third the quantity of Prawns exported from India comes from the backwaters of different maritime states; of which the Cochin's share is the largest. The two major species caught from these backwaters are Penaeus indicus and Metapenaeus dobsoni.

The commercial importance of prawns has prompted many researchers to carry out studies on various aspects of Prawns in India (Sreenivasan 1959; Velankar et al. 1961; Lekshmy and Pillai 1964; Karthiayani and Iyer 1975). However, the reported data on quantitative and qualitative aspects of the microflora of Prawns show lot of variations, both in number of bacteria present per gram or per square centimetre of fresh prawn and on the nature of the microflora present on them. Studies on the microflora of backwater Prawns in particular, are lacking and therefore a detailed study on both quantitative and qualitative aspects, including seasonal variations in the microflora associated with these two species of prawn has been carried out along with two other species of prawns, whose availability was not continuous during the fishing season.

While studying the quantitative and qualitative aspects of the microflora of any marine organism, it becomes necessary to use a suitable medium and incubation temperature so that maximum number of micro-organisms are recorded and also to ascertain the optimum growth temperature for these micro-organisms. Any mistake in this step may obviously result in low recoveries and faulty correlations.

Most of the experiments on the quantitative aspects of fresh prawns from Indian waters seem to have been carried out either at ambient (25° to 33°C) temperatures or at 37°C. The recent work on fish, however, shows that most of the marine bacteria are facultative psychrophils and do not tolerate temperatures above 32°C. Also in the context of extending the shelf life of Prawn products the need for manipulating the storage temperature need no emphasis. The spoilage of prawns at those temperatures are presumed to be caused by the bacteria growing at corresponding temperatures. In view of this, different incubation temperatures namely 8 ± 2°C, 25 ± 2°C, 37°C and 57°C have been tried for bacterial isolates obtained from fresh prawns, water samples and bottom sediments.

Pollution of the aquatic environments, particularly near the industrialized towns, is assuming greater importance from the public health point of view, in recent times. The Cochin backwaters which constitute a significant share in the total prawn catch of India, assumes a special significance in this context, since it receives considerable organic pollution due to inflow of sewage and oil pollution due to the presence of oil berths in the area.

In view of the above mentioned reasons, it was thought worthwhile to examine the Prawns, water and sediment samples collected from Cochin backwaters for the presence of bacteria of public health significance like Salmonella, Escherichia coli, Vibrio and Staphylococcus aureus. It may also be mentioned here that very little work seems to have been done on the occurrence of these organisms in prawns collected from backwaters of not only Cochin area but from other areas also.

Antibiotics have been used earlier to extend the shelf life of ice stored fish as well as for control of diseases in aquatic animals. Although the use of antibiotics for the preservation of fish has been banned by most of the countries, they are being used for control of diseases in food fishes as well as aquarium fishes (Oppenheimer 1955; Almeida et al. 1967). Culturing of fish and shell-fishes on a commercial scale is gaining lot of importance in recent times; so also the studies on microorganisms pathogenic to these marine organisms. In this context, it was thought worthwhile to study the effect of different antibiotics on marine bacteria isolated from prawns.

Not infrequently, bacteria have been considered as enemies to marine animals from the point of view of causing disease and spoilage after death. However, there are a few investigations which show that the bacteria can serve as food for different fishes (Margolis 1953; Shrivastava and Floodgate 1966; Trust and Sparrow 1974; and Mary et al. 1975). In fact the report of Trust and Sparrow (1974) indicated that feeding on microflora was essential for free living fishes, especially those which feed on debris that cannot be immediately digested. Surprisingly there seems to be no work done in this aspect with respect to prawns. Therefore, the possibility of bacteria acting as food for juvenile prawns has been examined in this study.

To summarise, the study deals with the bacterial flora of the Cochin backwaters, sediment and the prawns caught from these backwaters in quantitative and qualitative terms, their growth in relation to temperature, their susceptibility to various antibiotics, their role in terms of food for juvenile prawns and the presence of bacteria of public health significance. It is hoped that such a comprehensive study would be of some relevance to shell-fish processing industries apart from its academic value.

CHAPTER 2

MATERIAL AND METHODS

2. MATERIAL AND METHODS

In the peninsular India, Cochin Backwaters are located and connected with the Arabian Sea at Lat. 09° 58'N, Long. 76° 15'E. The sampling stations in Cochin Backwaters are shown in Fig.1. The backwater is a nursing ground for a number of commercially important prawns and contributes to rich prawn fishery in India (Kurian and Sebastian, 1976; Kumardas, 1980). Therefore, different species of prawns (Penaeus indicus, Metapenaeus dobsoni, M. monoceros, Macrobrachium idella) of commercial significance, the sediments and water samples from Cochin backwaters (collected from August 1979 to June 1980) were subjected to the present study. The prawns were indentified up to their species level using the identification chart published by Kurian and Sebastian (1976).

The prawns used in the present study were collected from the catches coming from cast nets and Chinese dip nets operated at the northern region of Cochin Backwaters. The samples (species wise) were transferred into sterilized steel containers using sterilized forceps.

Sediment samples were collected using Petersons grab. The centre portions of the samples were aseptically transferred to sterilized steel containers.

Water samples were collected using Casella water sampler. The water from the sampler bottle was transferred into a sterilized 250 ml capacity ground glass stoppered bottle. The temperature of surface water was recorded using a centigrade thermometer.

The box containing the above collected samples were transported to the laboratory and analysis was done immediately.

Sterilisation Procedure:

1. **Glasswares and steel equipments:**

Glasswares and equipments such as petri dishes, flasks, test tubes, pipettes, steel containers, scissors and forceps were cleaned first with teepol water and then thoroughly washed in tap water. The items were air dried and subjected to dry sterilisation in a hot air oven at 170°C for 2 hours as described in American Public Health Association (APHA, 1970). These sterile equipments and glasswares were used for the analysis.

2. Mortar, Pestle and Dissection Instruments:

These items were washed as described in the case of glasswares and sterilized by cleaning with alcohol and heating them over the flames of bunsen burners.

3. Sterilization of media:

All media except carbohydrate broths or those with other specifications were sterilized in an autoclave at 121°C for 15 minutes. Carbohydrate liquid medium was sterilized in an autoclave at 121°C for 10 minutes as described in APHA (1970) and Standard Methods (Anon, 1976).

Preparation of samples:

Prawn Samples:

Penaeus indicus and Metapenaeus dobsoni were dissected in laboratory under aseptic conditions using sterilized forceps to separate head (cephalothorax), gut (stomach) and the tail (abdomen) regions. In the case of Metapenaeus monoceros and Macrobrachium idella, whole specimens were utilized for the study.

For each analysis 8-12 prawns of P. indicus and M. dobsoni and 4-5 prawns in the case of M. monoceros and M. idella were used for the preparation of each sample. Such difference in the sampling size for each analysis was due to the non-availability of some of the prawn species in sufficient quantity. Ten grams of each sample was homogenised in 0.1% sterilised peptone water (W/V) as per Collins and Patricia (1976) and serial decimal dilutions were prepared as described in the Manual of Microbiological Methods (Pelczar, 1957) and APHA (1970).

Sediment:

Ten grams of each sediment sample was weighed in a sterile petri dish and suspended aseptically in 90 ml of 0.1% sterilised peptone water. The suspension was mixed thoroughly and allowed to settle for a while and the supernatant was then decimally diluted.

Water samples:

Ten ml of each water sample was diluted in 90 ml of 0.1% peptone water (APHA, 1970). One ml of the appropriate dilutions were used for the bacteriological investigation of the samples. Water samples were also analysed in the laboratory for salinity, using methods described by Strickland and Parsons (1972) and pH was determined using ELICO 322 pH meter.

Quantitative analysis of bacteria:

One ml of the inoculum from each serial decimal dilution was pipetted out into sterile petri dishes (marked with indentity) in duplicate, following the procedure of APHA (1970). Approximately 10 ml of Zo Bell's marine agar (Zo Bell 2216 e) cooled to around 40°C was poured into each petri dish following the procedures of Carroll et al.(1968), APHA (1970), AOAC (1970) and ISI (1977a, b). Immediately after pour plating, the petri dishes were rotated clock wise and anticlock wise for uniform mixing of the inoculum into medium, and allowed to solidify.

The American Public Health Association (1970) recommended incubating plates at 35°C, for 48 hours, for estimation of standard plate count of sea water and shellfish. However, in the present study all the plates were incubated in an inverted position (to prevent the condensation of moisture on the surface of agar medium) at 37°C for 48 hours as per ISI (1971, 1977a and b).

Following the incubation, only the plates containing 30-300 colonies were selected, as per the procedure described in the Manual of Microbiological Methods (Pelczar, 1957; APHA 1970; AOAC, 1970) and the colonies were counted with the aid of quebec colony counter. The dilution and the number of colonies found

per plate were recorded and expressed as number per gm/ml.

To compare the bacterial load in P. indicus and M. dobsoni over different months and body regions, data were subjected to statistical analyses using the variance technique. For the purpose of analyses, the bacterial counts were converted into their logarithmic values. To identify the months which gave significantly higher counts, the least significant difference (LSD) at 5% level was worked out. The mean bacterial load in log units were formed for different months.

Correlation matrix was constructed, to study the interdependency of the bacterial counts in sediments, water, P. indicus and M. dobsoni and their relationship with water temperature, salinity and pH. The formula used for the correlation coefficient is as follows:

$$r = \frac{\Sigma(x - \bar{x})(y - \bar{y})}{n \sigma_x \sigma_y}$$

where $\bar{x}$ = mean of x values = $\frac{\Sigma x}{n}$

$\bar{y}$ = mean of y values = $\frac{\Sigma y}{n}$

σ_x = S.D of x values = $\frac{\sqrt{(x - \bar{x})^2}}{n}$

σ_y = S.D. of y values = $\frac{\sqrt{(y - \bar{y})^2}}{n}$

n = No. of pairs of values.

The significance of the correlation coefficient was tested by using Student's 't' statistic,

$$t = r \frac{\sqrt{n-2}}{\sqrt{1-r^2}}$$, having degrees of freedom n - 2.

Qualitative analysis of bacterial flora in prawns, water and sediment.

The bacterial strains isolated from prawns (P. indicus and M. dobsoni), water and sediment, at 37°C, were subjected to study the qualitative aspects of bacterial flora. The pure cultures obtained by the streak plate technique were subjected to morphological and biochemical tests as described by Collins and Patricia (1976) and were identified up to their generic level following the scheme described by Sehewan et al. 1960 a & b) and Gilmour et al. (1976). The identity of cultures, based upon their morphological and biochemical characteristics, was also confirmed with the reference culture obtained from the Kings Institute, Madras. The percentage generic composition of bacteria on P. indicus, M. dobsoni, water and sediment samples were also subjected to analysis of variance technique and the results were presented in the appropriate context.

Enumeration of bacteria at different incubation temperature:

Fourteen prawns samples and twelve each of water ar

sediment samples collected from Cochin backwaters during August and September, 1979, were utilised for the estimation of total bacterial load at different incubation temperatures (8 ± 1°C, 25 ± 2°C, 37°C and 57°C). The samples were prepared as per the methods described earlier in this Chapter for quantitative analysis of bacteria. Since there was no significa-nt bacterial growth at 8 ± 1°C after 48 hours, the incubation period was further extended up to 20 days, following the procedure adopted by Karthiayani and Iyer (1967) and Thampuran and Iyer (1979), Thampuran et al(1981). The counts in this study were represented as total bacterial load per gram of prawns and sediment and per ml of water, with respect to different incubation temperatures. The results obtained were subjected to statistical analysis. To compare the total bacterial count at different incubation temperatures, the experimental data were analysed separately for bacterial count in prawns, water and sediment using analysis of variance technique. For the purpose of analysis, the bacterial counts were converted to log values after adding 1 to all observations wherever necessary.

Analysis of bacteria of public health significance:

The prawn samples as well as water and sediment

samples were also screened for bacteria of public health importance such as Salmonella, Escherichia coli, Vibrio and Staphylococcus aureus.

For isolation of bacteria of public health significance selective media were used. For Salmonella, detection methods described in AOAC (1975) was followed, for E. coli, Vibrios and Staphylococcus aureus, the methods described in the laboratory manual prepared by the Export Inspection Agency (unpublished) using Tergitol, agar, TCBS agar and Staphylococcus 110 agar were used respectively.

Effect of antibiotics on the bacteria isolated from prawns:

A total of 57 bacterial isolates obtained from prawns were tested for their growth against different antibiotic discs (Penicillin, Novobiocin, Oxytetracycline, Chlortetracycline, Chloramphenicol and Streptomycin) on nutrient agar (Difco) at 37°C for 48 hours. The diameter of the growth inhibiting zones were measured in millimetre as adopted by Almeida et al. (1967). Depending on the area of growth inhibiting zones caused by the antibiotics, the isolates were grouped (upto 5 mm, upto 10 mm, upto 15 mm, upto 20 mm, upto 25 mm, upto 30 mm, upto 35 mm).

The isolates whose growth was unaffected by the antibiotic discs showing the absence of zone of inhibition surrounding the antibiotics disc was considered as 'resistant'. The antibiotic discs were obtained from M/s. Tajal Corporation, Bombay.

Bacteria as food for prawn juveniles:

Selected bacteria belonging to Pseudomonas spp., Acinetobacter spp., and Aeromonas spp., based on their morphology and biochemical features, were isolated from Penaeus indicus collected from backwaters of Cochin and were cultivated and purified in the laboratory. Five specimens of juvenile Metapenaeus dobsoni, each measuring 15 to 20 mm (with known weight), were kept in separate sterile beakers of three litre capacity each containing 1 litre of sterilised brackishwater of salinity 19 parts per thousand (PPT). The control had the same composition but without bacteria.

The juvenile prawns before being used in the experiment were kept in the sterile brackish water for six hours, in order to minimise the interference of bacteria associated with the juveniles and the faecal matter of the juveniles to the extent possible and the juveniles were then transferred to experimental beakers at the end of six hours after giving 1-2 wash with sterile brackishwater.

The bacteria were purified and cultivated in the nutrient broth. The broth containing each group of bacteria was introduced aseptically into the experimental beakers so as to give the concentration above 1.5×10^5 of the bacteria per ml. of water. Bacterial concentration was measured using plate count technique.

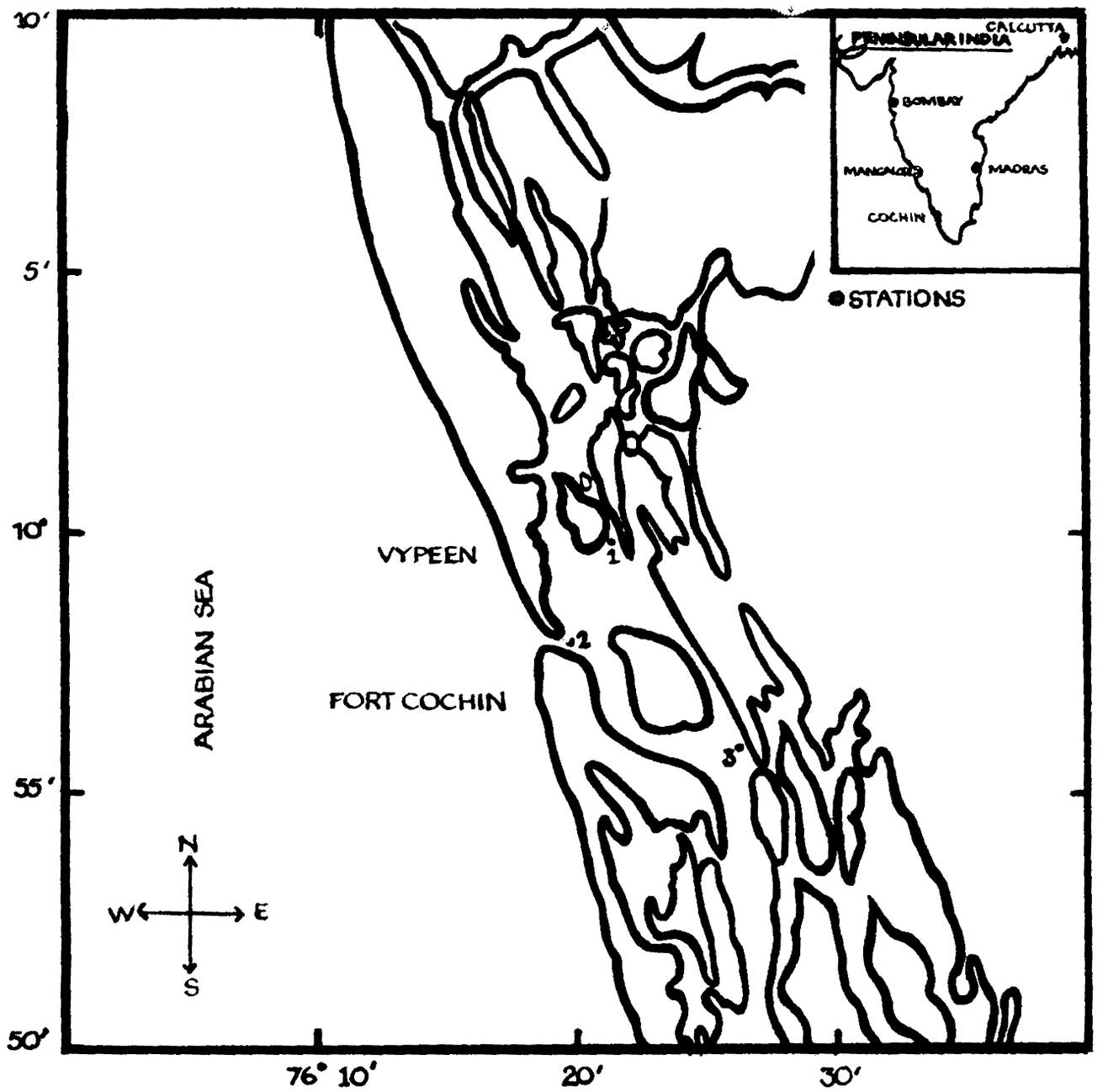
Both experimental and control beakers were covered with suitable plastic covers. The water was aerated throughout the period of observation (5 days). The pH of the water at the time of commencement of the experiment was 7.2 and the ambient temperature ranged between 28 and 31°C. Water in the experimental and control beakers was regularly exchanged with the water of same quality (salinity 19 ppt, pH 7.2) once in 24 hours keeping the bacterial density constant (by adding the known concentration of above 1.5×10^5 of bacteria per ml. of water) in experimental beakers. The survival and growth in terms of weight of juvenile prawns were recorded at the end of the experiment (5 days).

Fig. 1. Map of Cochin backwaters showing the location of sampling stations.

1. Vallarpadom

2. Cochin Harbour.

3. Thevara.


10'
5'
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VYPEEN
FORT COCHIN
ARABIAN SEA
1
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PENINSULAR INDIA
CALCUTTA
BOMBAY
MANGALORE
MADRAS
COCHIN
STATIONS

CHAPTER 3

QUANTITATIVE ASSESSMENT OF BACTERIAL POPULATION IN PRAWNS

3. QUANTITATIVE ASSESSMENT OF BACTERIAL POPULATION IN PRAWNS

3.1 Introduction:

Increasing global demand for prawn and prawn products has stimulated exploitation of the prawn fisheries. Modernisation of fishing methods and recent advances in food processing and preservation technology has created a greater demand and opened up export markets, which have become important sources of foreign exchange (Whitaker, 1970). To maintain the value of such an export it is essential that they are able to compete in quality, wholesomeness and price with similar products in the international market (Cann, 1977). The products must also meet the required standards of importing countries. Cann et al. (1971) opined that in order to arrive at realistic and practical standards, it is essential that there is a thorough understanding of the numbers and types of bacteria present in the raw material. Further, Cann (1977), suggested that the number and types of bacteria present in prawns will reflect the changes that have taken place in the initial flora and contamination that may occur during catching, handling and processing. Shewan (1961),

has reported that the flora recorded for fish from the same area or in areas with similar temperature conditions exhibit considerable differences. It was believed that this was a 'species effect' related to the constitution of the slime substratum, which may differ markedly from species to species (Wood, 1953).

The natural flora of marine fish reflects to a large extent that of its environment, the composition of which is governed by the seasonal temperature and the chemical constituents of water in which they exist (Venkataraman and Sreenivasan, 1952; 1954; Wood, 1953; and Shewan and Hobbs, 1967). Therefore, it is logical to infer that the microbial flora of prawns in different species may also vary in relation to different environmental conditions.

There is a volume of literature available on quantitative analysis of bacteria of prawns from different parts of the world (Green, 1949; Williams et al., 1952; Pieger, 1950; Pieger et al., 1958; Williams and Rees, 1952). Few studies were also made in India by Sreenivasan (1959); Iyer et al. (1970); Bose (1971) and Karthiayani and Iyer (1975).

The studies of Sreenivasan (1959) on the bacterial load of prawns at different stages of processing revealed the maximum bacterial load in the head region. Similar observations made by Green (1949) reported that the decapitation reduces bacterial load in shrimp. It was further reported by Sreenivasan (1959) that the removal of gut reduces the psychrophilic bacterial count. But it was not clear from where the prawns were collected, whether, it was immediately after trawling or from the processing plant. Further, the studies did not indicate any seasonal variations and there was no indication about the species used.

The studies on the bacterial quality of fresh prawns handled under tropical conditions were made by Iyer et al. (1970). The authors have reported the seasonal variation in bacterial population in raw material. It was also observed that the increase in total bacterial count of the raw material caused corresponding increase in the bacterial load on frozen prawn also. Although, detailed study was made on the bacterial load of prawns, the raw material used in the study was collected from the processing plant, thereby leaving no indication about the time lapse between the time of catch and reaching the processing plant. Further, there was no

indication about the bacterial load in freshly caught prawns and the level of extraneous contamination at different stages of handling. Also the authors have not indicated the identity of species used in the study.

Bose (1971) studied the bacterial load on slime, flesh and gut of prawns, trawled off Tuticorin coast and in sea water at the place of trawling. The author reported the bacterial load of 5.5×10^3 per sq. cm in slime, 5.1×10^3 per gram in flesh and 23.0×10^3 per gram in flesh and 23.0×10^3 per gram in gut samples. The author compared the results with that of fishes caught in the same area. However, the author has not indicated the names of species used in the study. Further, the study did not cover the different seasons during the year.

The studies carried out by Karthiayani and Iyer (1975) on the bacterial population of fresh prawns in relation to their environment reveal that the bacterial load varies from 9.3×10^3 to 2.3×10^5 per gram. The vein samples showed higher values, viz. 3.8×10^7 per gram. Eventhough, different species were used in the study, the results were pooled without indicating the bacterial load between the species used in the study. Further, the study was not in relation to season.

From the above studies it was evident that no work has been carried out on the bacterial load of freshly caught prawns in different regions of the body representing different seasons of the year. Besides this, the Cochin backwater was dredged and deepened for navigational purpose regularly. The importance of Cochin backwaters has grown considerably because of Cochin Harbour and Shipyard situated near its mouth (Balakrishnan and Shynamma, 1976). The Cochin backwater is a nursing ground for a number of commercially important prawns (Kurian and Sebastian, 1976). The presence of oil berths in this area has led to oil spillage during loading and unloading of oil, apart from draining of sewage and industrial waste in to these backwaters. Therefore, a study was undertaken to assess the bacterial load in different species of prawns, during different seasons, immediately after catch, and in different regions of the body. The study of bacterial load in water and sediment was also included since the bacterial flora of the prawns generally reflect the flora of the water and sediment in which they live.

3.2 Results:

Among the Penaeus indicus head samples tested,

the bacterial load varied between 1.5×10^3 and 1.6×10^6 per gram on an average. The distinct variation in the bacterial counts was noticed between monsoon months (June to October) with that of non-monsoon months (November to April) except for March, when slightly higher counts were recorded. The lowest counts were recorded in the month of January and highest in the month of June (Table 1).

Bacterial load in the gut samples of P. indicus in different months was indicatted in Table 2. The average bacterial counts during different months varied from 9.7×10^3 to 1.7×10^6 per gram. The lowest being in April and highest in June. Here also, as in the head samples of P. indicus the maximum counts were recorded during the monsoon season.

Table 3 indicates the bacterial population in the tail region of P. indicus during the different months. As in the case of gut samples of P. indicus lowest counts were seen during April (9.0×10^3 per gram) and June, as usual, recorded highest bacterial load (1.6×10^6 per gram) on an average. As in head and gut samples of P. indicus tail samples also contained higher bacterial load during monsoon season

and lower during non-monsoon season. In general, results are similar to those of the head and gut samples represented above, except in the month of March, which showed an average of 1.4×10^5 per gram, which was almost similar to that of the counts obtained during rainy season.

Log values of the average bacterial load in head, gut and tail samples of P. indicus in different months are indicated in Fig.2. It may be seen that the pattern of variation in bacterial load in different samples (head, gut and tail) during different months was almost similar. However, there was a marked difference in the bacterial load between monsoon and non-monsoon months. Except for the head and tail samples in the month of March, where the counts were found to be on the higher side and some what similar to counts of monsoon months.

As in the case of P. indicus, the head, gut and tail samples of M. dobsoni were also analysed. The results of the bacterial load in head region of M. dobsoni was presented in Table 4. It may be seen from the table that the average bacterial load for head region varied from 5.6×10^4 to 1.7×10^6 per gram. The season-wise results obtained for M. dobsoni head samples are comparable with that of P. indicus head

samples. The samples contained bacterial load on an higher side during rainy season than that of non-rainy seasons.

Table 5 indicates the results of the bacterial load of M. dobsoni gut samples in different months. Average bacterial load varied between 3.0×10^4 and 9.5×10^5 per gram. As usual the variation between the seasons (monsoon and non-monsoon) were noticed in the gut samples also. Much variation in bacterial load of head and gut samples of M. dobsoni and those of P. indicus was not noticed.

Bacterial load for M. dobsoni tail samples were given in Table 6. As in the case of other samples, seasonal variation in the bacterial load was noticed. The average monthly counts varied from 1.3×10^4 to 1.3×10^6 per gram. Unlike in other samples tested M. dobsoni tail samples indicated counts on the higher side during November. The gaps seen in the Table 1 to 6 indicate that the prawns were not available during the periods for the following reasons.

1. The prawn samples could not be obtained during July as there was no fishing due to bad weather and heavy rains. However, water and sediment samples were analysed for bacterial load during the month.

2. During other months i.e. February, April and May samples could not be obtained due to non-availability of prawns or appearance of insufficient number of them in catches, which was attributed to the seasonal and regional factors. In the P. indicus samples collected during January and February, only the head samples could be analysed (Table 1). Whereas, the results of the analysis of the gut samples during the above period (Table 2) and tail samples during February (Table 3), could not be presented as the samples were spoiled during the analysis. Further, the analysis could not be repeated owing to the dearth of the specimens during the above period.

The numerical variations in the bacterial load were also analysed statistically using analysis of variance technique. The results of the analysis between species, regions and months are represented in Table 7. It is evident from the table that there is no significant difference in the bacterial load between regions and between species at 5% level. However, the bacterial counts between months vary significantly at 1% level.

The analysis of variance of the bacterial counts was done separately for P. indicus and M. dobsoni and they are presented in Table 8 and Table 9 respectively. From Table 8, it is clear that there was no significant difference in the bacterial count between head, gut and tail regions at 5% level. The variation between months was significant at 1% level.

The least significant difference (LSD) worked out to identify the months which gave significantly higher counts showed that the bacterial load was significantly high during June, August and October and significantly less during April in P. indicus as reflected in the numerical estimation.

Table 9 represents the analysis of variance of the bacterial counts in M. dobsoni for different months and body regions. Here also there was no significant difference in the bacterial count over different regions (head, gut and tail), but the bacterial count varied significantly over August, September, October and November months were having significantly higher counts compared to other months.

Although species such as Metapenaeus monoceros and Macrobrachium idella which were not abundantly available and appeared rarely in the catches were also analysed for the bacterial load for the purpose of comparison and the results (counts/gram for the whole prawns) were represented in the Table 10. The samples contained the bacterial load similar to that of P. indicus and M. dobsoni samples.

Table 11 and 12 show the results of the bacterial load in sediment and water samples respectively collected during different months. It was evident from the tables that there was a relationship between the bacterial load of sediment with that of water in different months. It was seen that the increase in the bacterial load in sediment caused corresponding increase in water also. Here also there was marked difference between the bacterial load between monsoon and non-monsoon months.

The average hydrographical parameters of Cochin backwaters were represented in the Table 13. It was clear from the table that there was marked difference between the salinity of waters in monsoon and non-monsoon months.

The matrix of correlation was constructed to study the interdependency of the bacterial counts in sediments, water, P. indicus and M. dobsoni and their relationship with water temperature, salinity and pH is presented in Table 14. The results of the analysis from the correlation matrix are summarised as follows:

(1) There was significant positive correlation between bacterial counts in sediment and water and sediment and P. indicus. This indicates that as the bacterial

counts in sediment increases, there was significant increase in bacterial counts in water ($P < 0.01$) and in P. indicus ($P < 0.05$).

(2) The bacterial load in the water was significantly negatively correlated ($P < 0.01$) with salinity and water temperature ($P < 0.05$). This indicates that as salinity and water temperature increase bacterial count decreases. The bacterial count in water and P. indicus and that in water and M. dobsoni were significantly ($P < 0.05$) positively correlated indicating that as the bacterial count in water increases, the count in P. indicus and M. dobsoni also increases.

(3) Salinity was significantly positively correlated with water temperature ($P < 0.01$) and pH ($P < 0.05$). This indicated that with the increase in water temperature, salinity and pH also increases. Salinity was significantly negatively correlated with the bacterial count in P. indicus ($P < 0.01$) and M. dobsoni ($P < 0.05$). This indicates that as salinity increases, the bacterial load in P. indicus and M.dobsoni decreases.

(4) Water temperature is significantly positively correlated with pH ($P < 0.05$) and significantly negatively correlated with bacterial load in P. indicus

($P < 0.01$). This indicates that as water temperature increases, the bacterial load in P. indicus decreases.

3.3 Discussion:

In the present study, variations in the total bacterial counts were noticed in different species of prawns and between different regions of the body. The analysis of variance showed that the bacterial counts vary significantly at 1% level between regions and different species. But there is no significant difference at 5% level.

Generally, in the present study there was a marked difference in the bacterial counts between monsoon and non-monsoon months with the maximum during monsoon (Tables 11 & 12).

The observations made by Iyer et al. (1970) in raw and processed prawns obtained from the processing plants also indicated the seasonal variation in the bacterial load with more counts during rainy season. The higher counts during rainy months may be attributed to the seasonal effect due to inflow of more fresh water, as opined by Karthiayani and Iyer (1971). The variation in the bacterial density of prawns in general was in the range from 1.0×10^3 during January to 1.0×10^6 per gram in June in the present study. The results are comparable to that of the findings of Karthiayani and Iyer (1975) for prawns caught off Cochin

and Sandhya and Doctor (1983) for prawns off Bombay coast. However, the present results apparently differ from that of the observations of Bose (1971) for the prawns caught in the offshore waters of Tuticorin, where comparatively less bacterial counts were recorded. The probable reasons for the lower bacterial counts may be attributed to the environmental and seasonal parameters as opined by Shewan (1966) and Cann (1977). In the present study, as mentioned above, there was no significant difference in bacterial load between the different regions of the body of prawns. The present results differ from the observations of Williams et al. (1952) and Sreenivasan (1959), where it is reported that there will be reduction in the bacterial counts when they are beheaded or gutted.

Further, there was significant positive correlation between bacterial count in sediment and water, sediment and P. indicus, indicating that, when the bacteria in sediment increases, there was significant increase in the bacterial count of water ($P < 0.01$) and in P. indicus ($P < 0.05$). This shows that the flora of prawns are directly related to their aquatic environment as evidenced by Venkataraman and Sreenivasan (1952 & 1954), Wood (1953), Shewan and Hobbs (1967) and Cann (1971) for fishes and prawns. The bacterial load

was significantly negatively correlated with salinity ($P < 0.01$) and water temperature ($P < 0.05$), indicating that, as salinity and water temperature increase bacterial count decreases. The bacterial count in water and *P. indicus* and that in water and *M.dobsoni* were significantly positively correlated ($P < 0.05$), indicating, as the bacterial load in water increases, the bacterial load in prawns will also increase.

Salinity is also significantly positively correlated with temperature ($P < 0.01$) and pH ($P < 0.05$). This indicates that with the increase in water temperature, salinity and pH also increases. Salinity was significantly negatively correlated with the bacterial count in *P. indicus* ($P < 0.01$) and *M. dobsoni* ($P < 0.05$), thus indicating that when the salinity increases the bacterial load in *P. indicus* and *M. dobsoni* decreases. Such observations were also evident from the findings of Lakshmanaperumalsamy *et al.*(1981), where the increase in salinity caused decrease in bacterial load in water and sediment.

Water temperature was also significantly correlated with pH ($P < 0.05$) and significantly negatively correlated with bacterial load in *P. indicus* ($P < 0.01$). Thus, when the water temperature increases, pH also

increases. But as water temperature increases, the bacterial load in P. indicus decreases. This may be attributed to the mortality of some species for which the temperature might be critical or lethal as evidenced by Mohankumar et al. (1979) in the water samples collected from the Mangalore coast. Further, it was opined that the effect of temperature on organisms depend on the salinity of the environment (Cooper and Morita, 1972), and availability of growth supporting organic matter and micro nutrients (Sayler et al., 1975).

Variation in the bacterial density observed in the sediment samples during different months may be attributed to the tidal and soil factors besides environmental and seasonal parameter. These observations were comparable with that of the findings of Karthiayani and Iyer (1975) but at a higher magnitude.

Water samples had the average bacterial density of 1.9×10^4 per ml in the month of April and 6.0×10^5 per ml in the month of July. As in the sediment samples, the counts obtained for water samples in the present study were higher than the findings of Karthiayani and Iyer (1975) for sea water collected from the offshore region of Cochin.

3.4 Conclusion:

From the results, it may be said that the bacterial load of prawns seems to be autochthonous to it's environment. It appears that, the seasons played a significant role in determining the bacterial population, looking at the first few months' (August 1979 to December 1979) data from Tables 1 to 6. However, the seasonal variation of bacterial load for full annual cycle could not be obtained as explained earlier. Hence, the specific conclusions could not be drawn. Nevertheless, the earlier reports (Karthiayani and Iyer, 1970 and 1975) indicate that monsoon season favours the presence of great number of bacteria in oil sardines. In the present study, the decreasing order of bacterial load was noticed among the samples collected from Cochin backwaters was found to be prawn < water < sediment.

Table 1. The Bacterial Count in the head region of *Penaeus indicus* (1979-80).

Year	Month	No. of samples tested	Minimum bacterial count per gram	Maximum bacterial count per gram	Average bacterial count per gram
1979	August	16	3.0×10^4	1.4×10^6	4.0×10^5
"	September	12	1.9×10^4	1.4×10^6	3.5×10^5
"	October	14	3.0×10^4	1.6×10^6	6.8×10^5
"	[illegible]	15	2.0×10^4	1.2×10^5	5.7×10^4
"	[illegible]	13	5.0×10^4	5.8×10^5	5.3×10^4
[illegible]	January	12	1.0×10^3	2.0×10^3	1.5×10^3
"	February	11	5.5×10^3	1.7×10^4	1.1×10^4
"	March	19	7.0×10^3	2.5×10^5	3.2×10^5
"	April	13	9.7×10^4	1.3×10^4	1.2×10^4
"	May	-	-	-	-
"	June	12	1.5×10^6	1.7×10^6	1.6×10^6

Table 2. The bacterial count in the gut region of *Penaeus indicus* (1979-80).

Year	Month	No. of samples tested	Minimum bacterial count per gram	Maximum bacterial count per gram	Average bacterial count per gram
1979	August	15	3.0×10^4	1.4×10^6	6.5×10^5
"	September	17	1.2×10^5	1.4×10^6	4.9×10^5
"	October	14	3.3×10^5	6.3×10^5	4.4×10^5
"	November	13	4.7×10^4	7.3×10^5	4.7×10^5
"	December	14	4.3×10^5	9.2×10^5	7.2×10^5
1980	January	-	-	-	-
"	February	-	-	-	-
"	March	13	7.0×10^3	4.6×10^4	2.3×10^4
"	April	11	2.5×10^3	9.7×10^4	9.7×10^3
"	May	-	-	-	-
"	June	7	8.6×10^5	2.0×10^6	1.7×10^6

Table 3. The bacterial count in the tail region of *Penaeus indicus* (1979-80).

Year	Month	No. of Samples tested	Minimum bacterial count per gram	Maximum bacterial count per gram	Average bacterial count per gram
1979	August	16	1.5×10^5	1.6×10^6	6.3×10^5
"	September	12	8.0×10^4	6.5×10^5	2.5×10^5
"	October	14	3.0×10^5	1.4×10^6	6.8×10^5
"	November	14	2.2×10^4	1.9×10^5	7.7×10^4
"	December	13	1.5×10^4	5.7×10^4	3.2×10^4
1980	January	12	1.0×10^3	2.0×10^4	1.1×10^4
"	February	1	-	1.6×10^4	1.6×10^4
"	March	16	9.0×10^3	4.4×10^5	1.4×10^5
"	April	8	6.7×10^3	1.3×10^4	9.0×10^3
"	May	-	-	-	-
"	June	6	1.1×10^5	2.8×10^6	1.6×10^6

Table 4. The bacterial count of the head region of *Metapenaeus dobsoni* (1979–80).

Year	Month	No. of samples tested	Minimum bacterial count per gram	Maximum bacterial count per gram	Average bacterial count per gram
1979	August	12	9.1×10^4	1.7×10^5	1.4×10^5
"	September	16	7.1×10^4	3.5×10^5	1.2×10^5
"	October	14	4.4×10^4	8.0×10^5	4.8×10^5
"	November	15	1.8×10^4	2.8×10^5	1.4×10^5
"	December	12	1.1×10^4	9.0×10^5	5.6×10^4
1980	January	5	2.4×10^4	1.3×10^5	8.6×10^4
"	February	-	-	-	-
"	March	12	2.2×10^4	1.6×10^5	8.0×10^4
"	April	-	-	-	-
"	May	-	-	-	-
"	June	1	-	-	1.7×10^6

Table 5. The bacterial count in the gut region of *Metapenaeus dobsoni* (1979-80).

Year	Month	No. of samples tested	Minimum bacterial count per gram	Maximum bacterial count per gram	Average bacterial count per gram
1979	August	12	1.4×10^5	1.3×10^6	9.5×10^5
"	September	14	4.9×10^4	1.2×10^6	8.4×10^5
"	October	12	3.3×10^4	9.4×10^5	4.6×10^5
"	November	13	2.6×10^4	2.1×10^5	1.1×10^5
"	December	1	-	5.5×10^4	5.5×10^4
1980	January	12	1.3×10^4	4.6×10^4	3.0×10^4
"	February	-	-	-	-
"	March	12	4.7×10^4	1.1×10^5	7.5×10^4
"	April	-	-	-	-
"	May	-	-	-	-
"	June	-	-	-	-

Table 6. The bacterial count in the tail region of *Metapenaeus dobsoni* (1979-80).

Year	Month	No. of samples tested	Minimum bacterial count per gram	Maximum bacterial count per gram	Average bacterial count per gram
1979	August	12	1.8×10^5	5.4×10^5	3.6×10^5
"	September	15	2.2×10^4	1.6×10^5	8.9×10^4
"	October	16	1.6×10^4	5.0×10^5	3.0×10^5
"	November	15	3.8×10^4	2.3×10^6	1.2×10^6
"	December	14	1.2×10^4	1.3×10^5	9.5×10^4
1980	January	4	3.0×10^3	2.0×10^4	1.3×10^4
"	February	-	-	-	-
"	March	12	9.0×10^3	4.8×10^4	2.4×10^4
"	April	-	-	-	-
"	May	-	-	-	-
"	June	4	1.9×10^5	1.8×10^6	1.3×10^6

Table 7. Analysis of variance of bacterial count.

	Source	Sum of squares	Degrees of freedom	Mean square	Variance Ratio
	Total	10.5469	35		
Between	Species	0.0989	1	0.0989	0.63
Between	Months	5.6173	5	1.1235	7.18**
Between	Regions	0.6045	2	0.3023	1.93
	Error	4.2262	27	0.1565	

** Indicates significance at 1% level.

Table 8. Analysis of variance of bacterial count in *Penaeus indicus*.

	Source	Sum of squares	Degrees of freedom	Mean square	Variance Ratio
	Total	12.4399	23		
Between	Months	10.4270	7	1.4896	12.0226**
Between	Regions	0.2780	2	0.1390	1.1218
	Error	1.7349	14	0.1239	

Least significant difference at 5% level for months = 0.6165

Mean bacterial load for different months (in log values)

April	March	December	November	September	August	October	June
4.0067	4.6710	5.0259	5.0931	5.5441	5.7381	5.7695	6.2129

** Indicates significance at 1% level.

Table 9. Analysis of variance of bacterial count in *Metapenaeus dobsoni*.

	Source	Sum of squares	Degrees of freedom	Mean square	Variance Ratio
	Total	7.0779	20		
Between	Months	4.7134	6	0.78557	4.66*
Between	Regions	0.3435	2	0.17175	1.02
	Error	2.0210	12	0.16842	

L_t^e st significant difference at 5% level = 0.7301

The mean monthly count in log values.

December	January	March	September	November	August	October
4.48888	4.5016	4.7195	5.3176	5.5226	5.5600	5.6070

* Indicates significance at 5% level.

Table 10. The bacterial count in the species of seasonal prawns (1979-80).

Species	Months	No. of samples tested	Minimum bacterial count per gram	Maximum bacterial count per gram	Average bacterial count per gram
Metapenaeus monoceros	December-February	12	2.2×10^4	1.2×10^6	1.8×10^5
Macrobrachium idella	August-September	10	6.2×10^3	3.5×10^5	9.7×10^4

Table 11. The total bacterial count per gram of Sediment (1979-80).

Year	Month	No. of samples tested	Minimum bacterial counts per gram	Maximum bacterial counts per gram	Average bacterial counts per gram
1979	August	6	1.6×10^5	9.6×10^5	5.9×10^5
"	September	6	3.0×10^3	2.7×10^5	7.5×10^4
"	October	6	3.4×10^4	4.5×10^5	1.8×10^5
"	November	6	3.0×10^4	3.2×10^6	6.4×10^5
"	December	6	2.4×10^4	1.1×10^5	6.1×10^4
1980	January	6	1.0×10^5	9.6×10^5	5.1×10^5
"	February	6	2.4×10^4	9.2×10^4	4.8×10^4
"	March	6	2.4×10^4	1.0×10^5	7.2×10^4
"	April	6	2.1×10^4	7.3×10^4	4.3×10^4
"	May	6	1.8×10^4	8.4×10^4	4.1×10^4
"	June	6	2.2×10^5	5.2×10^5	3.4×10^5
"	July	6	1.5×10^5	7.3×10^5	4.0×10^5

Table 12. The bacterial count per ml of water (1979-80).

Year	Month	No. of samples tested	Minimum bacterial counts per ml.	Maximum bacterial counts per ml.	Average bacterial counts per ml.
1979	August	6	2.2×10^5	7.2×10^5	4.6×10^5
"	September	6	2.2×10^4	1.0×10^5	4.3×10^4
"	October	6	1.2×10^4	2.4×10^5	1.0×10^5
"	November	6	1.5×10^4	3.3×10^5	1.2×10^5
"	December	6	1.5×10^4	5.0×10^4	3.3×10^4
1980	January	6	1.0×10^4	3.5×10^4	2.5×10^4
"	February	6	4.2×10^3	3.2×10^4	2.0×10^4
"	March	6	2.0×10^3	4.8×10^4	2.3×10^4
"	April	6	3.0×10^3	3.8×10^4	1.9×10^4
"	May	6	6.0×10^4	1.1×10^5	8.2×10^4
"	June	6	1.2×10^5	3.1×10^5	3.3×10^5
"	July	6	3.0×10^5	8.9×10^5	6.0×10^5

Table 13. The average of the hydrographical parameters of three Stations viz. Vallarpadom, Cochin Harbour and Thevara in Cochin Backwaters (1979-80).

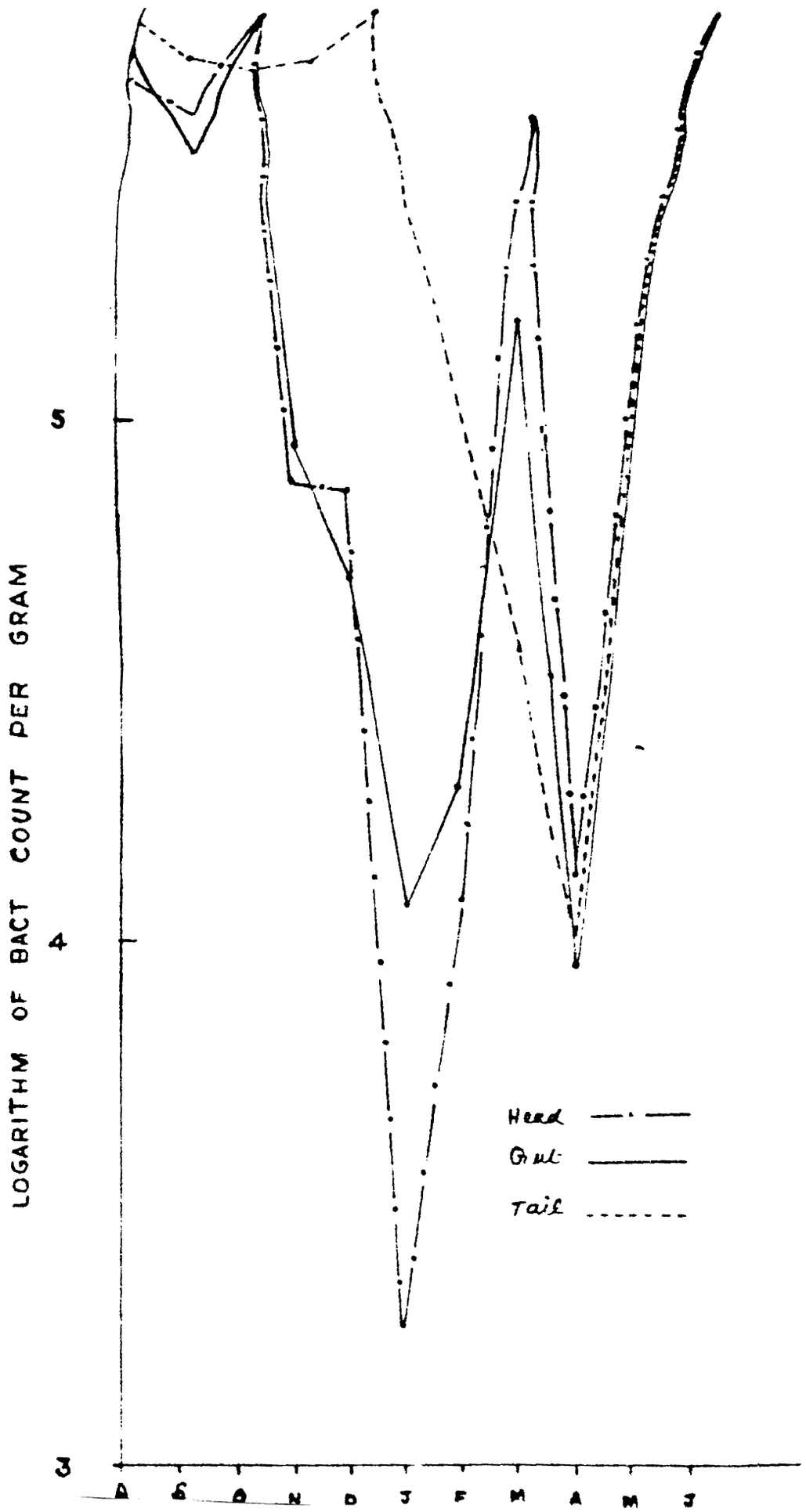
Year	Month	Average Salinity (‰)	Surface water Temperature (°C)	pH
1979	August	3.61	28.6	8.0
"	September	4.99	28.6	8.0
"	October	6.86	29.3	8.2
"	November	14.07	30.0	8.6
"	December	12.74	31.1	8.4
1980	January	27.33	30.8	8.4
"	February	29.10	31.0	8.6
"	March	30.23	32.0	8.4
"	April	32.00	33.0	8.4
"	May	16.47	31.9	8.6
"	June	8.47	29.0	8.4
"	July	6.42	28.2	8.2

Table 14. Matrix of correlation.

	Sediment	Water	Salinity	Water temp.	pH	*P. indicus*	*M. dobsoni*
Sediment	1.0000	.7133**	-.5803	.1771	-.0503	.8048*	.3900
Water		1.0000	-.8269**	-.7028*	-.3895	.8114*	.8043*
Salinity			1.0000	.8579**	.6288*	-.8980**	-.8670*
Water temp.				1.0000	.6659*	-.9050**	-.6957
pH					1.0000	-.4188	-.4107
P. indicus						1.0000	.7576*
M. dobsoni							1.0000

* = Significance at 5% level.
** = Significance at 1% level.

Fig.2. Log numbers of average bacterial population in head, gut and tail samples of *Penaeus indicus* in different months.


LOGARITHM OF BACT COUNT PER GRAM
5
4
3
A
S
O
N
D
J
F
M
A
M
J
Head
Gut
Tail

C H A P T E R 4

QUALITATIVE ASPECTS OF BACTERIAL FLORA OF PRAWNS

4. QUALITATIVE ASPECTS OF BACTERIAL FLORA OF PRAWNS

4.1 Introduction:

Prawns die very soon after catch, when compared to lobsters and crabs (Fieger and Novak, 1961). Deteriorative changes in prawns also start immediately after catch, because they contain greater amounts of amino acids than fish (Velankar and Govindan, 1957, 1958; Ranke, 1959). The authors further believed that the amino acids in prawns facilitate the bacterial growth, which presumably explains the ready spoilage. The quality deterioration of prawns was mainly by the action of bacteria, besides the action of its body enzymes (Fieger and Novak, 1961).

The significance of bacterial flora associated with prawns lies in the fact that it must explain the pattern and the probable extent of its spoilage (Castell and Mapplebeck, 1952; Venkataraman and Sreenivasan, 1954). The occurrence of high percentage of proteolytic types, points to the high spoilage potential. The occurrence of indole and hydrogen sulphide producers indicates the presence of putrefactive type of spoilage. Thus, the importance of various types of bacteria can be assessed on the basis of their biochemical activities, such as

liquefaction of gelatin, production of indole and hydrogen sulphide (Shewan, 1949; Castell and Mapplebeck,1952; Venkataraman and Sreenivasan, 1954).

The distribution and abundance of the bacterial flora of prawns are directly related to the flora of its aquatic environment (Venkataraman and Sreenivasan, 1952 and 1954; Shewan, 1961; Shewan and Hobbs, 1967). However, the observations of Wood (1953) and Colwell and Liston (1962) express the view that there was characteristic bacterial flora associated with a variety of animals. Further, it was believed that the characteristic flora of fish and prawn species was related to the constitution of slime substratum, which may differ from species to species. The composition of flora was also influenced by different [illegible] (Shewan, 1953; 1961; Liston, 1955; Geogala,1957 and 1958). Further, it was also believed that the geographical location was also known to cause variations in the generic distribution of the bacterial flora (Shewan, 1961).

In view of the above facts, it may not be surprising to note that there are only a few studies on the qualitative aspects of bacterial flora of prawns from Indian waters.

Sreenivasan (1959) isolated a few bacterial cultures from prawns and identified them up to their generic level. The author found the predominance of Micrococcus and Corynebacterium and to a lesser extent Bacillus, Achromobacter, Flavobacterium and Bacterium. It was opined by the author that the isolates studied were far too small to warrant any conclusion. Also, the prawns used for the studies were collected from the freezing plants. It was also not clear from where the prawns were caught, influence of the bacterial flora of the environment and the time gap between the time of catch and reaching the processing plant.

The studies on the bacterial flora of fresh prawns caught in the offshore waters of Tuticorin was carried out by Bose (1971). The author found that the flora of prawns mainly consisted of gram negative rods. It was 83% in slime; 54% in flesh and 53% in gut. Bacteria identified to generic level mainly composed of Achromobacter spp., Pseudomonas spp., Bacillus spp., Coryneforms and Vibrio spp. The bacterial flora of sea water consisted of Achromobacter spp., Vibrio spp., Bacillus spp., and Pseudomonas spp. Even though, the study gave a good account of the bacterial aspects of prawns caught off Tuticorin in relation to environment,

the author had not revealed the species identity. Further, the study did not reveal biochemical nature of bacterial flora.

The studies of Karthiayani and Iyer (1975) on the bacterial flora of prawns caught in Cochin waters showed the preponderance of Gram negative rods of the genera Pseudomonas spp., Vibrio spp. and Achromobacter spp. However, the authors have not indicated the biochemical characteristics of the bacterial groups. Further, even though different species of prawns were used for the study, there was no indication about the specieswise data.

There is a volume of literature indicating that the bacterial flora of prawns is directly related to the flora of its aquatic environment. Further, geographical location is also known to cause variation in the generic distribution of the bacterial flora (Shewan, 1961.) From the literature it is also evident that there are no comprehensive studies on the bacterial flora of prawns in relation to environment in Cochin region. Therefore, the study was undertaken to understand the qualitative nature of the bacterial flora of freshly caught prawns from Cochin backwaters, and their relation to the flora of water and sediment in the same area.

4.2 Results:

A total of 199 bacterial isolates, representing not less than 30 numbers from each region (Head, Gut and Tail) of Penaeus indicus and Metapenaeus dobsoni were pooled and tested for their morphological and biochemical characteristics. The cultures were also identified upto the generic level as described earlier. The results indicated the predominance of Pseudomonas, Acinetobacter, Moraxella, Bacillus and Micrococcus and to a lesser extent Flavobacter, Vibrio, Aeromonas and Enterobacter genera as shown in Figure 3.

The results of the biochemical characteristics of the isolates are shown in the Table 15. It may be seen from the table that 62% of the isolates were found to be motile. The Gram negative bacteria accounted for 72% (20.83% were Gram positive and 7.17% were Gram variable). The indole producers were 98.05%, Nitrate reducers 94.1%, Oxidase positive 91.04% and catalase positive isolates were 91.18%. 71% of the isolates were able to liquefy gelatin and 23.92% produced Hydrogen Sulphide (H_2S). Among the isolates tested, the lysine decarboxylisers were found to be 54.85%, Methyl red producers 58.26% and 8.53% were positive to voges-proskauer test. The percentage of

isolates fermenting different sugars namely D. Glucose, Lactose, Maltose, Mannitol, Sucrose were 48.84, 16.67, 44.85, 38.70 and 60.90 respectively.

From the results obtained above, it was very difficult to draw a definite conclusion on the pattern of bacterial flora in different species of prawns analysed. Therefore, in order to have an idea on the composition of the bacterial flora of different species present in some selected prawns namely P. indicus and M. dobsoni and their relation to the flora of water and the sediment during August and September months of the monsoon season, analysis was carried out and the results are presented in Table 16 and Figure 4. It is seen from the table that there was predominance of Pseudomonas, Vibrio and Acinetobacter in all the samples.

Among the different samples (P. indicus, M. dobsoni, water and sediment) tested, P. indicus yielded 30.61% Pseudomonas and 16.33% Vibrio genera. The generic composition of bacteria in M. dobsoni was almost similar to that of P. indicus with small variations. The major genera present were Pseudomonas, Vibrio and Acinetobacter.

However, higher percentage of Moraxella (17.02) was also noticed in M. dobsoni than in P. indicus (2.08). Water samples contained to a greater extent Pseudomonas (30.44%) and Vibrio (17.39%). The sediment harboured 33.33% Pseudomonas and 28.51% Vibrio and to a lesser extent other genera of bacteria Acinetobacter, Micrococcus, Corynebacter, Alcaligens, Enterobacter, Flavobacter, Aeromonas, Moraxella and Bacillus as indicated in Table 16 referred to above. Table 17 gives the analysis of variance of percentage generic composition of bacteria on P. indicus and M. dobsoni, water and sediment samples. It could be seen from the table that there was no significant difference between the samples but there was significant difference between genera at 1% level. The least significant difference for genera at 5% level was 5.2189. Pseudomonas spp., Vibrio spp. and Acinetobacter spp. were significantly high in the generic composition compared to others.

Table 18 shows the percentage biochemical characteristics of the bacterial cultures isolated from different samples (P. indicus, M. dobsoni, water and sediment) during August and September 1980. It was seen from the table that there was high percentage of nitrate reducers (91.49 to 100% and indole producers

(97.96 to 100%) in prawns (P. indicus, M. dobsoni), water and sediment samples. The percentage of H_2S producers were found in the range from 25.53 to 32.65% in prawns which was higher than in water and sediment samples (8.70 to 14.29%).

The gelatin liquefiers were 53.06% in P. indicus and 79.72% in M. dobsoni, 100% in water and 61.91% in sediment. The lysine decarboxylisers were found between 57.14 and 61.70 in prawns and 71.43% in sediment and to a lesser extent in water (43.48%). The variation in the biochemical characteristics of the isolates in different samples (P. indicus, M. dobsoni, water and sediment) are indicated in Fig. 5.

Eventhough the pattern of sugar fermentation (Acid from D. Glucose, Lactose, Maltose, Mannitol) among the isolates from different sources was almost similar,the number of isolates from water samples, fermenting sugars were on the higher side than in other samples. The percentage of sucrose fermenters from water samples were also on the higher side. Lactose fermenters were less in all the samples as compared to other sugars (D. Glucose, Malthse, Mannitol and Sucrose).

4.3 Discussion:

The present study records high percentage (62%) of motile isolates, similar to the observations made by Santhakumari (1966), working on Cochin backwaters. Gram negative organisms were found to be more in percentage (72%) than the Gram positive organisms (20.83%) and the remaining percentage being Gram variables. The isolates tested exclusively during monsoon months (August and September 1980) also indicated the high percentage of Gram negative organisms. The predominance of Gram negative bacteria in the water samples of Cochin backwaters were also recorded by Santhakumari (1966). The observation of Karthiayani and Iyer (1967, 1971 and 1975) for oil sardines and prawns caught in the offshore waters of Cochin, also indicated similar results with high percentage occurrence of Gram negative organisms.

The bacterial isolates obtained from prawn samples indicated the predominance of Pseudomonas and Acinetobacter groups followed by other genera such as Moraxella, Bacillus, Micrococcus, Coryneforms, Flavobacter, Enterobacter, Vibrio and Aeromonas. However, the bacterial isolates obtained exclusively during monsoon season (August-September, 1980) from P. indicus, M. dobsoni, water and sediment samples (Fig.4) indicated

significantly high percentage of Vibrio apart from Pseudomonas and Acinetobacter. A comparison of the flora in different samples (P. indicus, M. dobsoni, water and sediment) showed that there was virtually no difference in the qualitative composition but minor differences in the relative proportions of the different genera were seen which was significant at 1% level. The high percentage incidence of the genus Vibrio in different samples as in the present findings were also recorded by Surendran and Gopakumar (1983) in the bacterial isolates obtained from oil sardines and mackerels caught off Cochin, thus indicating the variation in the bacterial flora with respect to season in different samples as also evidenced by Karthiayani and Iyer (1971 and 1975). According to Shewan and Hobbs (1967) the seasonal differences in the relative proportions of the bacterial genera were related to temperature preference of various groups of bacteria and also changes in salinity caused by heavy rains during monsoon.

High percentage of isolates from prawns, water and sediment samples in general liquefied gelatin and produced indole. In a similar study made by Santhakumari (1966), also indicated high percentage of indole producers which was unique to the bacteria of purely marine origin.

From the literature it is known that *V. parahaemolyticus* can tolerate high salinity range (Collins and Patricia, 1976), while other strains are least tolerant to salinity, but during monsoon season there was heavy inflow of fresh water into the backwaters of Cochin, diluting the salinity to a considerable extent and the occurrence of high percentage of *Vibrio* genera may be attributed to the addition of *Vibrios* from the fresh waters.

It was further opined by various authors (Shewan, 1949; Castell and Mapplebeck, 1952) that the importance of various groups of bacteria can be assessed chiefly on the basis of their biochemical activities such as liquefaction of gelatin, production of indole and hydrogen sulphide. Venkataraman and Sreenivasan (1954) inferred that the predominance of proteolytic types points to a high spoilage potential and presence of indole and H_2S producers indicates the putrefactive types of spoilage. Thus, in the present study also high percentage occurrence of such isolates producing indole and liquefying gelatin indicates high potential for spoilage.

High percentage occurrence of nitrate reducers in the present study were comparable with the findings

of Santhakumari (1966) in the isolates obtained from water samples of Cochin backwaters and Karthiayani and Iyer (1971) in the oil sardines caught from the off-shore waters of Cochin.

Tests with carbohydrates (Glucose, Maltose, Sucrose) showed that the percentage of bacterial isolates from different samples (Prawns, water and sediment) fermenting sugars were higher in general. However, the percentage of mannitol and lactose fermenters were only moderate and low respectively (Tables 15 and 16).

The occurrence of lactose fermenting bacteria in prawns appears to be indicative of faecal contamination of human and non-human origin, as indicated by Santhakumari (1966). The pattern of carbohydrate fermentation by the isolates in the present study was comparable with the observations made by Karthiayani and Iyer (1971).

4.4 Conclusion:

Majority of the isolates being motile, gram negative and indole positive, indicates that the high percentage of bacteria in the Cochin backwaters are of marine origin.

The above study recorded significantly high percentage incidence of Gram negative bacteria belonging to genera Pseudomonas, Acinetobacter and Vibrio in prawns, water and sediment samples. The results also confirm that the bacterial flora of the water and sediments is reflected in the flora of prawns. High percentage occurrence of isolates with the ability to liquefy gelatin and produce indole indicated that the isolates had high spoilage potentials as opined by Venkataraman and Sreenivasan (1954).

Table 15. Biochemical characteristics of bacteria isolated from prawns (P. indicus, M. dobsoni) of Cochin Backwaters (1979-80).

Sl.No.	Characters	Percentage positive
1	Motility	62.00
2	Gram staining (-ve)	72.00
3	Nitrate reduction	94.10
4	Indole production	98.05
5	Gelatin liquefaction	71.00
6	H_2S production	23.92
7	M.R.	58.26
8	V.P.	8.53
9	Oxidase	91.04
10	Catalase	91.18
11	Lysine decarboxylation	54.85
12	Glucose fermentation	48.84
13	Lactose "	16.67
14	Maltose "	44.85
15	Mannitol "	38.70
16	Sucrose "	60.90

Table 16. Generic distribution of bacteria on P. indicus, M. dobsoni, water and sediment samples.

Name of sample	No. of isolates tested	Percentage of isolates											
		Pseudomonas	Vibrio	Acinetobacter	Micrococci	Coryneforms	Entrobacter	Flavobacter	Aeromonas	Alcaligens	Moraxella	Bacillus	Unidentified
P. indicus	49	30.61	16.33	12.25	6.12	6.12	6.12	6.12	4.08	4.08	2.08	0.00	6.12
M. dobsoni	47	25.33	12.77	10.64	4.23	2.13	2.13	4.23	6.38	8.51	17.02	2.13	4.23
Water	23	30.44	17.39	8.70	8.70	4.38	0.00	8.70	8.70	8.70	4.35	0.00	4.35
Sediment	21	33.33	28.51	9.52	0.00	4.72	0.00	4.76	4.76	9.52	4.76	0.00	0.00

Table:17. Analysis of variance of generic composition of bacteria on *P. indicus*, *M. dobsoni*, water and sediment samples.

Sources	Sum of squares	Degrees of freedom	Mean square	Variance Ratio (F)
Total	3432.3733	47		
Between treatments	1.2286	3	0.4095	0.03
Between Genera	2978.0346	11	270.7304	19.72**
Error	453.1101	33	13.7306	

** indicates significance at 1% level.

Table 18. Biochemical characteristics of bacteria isolated from *P. indicus*, *M. dobsoni*, water and sediment.

Percentage of the isolate indicating positive characteristics.

	No. of isolates tested	Nitrate Reduction	Indole Production	Gelatin liquefaction	H_2S Production	Lysine Decarboxylysers	Acid from D. Glucose	Acid from Lactose	Acid from Maltose	Acid from Mannitol	Acid from Sucrose
M. dobsoni	47	91.49	100.00	79.72	25.53	61.70	48.94	6.38	55.32	36.17	74.47
P. indicus	49	97.96	97.96	53.06	32.65	57.14	34.69	22.45	42.86	36.74	40.82
Water	23	100.00	100.00	100.00	8.70	43.48	91.30	30.44	78.26	39.13	69.57
Sediment	21	100.00	100.00	61.91	14.29	71.43	61.91	4.76	52.38	23.81	52.38

Fig. 3. Percentage composition of different genera of bacteria present in prawns in Cochin backwaters.

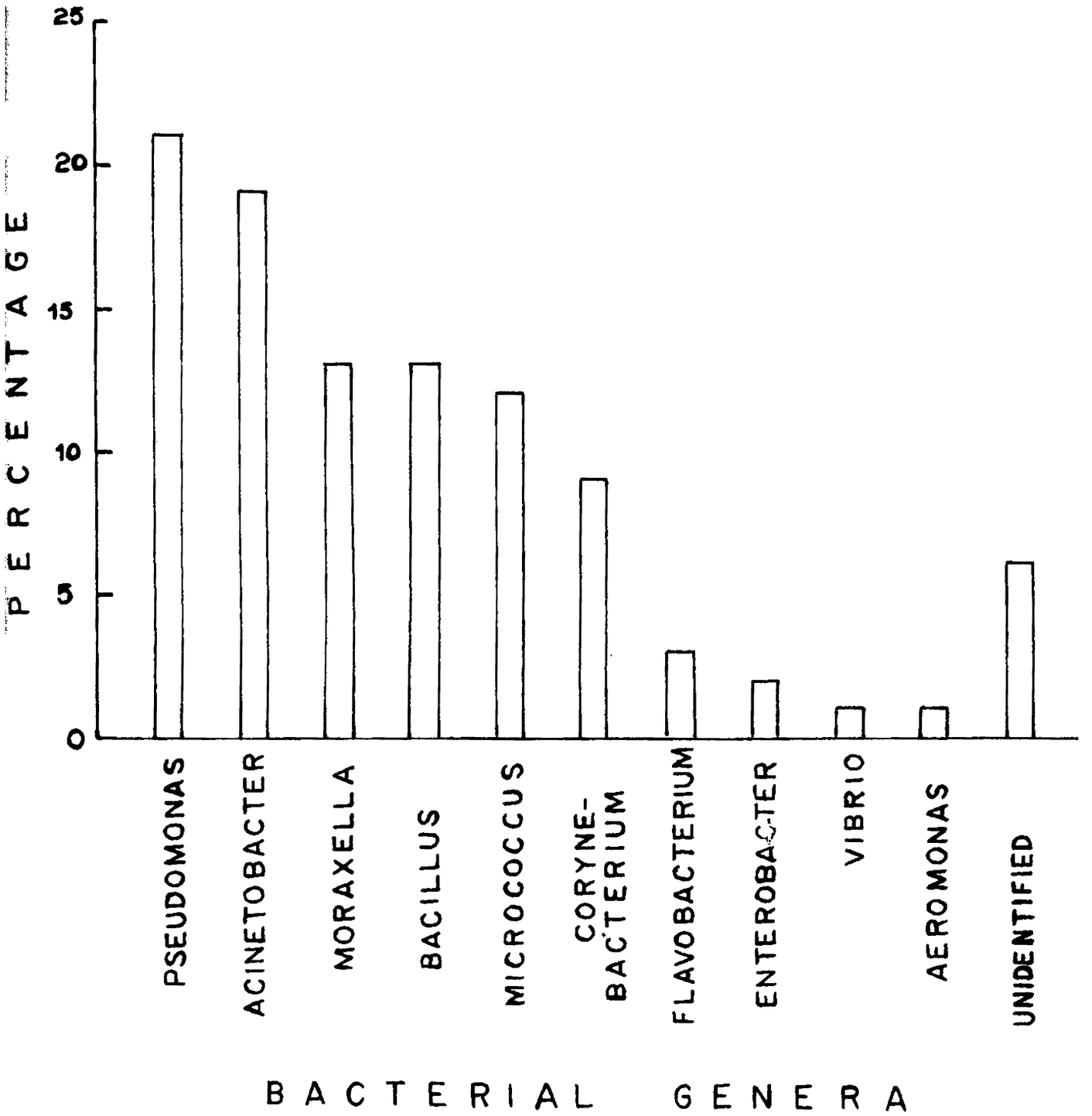

PERCENTAGE
25
20
15
10
5
0
PSEUDOMONAS
ACINETOBACTER
MORAXELLA
BACILLUS
MICROCOCCUS
CORYNE-BACTERIUM
FLAVOBACTERIUM
ENTEROBACTER
VIBRIO
AEROMONAS
UNIDENTIFIED
BACTERIAL GENERA

Fig. 4. Generic composition of bacteria present in *Penaeus indicus*, *Metapenaeus dobsoni*, water and sediment samples during monsoon months.

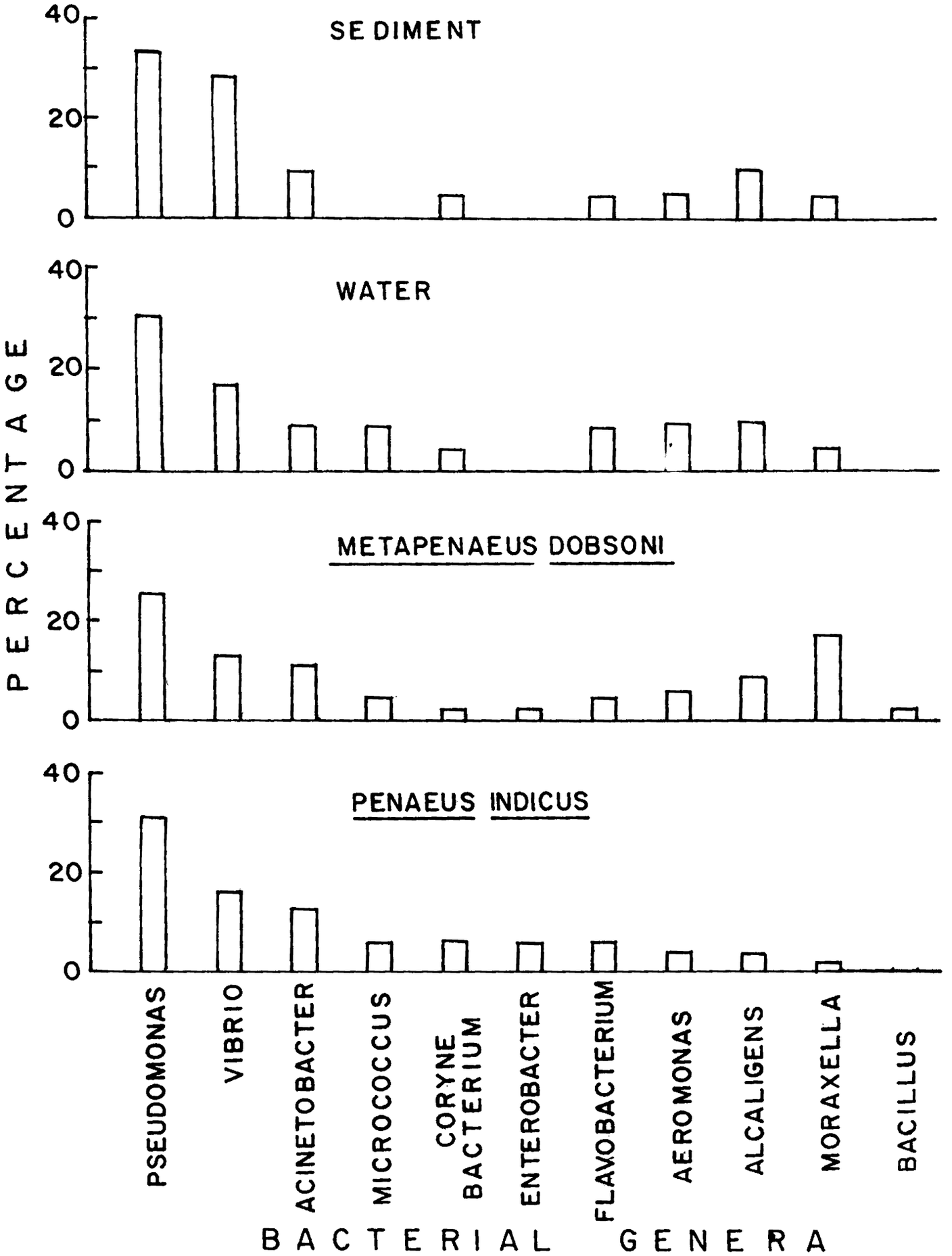
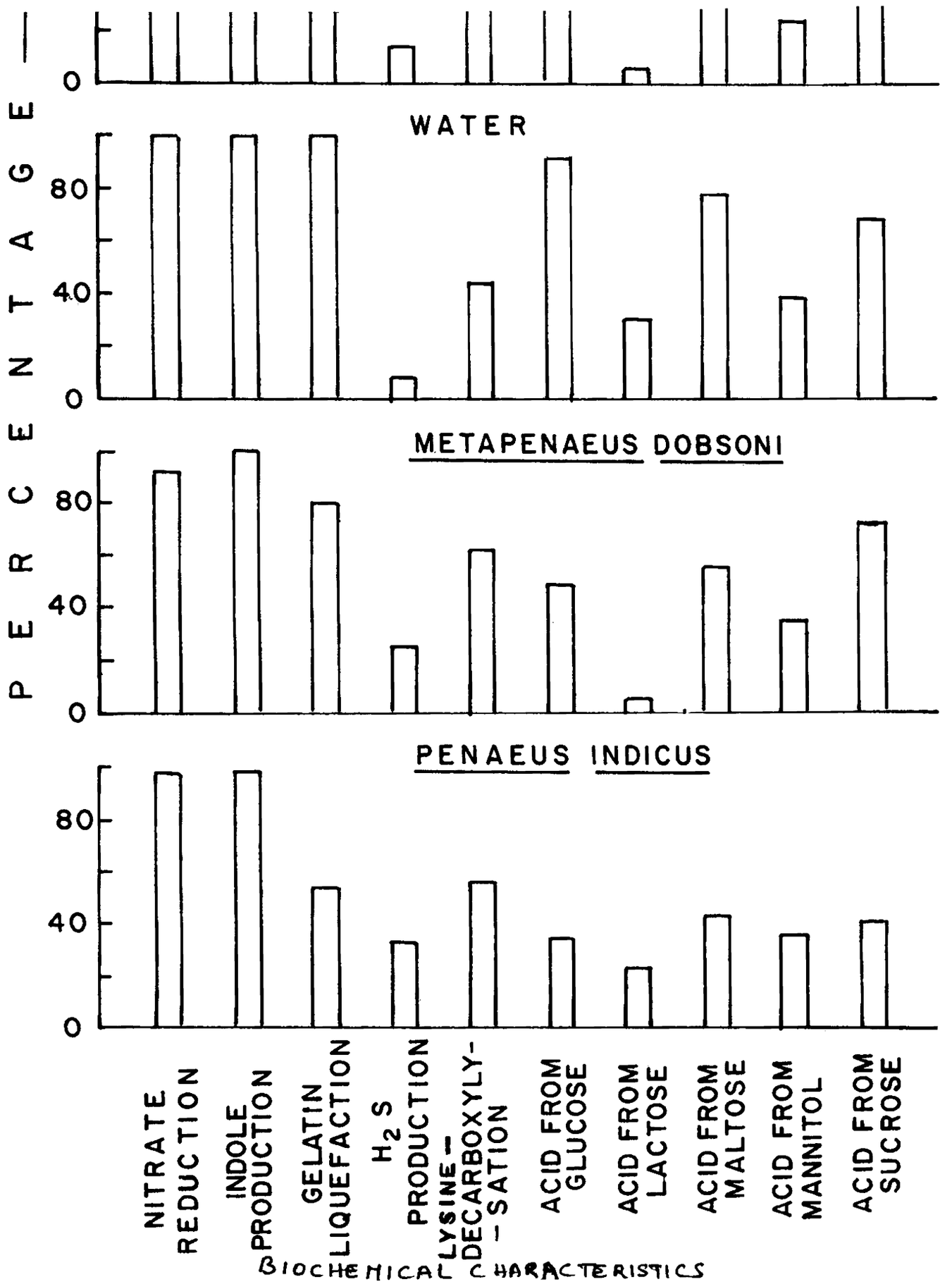

SEDIMENT
WATER
METAPENAEUS DOBSONI
PENAEUS INDICUS
40
20
0
PERCENTAGE
PSEUDOMONAS
VIBRIO
ACINETOBACTER
MICROCOCCUS
CORYNE BACTERIUM
ENTEROBACTER
FLAVOBACTERIUM
AEROMONAS
ALCALIGENS
MORAXELLA
BACILLUS
BACTERIAL GENERA

Fig.5. Biochemical characteristics of the bacterial isolate from different samples namely *Penaeus indicus*, *Metapenaeus dobsoni*, water and Sediment.


PERCENTAGE
0
WATER
80
40
0
METAPENAEUS DOBSONI
80
40
0
PENAEUS INDICUS
80
40
0
NITRATE REDUCTION
INDOLE PRODUCTION
GELATIN LIQUEFACTION
H_2S PRODUCTION
LYSINE-DECARBOXYLY-SATION
ACID FROM GLUCOSE
ACID FROM LACTOSE
ACID FROM MALTOSE
ACID FROM MANNITOL
ACID FROM SUCROSE
BIOCHEMICAL CHARACTERISTICS

C H A P T E R 5

QUANTITATIVE ANALYSIS OF BACTERIAL POPULATION IN RELATION TO INCUBATION TEMPERATURE.

5. QUANTITATIVE ANALYSIS OF BACTERIAL POPULATION IN RELATION TO INCUBATION TEMPERATURE.

5.1 Introduction:

Statistics of FAO (1981) reveal that the International Trade in tropical prawn products had raisen steadily. It was also known over a decade that there was vast scope for the export of prawn and prawn products from India (MPEDA, 1983). Improved fishing methods, intensive culture, increased use of refrigeration and advances in food processing technology enabled to develop export markets, which have become important to national economics (Cann, 1977). Prawns occupy an important place and the sea food industry heavily dependant on them as they constitute the major and most valued commodity of the marine products of export from India (Silas, 1980).

Since the prawn and prawn products had to meet the required quality standards of importing countries, there had been a few studies on the quantitative analysis of bacteria in prawns in India by Sreenivasan (1959), Velankar et al. (1961); Iyer et al. (1970); Bose (1971); Karthiayani and Iyer (1975), Sandhya and Doctor (1983). These studies were confined to the isolation of bacteria

either at ambient temperature or at 37°C. But the analysis of bacterial load with respect to different incubation temperatures was rather rare. The related literature include those of Harris (1963) on the influence of recovery medium and the incubation temperature on the survival of 'damaged' bacteria. Dowben and Weiden Mullar (1968) observed the adaptation of mesophylic bacteria to grow at elevated temperatures. Efthimion and Corpe (1969), reported the effect of temperature on the viability of Chromobacterium violaceum. Hobbs et al. (1971) in their studies on the bacteriology of 'Scampi' (Nephrops norvegicus) observed higher counts (10^4 per gram) at 20°C than at 37°C (10^3 per gram). Similar studies on the spoilage of 'Scampi' was made by Cann et al. (1971) who observed high counts at 20°C than at 37°C. The probable reason for higher counts at 20°C may be attributed to the effect of environmental temperature of the ecosystem in which these bacteria live. It was opined by Cann (1971 & 1977) and Shewan (1977) that the cold water harboured more Psychrophiles than other groups of bacteria (Mesophiles and Thermophiles).

While studying the bacterial load in different regions of prawns at different stages of processing, Sreenivasan (1959) took the counts at 37°C after 48 hrs

and at 4°C after 5 and 12 days and represented the bacterial load separately for mesophiles and Psychrophiles. However, the author has not taken the bacterial load, either at ambient temperature or at 57°C.

Bacterial load of oil sardines and mackerels at different incubation temperatures (36 ± 1°C for 3 days, 28 ± 2°C for 3 days, 8 ± 1°C for 10 days and 1 ± 1°C for 21 days) has been studied by Surendran and Gopakumar (1983). The authors inferred that the counts at room temperature (ambient temperature) represented psychrophiles and mesophiles and 36 ± 1°C represented only mesophiles.

Most of the experiments on the quantitative aspects of fresh prawns from Indian waters have been carried out either at ambient temperature (Karthaiyani and Iyer, 1975) or at 37°C (Sreenivasan, 1959 and Bose, 1971). However, no studies appear to have been made on the effect of different incubation temperatures on the bacterial load of prawns, sediment and water of Cochin backwaters. Hence, the present study was carried out to record the bacterial biomass on prawns, sediment and water samples collected from Cochin backwaters, at different incubation temperatures, such as 8 ± 2°C, 25 ± 2°C, 37°C and 57°C, to know the probable influence of incubation temperatures on the growth of the bacteria.

These incubation temperatures 8 ± 2°C, 25 ± 2°C, 37°C and 57°C were selected for the following reasons.

The optimum temperature for the growth of thermophiles lies arround 57°C (Rheinheimer, 1971; Collins and Patricia, 1976) and these thermophiles cause deterioration of food products stored at high temperature or with inadequate ventilation.

Aerobic mesophilic bacterial counts remain one of the most useful indicators of the microbiological status of food (Refai, 1979). Since 37°C is the optimum temperature for the growth of mesophiles (Thampuran and Iyer, 1979) the counts were taken at 37°C. This incubation temperature has further references to standards like AOAC (1970); APHA (1970) and ISI (1977 a & b).

Ambient (in situ) temperatures are known to fluctuate very widely during different times of the day and months of the year (Pelczar, 1957). Therefore, it was felt desirable to have counts at controlled temperature. Hence the plates were incubated at 25° ± 2°C as suggested by Cann (1977), at air-conditioned temperature for 48 hours.

Refrigeration as a means of food preservation is well recognised in the world. In this context, it was felt appropriate to have an idea about the Psychrophilic bacterial load at that temperature (8°C). Hence the bacterial load was recorded at refrigerator temperature using domestic refrigerator as suggested by Collins and Patricia (1976). Counts at 8°C were also recorded by Karthiayani and Iyer (1967) and Thampuran and Iyer (1979) for fishes.

5.2 Results:

The average total bacterial load obtained for prawns, water and sediment samples are shown in Table 19. It is seen that on an average, water samples contained the highest bacterial load (1.1×10^7 per ml) at 25°C and at 37°C (6.6×10^6 per ml) at the end of 48 hrs. The lowest counts (7.5×10^5 per ml) were noticed at 57°C after 48 hrs. and an intermediary count at 8°C (1.8×10^6 per ml) after 20 days incubation. The bacterial counts recorded individually for all the 12 water samples are shown in Table 20. The statistical analysis of the data obtained for water samples is presented in Table 21, which indicates that the variation between samples was not significant at 5% level but variation between incubation temperatures was significant

at 1% level. The least significant difference at 5% level worked out to be 0.8860 and the mean logarthemic count at 57°C, 17°C, 25 ± 2°C and 8 ± 1°C were 4.9169, 6.7661, 6.9740 and 5.9184 respectively. Significantly higher counts were observed in 25 ± 2°C at 48 hours and 37°C at 48 hours.

The sediment samples analysed for bacterial load at different temperatures indicated higher counts at 37°C (1.7×10^6 per gram) and at 25°C (1.4×10^6 per gram , Table No.19). The counts at 57°C after 48 hrs (1.8×10^5 per gram) and at 8°C at the end of 20 days (6.5×10^5 per gram) were on the lower side. The bacterial counts, at different incubation temperatures, for individual samples are given in the Table 22. Table 23, shows the analysis of variance of bacterial counts taken for sediment samples at different incubation temperatures. Here also, between samples, the variation was not significant at 5% level but between incubation temperatures, the variations was significant at 1% level. The least significant difference at 5% level for different incubation temperatures was 0.2197. The mean logarithimic count at 57°C, 37°C, 25°C and at 8°C were 5.0924, 6.1125, 6.0815 and 5.7852 respectively. Significantly higher counts were observed in 37°C at 48 hrs and 25°C at 48 hrs.

In general, prawn samples contained lesser bacterial load than sediment and water samples at all temperatures (Table 19). On an average bacterial load of 9.0×10^4 per gram was recorded at 57°C after 48 hrs. The bacterial load at other incubation temperatures 37°C, 25°C and 8°C were 3.0×10^5, 2.8×10^5 and 5.5×10^5 per gram respectively.

Table 24 indicates the bacterial counts at different incubation temperature for each of the 14 prawn samples. The statistical analysis of bacterial counts at different incubation temperatures for prawns are represented in the Table 25. It could be seen from the table that there was no significant difference in bacterial counts, between samples at 5% level. While there was significant difference ($P < 0.01$) in the bacterial count between incubation temperatures. The least significant difference at 5% level for the incubation temperature were worked out to be 0.9987. The mean logarithemic count in the four incubation temperatures were 3.0748, 5.2289, 4.9261 and 5.1591. Incubation at 37°C at 48 hrs. have shown higher counts followed by 8°C after 20 days and 25°C.

5.3 Discussion:

It was seen from the results that the prawn

samples exhibited lower bacterial counts at all incubation temperatures compared to water and sediment samples. The present results obtained at 37°C were comparable with the counts at the same temperature during monsoon as indicated in Chapter 3. The bacterial growth at 8°C was found to be absent in all the samples at the end of 48 hrs. But later (after 48 hrs.), the colonies appeared and a gradual increase in the number of colonies were found upto 20 days in all the samples. Similar observations were also made by Thampuran and Iyer (1979) on oil sardines, where the authors observed the appearance of bacterial colonies at 8°C after 12 to 14 days. Further, the authors indicated that the incubation at 8°C facilitated the recovery of Psychrophiles.

In the present study, the average bacterial counts obtained for water, sediment and prawn samples at 8°C after 20 days were comparable with the counts at 25°C and at 37°C after 48 hours, eventhough, there were variations in the bacterial counts among the different samples. The bacterial load in water and sediment samples at 8°C after 20 days were comparatively lower than at 25°C and at 37°C after 48 hours. Whereas, in prawns the counts at 8°C after 20 days were on the higher side compared to the counts at incubation temperatures of 25°C and 37°C after 48 hours, probably due to low water temperatures during August and September (1979), which was a peak monsoon period in Cochin region. The present results could not be compared with similar studies elsewhere in Cochin region due to the non-availability of related literature in fresh prawns.

Even though there were slight variations in bacterial counts between the samples and with respect to different incubation temperatures, the over-all pattern of bacterial load was comparable with the findings of Karthiayani and Iyer (1967) for oil sardines, where the counts at 8°C run almost parrallel with the counts obtained at ambient (room) temperature.

The counts recorded at 57°C for prawns, water and sediment were far less than those obtained at temperatures (37°C and 25°C) indicating the presence of less thermophiles in the samples. It was presumed that the appearance of thermophiles to a lesser extent in the samples was due to low environmental temperature during the months under study for the growth of thermophiles. This had reference to the works of Cann (1971) and Newell (1973) indicating that the environmental temperature had direct bearings on the bacterial load of crustaceans. The statistical analysis of the data also indicated significantly higher counts at 37°C and at 25°C in all the samples. In case of prawns also, 8°C showed higher counts as referred to above.

5.4 Conclusion:

In general, the bacterial load recorded for

prawns was less than these for water and sediment samples at all incubation temperatures. The bacterial counts were higher at 25°C and 37°C than at 57°C and 8°C except in the case of prawn samples where 8°C also recorded higher counts, indicating that the environmental temperature had direct effect on the bacterial load.

Table 19. The average total aerobic bacterial count at different incubation temperatures.

Samples	No. of samples tested	TEMPERATURE			
		57°C (48 hrs.)	37°C (48 hrs.)	25 ± 2°C (48 hrs.)	8 ± 1°C(20 days)
Water	12	7.5×10^5	6.6×10^6	1.1×10^7	1.8×10^6
Sediment	12	1.8×10^5	1.7×10^6	1.4×10^6	6.5×10^5
Prawns unpeeled	14	9.0×10^4	3.0×10^5	2.8×10^5	5.5×10^5

Table 20. Total aerobic bacterial count per ml. of water samples at different incubation temperatures.

Sample Nos.	57°C at 48 hrs.	37°C at 48 hrs.	25 ± 2°C at 48 hrs.	8 ± 2°C after 20 days.
1	12.0×10^5	4.8×10^6	1.6×10^7	1.8×10^6
2	3.0×10^5	9.2×10^6	1.5×10^7	0.8×10^6
3	7.0×10^5	4.7×10^6	0.7×10^7	1.6×10^6
4	5.0×10^5	2.6×10^6	0.8×10^7	0.3×10^6
5	-	1.8×10^6	0.4×10^7	0.05×10^6
6	-	3.8×10^6	1.6×10^7	0.03×10^6
7	5.0×10^5	7.4×10^6	2.1×10^7	2.2×10^6
8	8.1×10^5	9.9×10^6	0.5×10^7	3.3×10^6
9	18.1×10^5	9.4×10^6	1.8×10^7	2.5×10^6
10	12.5×10^5	8.7×10^6	1.0×10^7	1.5×10^6
11	12.1×10^5	7.5×10^6	0.5×10^7	0.6×10^6
12	7.2×10^5	9.4×10^6	0.6×10^7	6.2×10^6

' - ' denotes no growth of bacteria

Table 21. Analysis of variance of aerobic bacterial counts for water samples at different incubation temperatures.

Source	Sum of squares	Degrees of freedom	Mean square	Variance Ratio (F)
Total	97.0463	47		
Between samples	25.7356	11	2.3356	2.04
Between incubations	33.5912	3	11.1971	9.80**
Error	37.7195	33	1.1430	

** indicates significance at 1% level.

Table 22. Total aerobic bacterial counts per gram of sediment samples at different incubation temperatures.

Sample Nos.	57°C at 48 hrs.	37°C at 48 hrs.	25 ± 2° C at 48 hrs.	8 ± 2°C after 20 days
1	0.9×10^5	1.7×10^6	0.8×10^6	6.9×10^5
2	0.3×10^5	0.9×10^6	0.6×10^6	8.7×10^5
3	0.5×10^5	1.2×10^6	0.9×10^6	4.3×10^5
4	5.0×10^5	0.9×10^6	1.6×10^6	2.8×10^5
5	4.0×10^5	1.2×10^6	0.8×10^6	7.6×10^5
6	0.4×10^5	0.2×10^6	1.2×10^6	4.5×10^5
7	1.8×10^5	2.1×10^6	0.4×10^6	5.3×10^5
8	0.7×10^5	0.7×10^6	2.0×10^6	4.8×10^5
9	2.6×10^5	3.1×10^6	1.3×10^6	5.6×10^5
10	1.7×10^5	2.4×10^6	2.9×10^6	9.8×10^5
11	1.2×10^5	2.8×10^6	1.9×10^6	7.9×10^5
12	2.0×10^5	3.2×10^6	2.5×10^6	9.7×10^5

Table 23. Analysis of variance of aerobic bacterial count in sediment at different incubation temperatures.

Source	Sum of squares	Degrees of freedom	Mean square	Variance Ratio(F)
Total	12.3144	47		
Between samples	1.4411	11	0.1310	1.86
Between incubation	8.5534	3	2.8511	40.56**
Error	2.3199	33	0.0703	

** indicates significance at 1% level.

Table 24. Total aerobic bacterial counts per gram of prawns at different incubation temperatures.

Sample Nos.	57°C at 48 hrs.	37°C at 48 hrs.	25 ± 2°C at 48 hrs.	8 ± 2°C after 20 days
1	0	0.5×10^5	0.8×10^5	0
2	0	0.6×10^5	0.3×10^5	3.0×10^5
3	0.1×10^5	1.3×10^5	0.4×10^5	2.6×10^5
4	0.1×10^5	1.6×10^5	0.2×10^5	1.5×10^5
5	0.1×10^5	0.9×10^5	0.1×10^5	0.6×10^5
6	0	2.9×10^5	0.6×10^5	3.1×10^5
7	0	1.4×10^5	0.4×10^5	4.8×10^5
8	0	0.5×10^5	0.5×10^5	7.2×10^5
9	0.3×10^5	2.4×10^5	0.2×10^5	4.5×10^5
10	1.1×10^5	0.8×10^5	0.4×10^5	3.1×10^5
11	2.0×10^5	0.9×10^5	4.8×10^5	9.2×10^5
12	1.3×10^5	9.4×10^5	10.0×10^5	8.2×10^5
13	2.6×10^5	9.5×10^5	11.0×10^5	15.1×10^5
14	5.0×10^5	9.1×10^5	9.5×10^5	14.1×10^5

' 0 ' denotes no growth of bacteria.

Table 25: Analysis of variance of aerobic bacterial count in prawn samples at different temperatures.

Source	Sum of squares	Degrees of freedom	Means of square	Variance Ratio (F)
Total	160.5995	55		
Between samples	43.5604	13	3.3508	1.98
Between incubation	50.9676	3	16.9892	10.03**
Error	66.0715	39	1.6941	

** indicates significance at 1% level.

C H A P T E R 6

BACTERIA OF PUBLIC HEALTH SIGNIFICANCE PRESENT IN PRAWNS, WATER AND SEDIMENT.

6. BACTERIA OF PUBLIC HEALTH SIGNIFICANCE PRESENT IN PRAWNS, WATER AND SEDIMENT.

6.1 Introduction:

A great variety of pollutants produced by man reach the aquatic environment either directly or indirectly. The magnitude of pollution of the aquatic environment in various regions depends on the quantum of the pollutants and partly on the level of industrialisation (FAO, 1971).

Although most of the bacteria present in prawns of unpolluted waters are harmless, the pollution caused by raw or inadequately treated effluents from industries and domestic sewage can, however, introduce health hazard to man. Further, bacteria also contaminate the environment with noxious metabolic products.

An ideal organism indicating pollution, would be present in faeces, in large number. It may be absent in unpolluted environments. In case the indicators are present in an environment, they may thrive longer than the pathogens. No ideal indicator is known yet. However, coliforms especially, Escherichia coli, Enterococcus spp., Salmonella spp., etc. are indicators of not

only faecal contamination but also of the possible presence of other bacteria potentially pathogenic to man (Shrivastava, 1974).

With reference to the occurrence and behaviour of several human pathogens in aquatic environs and animals, Salmonella spp. was the most intensely studied human pathogen, because of typhoid fever and the incidence of human non-typhoid salmonellosis. Although the contaminated food remains the most important source of infection, the direct and indirect roles of water in spreading Salmonellosis are now widely appreciated.

Staphylococcus spp. may provide the best index of pollution from direct human contamination since the strains of this species are common inhabitants of human skin and nose. This organism may gain access to food materials by direct human contamination (Liston et al., 1971).

The ecology of Vibrio parahaemolyticus and other Vibrio spp. appears to be closely connected with sea water. Studies with regard to the occurrence of bacteria of public health significance, V.parahaemolyticus in particular, in fish and fishery products and their aquatic environment are rather rare from Indian waters.

The present study deals with the incidence of bacteria of public health significance, viz., *Salmonella* spp., *E. coli*, *Vibrio* spp. and *Staphylococcus aureus* in freshly caught prawns, water and sediment samples collected from the Cochin backwaters.

According to Kauffmann and Edwards (1952), the genus *Salmonella* consisted of three species, *S. typhosa*, *S. choleraesuis* and *S. enterica*, with the latter species serving as a repository for the approximately 1,500 sero types currently in existence. A similar three species system of nomenclature proposed by Ewing (1968) also designated the three species as *S. typhi*, *S. cholerae* and *S. enteritides*. Kauffmann's (1966) scheme is the principal tool on which a food microbiologist relies for *Salmonella* identification.

Buthiaux and Lewis (1953) isolated *Salmonella* from estuarine and marine environments. Free living *Salmonella* forms were isolated by Steiniger (1955) from the coasts of Helgoland. Grunnet and Brest Nielten (1969) reported the *Salmonella* types from the Gulf of Aarthas and compared them with the types obtained from contaminated food products, infected human beings and animals.

Cobet *et al.* (1970) studied the *Salmonella* contamination in cat fish caught 1½ miles away from a sewage treatment plant. The cat fish were found to excrete the pathogens for almost a month into holding tanks, indicating their growth in the intestine of the fish. The studies on the distribution and densities of indicator bacteria and *Salmonella* spp. in the surface waters of Sidney Lanier was made by Warren *et al.* (1978) who correlated the results with water quality. In north Yorkshire, Harbourne *et al.* (1978) reported the occurrence of *Salmonella* in industrial effluent waters and river sites and indicated their sensitivity to antibiotics. Smith *et al.* (1978) have studied the *Salmonella* pollution of shore waters off Lancashire and Cheshire. Liston *et al.* (1971) studied the growth characteristics of food poisoning bacteria including *Salmonella* in sea foods. Besides these, other reports include those of Slanetz *et al.* (1968), Brezenski (1971) and Yosphe-purer and Shuval (1972).

Important studies in India, on *Salmonella* from sea-foods include those of James and Iyer (1972), Joseph *et al.* (1976), Merkar *et al.* (1975) and Iyer *et al.* (1975). The studies of James and Iyer (1972)

on the isolation of *Salmonella* in the presence of high numbers of coliforms in fish, compared different enrichment broths and selective plating media for efficient detection of *Salmonella* from fish. The authors found that Dulicitol, Selinite and Selinite Cystine broths were equally efficient and selective plating media like Xylose Lysine Deoxycholate agar, Brilliant Green, Sulphadiazine agar and Brilliant Green agar were found to be superior in the performance to *Salmonella Shigella* agar and Bismuth sulphite agar. The other studies of Nerkar *et al.* (1975) and Iyer *et al.* (1975) were on the isolation of *Salmonella* in fish and frozen frog legs. With regard to the incidence of *Salmonella* spp. in prawns, sediment and water environment, the literature appears to be scanty.

Important earlier reports on *E. Coli* include those of Beard and Meadowcroft (1935), Carpenter *et al.* (1938), Katchum *et al.* (1949) and Vaccaro *et al.* (1950). Petrilli *et al.* (1974) conducted a survey of the pollution by *E. Coli* in the coastal area of the Tyrrhenion Sea. Warren *et al.* (1978) studied these indicator bacteria in surface waters of lake Sidney Lanier. The reports of Goldrich and Clarke (1966) on the occurrence of coliforms in water and mud signified the potential presence of other enteric pathogens in the same environment.

In India, the studies of Raveendran *et al.* (1977) on faecal contamination of Cherai beach in Kerala indicated that *E. coli* occurred during most of the months both in sea water and in sand. Quantitative studies on the occurance of *E. coli* in sediment and water samples from Cochin backwaters was studied by Gore *et al.* (1979). The authors found that the distribution of *E. coli* both in sediment and in water was erratic and the counts were high during June and October months.

The implications of *Vibrio* as the causative organism of diseased conditions in fish was first recognised by Bergman (1909). David (1927) isolated this organism from a carp and named *Vibrio piscium.* Wells and Zobell (1934) isolated it from diseased marine fish. Hodgkiss and Shewan (1950) isolated an organism from a diseased plaice, which was identified by them as *Pseudomonas ichtyodermis.* Later, Shewan *et al.* (1960) identified the organism as belonging to the genus *Vibrio.*

Fish pathogens belonging to the genus *Vibrio* have been isolated from Cod by Bagge and Bagge (1956). Moshina (1957) has conducted comprehensive studies on

Vibrio isolated from rain-bow trout. The association of chitinoclastic *Vibrios* with zooplankton and their significance in recycling of nutrients have been studied by Kaneko and Colwell (1973).

Zinnaka and carpenter (1965) and Chashi *et al.* (1972) studied the non-cholera *Vibrio* strains producing an enterotoxin, which was antigenically similar to *V. cholerae* enterotoxin and was thought to cause diarrhoea in humans.

Bose and Freda Chandrasekaran (1976) studied the occurrence of *Vibrio parahaemolyticus* in sea water and prawns of Nagapattinam region along the south-east coast of India. Natarajan *et al.* (1979 and 1980) studied the distribution of *Vibrio parahaemolyticus* and allied *Vibrios* in backwater and Mangrove biotypes of Porto Novo. Balakrishnan Nair *et al.* (1980) studied the marine *Vibrios* and related genera from Vellar estuary, Porto Novo.

Earlier works on the detection of *Staphylococcus aureus* in frozen foods and standardization of methods were made by Bergstrom (1955), Barnes (1956 a,b and 1959), Finelli and Ayres (1959) and Raj and Liston (1961) [illegible]. Lekshmy and Pillai (1964) enumerated *Staphylococci* in frozen prawn products and fresh prawns.

Several investigations have been carried out for determining the limits of tolerance pertaining to Staphylococcus aureus in various types of frozen products, particularly pre-cooked or partially cooked food by Fitzgerald (1947), Rayman et al. (1955) and Frechette and Michael (1961).

Warren et al. (1978) studied the occurrence of S. aureus in surface microlayers of fresh water lake and correlated it with water quality and concluded that the bacteria were in highest densities in areas directly influenced by urban and poultry processing wastes. Liston et al. (1971) studied the survival and growth of pathogenic bacteria in sea foods.

The present study is a record of observations on the incidence of Salmonella, E. Coli, Vibrios and Staphylococcus aureus in prawns, water and sediment during monsoon and summer periods.

6.2 Results:

Salmonella:

100 per cent of the water samples tested during monsoon, contained Salmonella, whereas only 25 percent of the samples indicated the presence of Salmonella

during summer (Table 26 and Fig.6). Among the sediment samples, 60.60 percent and 50 percent of the samples indicated the presence of Salmonella during monsoon and summer respectively. 75 percent of the prawn samples contained Salmonella during monsoon and 60 percent during summer.

Escherichia coli:

In the case of E. coli, 25% of the water samples tested were positive. On an average 30 numbers per ml were present during monsoon and 80% of the samples contained 84 cells per ml during summer (Table 27 and Figs.7 and 8).

In the monsoon, sediment samples did not contain E. coli, however during summer 100% of the sediment samples contained 60 cells on an average per gram (Table 27).

29.6 per cent of the prawn samples contained on an average 14 cells per gram during monsoon and 41.6% of the samples contained 148 cells per gram during summer (Table 27). In general, the incidence of Salmonella was more during monsoon when E. coli showed a declining trend.

Vibrios:

During monsoon, on an average, TPC of 1.0×10^5/ml of water was encountered and the Vibrio counts were 10 per ml (in 80% of the samples) and in summer TPC in water samples was 3.7×10^4/ml when Vibrio counts were 20 per ml (in 66.66% of the samples) as shown in Table 28.

In sediment, the TPC during monsoon, on an average, was 2.0×10^4 and Vibrio were 25 cells per gram (in 100% of the samples). During summer months TPC was 7.2×10^4 per gram and Vibrios were 3 per gram (in 60% of the samples). Vibrio counts were higher in water during summer and in sediment during monsoon (Table 28). Head region of prawn samples did not show Vibrios during monsoon as well as summer; however, the tail region contained 19 Vibrios per gram on an average during monsoon (in 43.75% of the samples) but not during summer.

Staphylococcus aureus:

Of the 29 samples of water, 29 samples of sediment and 57 samples of prawns tested, during monsoon and summer, only two samples of prawns during summer months indicated the presence of Staphylococcus aureus (Table 29).

6.3 Discussion:

Salmonella:

The observations on the incidence of Salmonella

in water, sediment and prawns were made during monsoon and summer periods from the Cochin backwaters of south-west coast of India. The results are presented in Table 26.

Percentage incidence of Salmonella in water, sediment and prawn samples varied during monsoon and summer periods. Comparatively, the incidences of Salmonella during monsoon were higher than in summer. Among the samples tested, 100% of the water samples contained Salmonella during monsoon. During summer the percentage incidence of Salmonella was more in prawns. There was a marked variation in the incidence of Salmonella with regard to the season. Since there are no references available with regard to the seasonal variation in Salmonella incidence on prawns, the present data can neither be compared with earlier works nor any defenite conclusions be drawn. However, the presence of Salmonella in water, sediment and prawn samples in both the seasons, although to different degrees, is noteworthy from the point of view of prawn processors and exporters.

The bacteria present in prawns at the time of catch also affect the end products (Iyer et al., 1970). It may be mentioned that monitoring of samples for the presence of indicators and pathogens seasonally would be an added advantage to the prawn processing industry.

Nevertheless, mere incidence of Salmonella in samples may not be hazardous (FAO/WHO, 1977), since the same can be destroyed easily by cooking (MPEDA 1979; Refai, 1979).

Escherichia coli:

E. coli showed seasonal variation in the fresh prawns tested from the Cochin backwaters and so also in the water and sediment from the same aquatic environment.

E. coli was not detected in the sediment samples during monsoon, whereas a mean incidence of 60 cells per gram in all the 15 samples (100%) was recorded during summer period.

All the sediment samples tested during summer contained E. coli. The average count in the sediment samples was 60 cells per gram, which agrees with the results of Gore et al. (1979), where the peak incidence was recorded during December 1975 to May 1976.

Among the water samples tested, only 25% contained E. coli during monsoon and 80% during summer. The average counts were 84 cells per ml during summer and 30 cells per ml during monsoon.

In the present investigation, higher count of E. coli was recorded in water than in sediment, which was similar to the findings of Gore et al. (1979). The percentage incidence was also higher in water than in sediment among the samples tested during monsoon months; whereas the percentage of incidents of E. coli were higher in sediment during summer than in water samples. Rittenberg et al. (1958) found higher counts of E. coli and Coliforms in sediments than in water, while analysing the California marine sewage out fall.

It was seen from the present findings that among the prawn samples tested 29.6 per cent during monsoon and 41.6 percent during summer contained E. coli. The average counts varied from 14 nos. per gram during monsoon to 148 per gram during summer. The overall incidences of E. coli were higher during summer than monsoon.

In the present study, seasonal variation of E. coli abundance was observed, which agrees with the observations of Iyer et al. (1970). However, it differs from the above findings of Iyer et al. (1970), by showing lesser incidence of E. coli during rainy season.

Vibrios:

Among the water, sediment and prawn samples tested during monsoon and summer months, the relationship between total aerobic bacterial count and Vibrio counts could not be established.

Comparatively, TPC of water, prawn head and tail samples were higher during monsoon than during summer showing average counts of 1.0×10^5 per ml, 1.0×10^5 per gram and 8.0×10^4 per gram respectively for the monsoon season (Table 28). The sediment samples showed higher Vibrio counts (25/gram in 100% of the samples)

during monsoon than in summer months (3/gram in 60% of the samples). Water samples contained 20 cells/ml (in 66.66% of the samples) on an average during summer and 10 cells/ml (in 80% of the samples) on an average during monsoon months.

Though there were reports on the incidence of Cholera and similar diseases in man due to cholera and non-cholera Vibrios, the quantitative aspects of bacteria causing illness and the appearance of symptoms of disease need be worked out in detail.

Staphylococcus aureus:

Of the water, sediment and prawn samples tested, water and sediment samples were devoid of Staphylococcus aureus during summer and monsoon. The incidence of Staphylococcus aureus was not noticed in any of the prawn samples tested during monsoon period. However, two of the 33 (6%) prawn samples showed the presence of S. aureus during summer months.

Cann (1977) detected no S. aureus in fresh prawns. However, the present study recorded S. aureus in 2 samples out of 33 during summer and no sample was found to contain

the organism during monsoon months which indicates the contamination of the prawns from the human source. Therefore, strict adherance of the prescribed sanitory practices in the prawn processing industry may eliminate the contamination of the product with *Staphylococcus aureus*.

6.4 Conclusion:

The present study recorded the incidence of bacteria of public health significance in fresh prawns, water and sediment samples from the Cochin backwaters. *Salmonella* and *E. coli* were observed during both monsoon and summer seasons in water, sediment and prawn samples with the latter having higher incidence in summer season. On the other hand, *Vibrio* species were also recorded in both the seasons in water and sediment samples, but its presence in prawn samples was noticed only in monsoon season. These observations will give an idea as to the level of initial contamination of prawns from the fishing grounds caught from Cochin backwaters and may be of great value for the prawn processing and exporting industries.

Table 26: Table showing the incidence of *Salmonella* in water, sediment and prawn samples.

Samples	Monsoon		Summer	
	No. of samples analysed	Percentage of samples containing *Salmonella*	No. of samples	Percentage of samples containing *Salmonella*
Water	14	100	15	25
Sediment	14	60.6	15	50
Prawns	24	75	33	60

Table 27. Table showing the incidence of E. coli in water, sediment and prawn samples.

Samples	Monsoon			Summer		
	No. of samples analysed	% of samples containing E. Coli	Average E.coli cells per ml/ per gram	No. of samples analysed	% of samples containing E.coli	Average E.coli cells per ml/per gram.
Water	16	25	30	15	80	84
Sediment	16	0	0	15	100	60
Prawn	27	29.6	14	24	41.6	148

Table 28: Average total plate count and *Vibrio* counts during monsoon and summer periods

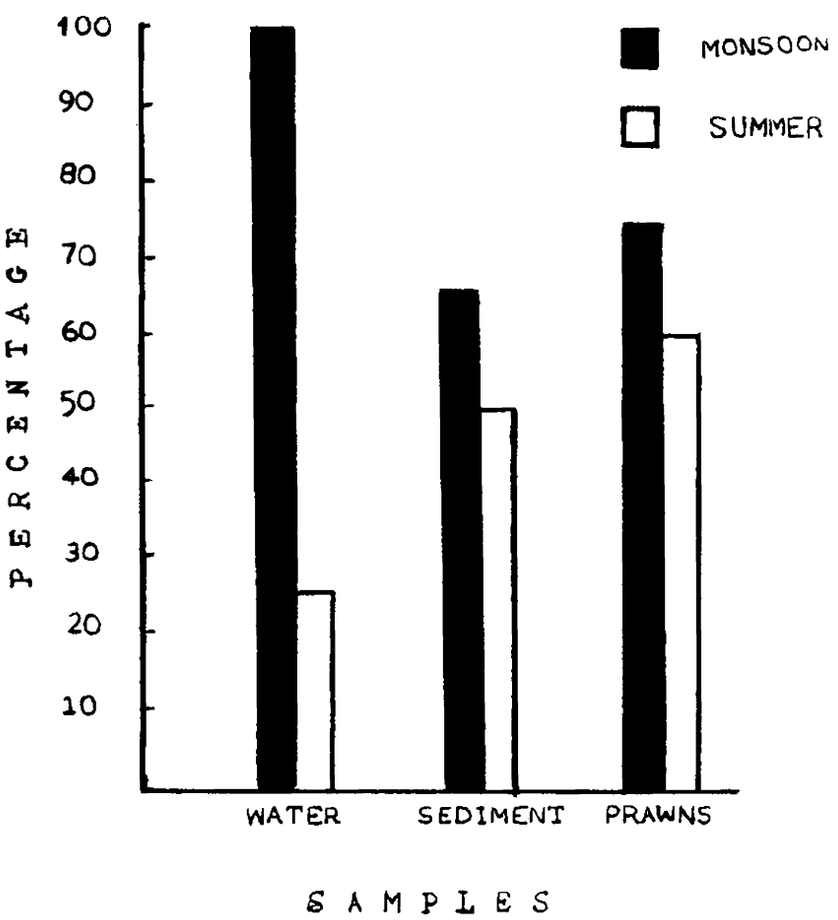
Samples	Monsoon				Summer			
	No. of samples tested	TPC/ gram or ml.	*Vibrio* counts per gram/ml.	Percentage of *Vibrio* positive samples	No. of samples tested	TPC/ gram or ml.	*Vibrio* counts per gram/ml.	Percentage of *Vibrio* positive samples
Water	15	1.0×10^5	10	80.00 (12)	15	3.7×10^4	20	66.66 (10
Sediment	15	2.0×10^4	25	100.00 (15)	15	7.2×10^4	3	60.00 (9)
Metapenaeus dobsoni Head region	16	1.0×10^5	Absent	0 (0)	16	4.0×10^4	Absent	0 (0)
Metapenaeus dobsoni Tail region	16	8.0×10^4	19	43.75 (7)	16	7.0×10^4	Absent	0 (0)

Values in paranthesis indicate the number of samples contained *Vibrio*

Table 29. The number of samples tested and the number of samples showing *Staphylococcus aureus*.

Samples	Monsoon		Summer	
	No. of samples tested	No. of samples containing *S. aureus*	No. of samples tested	No. of samples containing *S. aureus*
Water	14	Absent	15	Absent
Sediment	14	Absent	15	Absent
Prawns	24	Absent	33	2

Fig. 6. The percentage incidence of *Salmonella* among the water, sediment and prawn samples tested during summer and monsoon periods.


100
90
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50
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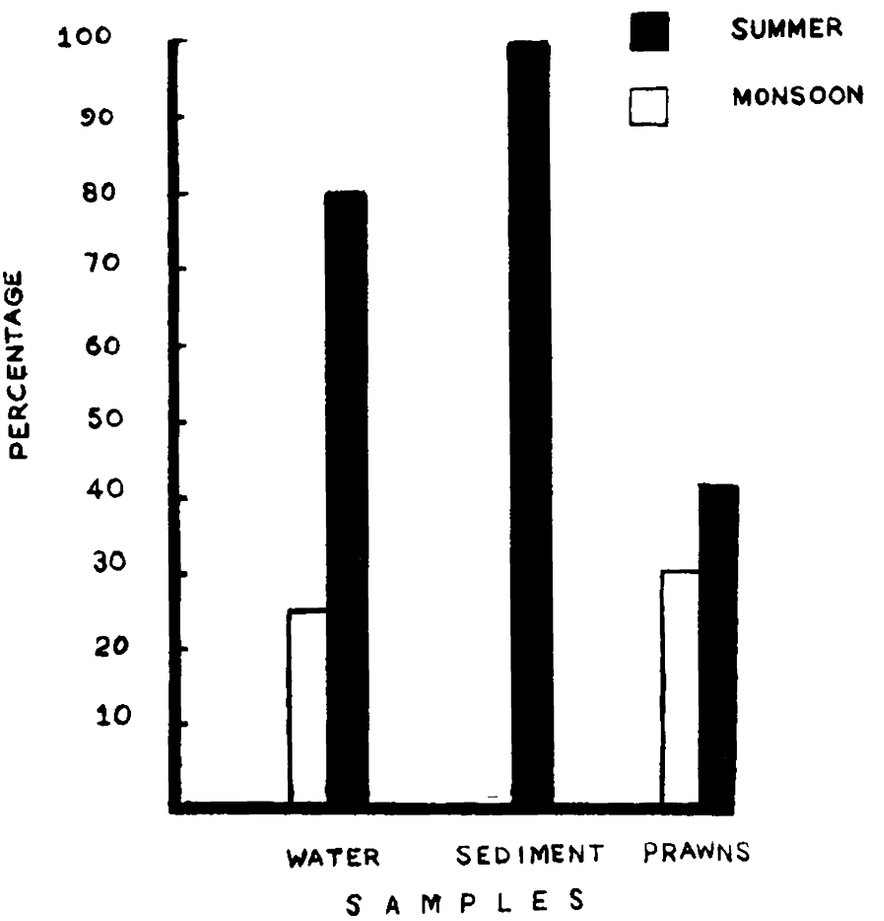
10
PERCENTAGE
MONSOON
SUMMER
WATER
SEDIMENT
PRAWNS
SAMPLES

Fig.7. The percentage incidence of *E. coli* among water, sediment and prawn samples tested during summer and monsoon periods.


SUMMER
MONSOON
100
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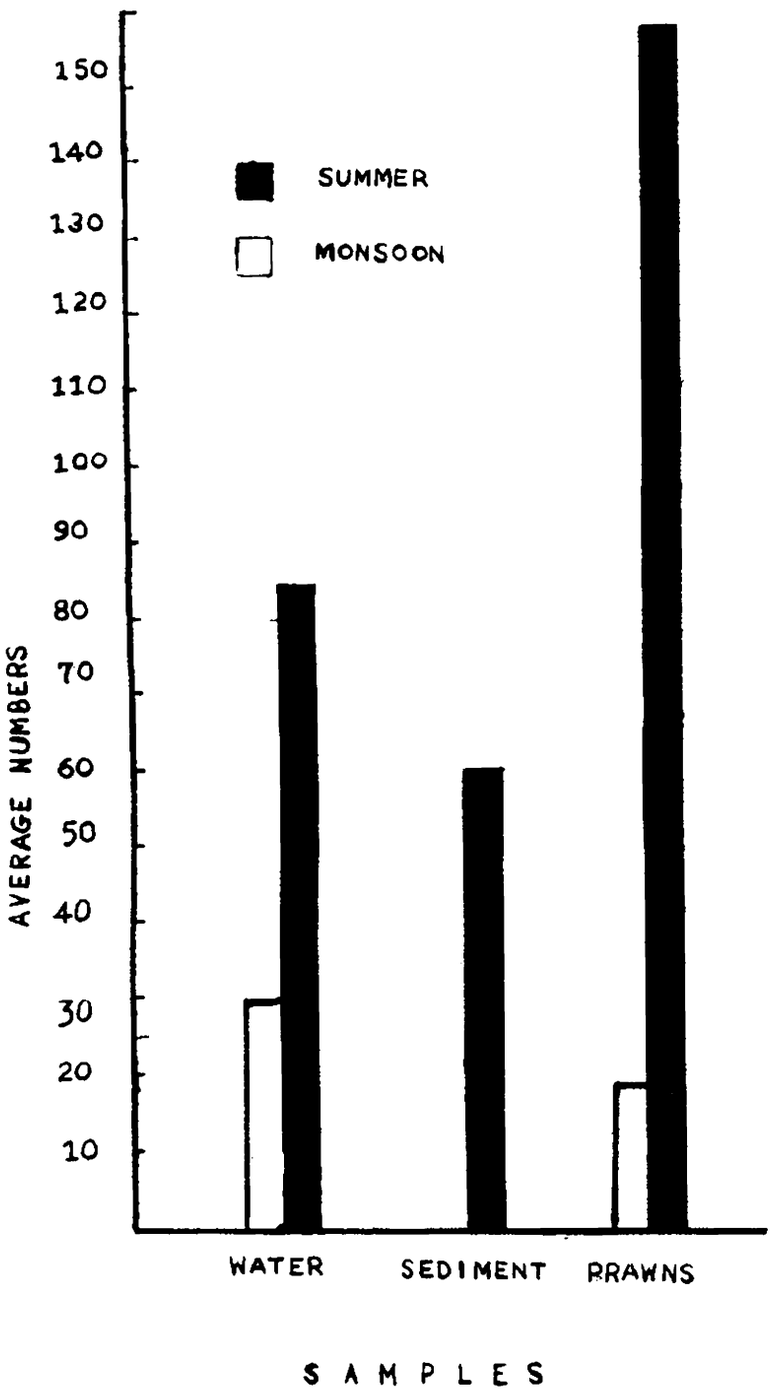
WATER
SEDIMENT
PRAWNS
S A M P L E S

Fig. 8. Average *E. coli* per gram of prawns, sediment and per ml. of water during summer and monsoon periods.


150
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100
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AVERAGE NUMBERS
SUMMER
MONSOON
WATER
SEDIMENT
PRAWNS
SAMPLES

C H A P T E R 7

EFFECT OF ANTIBIOTICS ON THE BACTERIA PRESENT IN PRAWNS

7. EFFECT OF ANTIBIOTICS ON THE BACTERIA PRESENT IN PRAWNS.

7.1 Introduction:

Even though antibiotics kill the bacteria or inhibit their growth, their use in food preservation is very much restricted in recent days. The studies on the chlortetracycline treatment of fish and prawns showed enhanced shelf life under refrigerated storage (Surendran and Gopakumar, 1981). It was believed by Surendran and Gopakumar (1976) that the effectiveness of antibiotics in the preservation of fish depends very much on the composition of the native flora. Also the authors opined that the flora of different types of fishes, though caught from the same waters, may show considerable variations in the generic composition and their behaviour towards antibiotics.

In order to study the role played by the antibiotics in the control of micro-organisms a few studies on the effectiveness of antibiotics on inhibiting the growth of marine bacteria were undertaken (Velankar, 1958; and Surendran and Iyer, 1971 & 1976; Oppenheimer, 1955). Oppenheimer (1955) in his studies on the

effect of marine bacteria on development and hatching of pelagic fish eggs,employed various antibiotics for controlling bacterial growth.

The inhibiting action of aureomycin in different concentrations (2, 5, 20 and 50 ppm) on the growth of number of bacterial population in sea water samples was studied by Velankar (1958). The author found seventy percent of the bacteria were sensitive to lesser concentration (< 5 ppm) of the antibiotics. But,the auther has confined his studies only to the bacterial flora of sea water.

The studies of Surendran and Iyer (1976) determined the nature and the rate of development of tolerance by the bacteria isolated from fresh sardines and prawns to chlortetracycline and indicated reluctance to the development of tolerance in the initial stages but subsequently the rate of build up of tolerance was rapid, until each culture reached its essential upper limit of tolerance and indicated the changes in bacterial flora of mackarel with respect to antibiotic treatment.

The experimental studies of Almeida et al.

(1967) proved that not all antibiotics (penicillin, Streptomycin, Chlortetracycline, Terramycin and Furacmycin) were equally effective in the control of pathogenic bacteria of fishes indicating variations in the effectiveness of antibiotics to specific pathogens.

There were a few studies on the effect of antibiotics on the bacterial flora of fishes as referred above; but, flora of different types of fishes may show considerable variations in their generic distribution and their behaviour towards antibiotics (Surendran and Gopakumar, 1981). Besides these, the studies with regard to bacterial growth inhibition in prawns was rather rare. Therefore, eventhough the use of antibiotics were not permitted in the food industry on health grounds, a preliminary study was done on the effectiveness of different antibiotics (Penicillin, Erythromycin, Oxytetracycline, Chlortetracycline, Chloramphenicol and Streptomycin) at lower concentration of 0.5 I.U., on the growth of bacteria isolated from prawns; so that the information generated would at least be useful in prawn culturing to control the diseases caused by bacteria.

7.2 Results:

The response of bacteria to different antibiotics

(Oxytetracycline, Novobiocin, Chloramphenicol, Streptomycin, Penicillin, Chlortetracycline) are shown in the Table 30 and Fig.9. It can be seen from the table that among the six antibiotics tested, bacterial isolates were least sensitive to penicillin.

Bacterial sensitivity to Chloramphenicol, Streptomycin, Chlortetracycline and Novobiocin was very high. Only one out of 57 isolates was found to be resistant to Chloramphenicol, Streptomycin and Chlortetracycline and four isolates were resistant to Oxytetracycline and three to Novobiocin.

7.3 Discussion:

From the findings presented in the Table 30, it was seen that the bacteria varied in their sensitivity to different antibiotics. Among the isolates tested, a large number of isolates were found to be resistent to penicillin (77.19%). In a similar study by Bose (1971), nearly 50% of the bacterial flora of fresh prawns caught off Tuticorin waters, also showed resistance to penicillin.

The very high sensitiveness of the isolates to chlortetracycline in the present study was in confirmity

with the findings of Velankar (1958), where 70% of the bacterial isolates were sensitive to chlortetracycline at lower concentrations (less than 6 mg/ml). The studies of Tarr et al. (1950, 1952) also indicated that chlortetracycline was more effective in controlling the bacterial growth in fishes and found that the chlortetracycline was more effective than oxytetracycline. The present study also records similar results where higher percentage of isolates (7.02%) is resistant to oxytetracycline than chlortetracycline (1.75%).

From the present observations it was noticed that the use of streptomycin was also found to be effective with regard to the growth of bacteria isolated from prawns. However, it differs from the observations of Tarr (1948) and Tarr and Deas (1948) where it was found that the streptomycin did not give significant protection to the bacterial spoilage of fish.

It was also evident from the results that the antibiotics like Novobiocin and Chloramphenicol were also effective in retarding the bacterial growth but comparatively chloramphenicol appeared to be more effective than Novobiocin.

4 Conclusion:

From the findings it can be concluded that the antibiotics retard the growth of bacteria present in wounds. But the sensitiveness of the isolates varied with different antibiotics. In general, most of the isolates were sensitive to one or other antibiotics.

Table: 30. BACTERIAL SENSITIVILY TO DIFFERENT ANTIBIOTICS

Antibiotics of equal strength (2.5 I.U)	SENSITIVITY IN MILLI METER								Total No.of bacterial isolates tried	Percentage Resistant	Percentage Sensitive
	Up to 5 mm	6 to 10 mm	11 to 15 mm	16 to 20 mm	21 to [illegible] mm	26 to 30 mm	31 to 35 mm	36 to 40 mm			
Oxytetra-cycline	4	13	29	8	3	0	0	0	57	7.02%	92.98%
Novobiocin	3	0	10	31	11	0	2	0	57	5.26%	94.74%
Chloramphe-nicol	1	0	13	28	14	1	0	0	57	1.75%	98.25%
Streptomycin	1	1	24	22	8	1	0	0	57	1.75%	98.25%
Penicillin	44	4	6	1	0	1	0	1	57	77.19%	22.81%
Chlortetra-cycline	1	8	15	25	7	1	0	0	57	1.75%	98.25%

Fig.9. Effect of antibiotics on bacteria.

1. Effect of Novobiocin on the bacterial of isolates of prawns as sensitivity in mm (Zone of inhibition).

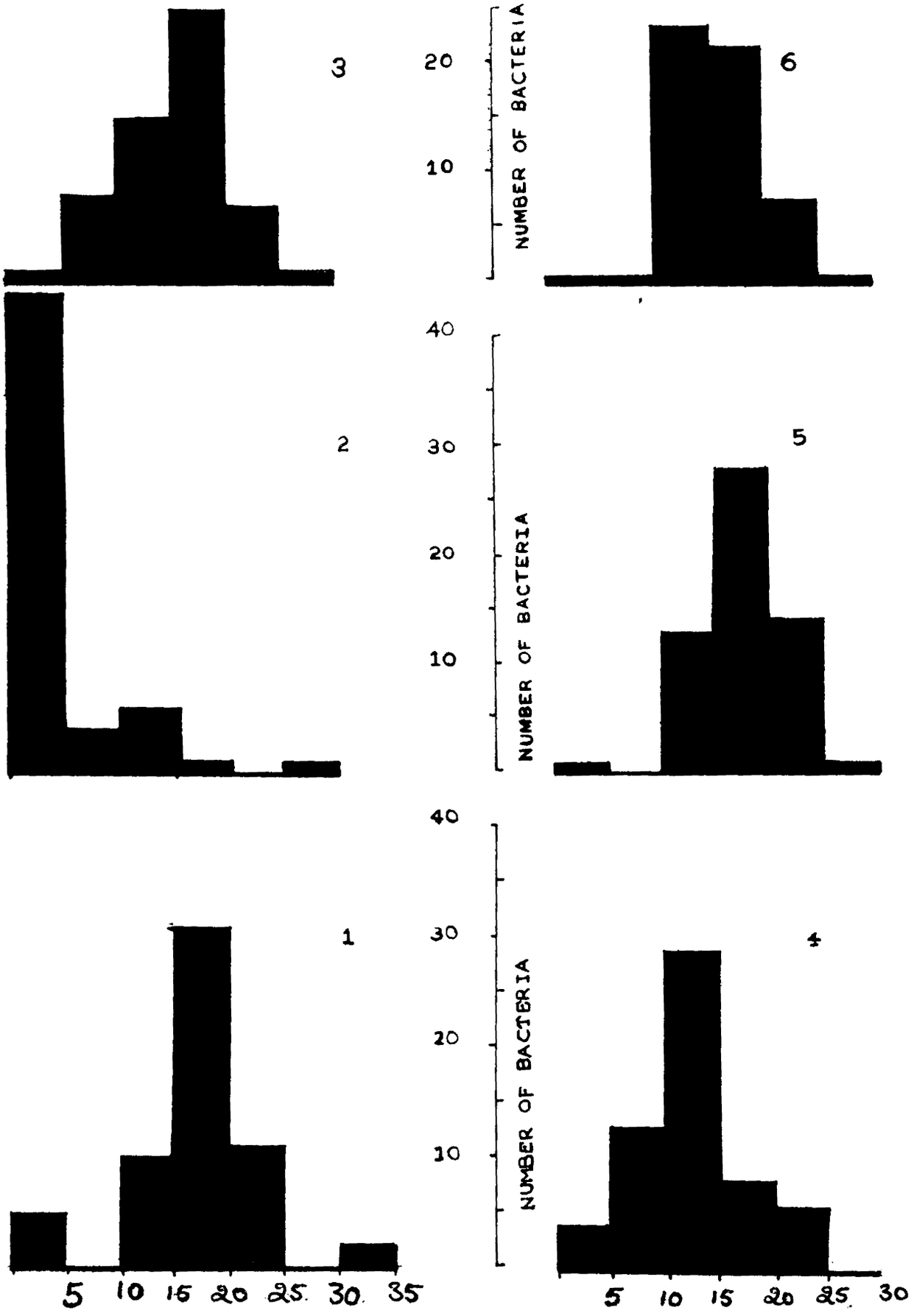
2. Effect of Penicillin on the bacterial isolates of prawns as sensitivity in mm.

3. Effect of Chlortetracycline on the bacterial isolates of prawns as sensitivity in mm.

4. Effect of Oxytetracycline on the bacterial isolates of prawns as sensitivity in mm.

5. Effect of Chloramphenicol on the bacterial isolates of prawns as sensitivity in mm.

6. Effect of Streptomycin on the bacterial isolates of prawns as sensitivity in mm.


3
6
2
5
1
4
NUMBER OF BACTERIA
20
10
40
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5 10 15 20 25 30 35
5 10 15 20 25 30
SENSITIVITY IN MM

CHAPTER 8

BACTERIA AS FOOD FOR THE JUVENILE PRAWNS

8. BACTERIA AS FOOD FOR THE JUVENILE PRAWNS

8.1 Introduction:

The previous chapters in the present study deals with the quantitative and qualitative aspects of bacteria with main emphasis to the quality of prawns. However, there are reports available that the bacteria can serve as food for the crustaceans and molluscs. Bacteria serving as food for aquatic organisms had already been indicated by many workers such as Cälic (1960), Zhukova (1963) and Krishnamurthy et al. (1968). It was reported by Krishnamurthy et al. (1968) that inspite of high rate of multiplication, the bacteria seem to maintain a constant level of density presumably indicating that the bacteria may serve directly as food for the aquatic organisms in the sea. Despite the above features there were many experimental evidences indicating bacteria as food.

The studies of Gayevskaya (1938) and Rodina (1946) proved that the micro-organisms form a necessary component of the nutrients of the aquatic inverterbrates. Gerburov (1946) linked the cellulose bacteria in the food chain in fresh water reservoirs. Romanenko (1966) compared the bacterial productivity with that

phytoplankton. Sorokin (1969) showed that the bacterioplankton can satisfy the nutritional requirements of aquatic invertebrates at their natural concentrations in fresh water reservoirs and assimilability of bacteria was not lower than that of planktonic algae.

Sorokin et al. (1970) reported that filter feeders, Mysidia and Paracalanus and Acartia are able to feed on dispersely distributed bacteria. According to their findings the natural bacterioplankton was consumed by 30 - 40% of bacterial cells on an aggregate, whose diameter exceeds 4 microns and were consumed with the same intensity as phytoplankton not only by copepods but also by coarse filter feeders. However, with regard to the work on bacteria as food for juveniles of prawns, the information available was extremely scanty.

The experimental studies of Cvilic (1960) revealed the role of bacteria as food for the free living crustaceans like copepods and the lobster larvae. As the juveniles of prawns are also of similar habitat as that of the crustaceans referred above and also feeds on microparticulate materials, it was logically presumed that bacteria may also serve as food for juvenile prawns.

As there were no studies indicating this aspect, it was felt desirable to make an attempt in studying the possible role of bacteria as food for juvenile prawns. Therefore, experimental studies were conducted on the juveniles of M. dobsoni with different suspensions of aquatic bacteria.

8.2 Results:

100% survival of M. dobsoni juveniles were recorded in the beakers containing different bacteria namely Pseudomonas spp., Acinetobacter spp., and Aeromonas spp. over a period of 5 days.

Since there was 100% survival of the prawn juveniles in the beakers containing Pseudomonas, Acinetobacter and Aeromonas during the experimental period, the growth of the juveniles were recorded as increase in body weight, which is presented in the table 31. It was seen from the table that there was increase in the body weight by 2.5 mgs. (0.17%) in the case of Pseudomonas, 2.0 mgs. (0.14%) in the case of Acinetobacter and 1.8 mgs. (0.12%) in the case of Aeromonas as food for the juveniles, at the end of 5th day. But, the control indicated a decrease in body weight by 40 mgs. (2.73%) in 5 days.

8.3 Discussion:

It was seen from the table 31 that 100% survival

was recorded in the Metapenaeus dobsoni juveniles in the presence of Pseudomonas spp. Acinetobacter spp. and Aeromonas spp. Further, it was observed that there was an increase in the body weight of M. dobsoni juveniles by 2.5 mg. (0.17%) in the case of Pseudomonas spp. as food. The studies of Sorokin et al. (1970) using Pseudomonas spp. as food, found that the bacteria served as food for Penilia, Paracalanus and Acartia. Similar studies by Yasuda and Taga (1980) also indicated that Pseudomonas strains acted as food for Brachionus Plicatilis. As in the case of Pseudomonas spp., Acinetobacter spp. and Aeromonas spp. also served as food for M. dobsoni juveniles in the present study; indicating an increase in the body weight by 0.14% and 0.12% respectively. The studies of Mary et al. (1975) also indicated that the gastro intestinal microflora may play a role in nutrition and growth of fishes. Similar studies by Krishnamurthy et al. (1968) also inferred that the bacteria served as food for bivalves such as Area indequivalvis and Donax cuneatus under laboratory conditions. Apart from this, the studies of Cvilc (1960) also established that the bacteria played an important role in the feeding of copepods and lobster larvae.

TABLE 31. GROWTH RATE OF *M. DOBSONI* JUVENILES IN THE PRESENCE OF BACTERIA

Sl.No.	Name of the Bacteria	No. of *M. dobsoni* juveniles used	Initial wt. of the juveniles (gms.)	Final wt. of the juveniles (gms.)	Weight difference (gms)	Percentage difference in weight
1.	*Pseudomonas* spp.	5	1.4700	1.4725	+ 0.0025	+ 0.17
2.	*Acinetobacter* spp.	5	1.4750	1.4770	+ 0.0020	+ 0.14
3.	*Aeromonas* spp.	5	1.4520	1.4538	+ 0.0018	+ 0.12
4.	Control (with no bacteria)	5	1.4650	1.4250	- 0.04	(-)2.73

\+ indicates increase in weight.

\- indicates decrease in weight.

CHAPTER 9

SUMMARY

9. SUMMARY

The present study dealt with the detailed aspects of bacterial flora of a few selected commercially important species of prawns and their environment (water and sediment) from the Cochin backwaters. Quantitative and Qualitative studies of bacteria, their reaction to different biochemical tests, effect of different incubation temperature on the bacterial load, the bacteria of public health significance, effect of antibiotics on the bacteria and the utility of bacteria as food for the juvenile prawns were, studied and presented in different chapters.

The studies on the bacterial load of prawns and their environment revealed that the aerobic bacterial load of prawns from Cochin backwaters varied from 10^3 to 10^6 per gram while that of water and sediment varied from 10^3 to 10^5 per ml and 10^3 to 10^6 per gram respectively. It was observed that the bacterial load was fluctuating depending on the season.

The bacterial load in the head region of *P. indicus* varied from 1.0×10^3 to 1.7×10^6 per gram, the gut contains 2.5×10^3 to 2.0×10^6 per gram and the tail region contains 1.0×10^3 to 2.8×10^6 per gram. The

imum counts were noticed during monsoon and the
imum during summer seasons.

Metapenaeus dobsoni showed 1.1×10^4 to 9.0×10^5 nts per gram in the head region, 1.3×10^4 to 1.3×10^6 the gut region and 3.0×10^3 to 2.3×10^6 per gram in tail region. As in P. indicus, the counts were less ing summer and were more during monsoon seasons.

Comparatively less bacterial load was recorded in case of Macrobrachium idella which was on an average, $\times 10^4$ per gram.

The sediment contains counts between 3.0×10^3 3.2×10^6 per gram. The water samples contain bacterial nt between 2.0×10^3 and 8.9×10^5 per ml.

Eventhough the bacterial load in different cies of prawns (P. indicus and M. dobsoni) showed minor iations, the difference in the bacterial count between ions was not significant at 5% level. However, the iation was significant between months at 1% level.

In view of the findings, it may be mentioned that prawns from Cochin backwaters (tropical waters) contain bacteria on higher side especially during monsoon months. Hence, it may be suggested that strict sanitary and quality control measures have to be adopted from the stage of harvest to the end product, to ensure the required quality standards of prawns.

Qualitative studies on the bacteria isolated from prawns from Cochin backwaters showed that most of the characteristics of the bacteria were typical to marine environment. There was significantly high percentage of Gram negative bacteria belonging to Pseudomonas and Acinetobacter genera. The other genera present being Moraxella, Bacillus, Micrococcus, Corynebacterium, Flavobacterium, Vibrio, Aeromonas, Enterobacter and Alcaligens.

Gelatin liquefaction was observed among 71% of the isolates tested, indicating their spoilage potential. Indole production was noticed in 98.05% of the isolates and H_2S in 23.92% pointing to the possible putrefactive types of spoilage. The presence of lactose fermenters indicated faecal contamination from human and other source.

It was observed from the studies on the bacterial load in relation to incubation temperatures that prawn samples showed lower counts than water and sediment samples at all incubation temperatures, while in the case of water and sediment the counts were significantly higher at 37°C and at 25 ± 2°C and lower at 57°C, indicating the presence of more mesophiles and Psychrophiles than thermophiles. At 8°C bacterial growth was found to be absent even at 48 hours of incubation but the same increased steadily afterwards. From these observations, it could be stated that any change in the temperature during handling and processing is likely to alter the bacterial growth considerably.

Among the bacteria of Public health significance Salmonella was found in 75% of the prawn samples during monsoon months and 60% in summer. 100% of water samples contain Salmonella during monsoon and 25% during summer months. The sediment samples indicated 60.6% and 50% of the samples positive for Salmonella during monsoon and summer months respectively. E. Coli was found in 29.6% of prawn samples with an average count of 14 cells per gram during monsoon and 41.6% with an average count of 148 cells per gram during summer.

25% of the water samples contain E. Coli during monsoon and 80% during summer months with an average

count of 30 and 84 cells per gram respectively. The sediment samples did not indicate the presence of E. coli during monsoon, but 100% of the samples analysed during summer indicated E. coli with an average count of 60 cells per gram. In general, the occurrence of Salmonella was more during monsoon when E. coli was less in percentage and vice versa, in summer. Vibrio counts were determined in relation to total aerobic counts in the present study.

The antibiotic sensitivity tests revealed that the bacteria were more sensitive to chloramphenicol, streptomycin and chlortetracyclin and least to penicillin of same strength (2.5 I.U.).

The studies on the utility of bacteria as food for prawn juveniles indicated increased body weight in 5 days. However, further studies on the subject would be an added advantage for the development of feed for juvenile prawns.

The present study showed that the prawns caught in the Cochin backwaters contain bacterial counts which were well within the acceptable limits. The species

composition includes mainly spoilage bacteria, which can be destroyed by the use of antibiotics. A few bacteria also act as food for the prawn juveniles indicating increase in body weight under laboratory conditions.

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Addendum:-

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* Not referred to in original.