STUDIES ON THE BACTERIA ASSOCIATED WITH *PENAEUS INDICUS* IN A CULTURE SYSTEM

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By

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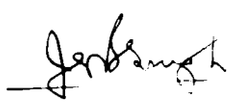
DECLARATION

I hereby declare that this thesis entitled 'Studies on the bacteria associated with *Penaeus indicus* in a culture system' has not previously formed the basis of the award of any degree, diploma or associateship in any U[redacted]rsity.



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October, 1986.

(I.S. BRIGHT SINGH)

CERTIFICATE

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

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ABBREVIATIONS

MTL	:	Mean total length
MW	:	Mean width
MFS	:	Mean length of longest pair of furcal setae
MCL	:	Mean carapace length
A1	:	Antennule
A2	:	Antenna
Mx1	:	Maxillule
Mx2	:	Maxilla
MxP	:	Maxilliped
MxP1	:	First maxilliped
MxP2	:	Second maxilliped
Md	:	Mandible

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3 BACTERIA ASSOCIATED WITH JUVENILES AND ADULT IN A CULTURE POND

INTRODUCTION

1 INTRODUCTION

1.1 PREFACE

Ocean persists as a rich and renewable source of cheap protein for the whole world. Among the prawns/shrimps landed from the Indian Ocean and her backwaters, more than 90% are exported to affluent countries. The overall export of marine products effected from India during 1985-1986 rose to an all time high of Rs 398.00 crores, of which frozen shrimp constituted 83% in value, and 60% in terms of quantity (Table 1).

The Indian white prawn Penaeus indicus, constitutes the major portion of the frozen shrimps exported from India every year. This was an out come of the widespread operation of trawlers and other mechanised crafts and gears. However a couple of years of continuous harvest resulted in a steady decline in the size of the prawns as well as in the quantity with respect to the effort put on. As per a study conducted by the Central Marine Fisheries Research Institute, there was a 40% reduction in catch per unit effort (CPUE) during 1979 for penaeids, of which 63.3% was contributed by P. indicus (CMFRI, 1980).

It is highly imperative to strife for perpetuating the position of India in the International markets. The only way left before us is to opt for culturing these animals in man made impoundments. India is estimated to possess along its coasts a total area of 2 million hectares suitable for brackishwater fish farming (Jhingran, 1982). But, only very little of this vast area (West Bengal, Kerala and in parts of Karnataka) is currently utilised.

Till recently, prawn 'fry' (advanced post larvae), were collected from their wild habitat and were grown in enclosed areas. This practice, in spite of being cumbersome, the non-availability of sufficient seeds is the major constraint for intensive prawn culture. To overcome this problem , hatcheries were set up with a view to supply any number of quality seeds with the least effort and expense.

Two penaeid prawns (Penaeus monodon and P. indicus) are most suitable for large scale culture in India, mainly due to their wide distribution along with Indian coastlines. Although P. monodon grows to a larger size than P. indicus and is at present preferred in India for prawn farming, it is believed that white prawn P. indicus is equally a good candidate species when compared to many other penaeids. For example, it is hardy, its larval and post larval stages

are short and survival rates are good (CMFRI, 1985). Also P. indicus adapts well to both extensive and intensive culture systems (Fish Farming International, 1986, 13(7):15).

The first commercial shrimp hatchery in the country was set up at Azhicode in 1979, adopting Hameed Ali's new system of larval rearing (Alikunhi, 1980). During 1979-1980 in this hatchery an overall survival of 43.8% of the post larvae was observed. Much of the mortality occurred during the mysis and post larval stages (Alikunhi, 1980). Further, while these animals are reared in grow out ponds from post larvae to marketable size, many instances of mortality have been observed (unpublished).

When animals are grown in confinement, many of the physical, chemical and biological factors prevailing in the culture system interfere singularly or synergestically with their health, more than what can happen in the open ocean or backwater. Lack of adequate quantity of oxygen, change of pH and temperature, increased unionized ammonia, hydrocyanic acid, heavy metals, phenol, pesticide, vitamin defficiency etc. and pathogenic protozoans, bacteria, fungi and viruses are reported as the biological factors (Conroy and Herman, 1970; Wedmeyer et al. 1976) hampering

the health of the animals. In most of the instances a disease outbreak is a result of an interaction between the host and the pathogens, and the physico-chemical factors of the environment (Wedmeyer *et al*. 1976). In the case of penaeids with increasing density and production level, diseases rapidly appear. Records of fungal infections (*Legenidium* and *Sieolpidiune*), bacterial attacks (*Vibrio* and *Aeromonas*) and Virus (Baculovirus) are frequent in hatcheries. In grow out phase, fungus (*Fusarium*), parasitic protozoa (*Microsporidia*), various bacterial infections (*Vibrio*, *Aeromonas*) and diseases often due to nutritional, environmental or toxic problems (ascorbic acid deficiency, cramped tail, muscle necrosis, toxic blue green algae, black gill disease) are recorded (Couch, 1978; Johnson, 1978; Aquacop, 1985). Further an infectious hypodermal and haematopoitic necrosis (IHHN) due to a virus has also been recently discovered with the juvenile stage of *P. stylirostris* leading to high mortality (Aquacop, 1985). Pathogenic bacteria claim thus much importance in a culture system, which are invariably heterotrophs. However all of them are not strict pathogens. In turn they are the principal agents taking part in the mineralisation and regeneration of nutrients (Leadbetter and Pointexter, 1985). Detrital food chains, based on the use of manures and compost have been used in aquaculture for centuries. Heterotrophic

bacteria convert organic detritus to proteins and thus constitute an important food source in ponds (Moriarty, 1985). However, inadequate nutrition, poor water quality and physico-chemical factors may exert a stress and can render prawns more susceptible to infections. Heterotrophic bacteria makes advantage of this situation and may pose health hazards to prawns.

Total heterotrophic bacteria in a culture system remains thus as a decisive component which can make the system either efficient or inefficient. The present study is aimed at gathering information on the total heterotrophic bacteria (THB) associated with P. indicus, with special reference to eggs, nauplii, zoeae, mysis, and post larvae in hatchery, and juveniles and adults in culture pond. Simultaneously, THB associated with P. indicus in its natural habitat also is studied for comparison. It is envisaged that this information will be highly useful for modifying the existing hatchery and pond management practices.

1.2 REVIEW OF LITERATURE

1.2.1 Bacteria associated with eggs and larvae of prawns/shrimps in hatchery

Information on bacteria associated with the early stages of the penaeid shrimps/prawns are meagre. Yasuda and Kitao (1980) studied the bacterial flora in the digestive tract of prawns Penaeus japonicus in their early stages. Number of bacteria ($1.8 \times 10^5.ml^{-1}$) at the zoea stage started declining from the end of mysis stage to post larvae. In the digestive tract of the zoea stage, which first started eating bacteria, Vibrio sp. was the dominant genus. Through out the larval stages, the generic composition of bacteria in the intestine and in the water of the culture tanks was similar, implying that the intestinal flora was influenced by external flora through out the larval stages.

Lewis et al. (1982) studied on the aggregation of penaeid shrimp larvae due to microbial epibionts. Quantitatively no difference was observed in the bacterial flora between the samples where aggregation and no aggregation were observed. But qualitatively remarkable difference was observed. Pseudomonas piscicida, Aeromonas formicans or Flavobacterium sp. were dominant in

both water and larval samples when clumping was observed. Aggregation of larvae was observed after 24 hrs incubation in suspensions that received sufficient bacteria (10^4 cells$\cdot$ml^{-1}) of P. piscicida or Flavobacterium sp. The majority of aggregates contained viable larvae. Larvae exposed to 10^5 or greater of A. formicans cells$\cdot$ml^{-1} died within 10 hrs where an aggregation of larvae was not observed. At the same time when the larvae were exposed to 10^6 cells$\cdot$ml^{-1} or greater P. piscicida, A. formicans, Flavobacterium sp. or Vibrio sp., mortality of the larvae was observed within 24 hrs.

Anderson and Stephens (1969) and Bauer (1979) observed appendages of crustaceans often populated with high concentrations of bacteria that are periodically cropped and served as food for the host. Microbial epibionts multiply over the surface of the egg membrane or larval gill membranes, either directly including respiratory surfaces or setting the stage for growth of filamentous bacteria or sessile protozoa and ultimately death of the host. Lewis et al. (1982) illustrated aggregation as another consequence of epibiotic fouling. Under experimental conditions, relatively high numbers of bacteria (10^4 cells$\cdot$ml^{-1}) were required to cause aggregation. Water quality parameters did not appear

unusual. Thus factors which allowed the ubiquitous organisms involved in the episode to become established and bring about the aggregation phenomenon remain unknown. Antibiotics and other drugs were found to diminish the viability and or activity of the organism even when the organisms were present at relatively high numbers.

From the work of Gilmour et al. (1975) it is observed that spore bearing Gram-positive rods of the genus Bacillus were the only bacterial inhabitants of Artemia cysts. Austin and Allen (1981,1982) showed that there is a greater species diversity than what had been recognised previously, with representatives of Bacillus, Micrococcus, Staphylococcus and Vibrio. It is understandable that endospore producing bacilli could survive for long periods in the relative dessication as might be experienced in a vacuum sealed can of cysts. Pigmented bacteria may have an ecological advantage in so far as they are capable of resisting the harmful effects of desication (Grinsted and Lacey, 1973). Colorni (1985) made studies on the bacterial flora of giant prawn Macrobrachium rosenbergii larvae fed with Artemia salina nauplii and could not establish that A. salina nauplii were a possible vector of a bacterial overload. On the contrary the intestinal flora of the prawn larvae appeared to be clearly different from the bacterial flora associated with

brine shrimp nauplii. Furthermore only one of the strains used to contaminate the food and water of the larvae upset the composition of the pre-existing intestinal flora. This indicate the relative capacity of the larvae to maintain an autochthonous homeostatic intestinal flora quite independent of the bacteria the larvae ingested. The difficulty which was met in infecting shrimps with vibrios was experienced by other workers also and this suggested a lack of primary pathogenicity and a close link of bacterial pathogenicity with environmental conditions. Although the cause of mortality of larvae were not established, during his investigation, the evidence presented here indicates that direct involvement of bacteria, protozoa and fungus observed cannot be ruled out.

1.2.2 Bacteria associated with prawns/shrimps in culture pond

Studies on the microflora of Gulf of Mexico and pond reared shrimp revealed low microbial load of pond shrimp than Gulf shrimp, P. azeticus (Vanderzant et al. 1970,1971). Bacillus, Lactobacillus, Coryneforms and Flavobacterium were the important genera in the pond reared shrimp. Pseudomonas contained 2 to 2.6% only. The bacterial flora of pond water usually was dominated by Coryneform bacteria and species of Flavobacterium, Moraxella and Bacillus.

Fluctuations in generic composition of bacteria was also recorded in water as well as prawns and this was due to the environmental conditions, handling to some extent and methodology (Harrison et al. 1969; Vanderzant et al. 1971).

Christopher et al. (1978) while working on the microbial flora of pond reared shrimp P. stylirostris, P. vannami and P. setiferus could not establish a significant relationship between the changes in the number and types of microorganisms in pond reared shrimp and pond water characters. They also found that Vibrio sp. were the predominant bacteria from freshly harvested pond shrimp and water. Vanderzant et al. (1973) found that freshly harvested penaeids consisted of Aeromonas, Pseudomonas and Vibrio. Vanderzant and Nickelson (1973) also observed V. parahaemolyticus from Gulf coast shrimp. P. azetecus died within 3 hrs of inoculation with 10^4-10^5 cells.ml^{-1} of V. parahaemolyticus.

Further importance of vibrios causing potentially serious disease problem during the culture of penaeid shrimps were reported by Sinderman (1970), Overstreet (1973), Lightner (1975) and Couch (1978). Pathogenenic vibrios like V. alginolyticus, V. anguillarum, V. parahaemolyticus

and Aeromonas and Pseudomonas species have been isolated from shrimp. But V. alginolyticus was the most prevalent organism isolated from shrimp that showed clinical signs of bacterimia (Lightner, 1975). Although reports on various species of vibrios associated with disease of shrimps are extensive (Sneizko and Taylor, 1947; Rosen, 1970; Bauman et al. 1971; Barkate, 1972; Cook and Lofton, 1973; Lightner, 1975,1985; Overstreet, 1973; Sinderman, 1974, Delves-Broughton and Poupard, 1975), their role as primary pathogen is doubtful. However they remain as a major problem in pond cultivation of shrimp.

The dominance of Gram-negative bacteria in the digestive tract of pond reared P. indicus in general and the frequent occurrence of Vibrio, Pseudomonas and Bacillus in particular reported by Palaniappan (1982) suggested that they form the native microflora of the digestive tract. At the same time Micrococcus, Aeromonas and Flavobacterium - Cytophaga group were found to be lysed upon by the digestive activity. Apart from these reports there is no systematic work available on the bacteriology of P. indicus reared in ponds.

1.2.3 Bacteria associated with prawns/shrimps in natural environment

According to the review of Chandrasekaran (1985)

earlier works on prawn bacteriology mostly relate to spoilage. Quantitative and qualitative studies on bacteria associated with live prawns are confined to temperate waters. The total viable bacterial counts on temperate water shrimps were between 10^3 and $10^7.g^{-1}$ (Cobb et al 1976; Cann, 1977). The bacterial flora of tropical species of prawns differ from that of cold water species due to an environmental temperature 25-30°C (Cann, 1971; Newell, 1973). Initial total viable counts in marine prawns were 10^3-$10^7.g^{-1}$ (Pillai et al. 1961,1965; Velanker, 1961; Karthiayani and Iyer, 1975; Surendran et al. 1983; Pradeep, 1986). However works on the bacteriology of estuarine prawns are limited to Ivy Thomas, 1982; ICAR Project Report 1983; Chandrasekaran et al 1984 and Chandrasekaran, 1985. It was found that the bacterial population varied from 10^6-$10^7.cm^2$ on the body surface, 10^6-$10^8.g^{-1}$ in gill and 10^6-$10^8.g^{-1}$ in intestinal content of P. indicus (ICAR Project Report 1983).

The major groups of bacteria associated with prawns are Micrococcus, Corynebacterium, Moraxella, Acinetobacter, Pseudomonas and to a lesser extent Flavobacterium/Cytophaga and Bacillus. The percentage composition varies widely. Williams et al. (1952) found that the main group in Mexican Gulf shrimps were (as a whole) Acinetobacter, Micrococcus, Pseudomonas and Bacillus. Harrison and Lee (1969) observed predominance of Acinetobacter-Moraxella in Pacific shrimp.

The freshly landed shrimp from North sea (Pandalus borealis, P. montagui and Crangon crangon) largely contained either Achromobacter sp. or Gram-positive Micrococcus and Coryneforms (Early, 1967). Gram-negative organisms of the Achromobacter and Pseudomonas groups were significantly present in cold water types but were of lower incidence in the tropical prawns (Penaeus spp., Metapenaeus spp.) from Gulf of Thailand and Parapenaeopsis spp. from Straits of Malacca (Cann, 1977).

Sreenivasan (1959) reported that Indian prawns contained predominantly Micrococcus and Corynebacterium. The predominance of Gram-negative, asporogenous, rod like organisms belonging to Pseudomonas, Achromobacter and Vibrio associated with marine animals (sardines, prawns, lobsters and seer fish) were reported by Karthiayani and Iyer, 1975. Similar dominance of Gram-negative bacteria was also found in P. indicus and P. monodon of Cochin backwater (ICAR Project Report 1983). Species of Vibrio were found to be dominant and other genera commonly present were Pseudomonas, Aeromonas, Acinetobacter, Alcaligenes, members of Enterobacteriaceae, Moraxella, Micrococcus, Bacillus and Coryneform group.

It is quite obvious from the above review that there is a big gap in our knowledge on the bacterial flora

associated with prawn at various developmental stages (eggs - adults). Hence the present study was undertaken to fill up the lacuna.

1.3 RESEARCH APPROACH

The present work was programmed to investigate the heterotrophic bacteria associated with various developmental stages viz. eggs, nauplii, zoeae, mysis, post larvae, juveniles and adults of P. indicus in a culture system (eggs to post larvae in a hatchery and juveniles to adults in a culture pond). Similarly, THB associated with eggs to adults in a natural habitat was also aimed for comparison. In the natural environment, the hatchings of eggs to nauplii and their metamorphosis to zoeae and mysis are taking place in the sea and the stages of post larvae to adult pass through in the estuary. It is impractical to collect sufficient eggs, nauplii, zoeae and mysis from the sea for analysis and hence, the investigation is restricted to the latter stages viz. post larvae, juveniles and adults obtained from the estuarine environment.

Eventhough it was intended to release the advanced post larvae from hatchery to culture pond in order to continue the investigation from eggs to adults in a culture system, sufficient number of seeds from a single brood was not

available at the Regional Shrimp Hatchery, Azhicode . So, the juveniles were collected from the natural environment, released into the culture pond and the bacteriological changes were studied upto harvest.

The present investigation was thus restricted to
a) the samples of eggs to post larvae in the hatchery,
b) juveniles to adults in the culture pond and
c) post larvae to adults in the natural environment.
The overall aim of the study was to gather information regarding the THB associated with P. indicus in order to build up a microbiological back up for its culture system. The main objectives of the study are the following:

1. To estimate quantitatively and qualitatively the total heterotrophic bacteria (THB) associated with the eggs and larvae in hatchery, the juveniles and adults in culture pond, and the post larvae, juveniles and adults in natural habitat.

2. To study the impact of environmental factors such as temperature, pH, salinity, dissolved oxygen, and nutrients on the bacterial population as well as their generic shift.

3. To study the impact of environmental factors as well as the microbiological parameters on the survival and metamorphosis of eggs and larvae in hatchery.

4. To evaluate the ability of the isolates to elaborate hydrolytic enzymes in order to assess their ecological niche.

5. To study the effect of NaCl, pH and temperature on the growth pattern of the isolates to assess the suitability of the environment for the multiplication and colonization of bacteria.

Table 1. Share of shrimps in Marine Products Exports from India

(1973-1985)

Q : Quantity in Tonnes
V : Value in Rs. '000

Year		Shrimps								Total Marine Products Exports
		Frozen		Canned		Dried		Total		
1973	Q :	35,895	(73. 6%)	2,199	(4. 5%)	284	(0. 6%)	38.378	(78. 7%)	48,785
	V :	6,58.122	(82. 7%)	52,362	(6. 6%)	3,230	(0. 4%)	7,13,721	(89. 7%)	7,95,763
1974	Q :	34,361	(73. 7%)	1,516	(8. 3%)	116	(0. 2%)	35,993	(77. 2%)	46,629
	V :	6,37,326	(83. 5%)	47,842	(6. 3%)	1,426	(0. 2%)	6,86,594	(90. 0%)	7,63,126
1975	Q :	46,831	(87. 7%)	261	(0. 5%)	99	(0. 2%)	47,191	(88. 4%)	53,412
	V :	9,43,386	(89. 9%)	5,999	(0. 6%)	1,132	(0. 1%)	9,50,517	(90. 6%)	10,49,063
1976	Q :	47,952	(77. 2%)	102	(0. 2%)	36	(*)	48,090	(77. 4%)	62,151
	V :	16,06,499	(89. 3%)	3,935	(0. 2%)	385	(*)	16,10,819	(89. 6%)	17,98.620
1977	Q :	47,239	(72.72%)	128	(0.20%)	235	(0.36%)	47,602	(73.28%)	64,964
	V :	15,62.206	(86.92%)	5,221	(0.29%)	1.711	(0.10%)	15,69,138	(87.31%)	17,97,374
1978	Q :	51,223	(65.07%)	204	(0.03%)	4	(*)	51.431	(66.00%)	77,946
	V :	17,90,644	(84.40%)	9,149	(0.43%)	75	(*)	17,99,868	(84.83%)	21,21,574
1979	Q :	53,511	(58.05%)	139	(0.15%)	19	(0.02%)	53,669	(58.22%)	92,184
	V :	22,31,273	(85.15%)	6,428	(0.24%)	222	(0.01%)	22,37,923	(85.40%)	26,20,292
1980	Q :	47,762	(64.07%)	365	(0.49%)	124	(0.17%)	48,251	(64.73%)	74,542
	V :	18,33,661	(83.78%)	15,794	(0.72%)	1.349	(0.07%)	18,50,804	(84.56%)	21,88,756
1981	Q :	54,538	(72.36%)	100	(0.13%)	56	(0.07%)	54,694	(72.56%)	75,375
	V :	24,85,210	(86.68%)	4.900	(0.17%)	809	(0.03%)	24,90,919	(86.88%)	28,67,128
1982	Q :	54,625	(72.70%)	73	(0.10%)	89	(0.12%)	54,787	(72.92%)	75,136
	V :	30,09,783	(87.94%)	4,740	(0.14%)	726	(0.02%)	30.15,249	(88.10%)	34,22,429
1983	Q :	53,603	(62.21%)	29	(0.03%)	28	(0.03%)	53,660	(62.27%)	86,169
	V :	31,03,724	(85.35%)	1,408	(0.04%)	454	(0.01%)	31,05,586	(85.40%)	36,23,231
1984	Q:	55,194	(61.39%)	38	(0.04%)	19	(0.02%)	55,251	(61.45%)	89.912
	V:	32,72,848	(84.90%)	2,917	(0.07%)	381	(0.01%)	32,76,146	(84.98%)	38,54,983
1985	Q:	50,349	(60.19%)	12	(0.014%)	73	(0.09%)	50,434	(60.29%)	83,651
	V:	32,981,87	(82.67%)	605	(0.02%)	548	(0.014%)	32,99,340	(82.90%)	39,79,998

Note: Figures in brackets represent the share in overall exports of marine products.

*Negligible

HATCHERY

2 BACTERIA ASSOCIATED WITH EGGS AND LARVAE OF P. INDICUS IN A HATCHERY

2.1 MATERIALS AND METHODS

2.1.1 Study area

The hatchery complex is situated on the northern bank of Periyar, at Azhikodu (long. 76°12'E and lat. 10°12'N) covering 2.7 hectares, approximately half a kilometer east of the point where the river enters the Arabian Sea (Fig. 1 and 2). Fresh tidal seawater is available at the hatchery site. The salinity of water drops with the commencement of the south-west monsoon in Jun. Till the end of Oct., the salinity does not reach the levels required for successful larval rearing of penaeid prawns. The effective, penaeid larval rearing season is, therefore, from late Nov./early Dec. to Jun. The tidal amplitude at the hatchery area is about 80 cm.

2.1.2 Setting-up of culture pools

Seawater for the hatchery pools is pumped out from a double in situ water filter, either directly or through an overhead tank using a 5 HP electric pump (Fig. 3). Aeration in the hatchery pools is provided by a 5 HP air compressor through PVC pipes and tubings. Freshwater

(salinity 5‰) from an open well, pumped into an overhead tank and distributed through pipes provide necessary freshwater for the adjustment of salinity for culture pools (Alikunhi et al. 1980). Portable PVC circular pools of 1.75 to 10.5 tons capacity were used for rearing purposes. Besides, several smaller pools and fibreglass tanks each of 1 ton capacity were also used.

The atmospheric temperature used to be comparatively low, during Jan. - Feb., in the shed (27.5° to 29°C) and the pools were kept outside, in open air where the temperature ranged from 27 to 31°C. Culture pools were arranged on the cement floor inside the shed, and also on either side of the shed, outside, with filtered seawater filled to 1/4 of their capacity to receive spawners.

2.1.3 Procurement of spawners

In order to procure the spawners alive, mechanised boat crew were given the necessary training to pick out fully gravid females from the trawl catches and bring to the hatchery without any damage. Only fully gravid prawns with opaque green ovaries were accepted.

2.1.4 Spawning procedure

Generally the procedure described by Hameed Ali (1978) was adapted. Seawater pumped from the in situ water filter

was kept at 30 cm depth in the spawning pool and aeration points at the rate of one per sq. m. area were provided, each with a diffusion stone. Ordinarily, one good spawner of P. indicus was sufficient for a 1.5 ton tank. However, when good spawners were not available, the number of spawners per pool was increased, to make sure that the resulting number of larvae would be adequate for commercial rearing.

Spawners were generally released into the spawning pools in the evenings, between 18 and 19 hrs. Moderate aeration was then provided without reducing the number of points. Spawning generally started around 20 to 22 hrs and continued for 4 to 6 hrs. The following morning the spawners were taken out of the pools, after assessing the extent of spawning, and they were measured and weighed. Microscopic examination of developing eggs generally showed very high fertilization and uniform development, and indicated that all specimens in the pool were spawning more or less at the same time.

2.1.5 Larval feeding

Artificial feeding was used throughout the larval and post larval stages as reported by Hameed Ali (1978). However in the present study, instead of Metapenaeus dobsoni,

Oratosquilla nepa (locally known as 'chelly') was used for the preparation of larval feed. The animals were cooked and kept frozen. The tissue suspension was prepared in an electric blender and the feed ration was decided on the basis of the population of larvae in the pool which was estimated every day. With the development of larvae, feed ration and feed particle size were increased. During zoea stages feed particle size ranged from 50 to 150μ. During mysis I and II stages particle size was increased to 200 to 300μ and to 400μ during mysis III and early post larval stages.

The quantity of feed dispensed varied with the stage of development of larvae as follows (g/1000 larvae/day).

Zoea I - 1.0
Zoea II - 1.25
Zoea III - 1.5
Mysis I - 1.75
Mysis II - 1.75
Mysis III - 2.0

On the above basis, the raw material required to feed the larval population in the particular pool for a day, was weighed out, processed, diluted to three times the weight of raw material and stored in a refrigerator. The day's ration was divided into 5 equal portions and fed every 5 hrs.

2.1.6 Larval rearing and hatchery management

After removing the spawned specimens, water level in the pool was raised to 50 cm depth. Effective aeration was given all over the pool. At water temperature ranging from 27 to 29°C developing eggs commenced hatching around at 11 a.m. and 30 to 34 hrs later they moulted into VIth nauplius stage.

Larval population in the pool was then estimated by counting the larvae in one litre water from three different location in the pool. In the following night the larvae moulted into the first zoea stage and commenced feeding. As soon as zoea appeared in water the first feed was given and continued at 5 hrs intervals. In the following morning larval population in the pool was estimated by sampling, water level raised to 60 cm, and feed ration adjusted according to the population. During zoea II and III stages water level was again raised to 70 to 80 cm.

On the 6th day after hatching, zoea III moulted into mysis I. Larval population was again estimated. Water level in the pool was then lowered to 20 cm by keeping a wire mesh filter box in the pool and gently siphoning out the water. Level of water in the pool was

slowly raised by gently adding fresh filtered seawater. Feed ration was estimated again based on the number of larvae. About 25% of the water was renewed every day during mysis II and III stages and normally on the 10th day, after hatching, the mysis III moulted into post larva I, completing the larval cycle.

Hatching pools, in which larvae were reared upto post larva I, were continued to rear them till the advanced post larval stage. Usually at post larva III, they start swimming at the bottom of the pool and on the sides. At this stage the animal is quite hardy for transportation. Considering this the post larvae were generally harvested at PL V stage. Approximately 400μ particle sized chelly meat was given to the post larvae at 5 hr intervals. Water was renewed daily by siphoning out its 25% and adding an equal volume of fresh filtered seawater.

2.1.7 Morphology of eggs and larvae

2.1.7.1 Egg:

Egg is opaque with a narrow perivitalline space, chorion with a purplish sheen (Fig. 4a) diameter of eggs varying from 0.25 to 0.27 mm and that of yolk mass 0.22 to 0.24 mm (CMFRI, 1978).

Thirteen hours after spawning all the three appendages are fully formed with long setae. The embryo occupies the entire space inside the egg and the movements are restricted to sudden jerks of appendages. The furcal setae first pierces the egg membrane and the nauplius wriggles out of the egg, 16 to 17 hrs after the eggs are spawned.

2.1.7.2 Nauplius:

Nauplius I

MTL: 0.30 mm; MW: 0.17 mm; MFS: 0.13 mm

An ocellus is present at the anterior median region of the body (Fig. 4b). Dorsal surface of the body bears posteriorly a small median denticle. A pair of dorsally curved caudal setae are present at the posterior end of the body. Three pairs of appendages are present. A1 is uniramous with 2 long setae of almost the same length and a small rudimentary spine like setae at its apex, 2 short setae on inner distal margin and one long setae on outer distal margin. A2 is biramous, endopod shorter than exopod, bearing 2 long setae and one rudimentary setae at apex, and 2 short setae along its inner margin. Exopod carries 5 long setae along inner margin and tip; Md is biramous, shorter than other appendages, bearing 3 long setae on endopod and exopod; setae of appendages are non plumose. Duration of this substage is 1 - 4 hrs.

After 15 hrs nauplius I moults through five substages with slight changes and reaches nauplius VI.

Nauplius VI

MTL: 0.48 mm; MW: 0.20 mm; MFS: 0.31 mm

Body is more elongated and the frontal organ and carapace are clearly demarcated (Fig. 4c). Appendages are not clearly segmented, but surface is with annular indentations. Furcal lobes are with 7 setae each. A1 is approximately with 5 indistinct segments. Endopod of A2 is with 3 long setae, and one short seta is seen terminally, and rudimentary seta is seen at the root of distal seta, on the inner lateral margin. Exopod is with 10 setae along the inner and distal margin. Duration of this substage is only 15 to 24 hrs. At the end of 24 hrs nauplius metamorphoses to zoea I.

2.1.7.3 Zoea:

Zoea I

MTL: 0.88 mm; MCL: 0.42 mm

Carapace is anteriorly rounded, with median notch. Frontal organs are present as protuberances (Fig. 4d). Developing compound eyes are covered with carapace. Body is divisible in 3 parts, carapace covered anterior region, 6 segmented thorax in the middle and posterior unsegmented

abdomen. Last abdominal somite and telson are not separated by a movable joint. Each load of the caudal furca is with 7 setae. Al is 3 segmented, basal segment with 3 subsegments, middle segment with 3 setae and distal segment with 2 setae. A2 is biramous endopod, 2 segmented and exopod 10 segmented, flattened without exopod and endopod. Mxl is with unsegmented protopod having 2 lobes. Mx2 is with protopode having 5 lobes on inner margin. Mxp is biramous with protopod 2 segmented. Mxp2 shorter than Mxpl with a protopod 2 segmented. Duration of this substage is from 24 to 48 hrs, at the end of which it moults into zoea III through zoea II.

Zoea III

MTL: 2.69 mm; MCL: 1.01 mm

Supraorbital spines are not bifurcated (Fig. 4e). Telson is demarcated from the 6th abdominal segment by an articulating joint. Abdomen segmented from 1 - 5 are with dorsomedian spines on posterior border. Sixth segment is devoid of posterior median dorsal spines. Caudal furca bear 8 setae each. An increase in length of biramous buds of thoracic legs is also noticed. Duration of this substage is 24 to 36 hrs. Thus the entire zoea stage extends for three to four days. Zoea III metamorphoses into mysis I.

2.1.7.4 Mysis:

Mysis I

MTL: 3.36 mm; MCL: 1.17 mm

Larvae assume more or less a shrimp like appearance in this stage (Fig. 4f). Rostrum is longer and curved extending beyond eye, devoid of rostral spines. Carapace covers thoracic region completely and thoracic appendages are well developed. Telson is broader distally with a median notch, each lobe bearing 2 lateral 6 terminal setae. A1 is with 3 segmented peduncle. A2 is with endopod unsegmented, and exopod also unsegmented but leaf like MT is asymmetrical. Exopod of Mx2 is with an exopod enlarged to form scaphognathite carrying 10 plumose setae. Uropod well developed, protopod with a large posteroventral spine, exopod with a prominent posterolateral spine followed by a short nonplumose seta and about 15 plumose setae along distal and inner margin, endopod with 14 plumose setae along inner and disterolateral margin. Duration of this substage is 48 to 72 hrs.

Mysis III

MTL: 3.90 mm; MCL: 1.26 mm

Mysis II which extends for 24 to 48 hrs moults into mysis III with the following characters (Fig. 4g). Two segmented pleopod bud distinguishes this substage from mysis I and II and no change in spination of carapace and abdomen is seen. But a very minute rudiment of rostral tooth may be seen. Duration of this substage is 24 to 48 hrs, at the end of which it metamorphoses into post larva I.

2.1.7.5 Post larva I:

MTL: 5.03 mm; MCL: 1.53 mm

Rostrum is with 1 or 2 dorsal spines, and supraorbital, hepatic and pterygostomial spines are present (Fig. 4h). Pleopods are well developed and telson is rectangular in shape carrying 3 pairs of lateral and 5 pairs of terminal setae. Duration of this substage is 24 to 30 hrs at the end of which it moults into post larva II.

2.1.8 Food and feeding habits

No food is needed during the nauplius stage. As soon as they metamorphose into zoea, they start feeding on phytoplankton. In laboratory cultures zoea are maintained in Thalassiosira sp. when the larva reaches mysis stage freshly hatched Artemia nauplii are given. Feeding the larvae in commercial cultures is explained in 2.1.5.

2.1.9 Collection of samples

Details regarding the frequencies of the collections made from hatchery are given in Table 2. Egg, nauplius, zoea, mysis and post larva were collected from the same pool when the nauplii metamorphosed to post larvae through zoea and mysis, using a hand net made of bolting silk. These eggs and larvae were then transferred to a sterile screw capped bottle. Water samples from pools were also collected in sterile bottles (500 ml) for the estimation of physico-chemical parameters and for total aerobic heterotrophic bacteria (THB). These bottles were kept in an ice box ($4^{o}C$) and transported to the laboratory.

2.1.10 Estimation of physico-chemical parameters

Physico-chemical parameters monitored in the present study were temperature, pH, salinity, dissolved oxygen and nutrients in the water samples.

Temperature of water was measured using a mercury bulb thermometer with $0.5^{o}C$ accuracy. pH of water was measured using a pH meter (Elico Li-10). Salinity was determined following the standard argentimetric titration (Harvey, 1955) with necessary correction. Dissolved oxygen was estimated by Winkler's titration (APHA, 1965). Inorganic and organic nitrogen and inorganic and organic phosphorus were determined following the procedures described by Klaus-Grasshoff (1979).

2.1.11 Bacteriological analysis

2.1.11.1 Processing of the sample:

Eggs and larvae were transferred to a sterile petridish and using sterile pasteur pipettes hundred numbers each were picked up and transferred to a sterile filter paper Whatman No.1 kept on a sterile glass funnel. The filter paper was punctured with a sterile glass needle and poured 5 ml of sterile 50% aged seawater into it. In this way the eggs and larvae could be collected in sterile tissue homogeniser where they were fully ground. Sterile suspension medium (10 ml and 9 ml) was prepared using 50% aged seawater as diluent and autoclaved. All the samples were serially diluted upto 10^{-6} using this diluent.

2.1.11.2 Plating procedures:

ZoBell's 2216e agar having the following composition was used for the enumeration of THB.

ZoBell's 2216e medium

Peptone	: 5.0 g
Yeast extract	: 2.5 g
FePO4	: 0.1 g
Agar (BDH)	: 20.0 g
Aged seawater	: 750 ml
Distilled water	: 250 ml
pH	: 7.4 - 7.6

Pour plate technique was employed. One ml aliquots of inoculum was introduced into each sterile petriplate from 10^{-2} to 10^{-6} dilutions. About 20 ml of the medium ($\approx 40^{\circ}C$) was poured into each petridish, and mixed thoroughly by rotating the dishes clockwise and anticlockwise for 4-5 times, and allowed to solidify. The plates were incubated in an inverted position at room temperature ($28 \pm 2^{\circ}C$) for 7-15 days. All the estimations were made in duplicate.

The plates showing 30-300 colonies were selected. Counts were made and expressed as number of colonies per ml of water and per gram of solid samples (wet wt.).

2.1.11.3 Isolation, maintenance and identification:

After recording the morphological characters, opacity and pigmentation twenty colonies were isolated randomly into nutrient agar slants from each sample.

Nutrient agar medium

Peptone	: 5.0 g
Beef extract	: 5.0 g
NaCl	: 15.0 g
Agar (BDH)	: 20.0 g
Tap water	: 1.0 l
pH	: 7.5 $\pm$ 0.2

The cultures were repeatedly streaked on nutrient agar plates, checked for their purity and were transferred to soft nutrient agar (0.5% agar) in small glass vials with rubber stopper and preserved under liquid paraffin at 20°C in a cold room.

The isolated cultures were grouped into various genera based on their morphological and biochemical characters as suggested by Shewan et al. (1960), Cowan (1974) and Buchanan and Gibbons (1974).

2.1.12 Production of hydrolytic enzymes

Bacterial isolates were divided into various physiological groups on the basis of their ability to elaborate different hydrolytic enzymes, such as amylase, protease (caseinase and gelatinase), lipase, urease and chitinase.

2.1.12.1 Amylase:

Amylase production was tested on the agar medium of Harrigan and McCance (1972) supplemented with starch as the substrate.

Composition of the medium

Peptone	: 10.0 g
Meat extract	: 10.0 g
Starch (soluble)	: 2.0 g
Sodium chloride	: 5.0 g
Agar (BDH)	: 20.0 g
Tap water	: 1.0 l
pH	: 7.2 $\pm$ 0.2

The medium was autoclaved and poured into sterilised plates. Isolates were streaked onto the agar plates and incubated at room temperature ($28\pm2^{o}C$) for 7 days. The production of amylase was detected by flooding the plates with iodine solution (Potassium iodide, 2 g; Iodine, 1 g and Distilled water, 300 ml). Unhydrolysed starch formed a blue colour with iodine. The amylolytic colonies developed clear zones around them.

2.1.12.2 Caseinase:

Caseinase production by the isolated cultures was detected by employing casein agar medium of Harrigan and McCance (1972) with slight modification in preparation as described below.

Composition of the medium (Basal)

Peptone	: 10.0 g
Meat extract	: 10.0 g
Sodium chloride	: 15.0 g
Agar (BDH)	: 20.0 g
Tap water	: 750 ml
pH	: 7.2 ± 0.2

The medium was autoclaved at 15 lbs pressure for 15 min. 30 g of casein in 250 ml of distilled water was sterilized separately and mixed with the above medium before pouring into plates. Cultures were inoculated into the medium by surface streaking and incubated at room temperature ($28\pm2^{o}C$) for 7 days. Caseinase enzyme production was detected by the presence of clear zones around the colonies.

2.1.12.3 Gelatinase:

Ability of the bacterial isolates to produce gelatinase was tested employing Frasier's gelatin agar (modified) medium of Harrigan and McCance (1972).

Composition of the medium

Peptone	: 10.0 g
Meat extract	: 10.0 g
Gelatin	: 4.0 g
Sodium chloride	: 15.0 g
Agar (BDH)	: 20.0 g
Tap water	: 1.0 l
pH	: 7.2 ± 0.2

The prepared medium was autoclaved and poured into sterilized petridishes. Isolates were inoculated by surface streaking on the solidified agar medium and incubated at room temperature ($28\pm2^{o}C$) for 7 days. The plates were flooded with 8-10 ml of the test reagent (mercuric chloride 15.0 g, concentrated HCl 20.0 ml, and distilled water 1.0 l). The hydrolysis was identified by clear halos around the colonies.

2.1.12.4 Lipase:

Production of lipase was tested on Tween agar of Harrigan and McCance (1972).

Composition of the medium	
Peptone	: 10.0 g
$CaCl_2$	: 0.1 g
Tween 80	: 10.0 g
Sodium chloride	: 15.0 g
Agar (BDH)	: 20.0 g
Tap water	: 1.0 l
pH	: 7.2 $\pm$ 0.2

The medium was autoclaved and poured into sterilized petriplates. Isolates were streaked onto the agar medium and incubated at room temperature ($28\pm2^{o}C$) for 7 days. Lipase production was detected by the appearance of a waxy material around the colonies indicating the liberation of insoluble oleic acid.

2.1.12.5 Urease:

Ability of the bacterial isolates to produce urease was tested with Christensen's urea agar medium (1946).

Composition of the medium (Basal)

Peptone	: 1.0 g
KH_2PO_4	: 2.0 g
D-glucose	: 1.0 g
Sodium chloride	: 15.0 g
Phenol red (0.2% solution)	: 6.0 ml
Agar (BDH)	: 20.0 g
Tap water	: 1.0 l
pH	: 6.8 $\pm$ 0.2

The medium was autoclaved, cooled to 50°C and added to 20 g of sterilized urea, which gave a final concentration of 2% urea in the medium. The medium was poured into sterile petriplates. Bacteria were inoculated onto the surface of the medium and incubated at room temperature (28 ± 2°C for 7 days. Ureolytic activity was detected by the change in colour of the medium from light yellow to pink.

2.1.12.6 Chitinase:

Chitinase production was detected by using the following medium.

Composition of the medium

Peptone	: 5.0 g
Beef extract	: 5.0 g
Sodium chloride	: 1.5 g
Agar (BDH)	: 20.0 g
Tap water	: 100 ml

Chitin precipitate was supplemented to the melted medium till the medium became turbid and pH was adjusted to 7.5 using 1 N NaOH or 1 N HCl. The medium was autoclaved and poured into sterilized petridishes. The cultures were streaked onto the surface of the agar medium and incubated at room temperature ($28\pm2^{o}C$) for 7-14 days. Colonies which developed clear zones around them were counted as positive.

2.1.13 Physiology of bacterial isolates

2.1.13.1 Preparation of inoculum:

Inoculum was prepared by suspending 24 hrs old bacterial culture in sterile seawater and optical density was adjusted to 0.2 at 600 nm. From this 0.2 ml was used as inoculum in the following experiments which were carried out in duplicate.

2.1.13.2 Measurement of growth:

From the prepared inoculum 0.2 ml was transferred to culture medium and incubated at varying conditions for 48 hrs.

Turbidity resulted due to growth of the inoculated bacterial culture was measured at 600 nm in a Hitachi model 200 UV-visible Spectrophotometer.

2.1.13.3 Effect of temperature on growth:

To test the effect of temperature on the growth of bacterial isolates, bacteria were grown in 5.0 ml aliquots of nutrient broth (peptone 0.5%, beef extract 0.5%, NaCl 2%, pH 8.5) at different temperatures (4,10,30,40 and 50°C) and growth was measured.

2.1.13.4 Effect of pH on growth:

Effect of pH on the growth of bacteria was determined by growing them in nutrient broth tubes in 5.0 ml aliquots prepared in various pH levels (2,4,7,9 and 11). The tubes were incubated at room temperature (28±2°C) and the growth was measured.

2.1.13.5 Effect of NaCl concentrations on growth:

Effect of NaCl on the growth of bacteria was tested by growing them at concentrations of 0,1,3,7 and 10% of NaCl. Nutrient broth with above mentioned sodium chloride concentrations were prepared dispensed into tubes in 5 ml aliquots, inoculated, incubated at room temperature (28±2°C) and the growth was measured.

2.1.14 Statistical analysis

2.1.14.1 Diversity index:

Diversity indices of the bacterial genera in eggs and larvae and the water collected along with them were found out using the formula

$$H' = -\Sigma Pi \log Pi \text{ (Shannon index)}$$

where $Pi = n/N$ ('n' = the number of isolates of one genus and 'N' = the total number of isolates in the same sample) (Pielou, 1975).

2.1.14.2 Simple correlation:

Correlation coefficients were found out using the formula

$$r = \frac{N\Sigma xy - (\Sigma x)(\Sigma y)}{\sqrt{[N\Sigma x^2 - (\Sigma x)^2]\,[(N\Sigma Y)^2 - (\Sigma y)^2]}}$$

between various parameters, following Snedecor and Cochran (1967). The calculations were made using a computer (PSI omni system).

2.1.14.3 Multiple regression:

The multiple regression model $Y = a_0 + a_1x_1 + a_2x_2 \ldots + a_8x_8$ (Snedecor and Cochran, 1967) was applied to determine the

dependence of survival and metamorphosis of eggs and larvae, on the environmental parameters as well as on the percentage of occurrence of various genera in eggs, larvae and water. Here 'Y' stands for the percentage survival and metamorphosis of eggs and larvae, 'x' for the parameters (both environmental and microbiological) and 'a' for the constants. The regression was worked out using a computer (Keltron Versa Ins.).

2.2 RESULTS

2.2.1 Physico-chemical parameters

Variations in physico-chemical parameters monitored in water during the study are given in Table 3. Temperature varied between 28 and 33°C. pH showed a variation between 7.32 and 8.85. Salinity was found to be moderate and ranged from 22.1 to $30.54.10^{-3}$. Oxygen content in the pools fluctuated widely (5.45 to 8.17 $ml.l^{-1}$). Of the chemical parameters estimated it was observed that inorganic phosphorus varied between 2.1 and 9.0$\mu g.l^{-1}$ and organic phosphorus from 1.0 to 7.0$\mu g.l^{-1}$. Inorganic nitrogen ranged from 1.25 to 9.8$\mu g.l^{-1}$ and organic nitrogen recorded more variation (21.0 and 237.0$\mu g.l^{-1}$).

2.2.2 Quantitative estimation of heterotrophic bacteria (THB)

Results of the quantitative estimation of THB associated with eggs, larvae and water collected along with the corresponding stages are presented in Fig.5 and App. Table 1.

Eggs and larvae:

The number of bacteria varied widely for the samples analysed. In eggs they fluctuated between 7.47 and 872.41 x $10^5.g^{-1}$ in nauplii 6.5 and 616.45 x $10^5.g^{-1}$,

in zoea 1.11 and 313.93 x $10^5.g^{-1}$, in mysis 13.51 and 1248.95 x $10^5.g^{-1}$ and in post larvae 5.2 and 84.41 x $10^5.g^{-1}$. In general, post larvae harboured a lesser population than other stages. A decrease in the number of bacteria was recorded for the stages from egg to zoea which shot up in the next stage (mysis). However, they declined in post larvae.

Water:

The water of the respective larval samples, showed a maximum population (28 x $10^5.ml^{-1}$) in pool 4 when zoea was collected. Lower population (0.012 x $10^5.ml^{-1}$) was observed in pool 6 when eggs were collected.

2.2.3 Qualitative analysis of THB

2.2.3.1 Gram-negative bacteria:

The percentage of Gram-negative bacteria and the percentage survival of eggs and larvae are given in Table 4. Of the 902 isolates tested majority (87.42%) of them were Gram-negative.

Eggs and larvae:

During the development of the larval stages, Gram-negative bacteria were found to be dominant (89.65%). They showed an increase from 84.34% (eggs) to 93.61% (post larvae).

Water:

Among the 453 isolates tested, majority (85.19%) of them were Gram-negative. Unlike in the respective larval stages, they exhibited fluctuations in percentage occurrence.

In general higher percentages of Gram-negative bacteria were found to be associated with eggs and larvae than in water. However the water sample of mysis stage contained a higher (94.70%) percentage of the Gram-negative bacteria than the larvae (mysis).

2.2.3.2 Distribution of various genera in eggs, larvae and water:

The bacterial strains were identified to various genera and the results are presented in Table 5.

Eggs and larvae:

Among 902 isolates taken from both larvae and water, Vibrio was found to be dominant (41.91%) followed by Acinetobacter and Pseudomonas (17.84% and 17.74% respectively). The other genera were represented in small percentages (0.22 to 9.31%). Vibrio recorded an increase from 10.42 to 85.39% from eggs to post larvae. All the other genera

exhibited a declining trend from eggs to post larvae. Genera such as Micrococcus, Coryneform group, Enterobacteriaceae were not found on post larvae.

Water:

Similar to eggs and larvae, water collected along with larvae also exhibited an increase of Vibrio from 12.24 to 67.06% (eggs to post larvae). All the other genera experienced a decline and few of them such as Enterobacteriaceae and Coryneform group were hardly recorded in the post larval stage.

2.2.3.3 Generic composition of bacteria associated with eggs, larvae and water:

Vibrio was the prominent flora present in all the stages and in all the pools except in nauplii of pool 4 and zoea of pool 6 (Fig. 6a - f, App. Table 2). But they could not be isolated from the water collected along with the eggs, nauplii, zoeae and mysis of pool 4 and nauplii and zoeae of pool 5. The incidence of Pseudomonas, Acinetobacter and Moraxella was much lesser in the latter stages (mysis and post larvae) than in eggs and in other early stages, in all the pools. Incidence of Gram-positive forms such as Micrococcus, Bacillus and Coryneform group, also showed similar pattern. In general Vibrio was the prominent flora followed by Acinetobacter and Pseudomonas.

Eggs and larvae:

In eggs, Pseudomonas (30.21%), Acinetobacter (28.13%), Vibrio (10.42%) and Moraxella (10.42%) formed the major flora and the rest of the groups ranged from 2.08% to 8.33%. An average of 91.06% of eggs could hatch out into nauplii. The genera occurred in higher percentage in nauplii were Acinetobacter (28+42%), Pseudomonas (20.00%), Vibrio (20.00%) and Moraxella (12.63%). The other genera ranged from 2.11% to 8.42%. An average of 80.27% nauplii metamorphosed to zoea. In zoea the prominent genera were Vibrio (54.22%), Acinetobacter (19.28%) and Pseudomonas (16.87%). The other genera ranged from 1.20% to 3.60%. An average of 58.47% zoea metamorphosed to mysis. The dominant bacteria were members of Vibrio (69.77%) followed by Pseudomonas (11.63%) and Acinetobacter (9.30%) in mysis. The percentage of other genera ranged from 1.16 to 3.49%. On average, only 35.37% of mysis could metamorphose to post larvae. In post larvae the only dominant genus was Vibrio (85.39%). The other genera ranged from 2.24 to 6.74%. It was observed that 29.98% of the larvae alone could metamorphose to the next stage.

Water:

Water samples collected along with eggs contained Acinetobacter (25.51%), Pseudomonas (24.49%), Vibrio (12.24%)

and Moraxella (11.22%) as prominent flora. Among the other genera Bacillus (10.20%) was the important one and the percentage of other genera ranged from 4.08 to 7.14%. Pseudomonas (24.18%), Acinetobacter (23.08%), Vibrio (13.19%) and Moraxella (21.98%) were the prominent flora in water samples collected along with nauplii. The other genera ranged from 1.09 to 5.49%.

In water samples collected along with zoea, Vibrio (41.30%), Pseudomonas (20.65%) and Acinetobacter (15.21%) were dominant. The percentage of other genera ranged from 1.09% to 9.78%. Vibrio (57.65%), Pseudomonas (12.94%) and Moraxella (15.29%) were present as the prominent flora in water samples collected along with mysis. The other genera ranged from 1.18% to 7.06%. In the water samples collected along with post larvae Vibrio (67.06%) was the only prominent flora. The other genera ranged from 1.18 to 12.94%.

Thus, among various genera of heterotrophic bacteria encountered in the hatchery system, Vibrio was found to increase from eggs (10.42%) to post larvae (85.39%). The other genera which were encountered in larval stages were Pseudomonas, Acinetobacter, Moraxella, Enterobacteriaceae, Micrococcus, Bacillus and Coryneform group. All these genera

showed a declining trend as the nauplii metamorphosed to post larvae through zoea and mysis, both in water as well as in larvae.

When the percentage of Vibrio was 10.42 of majority of the embryonated eggs (91.06%) hatched out into nauplii and of this 80.27% metamorphosed into zoea stage, while, Vibrio increased to 20.00%. When 58.47% zoea metamorphosed to mysis, the percentage of Vibrio increased further to 54.22%. From this, only 35.37% of mysis could enter into the next stage (post larva I) while the percentage of Vibrio rose to 69.77%. Post larva was found to harbour 85.39% of Vibrio and only 29.98% of the larvae could pass onto post larva II.

2.2.4 Generic Diversity Index

Indices of generic diversity calculated in eggs, nauplii, zoeae, mysis and post larvae and the water collected along with them are presented in Table 6. It could be observed that the diversity indices declined from eggs (0.697) to post larvae (0.266), and a sharp reduction was seen from mysis to post larvae (0.463 to 0.266). A reduction in the diversity indices could be observed in the water samples eventhough it was not as sharp as that was recorded in larvae.

2.2.5 Correlation coefficients

2.2.5.1 Environmental factors and THB in eggs and larvae:

A significant positive correlation existed between pH and THB in zoea, oxygen and THB in nauplius, and organic phosphorus and THB in post larvae. At the same time a significant negative correlation was observed between organic phosphorus and THB and organic nitrogen and THB in eggs (Table 7).

2.2.5.2 Environmental factors and THB in water:

A significant positive correlation was observed between pH and THB of water of the pools with eggs and organic phosphorus and THB of water with zoea. At the same time a significant negative correlation was seen between inorganic phosphorus and THB of the pool with zoea (Table 7).

2.2.5.3 Environmental factors and Gram-negative bacteria of eggs and larvae:

A significant positive correlation was observed between pH and Gram-negative bacteria of zoea, oxygen and Gram-negative bacteria of nauplius, inorganic nitrogen and Gram-negative bacteria of post larva. No significant negative correlation was observed between any of the parameters and the Gram-negative bacteria of larvae (Table 7).

2.2.5.4 Environmental factors and Gram-negative bacteria of water:

Only at one instance, i.e., between organic phosphorus and Gram-negative bacteria in the pool with mysis a significant positive correlation was found (Table 7). No significant relationship could be observed for the other variables.

2.2.5.5 Environmental factors and percentage survival and metamorphosis of eggs and larvae:

A significant negative correlation was observed between the temperature and the percentage survival of post larvae, pH and the percentage survival and metamorphosis of mysis, inorganic phosphorus and the percentage of mysis metamorphosed and the percentage of post larvae moulted into next stage, organic nitrogen and the percentage of eggs hatched out, and a significant positive correlation existed between inorganic nitrogen and the percentage survival of post larvae (Table 7).

2.2.5.6 Environmental factors and percentage genera in eggs and larvae:

Egg:

A significant negative correlation was observed between salinity and Coryneform group, pH and Bacillus,

oxygen and Moraxella, organic phosphorus and Pseudomonas and organic nitrogen and Pseudomonas. At the same time a significant positive correlation was observed between oxygen and Pseudomonas (Table 8).

Nauplius:

In this stage a significant negative correlation was seen between organic nitrogen and Acinetobacter and a positive correlation between oxygen and Pseudomonas.

Zoea:

A significant negative correlation was found between temperature and Vibrio, and pH and Micrococcus.

Mysis:

A significant negative correlation was observed between inorganic phosphorus and Pseudomonas, Moraxella, and Coryneform group. At the same time a significant positive correlation was found between Vibrio and pH, and inorganic phosphorus.

Post larva:

Only in one case, i.e., between the phosphorus and Pseudomonas, a significant negative correlation could be observed.

2.2.5.7 Environmental factors and the percentage genera in water:

Egg:

A significant negative correlation was observed between salinity and Coryneform group, pH and Bacillus, and inorganic nitrogen and Moraxella (Table 9). At the same time a significant positive correlation was observed between pH and Pseudomonas.

Nauplius:

A significant negative correlation was observed between inorganic phosphorus and Aeromonas. Whereas a significant positive correlation was seen between oxygen and Pseudomonas and Coryneform group.

Zoea:

A significant positive correlation between inorganic phosphorus and Vibrio was observed.

Mysis:

A significant negative correlation was observed between pH and Pseudomonas, inorganic phosphorus and Pseudomonas, organic phosphorus and Coryneform group, and inorganic nitrogen and Moraxella. At the same time a

significant positive correlation was observed between pH, inorganic nitrogen and Vibrio.

Post larva:

Only at one instance, that is, between organic phosphorus and Pseudomonas a significant negative correlation was seen.

2.2.5.8 THB of eggs and larvae and water:

Correlation coefficient values for THB of eggs and larvae and THB of water at different stages showed that there existed a significant positive relationship between THB of mysis and the THB of corresponding water sample ($r = 0.8389$). THB of other stages did not exhibit significant relationship.

2.2.5.9 Percentage survival and metamorphosis of eggs and larvae and THB and Gram-negative bacteria:

When THB and Gram-negative bacteria of individual stages were related against percentage survival and metamorphosis of that stage of eggs and larvae, a significant negative correlation could be observed between Gram-negative bacteria associated with mysis and percentage survival and metamorphosis of mysis (Table 10). No significant relationship was observed for other stages.

2.2.5.10 Percentage survival and metamorphosis of eggs and larvae and percentage of various genera associated with them:

A significant negative correlation was observed between the percentage survival and metamorphosis of eggs and Moraxella and Enterobacteriaceae, zoea and Coryneform group, mysis and Vibrio and post larva and Vibrio. Similarly a significant positive correlation was recorded between eggs and Acinetobacter and mysis and Pseudomonas and Coryneform group (Table 11).

2.2.5.11 Percentage survival and metamorphosis of eggs and larvae and various genera present in corresponding water samples:

A significant positive correlation was noticed between eggs and Acinetobacter, zoea and Micrococcus and mysis and Pseudomonas whereas eggs and Enterobacteriaceae, nauplii and Acinetobacter, zoea and Vibrio and mysis and Vibrio showed a negatively significant correlation (Table 12).

2.2.6 Multiple regression models

Multiple regression models were applied for determining the dependence of survival and metamorphosis of eggs and larvae to their next stage on the various environmental parameters, and the estimated regression

coefficients are presented in Table 13. The model was found useful for interpreting the dependence of percentage survival values on the corresponding environmental parameters, both at the eggs and nauplii stages. However the model was found partially suitable statistically, for interpreting this dependence in the third stage, zoea, when two out of the six sample values were deleted. In the case of mysis and post larval stages the model was not statistically fit.

The above results indicate that in egg, nauplius and to a certain extent in zoea stages, the percentage of survival and metamorphosis could be expressed as a linear function of various physico-chemical parameters. At the same time this could not be extended to the latter stages, mysis and post larvae.

Table 14 gives the results of a similar interpretation of the dependence of survival and metamorphosis on the percentage genera in eggs and larvae. Here too, the model was found statistically acceptable for the first three stages viz. eggs, nauplii and zoeae, but no meaningful interpretation could be made in the remaining two stages viz. mysis and post larva. Therefore, only in the first three stages the survival and metamorphosis could be interpreted as a linear function of the various genera. For the other stages no direct relationship was observed.

2.2.7 Hydrolytic properties of bacteria in eggs, larvae and water

2.2.7.1 Distribution of hydrolytic bacteria:

The isolates obtained from eggs and larval stages and corresponding water samples were tested for their ability to produce various extracellular hydrolytic enzymes such as amylase, caseinase, gelatinase, lipase, urease and chitinase. A total of 449 and 453 isolates from eggs and larvae and water respectively were subjected to the above tests and classified as amylolytic, proteolytic (caseinolytic and gelatinolytic), lipolytic, ureolytic and chitinolytic.

Eggs and larvae:

Of the 449 isolates tested majority (97.10%) of the isolates were ureolytic, followed by proteolytic and amylolytic. Less than 50% of the isolates were chitinolytic and lipolytic (Table 15 and 16). Most of the amylolytic, proteolytic and ureolytic bacteria were found associated with all the stages of eggs and larvae. More than 50% of lipolytic bacteria were found to be associated with zoea, and post larvae. Chitinoclasts were found increasing from egg to post larva and 84.27% were recorded in post larva. However within the variation least percentages of proteolytic, lipolytic and chitinolytic occurred in eggs, while amylolytic and ureolytic were recorded in mysis and zoea respectively.

Water:

A similar trend which was observed for eggs and larvae in maintaining higher percentage of hydrolytic bacteria was recorded among isolates (453) collected from water. However the maximum occurrence of all the hydrolytic forms tested were recorded in water collected along with post larva except lipolytic which was observed in zoea. Least percentages were recorded for all the groups in nauplii except lipolytic and amylolytic which were recorded in post larvae and zoeae respectively. Chitinoclastic bacteria showed an increase from eggs to post larvae and such trend was not in other hydrolytic bacteria both in water and in eggs and larvae.

2.2.7.2 Generic composition of various hydrolytic bacteria:

The isolates tested for various hydrolytic enzymes belonged to members of Vibrio, Pseudomonas, Aeromonas, Acinetobacter, Moraxella, Enterobacteriaceae, Micrococcus, Bacillus and Coryneform group (Table 17).

Eggs and larvae:

The hydrolytic groups of bacteria (amylolytic, proteolytic, lipolytic, ureolytic and chitinoclastic) were mainly composed of Vibrio, Acinetobacter and Pseudomonas.

Higher percentages of Vibrio elaborated all the hydrolytic enzymes. Pseudomonas showed a decreasing order among amylolytic, proteolytic and lipolytic groups. However proteolytic were more among Acinetobacter. Chitinoclastic group was mainly composed of Vibrio. Percentage composition of other genera in the different hydrolytic group was very small.

Water:

The percentage composition of various genera in different hydrolytic groups showed slight variations from that of the eggs and larvae. Eventhough the different hydrolytic groups were mainly composed of Vibrio, the order of dominance of other genera was not exactly the same as that of the animals. Among amylolytic, lipolytic and ureolytic groups, Pseudomonas was more prominent than Acinetobacter. In chitinoclastic group, the percentage of Vibrio was not as prominent as seen in larvae, and further, the percentage of Acinetobacter producing chitinase was higher than that in larvae.

The distribution of various hydrolytic bacteria belonging to individual genera occurred in eggs and larvae and corresponding water is presented in Table 18.

Eggs and larvae:

Nearly 73 to 100% of Vibrio were amylolytic, proteolytic, ureolytic and chitinolytic. But lipolytic vibrios ranged from 38 to 91%. Majority of the Pseudomonas were amylolytic (67 to 100%), 20.69 to 100% were proteolytic and more than 90% were ureolytic. Chitinoclastic forms were absent except for the small percentage (14.29%) in zoea. The lower percentage of proteolytic forms were seen in eggs and nauplii. Lipolytic Pseudomonas was absent in eggs and post larvae whereas in other samples they ranged from 15.79 to 85.71%. Among Acinetobacter 50 to 93% were amylolytic and 83 to 100% of them were proteolytic. In all the samples except zoea 100% of Acinetobacter strains were ureolytic whereas only 31.25% were recorded ureolytic in zoea. Chitinoclastic forms were absent except for a small percentage (6.25%) recorded in zoea. Lipolytic Acinetobacter ranged from 11.11 to 62.50% and absent in post larvae. The percentage occurrence of other genera were very low and not represented in all the stages.

Water:

More than 83% of Vibrio elaborated all the hydrolytic enzymes except lipase. Amylolytic Pseudomonas ranged from 55 to 92% and proteolytic from 4.55 to 100%. Lipolytic forms

were absent in the pool of post larvae and ranged from 9.09 to 73.68% in other samples. More than 68.42% were ureolytic. Chitinoclastic forms were 50% in water collected along with post larvae. Amylolytic Acinetobacter was absent in mysis, and, in other samples it ranged from 21.43 to 100%. More than 88% of all the Acinetobacter cultures were proteolytic. Lipolytic forms were present only in zoea. Ureolytic bacteria were very low (4.76%) in nauplii and ranged from 4.76 to 100% in all the samples. Chitinoclastic forms were absent in the pools of egg, and mysis while with other larval stages they ranged from 14.29 to 71.43%. Among Moraxella, amylolytic forms ranged from 36 to 60%, proteolytic from 45 to 100% and ureolytic from 82 to 100%. Lipolytic forms were absent in eggs and nauplii and in other samples it ranged from 40 to 60%. Chitinoclastic Moraxella were absent in all the samples except in nauplii. Very few numbers of other genera encountered were found to elaborate these enzymes.

The results of the percentage contribution of different genera for a particular hydrolytic enzyme are given in Table 19.

Eggs and larvae:

Except for chitinase and lipase, for all the enzymatic groups, in general, there were contributions from all bacterial genera other than Aeromonas encountered in eggs and in nauplii.

Acinetobacter showed maximum contribution towards all the enzymatic groups except for urease and lipase which were recorded by Pseudomonas (30.21%) in eggs and by Vibrio in nauplii. At the later stages viz., zoea, mysis and post larva, Vibrio was found to be the major source of all enzymatic groups and in all the stages about 75 - 100% of vibrios were chitinoclasts.

Water:

In corresponding water samples of eggs and nauplii, all the genera contributed to various enzymatic groups except lipase and chitinase. However, Vibrio and Pseudomonas together comprised the entire lipolytic group. Also Aeromonas exhibiting hydrolytic activity were seen only in water. In general, in zoea and in mysis, Vibrio recorded the maximum percentage of enzymatic groups followed by Pseudomonas and Acinetobacter. But in post larvae more than 60% of the different enzymatic groups were composed of Vibrio. Chitinase was mainly the contribution of Vibrio in all the stages except in nauplii where Acinetobacter (41.66%) dominated followed by Vibrio (33.33%).

2.2.8 Effect of NaCl concentrations, pH and temperature on the growth of bacteria

The bacterial isolates were grown at various environmental conditions such as varying temperature, pH and

NaCl concentrations. The results showed that maximum number of isolates showed a preference of 3 to 7% NaCl concentration, pH 7 to 9 and temperature 30-40°C as optimal conditions for maximum growth. No growth was observed at pH 2 and 4. Similarly, 4 and 50°C were totally unacceptable for any isolate as optimum (Table 20).

Eggs and larvae:

Out of 449 isolates, about 37 to 38% showed maximum growth at 3 and 7% NaCl concentrations respectively. Only 13.58% of isolates exhibited maximum growth at 10% NaCl and 9.58% and 0.89% were growing maximum at 1 and 0% NaCl respectively. In individual pools also, more or less this trend was observed, where 3 and 7% NaCl concentrations were the optimum for majority for isolates. About 80% of the isolates showed maximum growth at pH 7 and the rest (17.81% and 2.23%) at pH 9 and 11 respectively. Same trend was observed in individual pools also. Maximum growth for 60.13% of the isolates was observed at 30°C. At the same time 37.86% showed optimum growth at 40°C and just 2% only grew to maximum at 10°C. Similar trend was observed in individual pools also.

Water:

Among 453 isolates, 42.16% and 35.32% preferred 3 and 7% NaCl concentration respectively for optimum growth.

At the same time 1% NaCl was the optimum for 11.04% of isolates and 10% NaCl for 9.27% of isolates. Complete absence of NaCl was optimum for 2.21% of the isolates. More or less the same trend was observed in individual pools also. 62.03% of isolates showed maximum growth a pH 7 and the rest of them preferred pH 9 as optimum for maximum growth. Temperature 30° and 40°C were acceptable for about 52.31% and 45.60% of isolates respectively. Only 1.99% of the isolates preferred 10°C. In individual pools also almost the same trend was observed.

Effect of NaCl concentrations, pH and temperature on the growth of isolates obtained from eggs, larvae and water in general are presented in Table 21.

Eggs and larvae:

From eggs to post larvae a shift was seen in the highest percentage of the isolates showing maximum growth at NaCl concentrations ranging from 3 to 10%. While in eggs and nauplii 70 and 60% of the isolates respectively showed maximum growth at 3% NaCl, in zoea and mysis, 66.29 and 75.58% of the isolates respectively showed the maximum growth at 7% NaCl. In post larvae 57.30% of the isolates preferred 10% NaCl for maximum growth. pH 7 was the optimum for 68 to 93% of isolates from eggs to post larvae. The next preference was for pH 9 (6 to 31.39%) and the least

was for pH 11 (2 to 8.42%). In eggs, nauplii, zoeae and mysis, 30°C was the optimum for 60 to 78.31% of the isolates. But in post larvae 64.04% of the isolates preferred 40°C. In stages other than post larva 18 to 40% of the isolates preferred 40°C as the optimum.

Water:

Similar to eggs and larvae a shift was seen in the highest percentage of the isolates showing maximum growth at NaCl concentration from 3 to 10%. While with eggs and nauplii 62.24% and 59.09% respectively showed maximum growth at 3% NaCl, in the pools with zoea and mysis 55.43% and 65.88% of the isolates grew to maximum at 7% NaCl. At the sametime, in pools with post larvae, for 38.55% of the population 10% NaCl was the optimum and 37.34% of the isolates grew well at 3% NaCl and 22.89% at 7% NaCl. pH 7 was the optimum for 41.30 to 84.33% of the isolates in the pools with eggs to post larvae. At the same time pH 9 was chosen by 3.88 to 35.71% of the isolates. pH 11 was the optimum for a small percentage of isolates (8.16 to 21.74%) in the pools with eggs and zoeae. In the pools with mysis and post larvae no organism preferred pH 11. Temperature 30°C was chosen by maximum percentage of the isolates (52.75 to 64.29%) in pools with eggs to mysis. Rest of the population preferred 40°C. At the same time in pools with post larvae 80.72% of the isolates grew maximum at 40°C.

Effect of NaCl concentrations, pH and temperature on the growth of different genera are presented in Table 22.

Eggs and larvae:

Except among Vibrio, in all the other genera there were at least a small percentage of isolates showing maximum growth at 1% NaCl (6.67 to 80%). Acinetobacter, Enterobacteriaceae, and Micrococcus contained 2.38 to 10% cultures which could grow maximum at 0% NaCl. Only three genera such as Vibrio, Pseudomonas, and Acinetobacter contained a small percentage each (23.81, 4.0 and 9.52%) which could grow maximum at 10% NaCl. Thus 3 and 7% NaCl concentrations were found to be widely acceptable for all the genera except Bacillus (68.75%) which preferred 1% NaCl. Further 7% NaCl concentration was preferred highly, only, by Vibrio (64.76%) and Micrococcus (60.0%). pH 7 was favoured highly by all the genera (70 to 100%) except Enterobacteriaceae, Micrococcus and Bacillus which preferred pH 9. pH 11 was acceptable for only a small percentage (0.5 to 13.33%) of a few genera such as Vibrio, Pseudomonas Acinetobacter and Moraxella. Majority of Vibrio (67.14%), Acinetobacter (84.52%), Moraxella (60.0%), Enterobacteriaceae (80.0%) and Micrococcus (80.0%) recorded 30°C as optimum. For the other genera, Pseudomonas (73.33%), Bacillus (81.25%)

and Coryneform group (88.89%), 40°C was the optimum. Very small percentage of Vibrio (0.95%), Pseudomonas (2.67%), Moraxella (10%) and Bacillus (12.5%) grew to maximum at 10°C.

Water:

Majority of the isolates belonging to all genera except Vibrio grew maximum at 3% NaCl, while 67.27% of Vibrio preferred 7% NaCl as optimum. For Vibrio (19.04%), Pseudomonas (7.14%), Moraxella (7.41%), 10% NaCl was found to be favourable. In the absence of NaCl also small percentages of the genera such as Pseudomonas (3.57%), Acinetobacter (1.30%), Enterobacteriaceae (11.11%) and Micrococcus (21.74%) showed maximum growth. pH 7 was preferred by majority of genera such as Vibrio (54.76%), Pseudomonas (78.57%), Aeromonas (100%), Acinetobacter (74.08%), Moraxella (61.11%) and Coryneform group (66.70%). Rest of the percentage of these genera preferred pH 9. At the same time pH 9 was found to be optimum for large number of cultures belonging to Enterobacteriaceae (66.67%), Micrococcus (60.87%) and Bacillus (54.17%). For majority of Vibrio (57.74%), Acinetobacter (72.73%), Enterobacteriaceae (77.78%) and Micrococcus (52.17%) 30°C was the optimum. The other genera such as Pseudomonas (61.90%), Moraxella (53.70%), Bacillus (66.67%) and Coryneform group (58.33%) 40°C was the optimum. 10°C was preferred by a small percentage of Vibrio (1.79%), Pseudomonas (3.57%) and Moraxella (5.56%).

2.3 DISCUSSION

The microbial populations in aquatic systems are known to be influenced by a variety of physico-chemical and biological factors. In the present study to understand their influence on bacteria a number of factors were monitored. The correlation between the environmental parameters and bacteria were determined by a series of correlation coefficient matrices (Pearson correlation coefficient and multiple regression). The results show that correlation between different factors and bacteria associated with eggs and larvae and corresponding water samples were not similar in all the samples throughout the collection. Further, it could be seen that in certain instances physical factors like pH and oxygen or chemical factors like organic nitrogen and phosphorus were found to play a major role in determining the density of the population in water and also the associated bacteria. These differed from stages to stages of eggs and larvae and also from corresponding water samples. It is thus indicated that the influence of environmental parameters on the bacterial population cannot be generalised.

The results of the present investigation indicated that THB declined as the eggs hatched out and nauplii metamorphosed to post larvae, through zoeae and mysis.

Similar findings were reported by Yasuda and Kitao (1980). Decrease of THB, increase of Gram-negative bacteria and the percentage increase of Vibrio and the declining of the generic diversity index were the prominent bacteriological changes taken place in eggs and larvae as well as in water during the rearing period. But the environmental factors did not vary much and all of them did not influence significantly on the population and generic diversity of the heterotrophs.

Gram-negative bacteria increased steadily from eggs to post larvae, but such a gradual increase of Gram-negative forms could not be seen in the corresponding water samples. A significant positive correlation was observed between pH and Gram-negative bacteria of zoea, oxygen and the Gram-negative bacteria of nauplii and inorganic nitrogen and Gram-negative bacteria of postlarvae. The significant positive correlation existed between the Gram-negative bacteria and the organic phosphorus in the pools with mysis indicate that the increase in organic matter in the water column was one of the reasons for the increase of Gram-negative bacteria (Rheinheimer, 1980).

Vibrio increased steadily from eggs to post larvae. At the same time all the other genera declined. In water sample also such an increase of vibrios could be seen from eggs to post larvae but with lesser magnitude. Diversity

index of genera in eggs and larvae showed a remarkable sharp reduction towards the later stages of the larval life, which coincided with the increase of Vibrio. Generic diversity index of water collected along with eggs to post larvae also showed a reduction, which was not as sharp as that was observed in the case of larvae. Lesser magnitude observed in the percentage increase of Vibrio in water coincided with the above observation. A reduction in the generic diversity index was also observed from eggs to post larvae. This might be due to the increase of Vibrio and the remarkable reduction of the other genera. Vibrio was the predominant flora followed by Acinetobacter and Pseudomonas. During the early stages (eggs and nauplii) Pseudomonas, Acinetobacter were the dominant ones and in the later stages Vibrio took over.

Vibrio is reported to prefer an alkaline pH (Beuchat, 1975) Similar findings were also observed in the present investigation. A significant positive correlation existed between the pH and the percentage of Vibrio in mysis as well as in the water collected along with them support the earlier findings. Factors such as short generation time of Vibrio compared to other Gram-negative and Gram-positive forms (Ulitzur, 1974; Ivy Thomas, 1982), ability of marine bacteria for the uptake of substrate at low concentrations (Wright and Hobby, 1966; Wright, 1973), confinment of water for a longer time resulting

in the loss of interaction of bacteria and the environment (Brock, 1967) must have resulted in the increase of Vibrio in the system. The tendency for proliferation of vibrios seen from the beginning onwards progressed steadily and attained the peak at post larval stage. Yasuda and Kitao (1980) observed the dominance of vibrios in seawater after 126 days of cultivation.

Shellfish in their natural environment carry commensal bacterial flora, the composition of which may be governed by their feeding and the living conditions, the geography of area, the season, temperature and quality of water in which they exist (Cann, 1977). However, in addition to this, the larval body provide an excellent microenvironment for the highly competent genera. Attachment to surface provide an important fitness trait for aquatic bacteria. Ability of microbes to attach to and colonise particulate material affords them with a microenvironment higher in nutrient concentration than the surrounding water (Stevenson, 1978). That might be the reason for the sharp increase of Vibrio seen in larvae than in water. Simidu et al. (1971) compared the generic composition of aerobic bacterial flora of plankton with that of seawater and demonstrated that more than 70% of the bacterial flora obtained from plankton samples were Vibrio and Aeromonas, when they accounted for about 45% in seawater. Based on the difference between the generic composition of bacteria in zooplankton and seawater samples, Simidu et al. (1971) suggested that Vibrio

and Aeromonas are common indigenous marine bacteria and they are closely associated with marine organisms, in particular, animals. Sochard et al. (1979) demonstrated that marine copepods carry a bacterial flora both on their surface and in their guts, with the predominant bacterial groups being members of Vibrio. These observations were in part confirmed by SEM examinations of copepods collected from aquatic environments in Bangladesh (Colwell et al. 1980). Huq et al. (1983) showed that Vibrio sp. alone attached themselves to copepods whereas strains of Pseudomonas and E. coli did not adhere to them.

A significant negative correlation existed between the percentage of Vibrio and the percentage survival and metamorphosis of mysis and post larvae ($P<0.05$ and $P<0.01$ respectively). Results of the application of multiple regression model to examine the dependency of the survival and metamorphosis of eggs and larvae on their bacterial flora showed that the model was found to be statistically acceptable for the first three stages viz. eggs, nauplii and zoeae, but was not acceptable for the remaining stages viz. mysis and post larvae. Therefore only in the first three stages (eggs to zoeae) the survival and metamorphosis could be meaningfully interpreted as a linear function of the various genera. In the other stages (mysis and post larvae) where the survival and metamorphosis were not a linear function of the different genera of heterotrophs, much of the mortality occurred and a significant negative

correlation was obtained with the percentage of Vibrio. This shows that the percentage survival and metamorphosis of mysis and post larvae were not a linear function of various genera but a direct simple function of Vibrio which was shown by a significant negative correlation coefficient.

A significant negative correlation existed between pH and the survival and metamorphosis of mysis, and between temperature and survival of post larvae. A significant positive correlation existed between pH and the percentage of Vibrio in mysis. Muthu (1978) stated that the prawn larvae were adversely affected when the pH of the water raised beyond 8.5. It could be substantiated with the results of the present study that higher pH existing in the pool with mysis might have accelerated the multiplication of Vibrio and resulted in the larval mortality. No significant positive correlation was obtained between temperature and the percentage of Vibrio in post larvae. The higher temperature prevailed in the pools with post larvae must have weakened the larvae so that they could easily become the prey for Vibrio invasion. Temperature above 31^{o}C is not found to be suitable for larval rearing (Muthu, 1978). Results obtained on determining the dependence of survival and metamorphosis of eggs and larvae using the multiple regression model, showed that in egg, nauplius and to a certain extent in zoea stages the percentage of survival and metamorphosis could be expressed as a linear function of various physico-chemical parameters.

It is clear from the data that dominance of Vibrio in the larvae (mysis and post larvae) may be a reason for the low percentage of survival and metamorphosis of them. Yasuda and Kitao (1980) showed that the zoea of P. japonicus harboured more Vibrio in the intestine. Cultured adult prawn grew poorly when there had been more Aeromonas and Vibrio in the digestive tract. At the same time those with Pseudomonas were found to be healthy. It has already been proved that species of Vibrio caused mortality of oyster larvae in hatchery (Brown, 1973; Brown and Lose, 1978). Lightner (1985) showed that attack of Vibrio generally occur in penaeid hatcheries. Kungvankij (1985) stated that the most serious disease causing organisms among bacteria in the larval stages was Vibrio. Liao (1985) reported that Vibrio infect the post larvae invading haemolymph and midgut gland. Thus the low percentage survival and metamorphosis of mysis and post larvae observed in the study might be due to the invasion of Vibrio into the larvae from the water in which they were reared.

Generally the percentage of various hydrolytic enzyme producing bacteria were found to be slightly lesser in water than in the larvae. It is quite natural to observe higher percentage of ureolytic, proteolytic and amylolytic forms and lower percentage of chitinoclastic and lipolytic bacteria

in a marine or brackishwater system. Both in water and larvae the maximum percentage of hydrolytic bacteria was confined to post larvae and the minimum percentage in the early stages such as eggs and nauplii. In larvae, the important genera producing various hydrolytic enzymes were Vibrio, Pseudomonas and Acinetobacter. Towards the later part of the larval history, the higher percentage of hydrolytic enzyme producing bacteria were members of genus Vibrio and it could be seen that Vibrio was the only genus comprising the highest percentage of hydrolytic enzyme producers. Interestingly majority of Vibrio and a small percentage of Moraxella were chitinoclastic. In eggs and nauplii the percentage of hydrolytic enzymes producers were small concomitant with the low percentage of Vibrio in them. Among Gram-positive bacteria Bacillus recorded the maximal percentage of amylolytic and proteolytic forms.

In eggs and nauplii various hydrolytic forms other than chitinoclasts were contributed by all the genera. However the chitinolytic bacteria were mainly vibrios. Also in zoeae, mysis and post larvae, Vibrio was the main source for all the hydrolytic enzymes. Genera other than Vibrio were also equally potent enough to elaborate these hydrolytic enzymes. Even in eggs and nauplii chitinolytic bacteria were mainly of vibrios. Usually, when the larvae

moult and metamorphose, large quantity of exoskeleton is shed into the water which forms a good substrate for the chitinoclastic bacteria to attach and proliferate. Since vibrios are capable of elaborating this enzyme, they can multiply rapidly and consequently get attached to larvae where they can grow further. That might be one of the reasons for the dominance of chitinoclastic Vibrio towards the later period of larval history. Chitinoclastic vibrios are known to predominate in association with crustaceans (Seki and Taga, 1963; Ivy Thomas, 1982).

In water also, almost the same observations could be seen. Thus when the chitinoclastic vibrios proliferate and dominate the microbial population both in water and in larvae, they can cause serious health hazard to the latter, provided the organisms can get an entry into the larval body.

NaCl concentration of 3 and 7% were the optimum for majority of isolates from larvae and water. While in eggs and nauplius 3% NaCl was the optimum concentration for majority of the isolates, in zoea and mysis it was 7% NaCl. Majority of the isolates from post larvae preferred 10% NaCl. Thus a shift was observed in NaCl preference by the bacteria from 3 to 10% as the egg transformed into post larvae. In water of the corresponding larval stages also, similar observation was made. The shift in NaCl requirement can be

correlated with the generic wise preference for the NaCl. It was seen that Vibrio contained the highest percentage of isolates requiring 10% NaCl for maximum growth. Further, 7% NaCl concentration was preferred highly by Vibrio and then by Micrococcus. It was further seen that in all the other genera there were at least a small percentage of cultures showing maximum growth at 1% NaCl. When the total heterotrophs shifted from slightly halophilic to moderately halophilic correspondingly an increase in the percentage of vibrios could also be seen. Similar was the situation in water samples also. Salinity of water did not increase in pools from eggs to post larvae whereas declined slightly. Therefore the increase of moderately halophilic forms, when the pool became aged could not be correlated with the salinity changes. The halophilic vibrios might have increased due to factors other than the salinity. However it could be observed that there existed 3 - 10% salt requiring vibrios. It could be suggested that an increase in the salinity of the water may further enhance the proliferation of Vibrio which might further augment invasion and the consequent mortality of larvae.

Majority of the isolates preferred pH 7 in both larvae and water. Members of Vibrio, Pseudomonas, Acinetobacter, Moraxella and Coryneform group preferred pH 7

for maximum growth whereas Enterobacteriaceae, Micrococcus and Bacillus preferred pH 9 as their optimum. In the beginning of larval history bacteria which preferred pH 7 were more and was constituted by all the genera. However in water members of Bacillus, Micrococcus which preferred pH 9 were dominant.

Majority of the isolates being mesophilic preferred 30°C for maximum growth in eggs, nauplii, zoeae, mysis and water. However in post larval stages most of the isolates preferred 40°C. This could be attributed to the dominance of Vibrio which preferred 40°C for their maximal growth.

Table 2. Details regarding the frequency of the collections made from hatchery

Year	Periods of collection	Pool No.	Mode of sampling
1981	18 - 24, March	1	From eggs to post larvae as they hatch out and metamorphose in the same pool.
1981	1 - 12, April	2	-do-
1982	16 - 27, March	3	-do-
1984	25, March - 3, April	4	-do-
1984	8 - 19, January	5	-do-
1984	9, February - 3, March	6	-do-

Table 3. Physico-chemical parameters measured during the study period in hatchery

Eggs/ Larval stage	Pool No.	Salinity (10^{-3})	Temperature (^{o}C)	pH	Oxygen ($ml.l^{-1}$)	Inorganic phosphorus ($\mu g.l^{-1}$)	Organic phosphorus ($\mu g.l^{-1}$)	Inorganic nitrogen ($\mu g.l^{-1}$)	Organic nitrogen ($\mu g.l^{-1}$)	Percentage survival and metamorphosis
1	2	3	4	5	6	7	8	9	10	11
Eggs	1	28.53	28.00	7.90	7.33	8.00	2.00	9.34	34.66	97.14
	2	25.64	28.00	7.90	7.32	9.00	1.00	9.59	35.90	93.75
	3	25.50	29.00	7.60	6.03	2.10	3.58	3.00	166.50	92.85
	4	28.27	29.00	8.20	6.50	5.25	2.95	1.35	111.75	93.75
	5	28.08	29.00	8.50	6.40	5.25	2.90	1.30	111.65	88.88
	6	29.05	28.00	7.50	5.85	5.70	4.00	4.00	237.00	80.00
	Ave.	27.51	28.50	7.93	6.57	5.88	2.74	4.76	116.24	91.06

Table 3. Contd.

1	2	3	4	5	6	7	8	9	10	11
Nauplii	1	29.07	29.00	8.50	6.43	7.30	7.00	8.67	21.34	80.15
	2	27.16	28.00	8.00	6.31	7.30	3.20	9.67	21.30	73.33
	3	25.25	28.00	7.70	5.98	2.30	3.70	2.90	173.10	60.00
	4	28.08	28.00	8.25	6.12	5.39	2.98	1.26	111.94	97.33
	5	28.10	28.00	8.20	6.50	5.40	2.97	1.25	111.85	87.50
	6	29.07	28.00	7.49	5.98	5.69	4.50	4.00	236.00	83.33
	Ave.	27.79	28.17	8.02	6.22	5.56	4.10	4.60	112.59	80.27
Zoea	1	30.54	28.00	8.13	7.78	5.10	3.00	8.96	34.50	26.85
	2	29.80	28.00	8.30	8.17	6.00	2.96	8.87	37.12	48.19
	3	24.70	28.00	8.47	5.61	4.10	3.71	1.71	208.90	25.64
	4	25.90	28.00	7.87	5.85	2.33	5.03	1.67	208.33	89.04
	5	25.89	29.00	7.85	6.21	2.35	5.13	1.65	208.30	94.44
	6	24.66	29.00	7.32	6.20	4.25	2.50	4.07	130.93	66.66
	Ave.	21.92	28.33	7.99	6.64	4.02	3.72	4.49	138.00	58.47

Table 3. Contd.

1	2	3	4	5	6	7	8	9	10	11
Mysis	1	29.17	31.50	8.58	7.27	6.00	3.00	7.80	27.20	14.28
	2	30.17	31.00	8.60	7.19	5.60	3.65	9.79	23.25	9.49
	3	23.58	29.00	8.69	5.45	5.60	3.00	7.30	190.80	10.00
	4	22.28	29.00	7.89	5.45	4.53	4.32	2.75	128.50	53.85
	5	26.85	29.00	8.10	5.95	4.57	4.30	2.65	128.30	45.00
	6	22.56	28.00	7.53	6.05	2.40	2.20	4.25	175.26	80.00
	Ave.	25.76	29.58	8.23	6.23	4.80	3.40	5.80	112.21	35.43
Post larvae	1	27.60	33.00	8.85	7.33	5.40	2.10	6.10	23.40	14.00
	2	30.53	33.00	8.70	7.36	6.35	2.97	6.74	21.00	16.00
	3	22.10	30.50	8.66	5.78	5.70	5.70	6.88	185.20	17.00
	4	16.90	29.00	8.24	6.01	3.56	4.25	2.86	127.40	42.85
	5	19.54	30.00	8.29	6.30	3.53	5.73	4.00	182.75	50.00
	6	12.50	29.00	7.63	6.10	2.50	2.30	4.18	176.25	40.00
	Ave.	21.53	30.75	8.39	6.48	4.50	3.84	5.16	119.33	29.98

Table 4. Percentage occurrence of gram negative bacteria in eggs, larvae and water

Eggs/ Larval stage	Larvae			Water		
	Pool No.	Total no. of isolates	Gram negative	Total no. of isolates	Gram negative	Percentage survival and metamorphosis into next stage
Eggs	1	26	80.77	26	76.92	97.14
	2	22	86.36	20	85.00	93.75
	3	20	70.00	20	65.00	92.85
	4	10	80.00	16	81.25	93.75
	5	9	100.00	5	100.00	88.88
	6	9	88.89	11	81.81	80.00
		96	84.34	98	81.66	91.06
Nauplii	1	33	81.81	21	85.71	80.15
	2	21	95.24	21	85.71	73.33
	3	19	73.68	17	100.00	60.00
	4	10	90.00	9	88.89	97.33
	5	5	100.00	16	75.00	87.50
	6	7	85.71	7	85.71	83.33
		95	87.75	91	86.84	80.27
Zoeae	1	25	96.00	21	90.48	26.85
	2	21	95.24	28	92.85	48.19
	3	18	100.00	15	86.67	25.64
	4	5	100.00	9	55.56	89.04
	5	11	81.82	14	78.57	94.44
	6	3	66.67	5	60.00	66.66
		83	89.96	92	77.27	58.47
Mysis	1	17	100.00	20	90.00	14.28
	2	23	95.65	22	95.45	9.43
	3	21	100.00	21	95.24	10.00
	4	10	90.00	6	100.00	53.84
	5	10	90.00	8	100.00	45.00
	6	5	80.00	8	87.50	80.00
		86	92.60	85	94.70	35.45
Post larvae	1	19	100.00	22	95.45	14.00
	2	20	100.00	21	100.00	16.00
	3	20	95.00	17	100.00	17.00
	4	6	100.00	5	100.00	42.85
	5	3	66.67	3	33.33	50.00
	6	21	100.00	19	84.21	40.00
		89	93.61	87	85.50	29.98
Total		449	89.65	453	85.19	

Table 5. Generic composition of bacterial strains isolated from eggs, larvae and water (%)

Genera	Total no. of isolates	Sample					Total (%)
		Eggs	Nauplii	Zoeae	Mysis	Post larvae	
Vibrio	210	10.42*	20.00	54.22	69.77	85.39	47.96
	168	12.24**	13.19	41.30	57.65	67.06	38.29
Pseudomonas	75	30.21	20.00	16.87	11.63	3.37	16.42
	84	24.49	24.18	20.65	12.94	9.47	18.35
Aeromonas	-	-	-	-	-	-	-
	2		1.09		1.18		0.45
Acinetobacter	84	28.13	28.42	19.28	9.30	6.74	18.37
	77	25.51	23.08	15.21	7.06	12.94	16.76
Flavobacterium	-	-	-	-	-	-	-
Alcaligenes	-	-	-	-	-	-	-
Moraxella	30	10.42	12.63	3.61	3.69	2.25	6.52
	54	11.22	21.98	5.43	15.29	5.88	11.96
Enterobacteriaceae	10	5.21	4.21	-	1.16	-	2.12
	9	5.10	3.29	1.09	-		1.90
Staphylococcus	-	-	-	-	-	-	-
	-	-	-	-	-	-	-
Micrococcus	15	8.33	2.11	3.61	2.33	-	3.28
	23	7.14	4.39	9.78	2.35	1.18	4.97
Bacillus	16	5.21	8.42	1.20	-	2.24	3.41
	24	10.20	5.49	3.26	1.18	5.88	5.20
Coryneform group	9	2.08	4.21	1.20	2.33	-	1.96
	12	4.08	3.29	3.26	2.35		2.60
Total No. of isolates	449	96	95	83	86	89	902
	453	98	91	92	85	87	

* From eggs and larvae ** From water collected along with eggs and larvae

Table 6. Diversity index (generic) in eggs, larvae and water

	Eggs	Nauplii	Zoeae	Mysis	Postlarvae
Eggs/larvae	0.6966758	0.6995127	0.5628237	0.4625380	0.26612297
Corresponding water sample	0.6863554	0.6917481	0.5998574	0.5808149	0.49406270

Table 7. Correlation coefficients between various environmental factors and THB and Gram-negative bacteria associated with eggs, larvae and water

Source	Salinity (10^{-3})	Temperature	pH	Dissolved oxygen ($ml.l^{-1}$)	Inorganic phosphorus ($\mu g.l^{-1}$)	Organic phosphorus ($\mu g.l^{-1}$)	Inorganic nitrogen ($\mu g.l^{-1}$)	Organic nitrogen ($\mu g.l^{-1}$)
1	2	3	4	5	6	7	8	9
				THB of eggs and larvae				
Eggs	-0.3121	-0.2506	0.4716	0.7641	0.6148	-0.8372*	0.5560	-0.8054*
Nauplii	0.0658	0.2021	0.5401	0.8848*	0.5526	-0.0269	0.4779	-0.7537
Zoeae	0.3141	-0.5102	0.8723*	0.0402	0.4169	-0.1548	0.0477	0.0024
Mysis	-0.1111	-0.1502	0.5668	-0.3901	0.3834	-0.0582	0.2627	0.4420
Post larvae	-0.1008	-0.3137	0.1929	-0.6181	0.1081	0.9803**	0.0375	0.1711

df : 4
*Significant level at <0.05
**Significant level at <0.01

Table 7. Contd.

1	2	3	4	5	6	7	8	9
				Gram negative bacteria of eggs and larvae				
Eggs	0.2565	0.0667	0.7796	0.2153	0.3729	-0.2992	-0.1111	-0.2895
Nauplii	0.0657	0.2021	0.5130	0.8849*	0.5526	-0.0269	0.4779	-0.7537
Zoeae	0.0314	-0.5102	0.8723*	0.0411	0.4169	-0.1548	0.0477	0.0024
Mysis	-0.1110	-0.1496	0.5668	0.3901	-0.3934	0.0582	0.2672	0.4421
Post larvae	-0.1008	-0.3145	0.1929	-0.1929	-0.6181	0.1081	0.9803**	0.6128
				THB of water				
Eggs	-0.0451	0.4170	0.8364*	0.0871	-0.4921	-0.2242	-0.3156	-0.2578
Nauplii	-0.0473	-0.3036	0.2916	0.6419	0.0415	-0.5023	-0.3911	-0.1426
Zoeae	-0.3104	-0.5613	-0.3804	-0.4673	-0.8602*	0.9507**	-0.6361	0.6698
Mysis	-0.0410	-0.2118	0.4049	-0.5066	0.3461	0.4029	-0.0955	0.3863
Post larvae	0.4150	0.2675	0.4864	-0.1323	0.7008	0.4855	0.7399	0.0657

Table 7. Contd.

1	2	3	4	5	6	7	8	9
				Gram negative bacteria of water				
Eggs	0.3725	0.0364	0.7464	0.1311	0.3774	-0.2138	-0.1784	-0.1788
Nauplii	-0.6721	-0.0688	-0.3863	-0.7440	-0.6192	0.0499	0.0052	0.2575
Zoeae	0.6387	-0.3912	0.6129	0.6243	0.6059	-0.3011	0.5867	-0.5299
Mysis	-0.0399	-0.1004	0.1195	-0.4267	0.2986	0.9435**	-0.3605	0.0619
Post larvae	0.2807	0.2789	0.2800	0.1557	0.4587	-0.4359	0.3905	-0.4567
				Percentage survival and metamorphosis of eggs and larvae				
Eggs	-0.4030	0.1393	0.3020	0.7277	0.2224	-0.6489	0.3896	-0.8359*
Nauplii	0.6622	0.0005	0.4269	0.2636	0.4200	-0.4200	-0.3589	-0.0041
Zoeae	-0.4075	0.5715	-0.6166	-0.3838	-0.7595	0.6654	-0.5397	0.5210
Mysis	-0.6893	-0.7733	-0.8306*	-0.3075	-0.8237*	-0.4224	-0.4264	0.4090
Post larvae	-0.8029	-0.8093*	-0.7818	-0.5247	-0.9144*	0.2512	0.9368*	0.6058

Table 8. Correlation coefficients between various environmental factors and the percentage genera in eggs and larvae

Parameters	Vibrio	Pseudomonas	Acinetobacter	Moraxella	Enterobacteriaceae	Micrococcus	Bacillus	Coryneform group
1	2	3	4	5	6	7	8	9
				Eggs				
Salinity	0.7872	-0.1927	-0.4774	0.1950	0.4911	-0.1677	0.0080	-0.8061*
Temperature	-0.1239	0.0930	0.4386	0.2948	-0.4472	0.5180	-0.5798	0.0734
pH	0.0734	0.7266	0.6213	0.1423	-0.5700	0.1213	-0.8611*	-0.4879
Dissolved oxygen	-0.1726	0.8244*	0.5714	-0.8065	-0.5612	-0.0219	-0.0749	0.0724
Inorganic phosphorus	-0.0423	0.5184	0.0728	-0.4931	-0.0405	-0.3022	-0.0287	-0.3288
Organic phosphorus	0.3972	-0.8193*	-0.5859	0.6800	0.5640	0.0473	0.2054	-0.1010
Inorganic nitrogen	-0.1159	0.3284	0.0978	-0.6583	-0.0988	-0.3216	0.4749	0.2916
Organic nitrogen	0.2115	-0.9407**	-0.7730	0.7731	0.7604	-0.0915	0.2549	-0.1144

df : 4 *Significant level at <0.05 **Significant level at <0.01

Table 8. Contd.

1	2	3	4	5	6	7	8	9
			Nauplii					
Salinity	0.0382	-0.1533	-0.6755	0.1281	0.4201	-0.6400	0.1650	-0.2276
Temperature	0.6816	0.0408	0.0992	-0.5457	-0.2000	-0.2955	0.4057	0.3578
pH	0.2605	0.6648	0.5229	0.0465	-0.6986	0.0717	-0.4591	-0.0611
Dissolved oxygen	0.5789	0.8224*	0.3670	-0.2886	-0.5198	-0.4755	-0.4225	-0.2459
Inorganic phosphorus	0.3626	0.1412	0.2120	-0.3294	-0.0338	-0.4898	-0.0566	-0.5774
Organic phosphorus	0.6743	-0.2871	-0.1832	-0.6473	0.1393	-0.3869	0.7035	0.4011
Inorganic nitrogen	0.6326	-0.0992	0.4327	-0.7646	-0.0830	-0.5500	0.3994	0.0763
Organic nitrogen	-0.4327	-0.5871	-0.8126*	0.2876	0.7169	0.1754	0.3268	0.0442

Table 8. Contd.

1	2	3	4	5	6	7	8	9
			Zoeae					
Salinity	0.4082	-0.0141	-0.2797	-0.1038	-	-0.4202	-0.1938	0.0716
Temperature	-0.9559**	0.4906	0.4934	-0.5081	-	0.7951	0.6324	-0.4909
pH	0.6619	0.0332	-0.7916	0.7629	-	-0.8359*	-0.1957	0.6291
Dissolved oxygen	0.2067	-0.0445	-0.7085	-0.1519	-	-0.1603	-0.5574	-0.0804
Inorganic phosphorus	0.1571	-0.3962	0.1683	0.3167	-	0.0191	0.6151	0.2585
Organic phosphorus	0.1642	0.5286	-0.5995	-0.1850	-	-0.4326	-0.3914	-0.2106
Inorganic nitrogen	0.2055	-0.2319	0.0845	-0.0659	-	-0.0817	0.4066	0.0587
Organic nitrogen	-0.1367	0.2618	-0.1810	0.1050	-	-0.0140	0.5887	-0.0228

Table 8. Contd.

1	2	3	4	5	6	7	8	9
			Mysis					
Salinity	0.5450	-0.4429	0.1217	-0.2472	-0.4959	-0.2220	-	-0.3701
Temperature	0.6989	-0.7479	0.5399	-0.5753	-0.2105	0.0117	-	-0.6605
pH	0.9853**	-0.7958	0.2322	-0.7323	-0.3579	-0.1886	-	-0.7846
Dissolved oxygen	0.3207	-0.3359	0.2142	-0.0643	-0.4662	-0.2130	-	-0.1845
Inorganic phosphorus	0.9602**	-0.8875*	0.4827	-0.8783*	-0.0946	-0.0378	-	-0.9075**
Organic phosphorus	0.1694	-0.2934	0.3051	-0.4275	0.5335	0.5899	-	-0.4399
Inorganic nitrogen	0.7325	-0.6388	0.1434	-0.3675	-0.4977	-0.2057	-	-0.4944
Organic nitrogen	-0.4327	0.5719	-0.5065	0.3587	0.1100	-0.1517	-	0.4729

Table 8. Contd.

1	2	3	4	5	6	7	8	9
			Post larvae					
Salinity	0.6229	0.3686	-0.5427	-0.4417	-	-	-0.1415	-
Temperature	0.5970	0.5848	-0.4771	-0.3945	-	-	-0.2140	-
pH	0.6524	0.6524	0.0453	-0.6076	-	-	-0.0731	-
Dissolved oxygen	0.3695	0.7445	-0.1793	-0.6170	-	-	-0.2060	-
Inorganic phosphorus	0.7814	0.2095	-0.5677	-0.1417	-	-	-0.2627	-
Organic phosphorus	-0.3161	-0.9292**	-0.4957	0.1388	-	-	0.6621	-
Inorganic nitrogen	0.6800	0.3786	-0.6441	0.2361	-	-	-0.2584	-
Organic nitrogen	-0.6105	-0.6758	-0.1769	0.6091	-	-	0.4683	-

Table 9. Correlation coefficients between various environmental factors and the percentage genera in water collected along with eggs and larvae

Parameters	Vibrio	Pseudomonas	Aeromonas	Acinetobacter	Moraxella	Enterobacteriaceae	Micrococcus	Bacillus	Coryneform group
1	2	3	4	5	6	7	8	9	10
				Eggs					
Salinity	0.1140	-0.1688	-	-0.5768	0.3806	0.4911	-0.2108	0.0675	-0.8262*
Temperature	-0.2153	0.1596	-	0.0601	0.6124	-0.4472	0.5082	-0.5224	0.0527
pH	-0.1768	0.8338*	-	0.1790	0.3922	-0.5700	0.1030	-0.8843*	-0.4853
Dissolved oxygen	-0.2419	0.6942	-	0.7010	-0.5726	-0.5612	-0.0473	-0.2252	0.0978
Inorganic phosphorus	-0.1064	0.5038	-	0.1878	-0.4146	-0.0405	-0.2970	-0.1290	-0.2974
Organic phosphorus	0.2544	-0.7618	-	-0.6585	0.5229	0.5640	0.0238	0.3475	-0.1355
Inorganic nitrogen	0.6598	0.1724	-	0.4937	-0.8537*	-0.0988	-0.2950	0.3479	0.3169
Organic nitrogen	0.2830	-0.8208*	-	-0.8005	0.4675	0.7604	-0.1066	0.4036	-0.1360

df : 4 *Significant level at <0.05 **Significant level at <0.01

Table 9. Contd.

1	2	3	4	5	6	7	8	9	10
				Nauplii					
Salinity	-0.0125	-0.0329	-0.4807	-0.5834	0.1210	0.4201	-0.0672	0.5647	-0.0439
Temperature	0.2411	0.0748	-0.2000	0.2217	-0.2248	-0.2000	-0.3993	0.3279	0.3564
pH	-0.3677	0.4405	-0.4235	-0.4057	0.1902	-0.6986	0.4255	-0.2007	0.5953
Dissolved oxygen	-0.1499	0.8696*	-0.5198	-0.2477	-0.3686	-0.5198	0.4295	0.1194	0.9590**
Inorganic phosphorus	0.5134	0.2795	-0.8733*	-0.4517	-0.2588	0.0338	0.0809	0.5032	0.5784
Organic phosphorus	0.4740	-0.1318	-0.1130	0.2076	-0.3340	0.1393	-0.6587	0.5429	0.1368
Inorganic nitrogen	0.7330	0.0436	-0.2293	0.2867	-0.5348	-0.0831	-0.5199	0.3175	0.4107
Organic nitrogen	-0.0057	0.4407	0.3515	-0.1855	0.1187	0.7169	-0.1844	0.1940	-0.7070

Table 9. Contd.

1	2	3	4	5	6	7	8	9	10
				Zoeae					
Salinity	-0.5483	-0.2411	-	0.4525	0.1822	-0.4266	-0.4521	-0.1744	-0.1755
Temperature	-0.5754	0.7046	-	-0.6217	-0.7536	0.6325	0.2584	0.5263	-0.4325
pH	0.6782	-0.3867	-	0.4780	0.5983	-0.8061	-0.5823	-0.7352	0.6425
Dissolved oxygen	-0.6157	-0.2471	-	0.1387	-0.3839	-0.2003	-0.5097	0.0526	-0.2858
Inorganic phosphorus	0.9156**	-0.6697	-	-0.2144	-0.1347	0.0761	-0.7774	0.2938	0.1557
Organic phosphorus	-0.6857	0.6503	-	0.4905	0.2613	-0.5336	0.6269	-0.7023	-0.1185
Inorganic nitrogen	0.6892	-0.4318	-	0.0861	-0.3151	-0.0576	-0.5785	0.2070	-0.1642
Organic nitrogen	-0.6651	0.4329	-	-0.0917	0.3674	-0.0409	0.5604	-0.3024	0.1977

Table 9. Contd.

1	2	3	4	5	6	7	8	9	10
				Mysis					
Salinity	0.6933	-0.4529	-0.3111	-0.0704	-0.6117	-	0.2319	0.4836	-0.2626
Temperature	0.7194	-0.7713	-0.2105	0.3461	-0.5453	-	0.2248	0.6919	-0.2948
pH	0.8506*	-0.8109*	0.4801	0.1990	-0.6597	-	0.6860	0.3649	-0.5890
Dissolved oxygen	0.6629	-0.4419	-0.4662	0.0600	-0.6494	-	0.0699	0.6263	0.1445
Inorganic phosphorus	0.6786	-0.8328*	0.3049	0.3685	-0.4199	-	0.4820	0.4543	-0.7083
Organic phosphorus	-0.3737	-0.0292	-0.2418	0.4934	0.5793	-	-0.0872	-0.2418	-0.8084*
Inorganic nitrogen	0.9317**	-0.7903	0.2555	0.2173	-0.8663*	-	0.7222	0.3383	-0.1141
Organic nitrogen	-0.4975	0.5712	0.5357	-0.3957	0.3647	-	-0.3559	-0.5796	0.1979

Table 9. Contd.

1	2	3	4	5	6	7	8	9	10
				Post larvae					
Salinity	0.2872	-0.5809	-	0.0986	-0.3507	-	-0.1451	-0.4023	-
Temperature	0.0944	0.2180	-	0.3274	-0.1189	-	-0.1999	-0.3439	-
pH	0.4861	-0.2586	-	-0.1922	-0.6278	-	-0.1155	-0.4521	-
Dissolved oxygen	-0.1336	0.3997	-	0.4225	0.0970	-	-0.1277	-0.1756	-
Inorganic phosphorus	0.5141	-0.1857	-	0.0589	-0.4057	-	-0.3155	-0.5911	-
Organic phosphorus	0.2517	-0.8822*	-	-0.8344	-0.7272	-	0.5660	0.2799	-
Inorganic phosphorus	0.2086	0.1046	-	0.3794	-0.0558	-	-0.3307	-0.4291	-
Organic nitrogen	-0.1947	-0.3198	-	-0.3525	-0.0228	-	0.3975	0.4912	-

Table 10. Correlation coefficient between percentage survival and metamorphosis of eggs and larvae and THB and Gram-negative bacteria

Variables	Percentage survival and metamorphosis of eggs and larvae				
	Eggs	Nauplii	Zoeae	Mysis	Postlarvae
THB of eggs and larvae	0.4372	-0.0782	-0.6378	-0.5170	0.1711
THB of water	-0.0092	0.3281	0.8050	-0.0365	-0.5868
Gram-negative bacteria of eggs and larvae	-0.1479	0.5888	-0.3027	-0.9586**	-0.5645
Gram-negative bacteria of water	-0.2695	-0.6126	-0.6878	-0.1437	-0.6741

df : 4

*Significant level at <0.05

**Significant level at <0.01

Table 11. Correlation coefficients between the percentages of various bacterial genera in eggs and larvae and their percentage survival and metamorphosis

	Eggs	Nauplii	Zoeae	Mysis	Post larvae
Vibrio	-0.2780	-0.4259	-0.3068	-0.9879**	-0.8957*
Pseudomonas	0.7889	0.1730	0.3699	0.8324*	-0.4821
Acinetobacter	0.8668*	-0.1980	0.0092	-0.2789	0.3585
Moraxella	-0.8559*	0.6015	-0.7474	0.7525	-0.1150
Enterobacteriaceae	-0.8992*	0.1175	-	0.1621	-
Micrococcus	0.4673	0.2380	0.2833	0.1194	-
Bacillus	-0.1391	-0.5139	0.5887	-	0.5629
Coryneform group	0.4462	-0.7455	-0.8213*	0.8155*	-

df : 4

*Significant level at <0.05

**Significant level at <0.01

Table 12. Correlation coefficients between the percentages of various bacterial genera in water and the percentage survival and metamorphosis of eggs and larvae

Genera / Sample	Eggs	Nauplii	Zoeae	Mysis	Post larvae
Vibrio	-0.4453	-0.2500	-0.8765*	-0.8379*	-0.4051
Pseudomonas	0.5786	0.0856	0.7551	0.8409*	-0.3068
Acinetobacter	0.9304**	-0.8160*	-0.0833	-0.2714	-0.4233
Moraxella	0.4675	0.4780	-0.3396	0.6359	0.0371
Enterobacteriaceae	-0.8991*	0.1175	0.1341	-	-
Micrococcus	-0.4871	0.6679	0.8665*	-0.6877	0.6116
Bacillus	-0.2717	0.1542	-0.0200	-	0.7001
Coryneform group	0.4513	0.0617	-0.7278	0.6114	-

df : 4

*Significant level at <0.05

**Significant level at <0.01

Table 13. Multiple regression between environmental parameters and the survival and metamorphosis of eggs and larvae

Stages		Observed value Y	Calculated value Y	$\chi^2 = \frac{\Sigma(O-E)^2}{E}$	$\chi^2(5)$ Table value	a_0	a_1	a_2	a_3	a_4	a_5	a_6	a_7	a_8
Egg	1	97.14	98.0523	1.6239	11.07	89.1534	-0.00389337	-0.00501269	-0.00747549	0.0660474	397.4443	-644.6403	811.578	-23.44437
	2	93.75	99.3011											
	3	92.85	86.3976											
	4	93.75	87.8908											
	5	88.88	88.0108											
	6	80.00	86.7088											
Nauplius	1	80.15	75.90612	11.0804	11.07	80.414	-0.00887179	0.0069716	-0.02275872	-0.00440015	-469.2715	-7.607422	-161.75	31. 29956
	2	73.33	75.7955											
	3	60.00	84.0113											
	4	97.33	80.8733											
	5	87.50	80.8727											
	6	83.33	84.1746											
Zoea	1	26.85	21.53318	80.98547	11.07	67.00294	-0.094207	0.000104452	0.0314433	-0.731535	-1637.142	1262.834	-4192.579	133.3766
	2	48.19	19.99083											
	3	25.64	79.59976											
	4	89.04	83.8966											
	5	94.44	83.8966											
	6	66.66	56.89839											
Mysis	1	14.28	-130.9752	N.A	-	95.8758	-0.747806	-0.1249722	-0.2335329	-3.164821	-6142.999	1321.697	-22736.57	1255.512
	2	9.43	-177.3912											
	3	10.00	91.658											
	4	53.84	133.1886											
	5	45.00	129.8901											
	6	80.00	166.1749											
Postlarva	1	14.00	-26.1936	N.A.	-	46.62254	-0.733147	-0.1009215	-0.215428	-0.932772	-3055.636	3963.72	-6678.245	359.2646
	2	16.00	-32.9281											
	3	17.00	45.8504											
	4	42.85	56.5628											
	5	50.00	72.4752											
	6	40.00	64.0806											

N.A. - Model not applicable

Table 14. Multiple regression between selected genera and the survival and metamorphosis of eggs and larvae

Stages		Observed value Y	Calculated value Y	$\chi^2 = \frac{\Sigma(O-E)^2}{E}$	$\chi^2(5)$ Table value	a_0	a_1	a_2	a_3	a_4	a_5	a_6	a_7	a_8
Egg	1	97.14	92.10	1.323	11.07	92.382	-0.0995207	-0.04844093	0.004092097	-0.03584385	-0.1025164	-0.0888595	-0.1731071	1.272552
	2	93.75	94.11											
	3	92.85	100.35											
	4	93.75	87.92											
	5	88.88	89.00											
	6	80.00	82.86											
Nauplius	1	80.15	67.66	6.188	11.07	78.94203	-0.0830933	0.2282588	0.1186617	0.2279124	0.02081132	-0.30288946	-0.04393482	-2.804329
	2	73.33	89.84											
	3	60.00	56.32											
	4	97.33	92.89											
	5	87.50	93.34											
	6	83.33	81.57											
Zoea	1	26.85	41.34	9.283	11.07	55.08509	0.0579633	0.2368549	0.003862085	-3.795559	287.848	-0.02443674	1.635812	-5.016143
	2	48.19	48.54											
	3	25.64	18.56											
	4	89.04	93.95											
	5	94.44	91.03											
	6	66.66	57.38											
Mysis	1	14.28	- 8.094	N.A	11.07	59.06559	-0.4128609	0.0772016	-1.15977	0.636779	4.589804	2.94646	156.3091	1.33937
	2	9.45	17.549											
	3	10.00	3.384											
	4	53.84	76.27											
	5	45.00	45.25											
	6	80.00	78.17											
Postlarva	1	14.00	44.62	1358.557 N.A	11.07	25.04895	0.0549308	3.602851	-2.279408	-2.297515	29.2314	35.76303	-0.4888603	7.205139
	2	16.00	41.84											
	3	17.00	28.05											
	4	42.85	1.31											
	5	50.00	51.78											
	6	40.00	12.23											

N.A. - Model not applicable

Table 15. Percentage occurrence of different hydrolytic forms in eggs, larvae and water

Sample	Total No.	Amylolytic	Caseinolytic	Gelatinolytic	Lipolytic	Ureolytic	Chitinolytic
Eggs	96*	80.21	61.46	59.38	20.83	100.00	15.63
	98**	84.69	77.55	69.39	13.27	83.67	14.29
Nauplii	95	81.05	76.84	73.68	26.32	100.00	21.05
	91	71.43	64.84	53.85	13.17	76.92	39.56
Zoeae	83	92.77	93.97	91.57	77.11	86.75	60.24
	92	66.30	94.57	95.65	32.60	92.39	52.17
Mysis	86	69.76	98.84	97.67	39.53	98.84	66.28
	85	76.47	90.59	94.12	27.06	97.65	56.47
Post larvae	89	92.13	98.88	98.88	59.55	98.88	84.27
	87	90.80	100.00	100.00	6.89	100.00	79.31
Total	449	83.07	85.30	83.52	43.65	97.10	48.33
	453	77.92	85.21	82.11	18.54	89.85	47.46

* Eggs and larvae

** Corresponding water sample

Table 16. Poolwise percentage distribution of different hydrolytic enzyme producing bacteria in eggs, larvae and water

Eggs

Pool No.	Total no. of isolates	Amylolytic	Caseinolytic	Gelatinolytic	Lipolytic	Ureolytic	Chitinolytic
1	2	3	4	5	6	7	8
1	26*	96.15	53.85	53.85	30.77	100.00	15.38
	26**	96.15	65.38	63.38	15.38	65.38	15.38
2	22	95.45	45.45	45.45	18.18	100.00	18.18
	20	95.00	80.00	80.00	15.00	100.00	20.00
3	20	80.00	40.00	40.00	40.00	100.00	20.00
	20	95.00	65.00	65.00	15.00	65.00	15.00
4	10	40.00	90.00	100.00	-	100.00	10.00
	16	50.00	100.00	81.25	-	100.00	-
5	9	55.55	100.00	100.00	-	100.00	-
	5	60.00	60.00	60.00	60.00	100.00	16.67
6	9	66.67	100.00	66.67	-	100.00	11.11
	11	81.82	100.00	54.55	-	100.00	18.18
Total	96	80.21	61.46	59.38	20.83	100.00	15.63
	98	84.69	77.55	69.39	13.27	83.67	17.35

*Eggs and larvae **Corresponding water samples

Table 16. Contd.

Nauplii

1	2	3	4	5	6	7	8
1	33*	96.96	81.81	84.85	39.39	100.00	36.30
	21**	100.00	76.19	61.90	19.05	71.43	47.62
2	21	100.00	80.95	80.95	28.57	100.00	19.05
	21	95.24	66.67	57.14	23.81	76.19	23.81
3	19	89.47	42.11	42.11	26.32	100.00	15.79
	17	94.12	88.24	64.71	5.88	41.18	58.82
4	10	20.00	90.00	90.00	-	100.00	20.00
	9	11.11	100.00	88.38	-	100.00	77.78
5	5	40.00	100.00	100.00	20.00	100.00	-
	16	18.75	31.25	31.25	12.50	100.00	12.50
6	7	42.86	100.00	42.86	-	100.00	14.29
	7	57.14	-	-	-	100.00	28.57
Total	95	81.05	76.84	73.68	26.32	100.00	21.05
	91	71.43	72.53	58.24	13.19	76.92	39.56

Table 16. Contd.

Zoeae

1	2	3	4	5	6	7	8
1	25*	96.00	96.00	96.00	96.00	88.00	60.00
	21**	66.67	95.24	100.00	33.33	85.71	47.62
2	21	95.24	85.72	80.95	80.95	80.95	61.90
	28	88.14	96.43	96.43	25.00	92.59	67.86
3	18	100.00	94.44	88.89	77.78	88.89	77.78
	15	10.00	93.33	100.00	46.67	93.33	53.33
4	5	80.00	100.00	100.00	-	100.00	80.00
	9	22.22	88.89	77.78	-	90.00	44.44
5	11	90.90	100.00	100.00	90.90	100.00	27.27
	14	64.29	100.00	100.00	64.29	100.00	35.71
6	3	33.33	100.00	100.00	-	100.00	33.33
	5	80.00	80.00	80.00	-	100.00	40.00
Total	83	92.77	93.97	91.57	77.10	86.74	60.24
	92	66.30	94.56	95.65	32.61	92.39	52.17

Table 16. Contd.

Mysis

1	2	3	4	5	6	7	8
1	17*	100.00	100.00	94.12	35.29	94.12	76.47
	20**	80.00	95.00	95.00	20.00	100.00	75.00
2	23	95.65	95.65	95.65	10.04	100.00	78.26
	22	77.27	95.45	95.45	27.27	100.00	72.73
3	21	9.53	80.95	80.95	80.95	23.80	71.43
	21	80.95	95.23	90.47	38.09	90.47	61.90
4	10	-	100.00	100.00	-	100.00	50.00
	6	16.67	23.82	100.00	-	100.00	-
5	10	90.00	100.00	100.00	80.00	100.00	50.00
	8	75.00	100.00	100.00	62.50	100.00	25.00
6	5	80.00	100.00	100.00	-	100.00	100.00
	8	100.00	50.00	50.00	-	100.00	25.00
Total	86	69.76	98.83	97.67	39.53	98.83	66.27
	85	76.47	90.59	94.12	27.06	97.65	56.47

Table 16. Contd.

Post larvae

1	2	3	4	5	6	7	8
1	19*	94.74	94.74	100.00	89.47	100.00	89.47
	22**	96.91	100.00	100.00	9.09	100.00	77.27
2	20	100.00	100.00	100.00	90.00	100.00	90.00
	21	90.48	100.00	100.00	14.28	100.00	98.48
3	20	90.00	100.00	100.00	90.00	100.00	90.00
	17	100.00	100.00	100.00	5.88	100.00	94.12
4	6	83.30	100.00	83.33	-	100.00	83.33
	5	100.00	100.00	100.00	-	100.00	80.00
5	3	100.00	100.00	100.00	-	66.67	100.00
	3	50.00	100.00	100.00	-	100.00	100.00
6	21	85.71	100.00	100.00	-	100.00	66.67
	19	89.47	100.00	100.00	-	100.00	52.68
Total	89	92.13	92.13	92.13	59.55	98.87	84.26
		90.80	100.00	100.00	6.89	100.00	79.31

Table 17. Percentage distribution of various hydrolytic enzyme producers in different genera isolated from eggs, larvae and water

Hydrolytic groups	Total No. of isolates positive	Vibrio	Pseudomonas	Aeromonas	Acinetobacter	Moraxella	Enterobacteriaceae	Micrococcus	Bacillus	Coryneform group
Amylolytic	373*	51.74	16.89	-	19.03	5.36	0.80	0.27	4.03	1.88
	353**	45.89	18.41	0.28	15.86	8.22	1.70	1.98	5.68	1.98
Caseinolytic	383	54.03	10.70	-	21.67	4.96	2.61	1.04	4.18	0.79
	386	43.00	12.69	0.52	19.95	11.66	1.29	4.15	5.96	0.78
Gelatinolytic	375	55.20	11.20	-	21.33	4.53	0.80	1.60	4.27	1.07
	372	44.62	13.44	0.52	19.35	10.48	-	4.03	6.18	1.38
Lipolytic	196	70.92	10.20	-	13.27	5.10	-	-	-	0.51
	84	52.38	28.51	-	3.57	14.29	-	-	-	1.19
Ureolytic	436	48.17	16.97	-	16.72	6.88	2.29	3.44	3.45	2.06
	407	41.28	18.92	0.48	11.06	12.53	2.21	5.16	5.90	2.46
Chitinolytic	217	92.63	0.92	-	0.46	4.61	-	-	0.46	0.92
	215	77.20	2.70	-	11.16	3.26	-	4.65	0.47	0.47

*Eggs and larvae **Water collected along with eggs and larvae

Table 18. Generic wise distribution of hydrolytic bacteria in eggs, larvae and water

Sample	No. of isolates	Percentages of hydrolytic forms					
		Amylolytic	Cascinolytic	Gelatinolytic	Lipolytic	Ureolytic	Chitinoclastic
1	2	3	4	5	6	7	8
				Vibrio			
Eggs	10*	100.00	100.00	100.00	70.00	100.00	100.00
	12**	100.00	100.00	100.00	83.33	100.00	100.00
Nauplii	19	94.74	84.21	82.21	78.95	100.00	78.95
	12	100.00	83.33	83.33	83.33	100.00	100.00
Zoeae	45	100.00	100.00	100.00	91.11	100.00	100.00
	38	44.73	100.00	100.00	23.68	100.00	100.00
Mysis	60	73.33	100.00	100.00	38.33	100.00	95.00
	49	97.96	100.00	100.00	22.45	100.00	97.96
Post larvae	76	100.00	100.00	100.00	69.74	100.00	97.37
	57	94.74	100.00	100.00	7.02	100.00	98.25

*Eggs and larvae

**Corresponding water samples

Table 18. Contd.

1	2	3	4	5	6	7	8
				Pseudomonas			
Eggs	29*	82.75	20.69	20.69	-	100.00	-
	24**	91.67	50.00	50.00	12.50	100.00	4.17
Nauplii	19	98.95	57.89	63.16	15.79	100.00	-
	22	54.54	4.55	4.55	9.09	100.00	-
Zoeae	14	100.00	85.71	85.71	85.71	100.00	14.29
	19	89.47	94.74	100.00	73.68	68.42	5.26
Mysis	10	80.00	100.00	90.00	50.00	90.00	-
	11	63.64	90.91	90.91	45.45	90.91	-
Post larvae	3	66.67	66.67	100.00	-	100.00	-
	8	89.50	100.00	100.00	-	100.00	50.00

Table 18. Contd.

1	2	3	4	5	6	7	8
			Aeromonas				
Eggs	-*	-	-	-	-	-	-
	-**	-	-	-	-	-	-
Nauplii	-	-	-	-	-	-	-
	1	100	100	100	-	100	-
Zoeae	-	-	-	-	-	-	-
	-	-	-	-	-	-	-
Mysis	-	-	-	-	-	-	-
	1	-	100	100	-	100	-
Post larvae	-	-	-	-	-	-	-
	-	-	-	-	-	-	-

Table 18. Contd.

1	2	3	4	5	6	7	8
			Acinetobacter				
Eggs	27*	92.59	100.00	100.00	29.63	100.00	-
	25**	88.00	100.00	88.00	-	52.00	-
Nauplii	27	92.59	100.00	96.29	11.11	100.00	-
	21	95.24	100.00	15.24	-	4.76	71.43
Zoeae	16	81.25	93.75	87.50	62.50	31.25	6.25
	14	21.43	100.00	92.86	21.43	100.00	14.29
Mysis	8	62.50	100.00	100.00	62.50	100.00	-
	6	-	100.00	100.00	-	100.00	-
Post larvae	6	50.00	100.00	83.33	-	100.00	-
	11	100.00	100.00	100.00	-	100.00	63.64

Table 18. Contd.

1	2	3	4	5	6	7	8
				Moraxella			
Eggs	10*	90.00	50.00	50.00	50.00	100.00	50.00
	11**	36.56	72.73	72.73	-	81.82	-
Nauplii	12	50.00	50.00	50.00	33.33	100.00	33.33
	20	55.00	90.00	45.00	-	95.00	35.00
Zoeae	3	100.00	100.00	33.33	-	100.00	33.33
	5	60.00	100.00	80.00	60.00	100.00	-
Mysis	3	66.67	100.00	100.00	33.33	100.00	-
	13	61.54	69.23	100.00	53.85	100.00	-
Post larvae	2	-	100.00	100.00	-	100.00	-
	5	60.00	100.00	100.00	40.00	100.00	-

Table 18. Contd.

1	2	3	4	5	6	7	8
				Enterobacteriaceae			
Eggs	5*	40.00	100.00	40.00	-	100.00	-
	5**	100.00	100.00	-	-	100.00	-
Nauplii	4	25.00	100.00	-	-	100.00	-
	3	33.33	-	-	-	100.00	-
Zoeae	-	-	-	-	-	-	-
	1	-	-	-	-	100.00	-
Mysis	1	-	100.00	100.00	-	100.00	-
	-	-	-	-	-	-	-
Post larvae	-	-	-	-	-	-	-
	-	-	-	-	-	-	-

Table 18. Contd.

1	2	3	4	5	6	7	8
				Micrococcus			
Eggs	8*	-	12.50	25.00	-	100.00	-
	7*	71.43	42.86	42.86	-	85.71	-
Nauplii	2	-	-	50.00	-	100.00	-
	4	25.00	75.00	75.00	-	100.00	50.00
Zoeae	3	33.33	66.67	66.67	-	100.00	-
	9	11.11	88.89	88.89	-	100.00	77.78
Mysis	2	-	50.00	50.00	-	100.00	-
	2	-	50.00	-	-	50.00	-
Post larvae	-	-	-	-	-	-	-
	1	-	100.00	100.00	-	100.00	100.00

Table 18. Contd.

1	2	3	4	5	6	7	8
			Bacillus				
Eggs	5*	100.00	100.00	100.00	-	100.00	-
	10**	100.00	100.00	100.00	-	100.00	-
Nauplii	8	100.00	100.00	100.00	-	100.00	-
	5	100.00	80.00	80.00	-	100.00	-
Zoeae	1	100.00	100.00	100.00	-	100.00	-
	3	33.33	100.00	100.00	-	100.00	-
Mysis	-	-	-	-	-	-	-
	1	-	100.00	100.00	-	100.00	-
Post larvae	2	50.00	100.00	100.00	-	50.00	-
	5	80.00	100.00	100.00	-	100.00	20.00

Table 18. Contd.

1	2	3	4	5	6	7	8
				Coryneform group			
Eggs	2*	100.00	-	-	-	100.00	-
	4*	75.00	25.00	25.00	-	75.00	25.00
Nauplii	4	100.00	25.00	25.00	-	100.00	25.00
	3	66.67	33.33	33.33	-	100.00	-
Zoeae	1	-	-	100.00	100.00	100.00	100.00
	3	-	33.38	100.00	33.33	66.67	-
Mysis	2	50.00	100.00	100.00	-	100.00	-
	2	100.00	-	-	-	100.00	-
Post larvae	-	-	-	-	-	-	-
	-	-	-	-	-	-	-

Table 19. Percentage contribution of different genera for a particular hydrolytic enzyme

Hydrolytic forms	Total no. of isolates positive	Vibrio	Pseudomonas	Aeromonas	Acinetobacter	Flavobacterium	Alcaligenes	Moraxella	Enterobacteriaceae	Staphylococcus	Micrococcus	Bacillus	Coryneform group
1	2	3	4	5	6	7	8	9	10	11	12	13	14
						Eggs							
Amylase	77*	12.99	31.17	-	32.47	-	-	11.69	2.59	-	-	6.49	2.59
	83**	14.46	26.51	-	26.51	-	-	4.82	6.02	-	6.02	12.05	3.62
Caseinase	59	16.95	10.17	-	45.76	-	-	8.47	8.47	-	1.69	8.47	-
	76	15.79	15.79	-	32.89	-	-	10.53	6.58	-	3.95	13.16	1.32
Gelatinase	57	17.54	10.53	-	47.37	-	-	8.78	3.51	-	3.51	8.78	-
	68	17.65	17.65	-	32.35	-	-	11.76	-	-	4.41	14.71	1.47
Lipase	20	35.00	-	-	40.00	-	-	25.00	-	-	-	-	-
	13	76.92	28.07	-	-	-	-	-	-	-	-	-	-
Urease	96	10.42	30.21	-	28.13	-	-	10.42	5.21	-	8.33	5.21	2.68
	82	14.63	29.27	-	15.85	-	-	10.98	6.09	-	7.32	12.19	3.65
Chitinase	15	66.67	-	-	-	-	-	33.33	-	-	-	-	-
	14	85.71	7.14	-	-	-	-	-	-	-	-	-	7.14

*Eggs and larvae

**Corresponding water samples

Table 19. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Nauplii								
Amylase	77*	23.37	19.48	-	32.47	-	-	7.79	1.29	-	-	10.39	5.19
	65**	18.46	18.46	1.54	30.77	-	-	16.92	1.54	-	1.54	7.69	3.08
Caseinase	73	21.92	15.07	-	36.99	-	-	8.22	5.48	-	-	10.95	1.37
	59	16.94	1.69	1.69	35.59	-	-	30.51	-	-	5.08	6.78	1.69
Gelatinase	70	22.86	17.14	-	37.14	-	-	8.57	-	-	1.43	11.43	1.43
	49	20.41	2.04	2.04	40.82	-	-	18.36	-	-	6.12	8.16	2.04
Lipase	25	60.00	12.00	-	12.00	-	-	16.00	-	-	-	-	-
	12	83.33	16.67	-	-	-	-	-	-	-	-	-	-
Urease	95	20.00	20.00	-	28.42	-	-	12.63	4.21	-	2.11	8.42	4.21
	70	17.14	31.43	1.43	1.43	-	-	27.14	4.28	-	5.71	7.14	4.28
Chitinase	20	75.00	-	-	-	-	-	25.00	-	-	-	-	-
	36	33.33	-	-	41.66	-	-	19.44	-	-	5.56	-	-

Table 19. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Zoeae								
Amylase	77*	58.44	18.18	-	16.88	-	-	3.89	-	-	1.29	1.29	-
	61**	59.02	27.86	-	4.92	-	-	4.92	-	-	1.64	1.64	-
Caseinase	78	57.69	15.38	-	19.23	-	-	3.85	-	-	2.56	1.28	-
	87	43.68	20.69	-	16.09	-	-	5.75	-	-	9.19	3.45	1.15
Gelatinase	76	59.21	15.79	-	18.42	-	-	1.32	-	-	2.63	1.32	1.32
	88	43.18	21.59	-	14.77	-	-	4.55	-	-	9.09	3.41	3.41
Lipase	64	64.06	18.75	-	15.63	-	-	-	-	-	-	-	1.56
	30	30.00	46.67	-	10.00	-	-	10.00	-	-	-	-	3.33
Urease	72	62.50	19.44	-	6.94	-	-	4.17	-	-	4.17	1.39	1.39
	85	44.71	15.29	-	16.47	-	-	5.88	1.18	-	10.59	3.53	2.35
Chitinase	50	90.00	4.00	-	2.00	-	-	2.00	-	-	-	-	2.00
	48	79.17	2.08	-	4.17	-	-	-	-	-	14.58	-	-

Table 19. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Mysis								
Amylase	60*	73.33	13.33	-	8.33	-	-	3.33	-	-	-	-	1.67
	65**	73.85	10.77	-	-	-	-	12.31	-	-	-	-	3.08
Caseinase	85	70.58	11.76	-	9.41	-	-	3.53	1.18	-	1.18	-	2.35
	77	63.63	12.99	1.29	7.79	-	-	11.69	-	-	1.29	1.29	-
Gelatinase	84	71.43	10.71	-	9.53	-	-	3.57	1.19	-	1.19	-	2.38
	80	61.25	12.50	1.25	7.50	-	-	16.25	-	-	-	1.25	-
Lipase	34	67.65	14.71	-	14.70	-	-	2.94	-	-	-	-	-
	23	47.82	21.73	-	-	-	-	30.43	-	-	-	-	-
Urease	85	70.59	10.58	-	9.41	-	-	3.53	1.18	-	2.35	-	2.35
	83	59.04	12.05	1.20	7.23	-	-	15.66	-	-	1.20	1.20	2.41
Chitinase	57	100.00	-	-	-	-	-	-	-	-	-	-	-
	48	100.00	-	-	-	-	-	-	-	-	-	-	-

Table 19. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
						Post larvae							
Amylase	82*	92.68	2.43	-	3.66	-	-	-	-	-	-	1.22	-
	79**	68.35	8.86	-	13.92	-	-	3.79	-	-	-	5.06	-
Caseinase	88	86.36	2.27	-	6.82	-	-	2.27	-	-	-	2.27	-
	87	62.06	9.19	-	12.64	-	-	5.75	-	-	1.15	5.75	-
Gelatinase	88	86.36	3.41	-	5.68	-	-	2.27	-	-	-	2.27	-
	87	65.52	9.19	-	12.64	-	-	5.75	-	-	1.15	5.75	-
Lipase	53	100.00	-	-	-	-	-	-	-	-	-	-	-
	6	66.67	-	-	-	-	-	33.33	-	-	-	-	-
Urease	88	86.36	3.41	-	6.82	-	-	2.27	-	-	-	1.14	-
	87	65.52	9.19	-	12.64	-	-	5.75	-	-	1.14	5.75	-
Chitinase	75	98.67	-	-	-	-	-	-	-	-	-	1.33	-
	69	81.16	5.79	-	10.14	-	-	-	-	-	1.44	1.44	-

Table 20. Percentage of isolates in various pools showing maximum growth at various NaCl concentration pH and temperature

Pool No.	Total no. of isolates	NaCl concentrations (%)				
		0	1	3	7	10
1	120*	-	8.33	32.50	44.17	15.00
	110**		12.73	24.55	50.91	11.82
2	107	-	7.48	31.78	39.25	21.49
	112		4.46	34.82	46.43	14.28
3	98	-	5.10	30.61	47.96	16.32
	90		8.89	46.67	44.44	-
4	41	-	4.88	56.09	34.14	4.87
	45		6.67	73.33	4.44	15.56
5	38	2.63	15.79	42.11	34.21	5.26
	46	17.39	17.39	34.78	17.39	13.04
6	45	6.67	26.67	66.67	-	-
	50	4.00	24.00	68.00	4.00	
Total	449	0.89	9.58	38.30	37.64	13.58***
	453	2.21	11.04	42.16	35.32	9.27***

*Eggs and larvae

**Corresponding water samples

***Percentage calculated from the total isolates showing maximum growth

Table 20. Contd.

Pool No.	Total no. of isolates	pH				
		2	4	7	9	11
1	120* 110**	-	-	90.83 48.18	9.17 51.82	-
2	107 112	-	-	76.63 73.21	23.36 26.78	-
3	98 90	-	-	79.59 54.44	20.41 45.56	-
4	41 45	-	-	58.54 40.00	17.07 20.00	24.39 40.00
5	38 46	-	-	78.95 76.09	21.05 23.91	-
6	45 50	-	-	80.00 88.00	20.00 12.00	-
Total	449 453	-	-	79.96 62.03	17.81 33.99	2.23 3.97***

Table 20. Contd.

Pool No.	Total no. of isolates	Temperature (°C)					
		4	10	30	40	50	
1	120*	-	0.83	47.50	51.67	-	
	110**		9.09	53.63	45.45		
2	107	-	0.93	63.55	35.51	-	
	112		-	46.42	53.57		
3	98	-	7.14	63.26	29.59	-	
	90		8.89	56.67	34.44		
4	41	-	-	70.73	29.27	-	
	45			44.44	55.55		
5	38	-	-	50.00	50.00	-	
				41.30	58.69		
6	45	-	-	77.77	22.22	-	
	50			72.00	28.00		
Total	449	-	2.00	60.13	37.86	-	***
	453		1.99	52.31	45.60		

Table 21. Percentage of isolates from eggs, larvae and water showing maximum growth at various NaCl concentrations, pH and temperature

Larval stages	No. of isolates	NaCl concentrations (%)					pH					Temperature (°C)				
		0	1	3	7	10	2	4	7	9	11	4	10	30	40	50
Eggs	96*	-	12.50	69.79	17.71	-	-	-	78.13	21.88	-	-	-	60.41	39.58	-
	98**	-	14.29	62.24	21.43	2.04	-	-	56.12	85.71	8.16	-	-	64.29	35.71	-
Nauplii	95	1.05	16.84	60.00	20.00	2.10	-	-	76.84	14.73	8.42	-	-	63.15	36.84	-
	91	3.29	21.98	56.09	14.29	4.39	-	-	72.52	18.68	8.79	-	3.29	52.75	43.90	-
Zoeae	83	3.61	2.40	18.07	66.29	9.64	-	-	83.13	14.45	2.40	-	3.61	78.31	18.07	-
	92	6.52	7.61	30.43	55.43	-	-	-	41.30	56.52	21.74	-	5.43	61.96	32.61	-
Mysis	86	-	9.30	15.11	75.58	-	-	-	68.60	31.39	-	-	3.48	67.44	29.06	-
	85	-	5.88	23.53	65.88	4.71	-	-	61.18	3.88	-	-	1.18	57.64	41.18	-
Post larvae	89	-	5.62	22.47	14.61	57.30	-	-	93.25	6.74	-	-	3.37	32.58	64.04	-
	87	1.20	4.81	37.34	22.89	38.55	-	-	84.33	20.48	-	-	-	24.09	80.72	-
Total	449	0.89	9.58	38.30	37.64	13.58	-	-	79.96	17.81	2.23	-	2.00	60.13	37.86	-***
	453	2.21	11.04	42.16	35.32	9.27	-	-	62.03	33.99	3.97	-	1.99	52.31	45.69	-

*Eggs and larvae **Corresponding water samples

***Percentage calculated from the total isolates showing maximum growth

Table 22. Growth response of different genera to NaCl concentration, pH and temperature

Genera	No. of isolates	NaCl concentrations (%)					pH					Temperature (°C)				
		0	1	3	7	10	2	4	7	9	11	4	10	30	40	50
Vibrio	210*	-	-	11.43	64.76	23.81	-	-	86.67	12.86	0.48	-	0.95	67.14	31.91	-
	168**	-	0.59	13.09	67.27	19.04	-	-	54.76	45.24	-	-	1.79	57.74	40.48	-
Pseudomonas	75	-	6.67	69.33	20.00	4.00	-	-	88.00	10.67	1.33	-	2.67	24.00	73.33	-
	84	3.57	10.71	45.23	33.33	7.14	-	-	78.57	21.43	-	-	3.57	34.52	61.90	-
Aeromonas	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	50.00	50.00	-	-	-	-	100	-	-	-	-	50.00	50.00	-
Acinetobacter	84	2.38	13.10	66.67	8.33	9.52	-	-	85.71	9.52	4.76	-	-	84.52	15.48	-
	77	1.30	3.89	81.82	12.99	-	-	-	74.03	18.18	7.79	-	-	72.73	27.27	-
Flavobacterium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alcaligenes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Moraxella	30	-	16.67	80.00	3.33	-	-	-	70.00	16.67	13.33	-	10.00	60.00	30.00	-
	54	-	20.37	70.37	1.85	7.41	-	-	61.11	16.67	22.22	-	5.56	40.74	53.70	-
Enterobacteriaceae	10	10.00	80.00	10.00	-	-	-	-	10.00	90.00	-	-	-	80.00	20.00	-
	9	11.11	88.89	-	-	-	-	-	33.33	66.67	-	-	-	77.78	22.22	-
Staphylococcus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Micrococcus	15	6.67	6.67	26.67	60.00	-	-	-	26.67	73.33	-	-	-	80.00	20.00	-
	23	21.74	21.74	30.43	26.01	-	-	-	39.13	60.87	-	-	-	52.17	47.83	-
Bacillus	16	-	68.75	25.00	6.25	-	-	-	25.00	75.00	-	-	12.50	6.25	81.25	-
	24	-	45.83	50.00	4.17	-	-	-	45.83	54.17	-	-	-	33.33	66.67	-
Coryneform group	9	-	22.22	77.77	-	-	-	-	100	-	-	-	-	11.11	88.88	-
	12	-	8.33	83.33	8.33	-	-	-	66.70	33.33	-	-	-	41.67	58.33	-
Total	449	0.89	9.58	38.30	37.64	13.58	-	-	79.96	17.81	2.23	-	2.00	60.13	37.86	-***
	453	2.21	11.04	42.16	35.32	9.27	-	-	62.03	33.99	3.97	-	1.99	52.31	45.69	-***

*Eggs and larvae **Corresponding water samples

***Percentage calculated from the total isolates showing maximum growth

Fig. 1. Cochin backwater showing the location of Hatchery (H), Station 1 (I), Station 2 (II) and the culture pond (C)

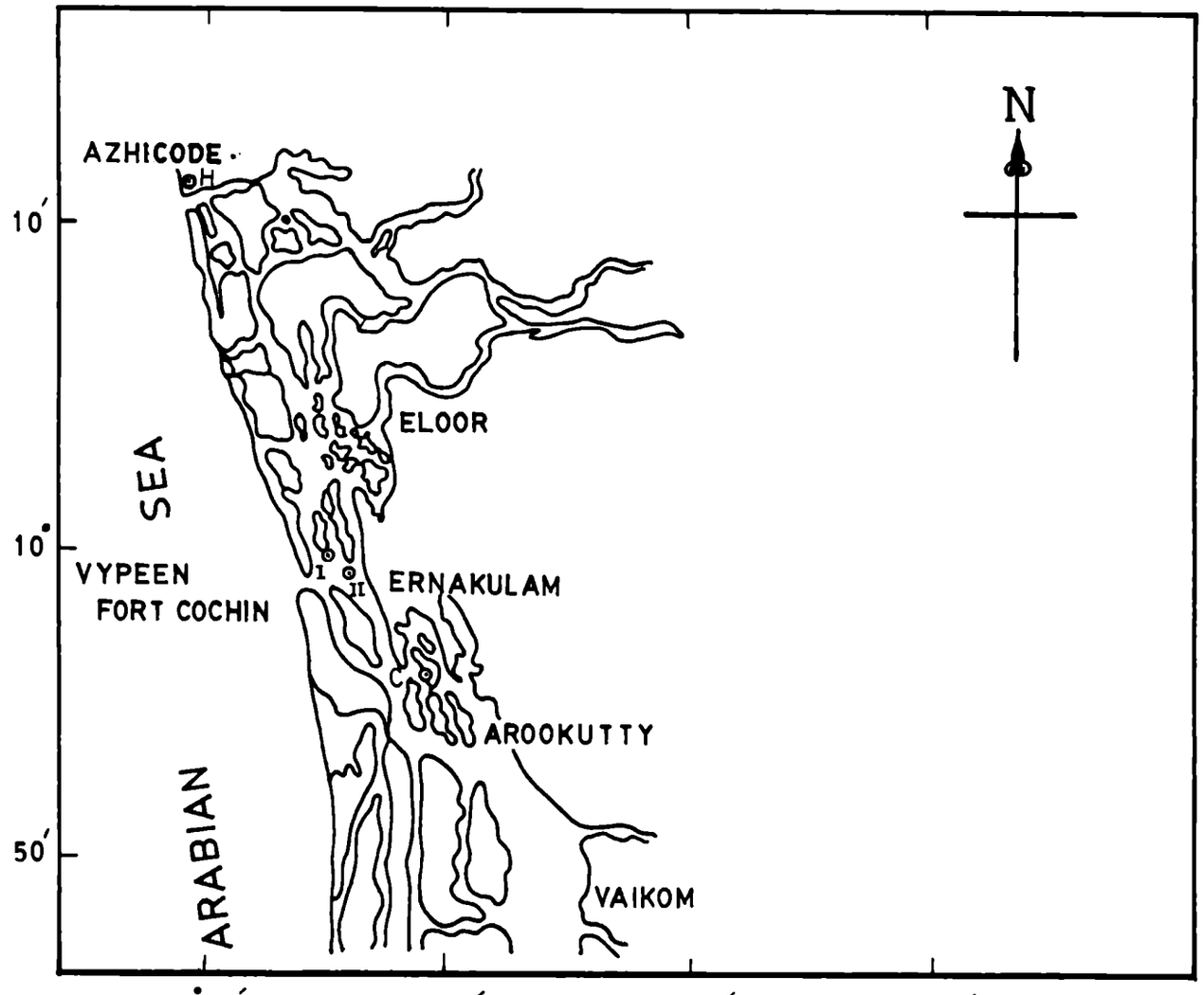

N
AZHICODE
H
10′
10°
50′
SEA
ARABIAN
ELOOR
VYPEEN
FORT COCHIN
I
II
ERNAKULAM
AROOKUTTY
VAIKOM
76°10′
20′
30′
40′

FIG. 1.

Fig. 2. Hatchery complex showing

1) Office and Laboratory 2) Macrobrachium Hatchery

3) 'In Situ' seawater filter 4) Pump

5) Overhead tank platform 6) 7) Jetty

8) Freshwater well 9) Overhead tank (Freshwater)

10) Dormitory and Stores 11) Fish breeding unit: Machinery 12) Raceway and Culture tanks

13) Raceway 14) Machinery room and overhead seawater tank 15) 'In Situ' seawater filter

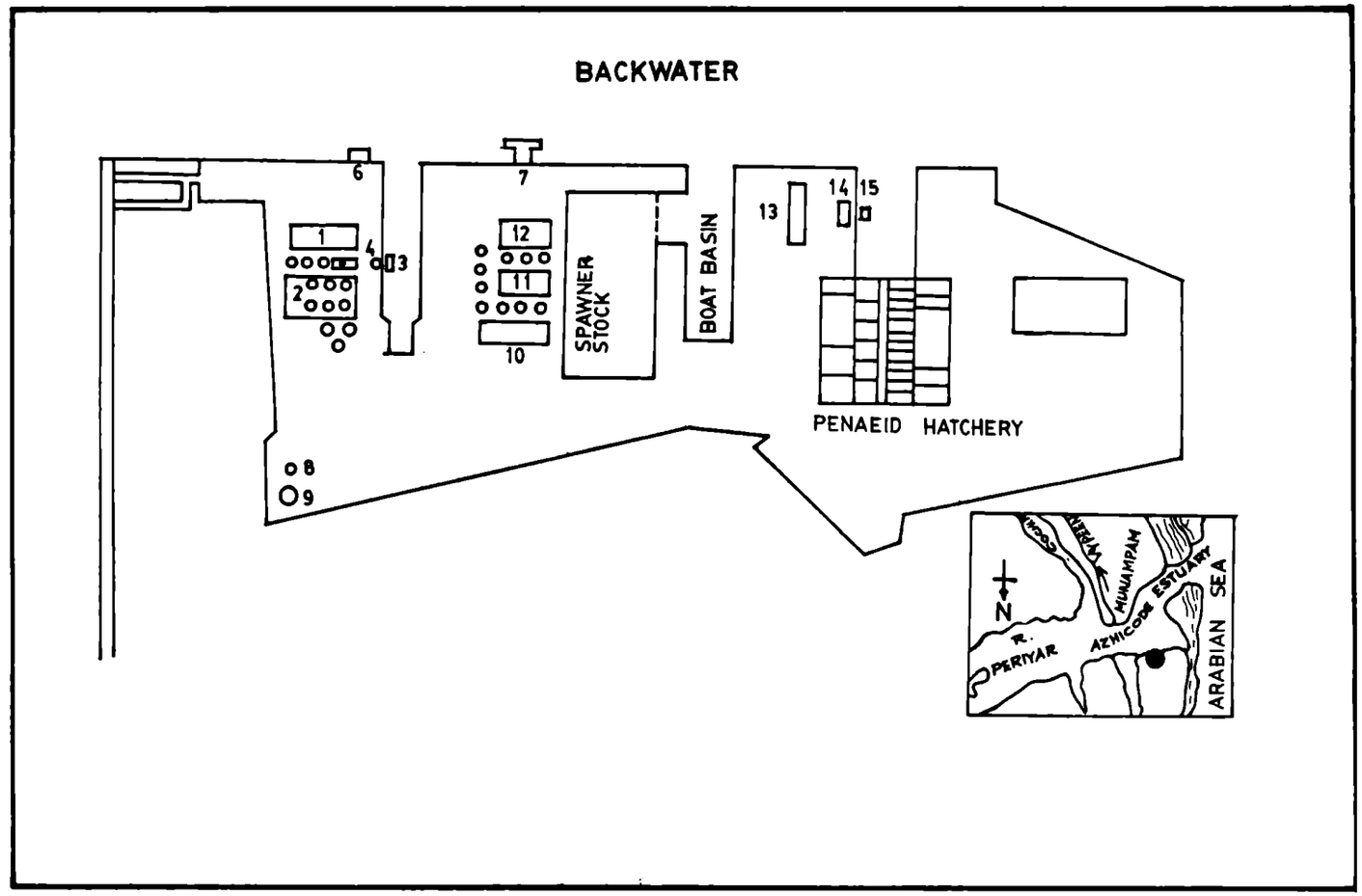
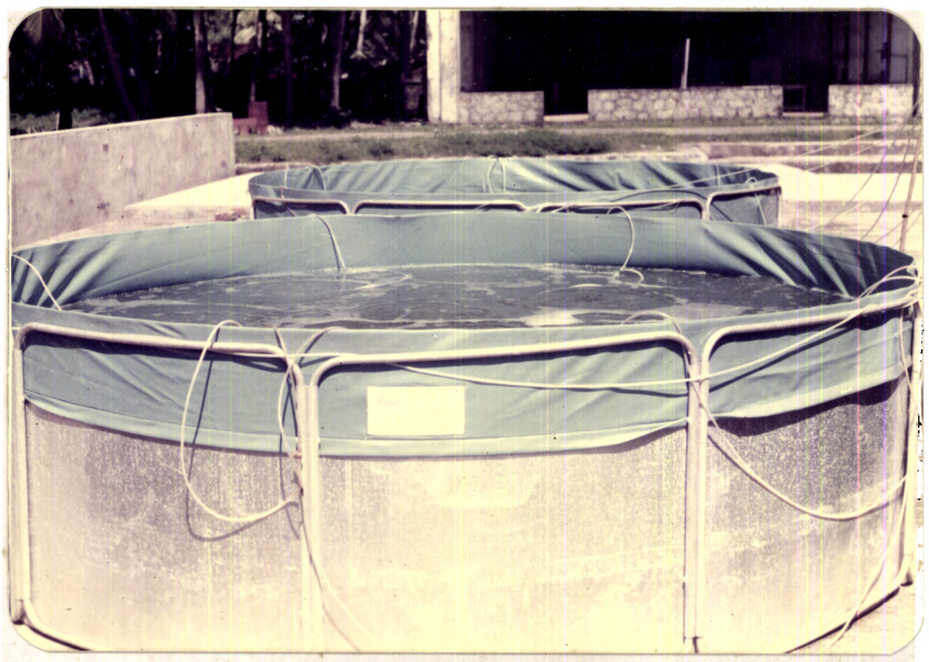

BACKWATER
1
2
3
4
6
7
8
9
10
11
12
13
14
15
SPAWNER STOCK
BOAT BASIN
PENAEID HATCHERY
N
R. PERIYAR
AZHIKODE
MUNAMPAM
ESTUARY
ARABIAN SEA

FIG.2.

Fig. 3. Functional culture pools



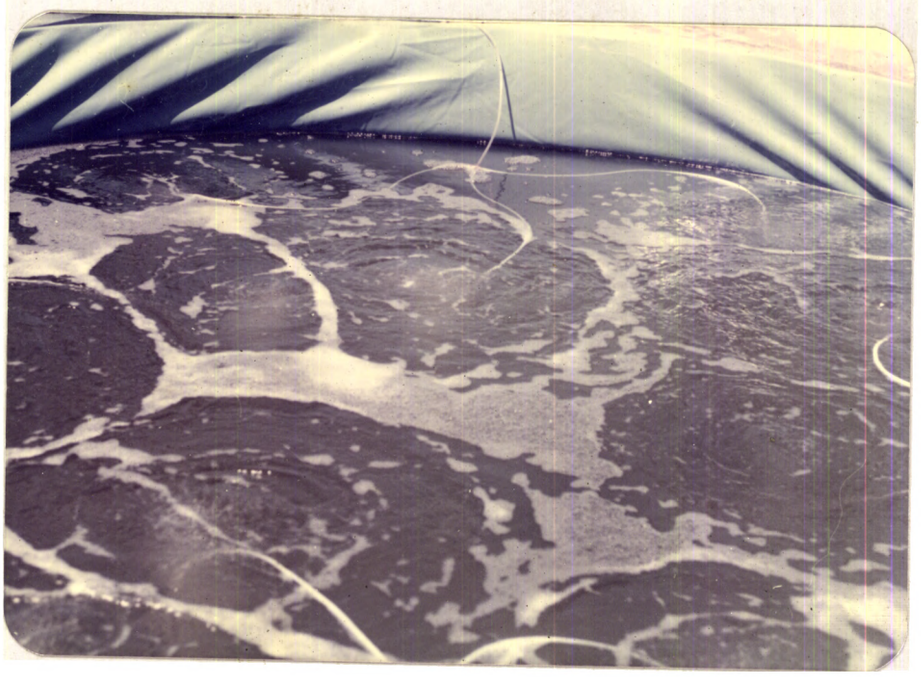


Fig. 4. Morphology of eggs and larvae

a,b : Eggs

c : Nauplius I

d : Nauplius VI

e : Zoea I

f : Zoea III

g : Mysis I

h : Mysis III

i : Postlarva I

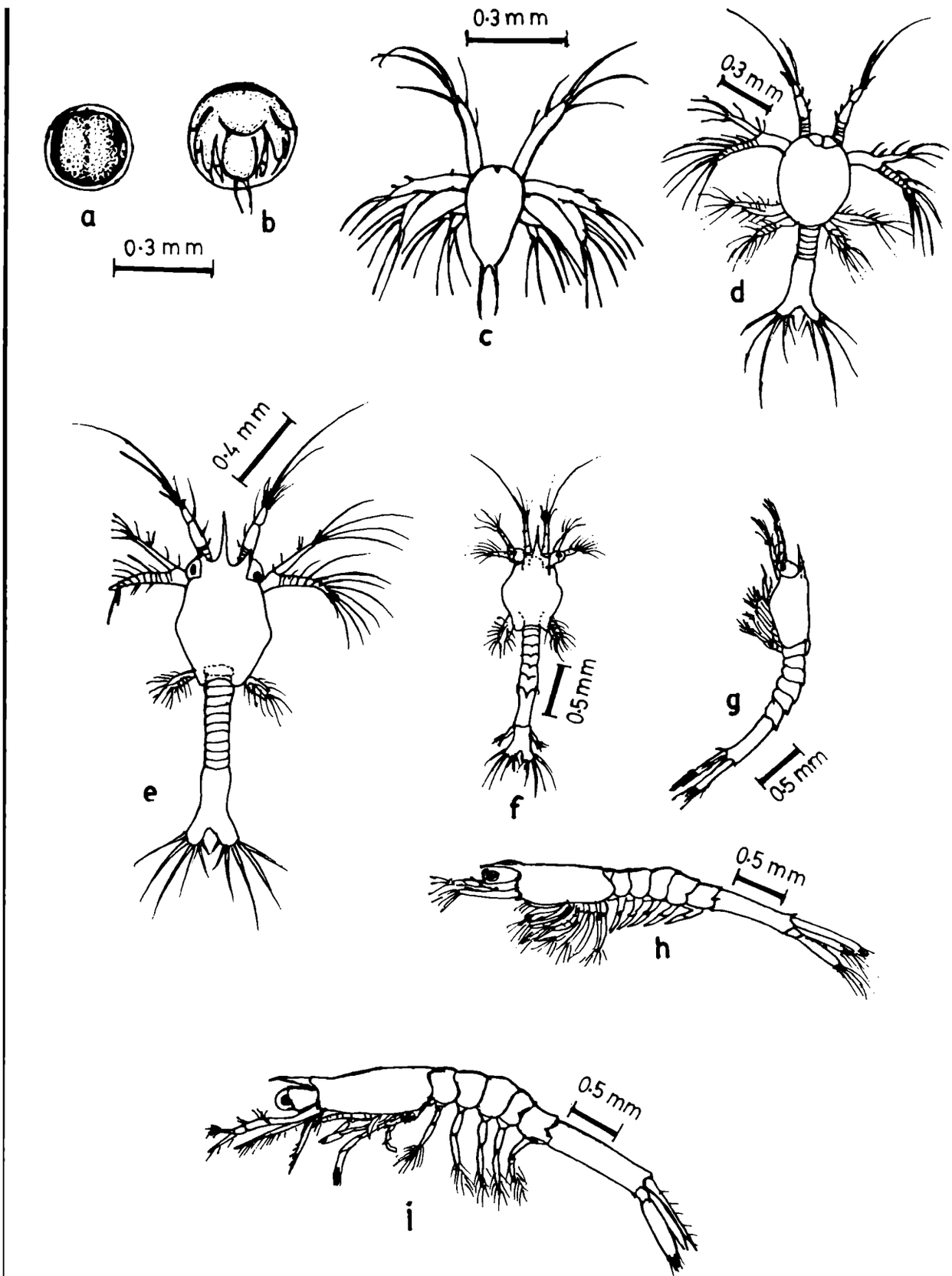


FIG. 4.

Fig. 5. Quantitative estimation of total heterotrophic bacteria in different pools

 larvae

 water

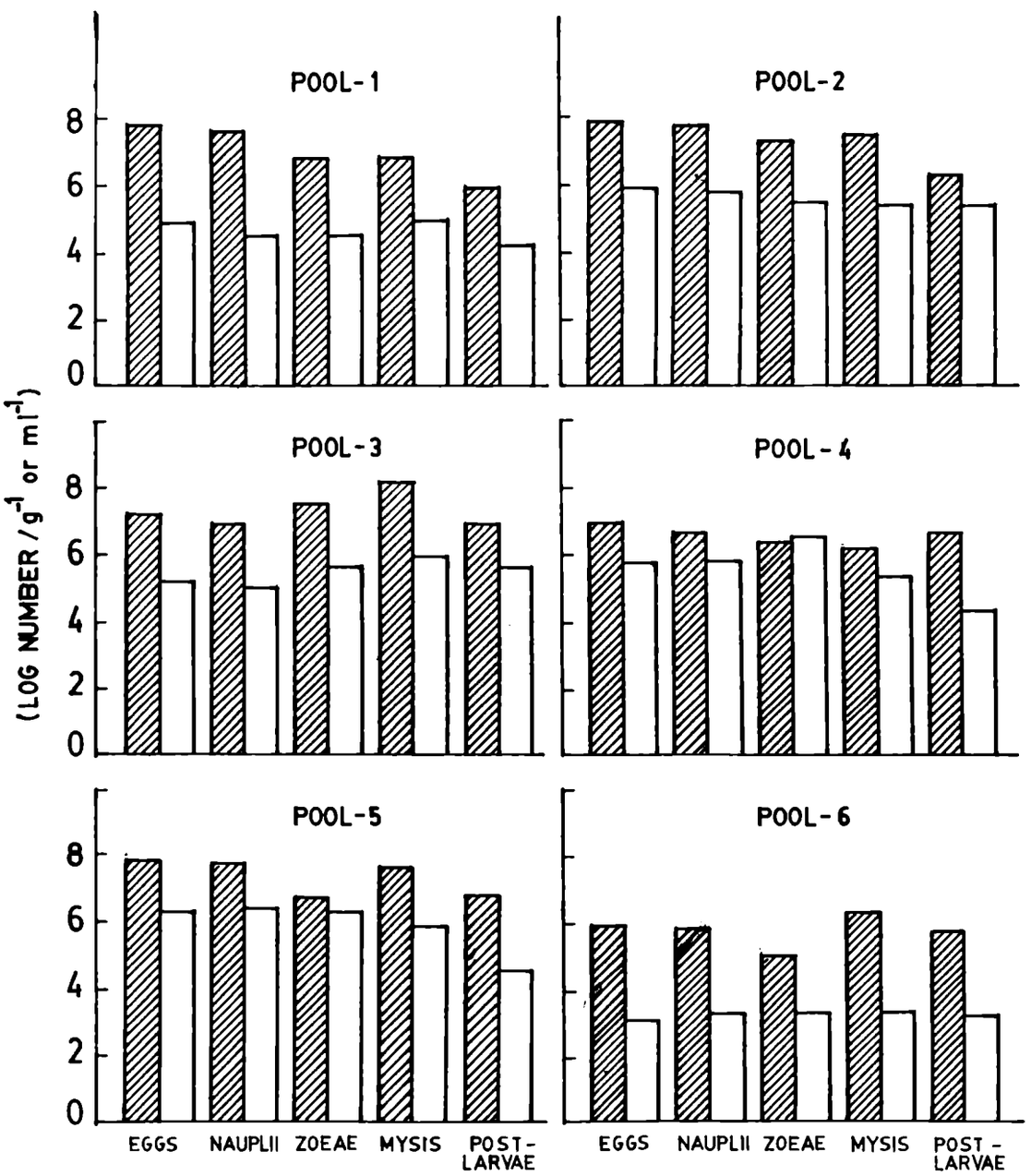
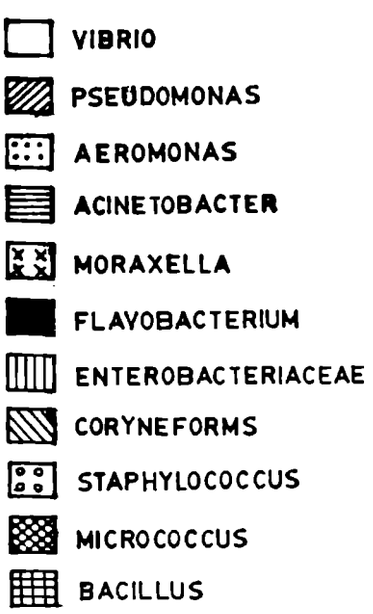

POOL-1
POOL-2
POOL-3
POOL-4
POOL-5
POOL-6
(LOG NUMBER / g⁻¹ or ml⁻¹)
0
2
4
6
8
EGGS
NAUPLII
ZOEAE
MYSIS
POST-LARVAE
SAMPLE

FIG. 5.

Fig. 6a. Distribution of different genera of bacteria isolated from various stages from pool No. 1



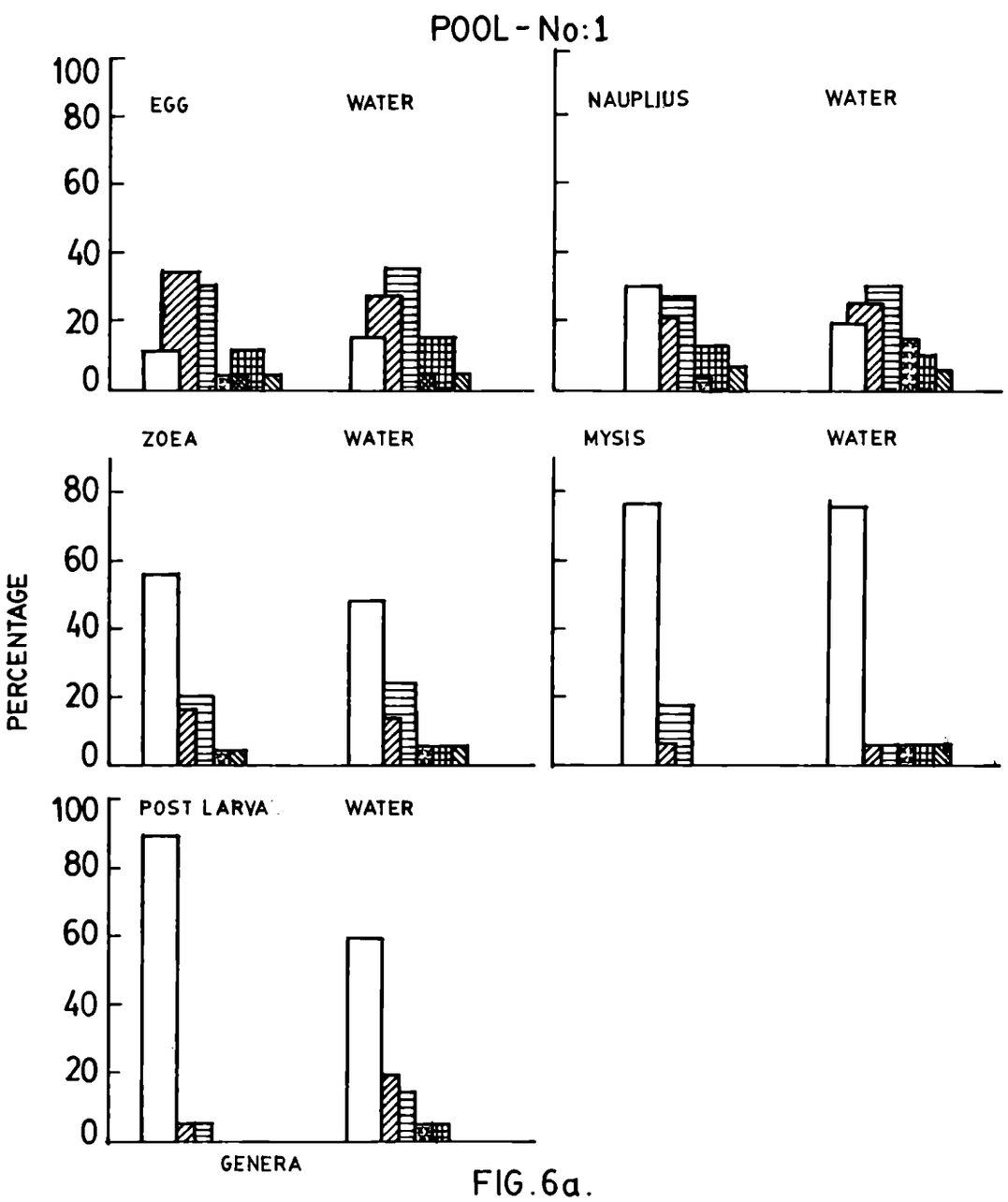
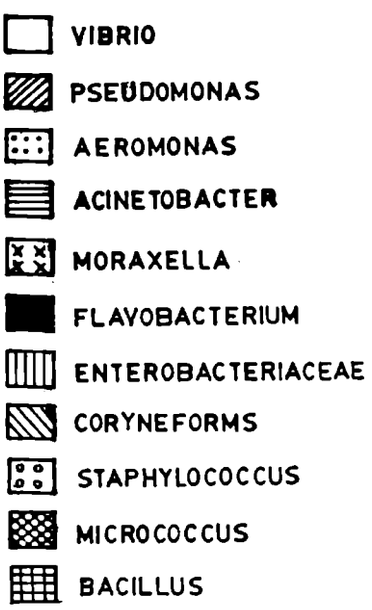

POOL - No:1
EGG
WATER
NAUPLIUS
WATER
ZOEA
WATER
MYSIS
WATER
POST LARVA
WATER
PERCENTAGE
GENERA
100
80
60
40
20
0

FIG. 6a.

Fig. 6b. Distribution of different genera of bacteria isolated from various stages from pool No.2



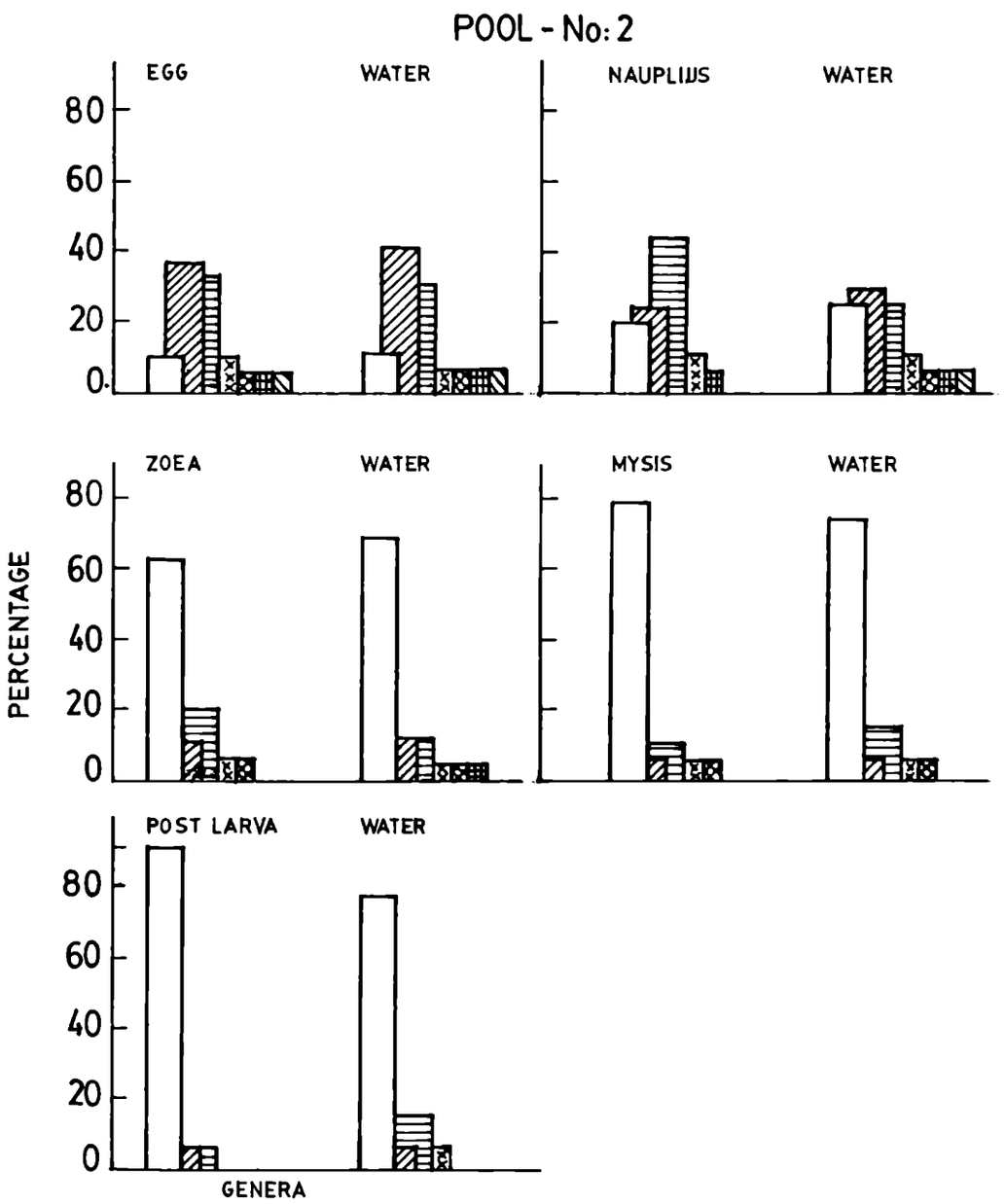
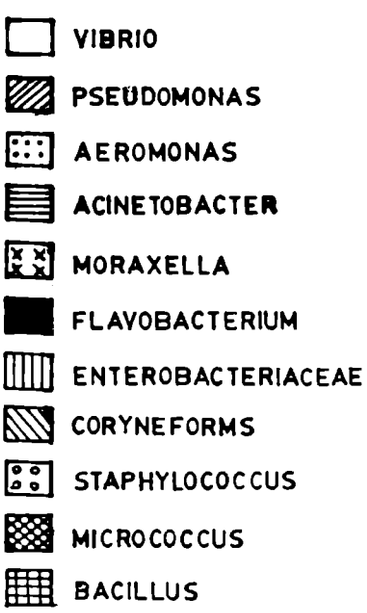

POOL - No: 2
EGG
WATER
NAUPLIUS
WATER
ZOEA
WATER
MYSIS
WATER
POST LARVA
WATER
80
60
40
20
0
PERCENTAGE
GENERA

FIG. 6b.

Fig. 6d. Distribution of different genera of bacteria isolated from various stages from pool No. 4



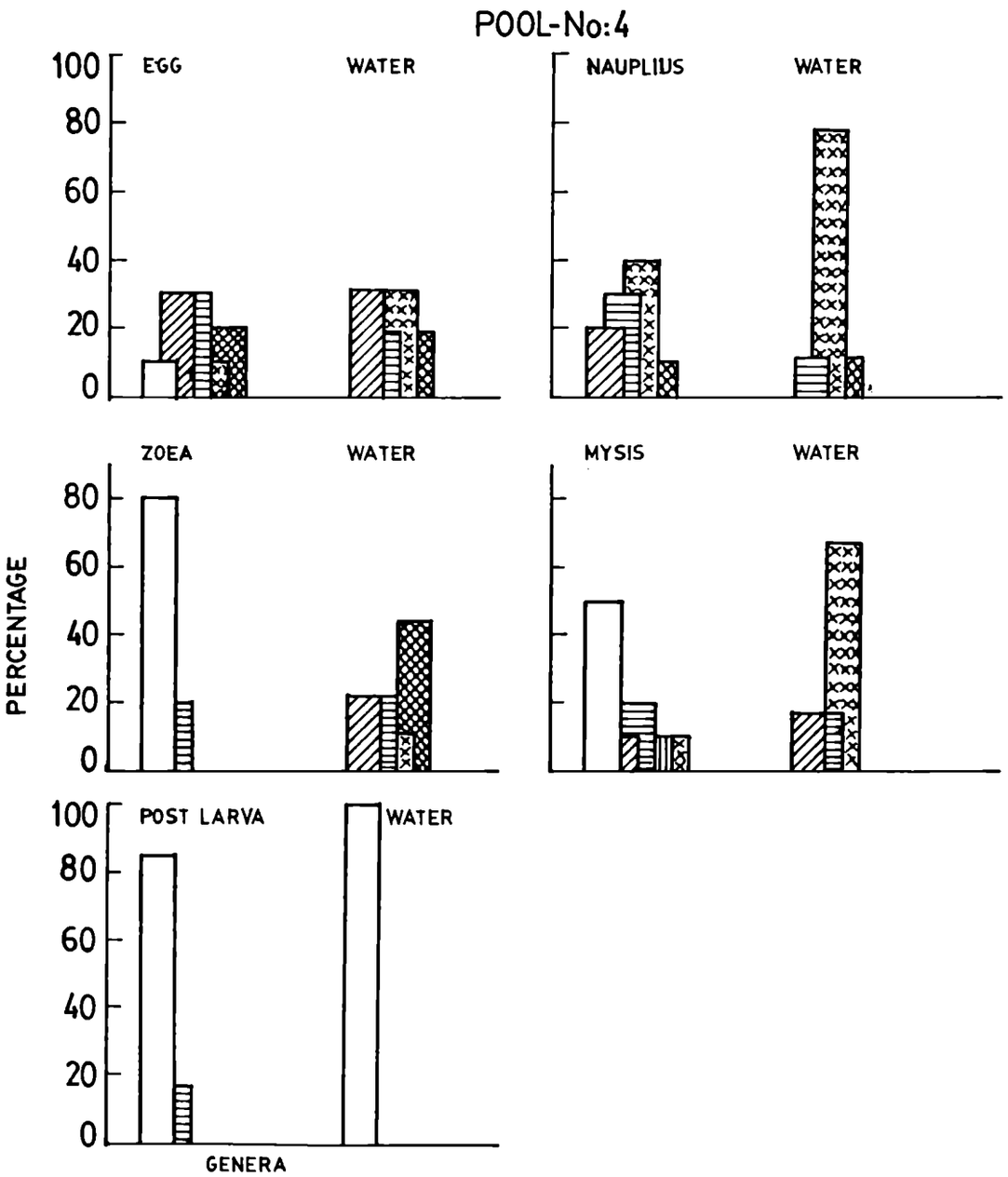
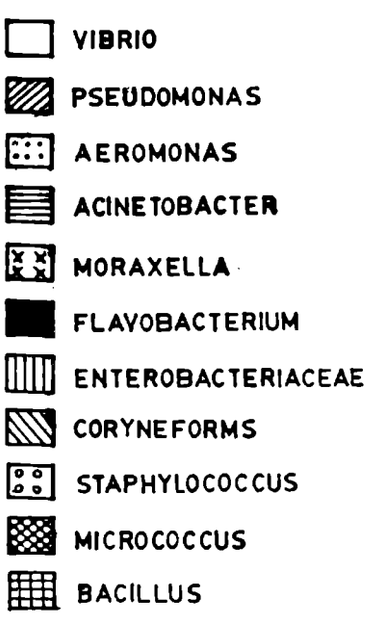

POOL-No:4
EGG
WATER
NAUPLIUS
WATER
ZOEA
WATER
MYSIS
WATER
POST LARVA
WATER
100
80
60
40
20
0
PERCENTAGE
GENERA

FIG.6d.

Fig. 6e. Distribution of different genera of bacteria isolated from various stages from pool No. 5



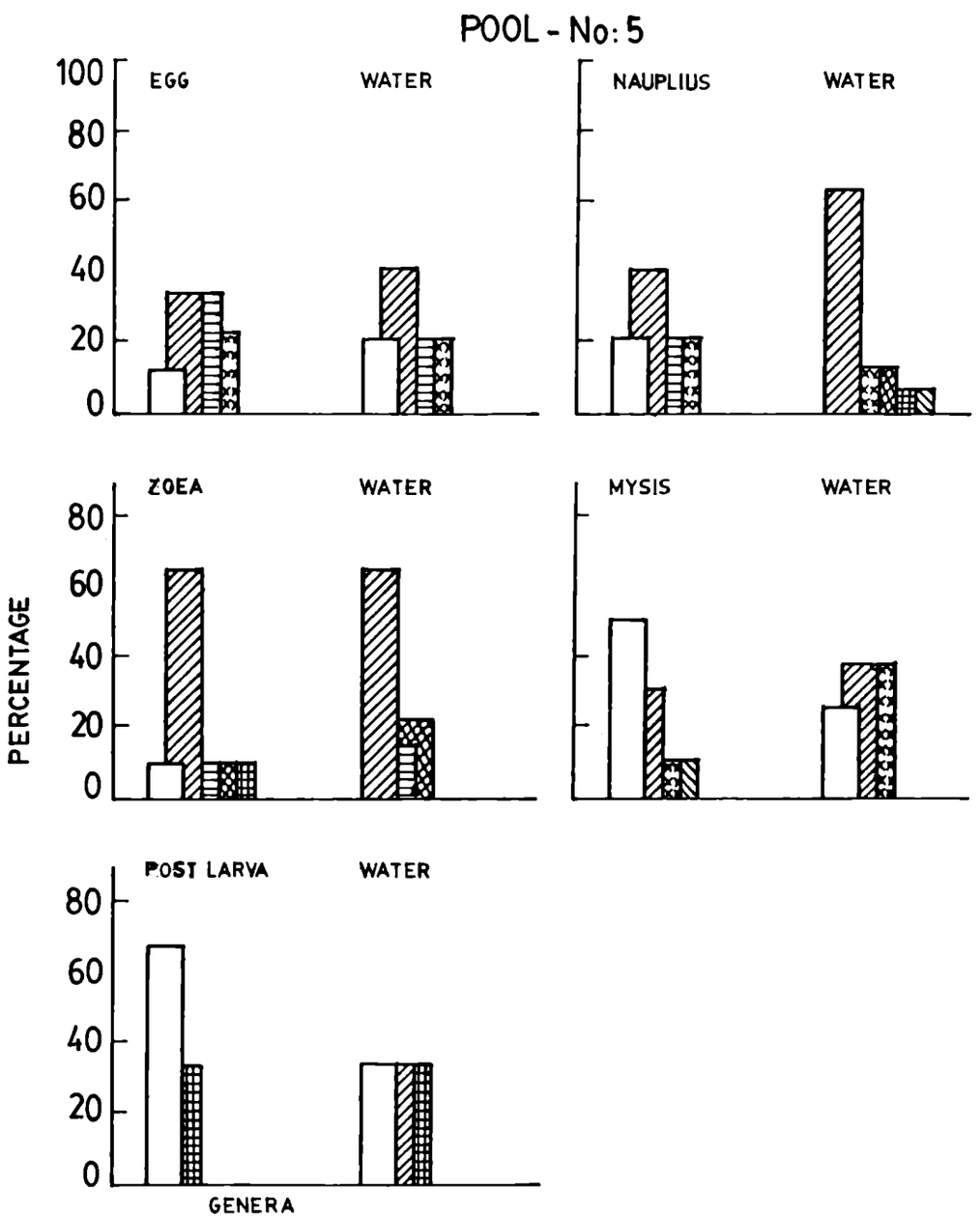
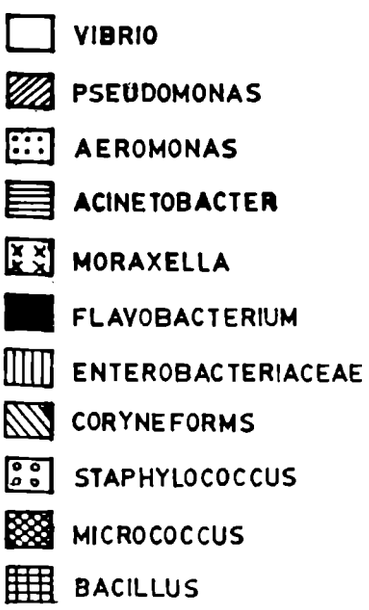

POOL - No: 5
EGG
WATER
NAUPLIUS
WATER
ZOEA
WATER
MYSIS
WATER
POST LARVA
WATER
100
80
60
40
20
0
PERCENTAGE
GENERA

FIG. 6e.

Fig. 6f. Distribution of different genera of bacteria isolated from various stages from pool No. 6



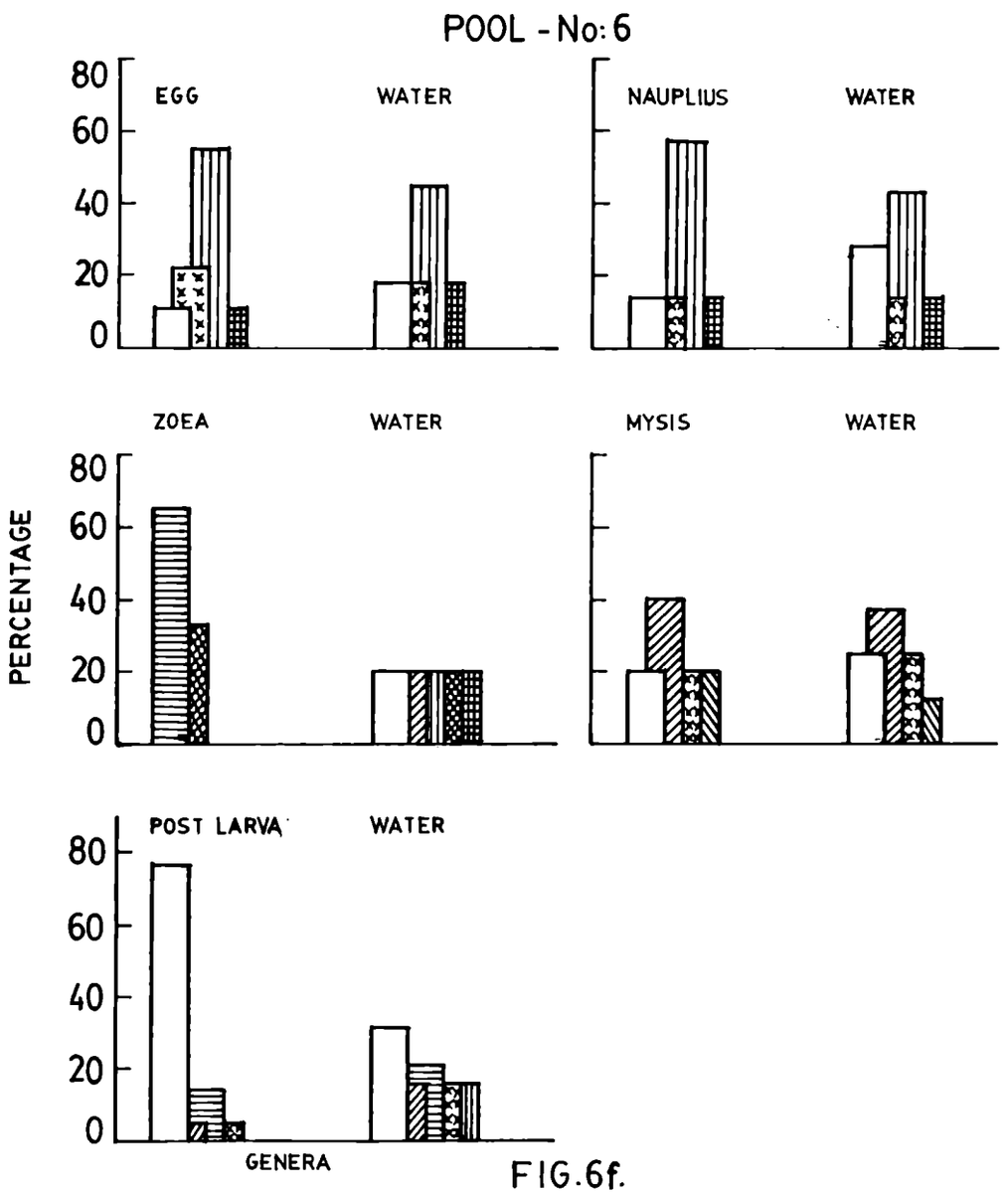

POOL - No: 6
EGG
WATER
NAUPLIUS
WATER
ZOEA
WATER
MYSIS
WATER
POST LARVA
WATER
PERCENTAGE
80
60
40
20
0
GENERA

FIG.6f.

CULTURE POND

3 BACTERIA ASSOCIATED WITH JUVENILES AND ADULTS OF *P. INDICUS* IN A CULTURE POND

3.1 MATERIALS AND METHODS

3.1.1 Study area

The culture pond is located in the premises of College of Fisheries, Kerala Agricultural University at Pananagad on the right bank of Vembanad Lake between lat. $9^{o}55'N$ and long. $76^{o}19'E$ (Fig. 1,7 and 8). It had a total area of 8 x 8 m and a depth of 1.0 m. Clayey bottom was with shallow furrows. The pond had access to the backwater via a feeder canal through sluice gate which was protected with wooden planks and nylon screen.

3.1.2 Preparation of pond

Water from the pond was completely drained off and allowed to be exposed to sunlight for a few days. In order to eradicate weed fishes and other predators Mahwa oil cake was applied at a rate of 250 ppm. Following this, lime was applied (1000 kg per hectare). Sluice gate of the pond was kept closed for three more days in order to prevent the entry of water so that the Mahwa applied was not diluted, reducing it's cidal effect on predatory fishes. The nylon mesh screen

of the sluice gate prevented the entry of any animal when the gate was opened. After the collection of enough water (45 cms depth) in the pond the sluice gate was closed.

In order to supply sufficient nutrients for the growth of phytoplankton, cowdung at a rate of 500 kg/hectare/year, mussouri phosphate 600 kg/hectare/year in equal monthly doses were applied. Both cowdung and mussouri phosphates were mixed with water and spreaded completely over the pond.

3.1.3 General maintenance of the pond

Entry and exit of water was made by lifting the planks of sluice gate once in every month before the application of manure. Level of water was always tried to be maintained at about 45 cms depth.

3.1.4 Sample

Penaeus indicus H Milne - Edwards, 1837

Systematic position

Phylum : Arthropoda

Class : Crustaceae

Order : Decapoda

Sub order : Natantia

Family : Penaeidae

Genus : Penaeus

Species : indicus

Biology:

Eggs of P. indicus have been reported to occur in large numbers associated with subsurface plankton in marine environment during breeding seasons (CMFRI, 1977). During early hours of morning, advanced larval forms could be collected from the inshore subsurface waters. Estuarine environment forms a nursery ground for the post larval stages. Juveniles (30 to 120 mm total length) spend their life mostly in estuaries and backwaters. On the southwest coast of India, they support the commercial fishery in the backwaters (including paddy fields) where they develop into adult (lengths of 100 to 120 mm) before returning to the sea. Juveniles are bottom dwellers and are obtained from the estuarine environment. Sexually mature adults occur in shallow coastal regions and muddy sea bottom which are subjected to changes due to the physical conditions of the coastline and the nutrient obtained from the land and rivers.

3.1.5 Distinguishing characters of the species

3.1.5.1 Juveniles and adults:

Body is completely glaborus, with a slender long rostrum and a distinct double curve, which is 1/2 to 2 times in length of carapace in the juvenile stage (Fig. 9). First five dorsal teeth of rostrum are very much close together,

and penultimate, as well as distal teeth are widely separated. The position of the latter teeth are much variable. Rostrum becomes shorter with the increase in the size of animal, equalising with carapace in animals of 80 mm, where the former appears straight and with higher blade. When rostrum extend beyond the tip of antenular scale in larger prawns, the blade does not form a triangular crest. Rostral groove is shallow, decreasing in depth backwards upto epigastric tooth. Eight to nine (sometimes seven) dorsal and four to five ventral teeth are found altogether on rostrum. The carapace is glaborus and thin; sulcus and carina feebly defined. Gastro-orbital carina occupies the posterior 1/4 distance between hepatic spine and orbital angle. Orbito-antenal sulcus is wide and ill-defined. Post antenular spine continue as an oblique ridge to the hepatic spine. Sub-hepatic ridge is absent. Four to five abdominal segments are keeled and keel on the sixth segment ends acutely. Telson is grooved, without any lateral spine. Second and third joints of the first leg and second joint of the second leg are provided with a spine.

General colour of live specimen is translucent whitish with numerous small, brownish, greyish or greenish chromatophores scattered over the carapace and abdomen. The upper half of the rostrum, base of eye stalks, dorsal

carina of the last three abdominal somites, telson and isopods are deeply pigmented, with maroon and dull brown chromatophores.

3.1.6 Food and feeding habits of juveniles and adults in culture environment

Being omnivorous P. indicus subsist on the planktonic mass which is produced in the pond. During its juvenile and adult life it settles at the bottom and feed on the detritus matter containing both phyto and zooplankton. Phytoplankton usually encountered in the pond were Chlorococcum among green algae, Synechococcus sp. among blue green algae and Nitzschia sp. and Navicula sp. among diatoms. Zooplankton was composed of copepods, rotifers, cladocerans, nauplii etc. Moreover the animal was found to eat on the green algae seen at the bottom of the pond also.

3.1.7 Collection of juveniles from the natural environment

Juveniles of P. indicus were collected from the natural environment, especially from creake and shallow areas, using happa net. After seggregating from the other species of penaeid and non-penaeid prawns, they were transported to the grow out pond area in plastic buckets.

3.1.8 Acclimatization in happa

The collected juveniles were transferred to a nylon happa suspended in a pond, which was well prepared for stocking and allowed to get acclimatized with the new environment.

3.1.9 Release of juveniles

Before releasing juveniles, which were collected and kept in a happa, into the grow out pond; pH, salinity, temperature, dissolved oxygen and the type and abundance of both phyto and zooplankton were analysed to ascertain whether the ecological conditions of the pond were quite congenial for P. indicus. The pond was then stocked at the rate of 10,000 juveniles of P. indicus per hectare during the early hours of the day.

3.1.10 Feeding

The animals were fed with a supplementary diet prepared from ground nut oil cake, fish meal, rice bran, cassawa powder, vitamins and calcium lactate which gave a final protein content of 38.5% (Ahamed Ali, 1982). This was given in the form of pellets daily at a rate of 5% of the total body weight of the animals. The quantity of the feed was thus increased once in every fortnight after assessing the growth. The feed pellets were broad-casted out the pond.

3.1.11 Collection of samples

Twenty of the juveniles of P. indicus which were collected from natural environment, were transferred to a sterile glass bottle and the remaining juveniles were released into the happa. After about 18 hrs, twenty of them were removed to sterile glass bottle. Water samples (500 ml) from happa were also collected whenever the samplings were made. Before the release of juveniles into the pond, water (500 ml) and sediment (100 g) samples were also collected. Later, periodical collections were made for prawns, water and sediment from the pond until harvest. All the above samples were transferred to sterile glass bottles separately and transported to the laboratory in ice box.

3.1.12 Estimation of physico-chemical parameters

Estimations of temperature, pH, salinity, dissolved oxygen and nutrients have been performed as detailed under section 2.1.10.

3.1.13 Bacteriological analysis

3.1.13.1 Processing of samples:

The juveniles (10 Nos.) were ground well in a sterile homogeniser using 10 ml sterile 50% aged seawater. From adults of P. indicus caught from the culture pond, body surfac gill, stomach, anterior intestine and posterior intestine

formed the samples for bacteriological assay. Exoskeleton of 5 animals drawn for sampling, were dissected out and introduced into 10 ml sterile 50% aged seawater blanks. After removing the carapace, gills were cut away, weighed and ground well in tissue homogenizer with 10 ml sterile 50% aged seawater. Surface of the animals were then sterilized with 20 ppm sodium hypochlorite, for 10 minutes and the latter was washed away thoroughly with sterile distilled water. Alimentary canal was then dissected out and divided into three regions viz. stomach, anterior intestine and posterior intestine as shown in Fig. 9. The terminology adopted here corresponds to proventriculus, anterior midgut and the posterior midgut alongwith the whole hindgut. Their contents were then squeezed out into petridishes and weighed, before diluting with 10 ml aged 50% seawater blank.

From the water sample collected from happa and culture ponds, 1 ml each were pipetted out into sterile 9ml 50% aged seawater blanks. Two grams of sediment weighed previously was introduced into 10 ml blank.

Samples such as exoskeleton, gill, stomach, anterior and posterior intestine, sediment and water were then submitted for serial dilution using 50% aged seawater blanks dispensed in 9 ml aliquots in test tubes. All the samples were diluted upto 10^{-6} using this diluent.

Details of the composition of the medium used, method of plating, incubation temperature, duration of incubation, enumeration, isolation and maintenance of cultures, identification, testing the production of hydrolytic enzymes and effect of environmental factors, such as temperature, pH and sodium chloride concentration on growth are given in 2.1.11, 2.1.12 and 2.1.13.

3.1.14 Statistical analysis

Correlation coefficients between the percentage of various genera in the animal and that of its environment were determined as described in section 2.1.14.2.

3.2 RESULTS

3.2.1 Physico-chemical parameters

Physico-chemical parameters measured from happa as well as pond during a period of three months are given in Table 23.

In happa the envirohmental variables such as temperature, pH, salinity, dissolved oxygen, were high in third collection (7.4.1982). Inorganic nitrogen, organic nitrogen, and inorganic phosphorus in the second collection, and organic phosphorus in the first collection were high. However in all the above mentioned instances the magnitude of the difference was very small.

In pond (Apr. to Jun.) there was a slight variation in temperature (28 - 31^{o}C). Highest pH (8.3) was recorded in Apr. and lowest (7.5) in Jun. Salinity varied from 10.25 to 16.60 x 10^{-3}. Dissolved oxygen varied between 3.62 and 4.81 ml.l^{-1}. The minimum concentration (4.5µg.l^{-1}) of inorganic nitrogen was recorded in Jun. and the maximum 12.5µg.l^{-1}, during the latter half of Apr. Organic nitrogen was low (2.5µg.l^{-1}) during the later part of May, and high (34.0µg.l^{-1}) during the later half of Apr. Inorganic phosphoru

was least during Jun. ($5.6\mu g.l^{-1}$) and high ($13.5\mu g.l^{-1}$) during the later half of May. Organic phosphorus was low ($5.8\mu g.l^{-1}$) during Jun. and high ($14.0\mu g.l^{-1}$) during the later half of May.

3.2.2 Quantitative distribution of heterotrophic bacteria in juveniles, adults, water and sediment

Quantitative distribution of heterotrophic bacteria associated with juveniles (before and after stocking in happa), on the body surface, gills, stomach, anterior intestine and posterior intestine of adults, water and sediment from the pond is presented in Table 24.

THB of juveniles in happa showed a lesser magnitude than that of juveniles from natural environment. THB of water from happa ranged from 0.21 to 33.65 x $10^5.ml^{-1}$.

Alimentary canal of adult harboured the highest population (149.85 to 1135.83 x $10^5.g^{-1}$). Stomach contents had lower population than that of anterior and posterior intestine (Table 25). During the beginning of May, various regions of alimentary canal possessed almost the same number of bacteria. THB of body surface and gill ranged from 0.01 to 0.18 x $10^5.cm^2$ and from 29.18 to 367.87 x $10^5.g^{-1}$ respectively. In general increase of population was observed from body surface to gill and to intestine.

Water from pond always contained lesser population ranging from 0.14 to 25.16 x $10^5.ml^{-1}$ than sediment (1.49 to 54.35 x $10^5.g^{-1}$). In both the cases the lowest population was observed during the beginning of Apr. and the highest during the first half of May.

3.2.3 Qualitative analysis of THB in juvenile, adult, water and sediment

3.2.3.1 Distribution of Gram-negative bacteria:

Table 26 gives the percentage of Gram-negative bacteria occurred in various samples. Among 644 isolates, 68.79% were Gram-negative and the rest were Gram-positive. Gram variables were not observed. In all the samples, except in sediment, more than 60% of the isolates were Gram-negative. Juveniles from natural environment contained the highest percentage (84.93%) of Gram-negative bacteria. Eventhough it declined to 67.90% in the juveniles from happa it was high (81.82%) in water. On one occasion (7.4.1982) water collected from happa as well as from pond showed very high percentages of Gram-negative bacteria, 93.75% and 84.22% respectively.

Gram-negative forms varied from 27.27 to 100% in prawn. In general there was an increase of Gram-negative bacteria from stomach to posterior intestine. Gram-negative forms were more (50 to 88.89%) in water than in sediment (5.26 to 60%).

3.2.3.2 Distribution of various genera:

The temporal changes in percentage composition of different genera in various samples are presented in Table 27. Juveniles from natural environment contained Vibrio, Pseudomonas, Micrococcus, Bacillus and Coryneform group. Vibrio, Pseudomonas, Micrococcus and Coryneform group were found to be associated with juveniles in happa in all the three collections. At the same time, water in happa contained Vibrio, Pseudomonas and Coryneform group in all the collections. The results indicate that juveniles from natural environment harbour members of Bacillus, besides other genera encountered in water and juveniles in happa.

Pseudomonas was the only genus isolated from the body surface of the animal throughout the period. The only organism present continuously in gills was Coryneform group. Vibrio and Pseudomonas were present in stomach contents throughout. In anterior intestine Vibrio and Coryneform group existed continuously. Vibrio, Micrococcus and Coryneform group were present in posterior intestine during all the collections. Thus the important genera in the juveniles of happa were Vibrio, Pseudomonas, Micrococcus and Coryneform group. Pseudomonas in water and Bacillus and Coryneform group in sediment of pond were the genera seen through out the period of study.

3.2.3.3 Generic composition:

The percentage occurrence of different genera encountered in various samples are shown in Table 28. In general Gram-negative bacteria of the genera Vibrio and Pseudomonas and Gram-positive forms of the genera Coryneform group, Bacillus and Micrococcus constituted more than 88%. Alcaligenes was totally absent. The other genera were found in small percentages.

The juveniles procured from natural environment contained more Vibrio (65.75%). Pseudomonas and Coryneform group constituted 8.22 and 9.59% respectively. Vibrio was dominant (48.15%) in juveniles from happa followed by Micrococcus (14.82%) and Moraxella (12.35%). Bacillus and Coryneform group were 8.64% each. Pseudomonas was least (7.41%) in occurrence. Other genera were totally absent. Water from happa contained Acinetobacter and Enterobacteriaceae in addition to those recorded in juveniles. Pseudomonas and Coryneform group were 11.36% each.

Pseudomonas was the dominant flora (40.63%) on the body surface of adults from ponds followed by Vibrio and Coryneform group (17.19% each). In gills also Pseudomonas was the dominant flora (33.96%) followed by Coryneform group (20.75%), Vibrio (18.36%) and Micrococcus (15.09%). In stomach Vibrio was the dominant flora (37.73%) followed by

Pseudomonas (20.78%) and Micrococcus (18.87%). Aeromonas, Flavobacterium and Alcaligenes were absent. Other genera occurred in very small percentages. The major flora of anterior intestine was Vibrio (64.06%). Coryneform group was 15.60%. Aeromonas, Acinetobacter, Flavobacterium, Alcaligenes and Staphylococcus were absent. The other genera were very small in percentage. In the posterior intestine also Vibrio was the dominant flora (59.76%) followed by Coryneform group (12.98%) and the other genera occurred in small percentages. Aeromonas, Flavobacterium, Alcaligenes, and Staphylococcus were absent.

In water at the same time Pseudomonas was dominating (55.93%) followed by Coryneform group (11.56%) and Vibrio (10.17%). The other available genera were very small in percentages. Aeromonas, Flavobacterium, Alcaligenes and Staphylococcus were absent.

In sediment of pond, Bacillus was the dominant flora (53.95%) followed by Coryneform group (14.47%) and Pseudomonas (13.15%). Flavobacterium, Alcaligenes and Staphylococcus were absent.

In general, the body surface and gills of the animal harboured more Pseudomonas and less Vibrio among Gram-negative bacteria, more Coryneform group than Micrococcus among Gram-positive bacteria. In the other regions of prawns

(stomach, anterior and posterior intestine) Vibrio was found to be dominant than Pseudomonas among Gram-negative. Among the Gram-positive bacteria Micrococcus was more than Coryneform group in stomach and in intestine it was reverse. In water Pseudomonas was the dominant (55.93%) genus. Bacillus (53.95%) dominated in sediment.

3.2.4 Statistical analysis

Results of the interrelationship (simple correlation coefficient('r') between the percentage of bacterial genera occurred in the various regions of prawns and those of water and sediment are presented in Table 29. The Micrococcus present in sediment showed a positive significant correlation with the Micrococcus occurred in gills. Pseudomonas of stomach and that of sediment showed a significant positive correlation. At the same time a significant negative correlation was found among Micrococcus present in anterior intestine and water. No other genera recorded a significant relationship in all the samples with other parameters tested.

3.2.5 Hydrolytic properties of bacteria in juveniles, adults, water and sediment

3.2.5.1 Distribution of hydrolytic bacteria:

The bacterial isolates taken from juveniles, adults, water and sediment collected from pond were screened for their

ability to produce various hydrolytic enzymes and the results are summarised in Table 30. Among 644 isolates tested 99.38% were ureolytic and found to be the dominant enzymatic group. About 94% of the isolates elaborated protease and about 40% of the total isolates were found to be chitinolytic while 45.65% were amylolytic, 52.48% were lipolytic It could be observed from the results that there was no uniform pattern of distribution of various enzymatic groups in the samples. Higher percentage of chitinoclastic bacteria were found in the anterior intestine of prawn and also in association with the juveniles from natural environment.

Temporal changes in the percentage occurrence of hydrolytic forms in juveniles, adults, water and sediment are given in Fig. 10a - k, App. Table 3. In the juveniles from natural environment, and water and juveniles from happa the percentage of ureolytic and proteolytic bacteria were high without much variations, with respect to time. Lipolytic bacteria in juveniles from natural environment showed a decreasing trend and in the water from happa an increasing trend. But, in the juveniles from happa lipolytic forms fluctuated. Amylolytic bacteria in water showed an increasing trend, while such a trend was absent in animals from happa. But, in the juveniles from natural environment the percentage of amylolytic bacteria in the second collection was lesser (63.69%) than that of the first one (77.50%). Chitinoclastic

bacteria in the water from happa showed a clear increasing trend (14.28 - 68.75%). Such an orderly pattern of increase was not seen in other samples. Proteolytic and ureolytic bacteria did not undergo marked changes in the samples from pond except certain isolated situations. Temporal changes of amylolytic bacteria did not show an increase or decrease in all the samples except in stomach were a decrease in their percentage was seen (47.06 to 25%). Lipolytic bacteria experienced a reduction in percentage in the anterior intestine (100 to 42.86%) posterior intestine (100 to 64.70% and the alimentary canal in general (81.03 to 48.72%). But, in other samples they varied widely. Chitinoclastic forms were not observed during all the samplings especially on the surface, gills, water and sediment. However a reduction in the percentage of chitinoclastic bacteria was seen in the anterior intestine (68.42 to 50%), posterior intestine (71.42 to 52.94%) and the alimentary canal in general (58.62 to 43.59%), from the beginning to end of the rearing period.

Thus the proteolytic and ureolytic bacteria did not undergo much alterations during the culture period. Lipolytic and chitinoclastic forms declined from Apr. to Jun. At the same time an increase of amylolytic and chitinoclastic forms could be seen in water of happa, during the period.

3.2.5.2 Generic composition of various hydrolytic bacteria:

Percentage distribution of various hydrolytic forms in different genera isolated from juveniles, adults, water and sediment are presented in Table 31. Majority of the isolates belonging to various genera of Gram-negative bacteria were proteolytic. Vibrio comprised the highest percentage of amylolytic (75.92) and lipolytic (84.49) forms than any other genera. Among Vibrio more than 97.96% were chitinoclastic and among other genera when present they were in low percentages

Among Micrococcus, Bacillus and Coryneform group more tha 72.29% were proteolytic, and above 94.41% were ureolytic. But amylolytic forms were very small in percentage (9.63 to 55.93%) and similar was the case with lipolytic forms (20.34 to 36.14%). None of the isolates of Micrococcus was chitinoclastic and among Bacillus and Coryneform group they were less in number (11.86 and 8.43% respectively).

Generic wise distribution of hydrolytic bacteria in various samples is given in Table 32. Among the eleven genera encountered in the culture pond environment, Vibrio, Pseudomonas, Coryneform group, Micrococcus and Bacillus were the important ones in the decreasing order of dominance. Therefore the ensuing description is restricted to only these genera.

Vibrio

All the isolates of Vibrio were proteolytic and ureolytic. Juveniles and water from happa harboured the highest percentage of amylolytic vibrios (90 to 95%). In other samples it ranged from 33 to 78%. Body surface did not contain amylolytic vibrios. Juveniles from natural environment contained the least percentage of lipolytic vibrios (43.75%). They ranged from 82.05 to 100% in other samples. The lowest percentage of chitinoclastic forms (72.73%) was observed on the body surface. In others they ranged from 83.33 to 100%.

Pseudomonas

Majority of the isolates of Pseudomonas were proteolytic and ureolytic. In juveniles and water from happa and the posterior intestine of adults, amylolytic Pseudomonas were not found. But in other samples it ranged from 15.38 to 50%. Higher percentages of lipolytic Pseudomonas were seen in water. In the anterior intestine of animals lipolytic Pseudomonas was absent. In other samples it varied from 9.09 to 87.88%. Chitinoclastic Pseudomonas were present only in anterior intestine (50%), water (15.15%) and sediment (40%).

Micrococcus

Proteolytic Micrococcus varied from 41.67 to 100% in different samples. At the same time percentage of ureolytic Micrococcus ranged from 66.67 to 100%. Amylolytic strains were absent in many samples and when present they ranged from 25 to 100%. Similarly lipolytic strains of Micrococcus were absent in many samples too and when present ranged from 20 to 100%. Chitinoclastic forms were totally absent.

Bacillus

Majority of Bacillus exhibited proteolytic and ureolytic activity. About 78% of them were amylolytic, 29.26% were lipolytic and 14.63% were chitinoclastic in sediment.

Coryneform group

Amylolytic Corneform group were very small in percentage and they were observed only in a very few samples such as juveniles from happa, water and sediment from pond. Majority of the isolates of Coryneform group were proteolytic and ureolytic in all the samples. At the same time in many samples gelatinolytic forms were lesser than caseinolytic forms. Lipolytic Coryneform group were absent in juveniles in happa and in the corresponding water samples. In other

samples it ranged from 18.18 to 71.43%. Chitinoclastic strains of Coryneform group were seen only in water and sediment.

The above results showed that most of the isolates of vibrios were elaborating all the tested hydrolytic enzymes. Proteolytic activity was recorded almost uniformly in all the genera. Percentage of the strains elaborating amylase and lipase were found to occur in lesser magnitude in Pseudomonas, Micrococcus, Bacillus and Coryneform group than Vibrio. Chitinase was mainly elaborated by vibrios.

Percentage contribution of different genera for a particular hydrolytic enzyme in a sample is presented in Table 33. All the hydrolytic enzymes producers were mainly members of Vibrio and the sole group of organisms elaborating chitinase was Vibrio. Vibrio (80.77%) and Coryneform group (19.23%) alone were exhibiting lipolytic activity. Except these two groups, the participation of other genera in the production of potential hydrolytic enzymes was less than 10% in the juveniles from natural environment.

More than 90% of amylolytic, lipolytic and chitinoclast forms were Vibrio in the juveniles from happa. But only 54 to 58% of Vibrio were proteolytic. Rest of the proteolytic bacteria were members of Pseudomonas, Moraxella, Micrococcus, and Coryneform group. Chitinase was elaborated by Vibrio alon

In the water from happa Vibrio formed the source of more than 90% of potential amylase, lipase and was the sole source of chitinase. 47 to 53.85% of the protease and urease were contributed by Vibrio and the rest by Pseudomonas, Acinetobacter, Moraxella, Enterobacteriaceae, Micrococcus, Bacillus and Coryneform group.

In the body surface except for chitinase and lipase, contribution of Vibrio for the potential hydrolytic enzymes was not prominent, whereas for the potential chitinoclastic activity, the sole source was Vibrio and 52.38% of the lipase production also was from the same genus. In the latter case the rest was shared by Pseudomonas, Flavobacterium, Moraxella, Enterobacteriaceae, Micrococcus and Coryneform group. Among caseinolytic, gelatinolytic and ureolytic forms 34 to 42% were Pseudomonas and the next prominent genus was Vibrio (17 to 19%); the rest being shared by Acinetobacter, Flavobacterium, Moraxella, Enterobacteriaceae, Micrococcus and Coryneform group.

In gills the major share of amylase, caseinase, gelatinase and urease was from Pseudomonas. The rest was contributed by Vibrio, Acinetobacter, Moraxella, Micrococcus, Bacillus and Coryneform group. At the same time 50% of the lipase producers were Vibrio and the rest by Pseudomonas, Moraxella, Micrococcus and Coryneform group. More than 88% of the potential chitinoclastic bacteria were members of Vibrio and the rest were Aeromonas.

In stomach among the various hydrolytic groups 37 to 71% and the entire chitinoclastic forms were composed of Vibrio. Rest of the hydrolytic groups were composed of Pseudomonas, Acinetobacter, Enterobacteriaceae, Staphylococcus, Micrococcus, Bacillus and Coryneform group.

In anterior intestine Vibrio was the main source (62 to 95%) for all the potential hydrolytic enzymes and the rest was from Pseudomonas, Moraxella, Enterobacteriaceae, Micrococcus and Coryneform group. But in the case of chitinase, apart from Vibrio 5% of Pseudomonas also elaborated the enzyme.

Vibrio contributed 59 to 100% of all the hydrolytic groups and the rest was by Pseudomonas, Moraxella, Enterobacteriaceae, Micrococcus, and Coryneform group in the posterior intestine. But the chitinase was mainly from Vibrio.

In the alimentary canal Vibrio was the main source of all the potential hydrolytic enzymes tested (55.61 to 98.11%). This was specifically true in the case of chitinase.

Pseudomonas was the predominant flora (33 to 66%) in determining the extent of potential hydrolytic groups in water. Vibrio stood second (10 to 33%). The rest was shared by Moraxella, Enterobacteriaceae, Micrococcus, Bacillus,

and Coryneform group. Interestingly 20% of the chitinoclastic forms were composed of Coryneform group, while 33.33% each were Vibrio and Pseudomonas.

Majority of all the hydrolytic groups were composed of Bacillus (33 to 71%), Coryneform group (8 to 22%) and Vibrio (2 to 16%).

3.2.6 Effect of NaCl concentrations, pH and temperature on the growth of bacteria

The bacterial isolates were grown at different environmental conditions such as varying temperature, pH and sodium chloride concentrations. The results showed that maximum growth of all the isolates was not confined to any one concentration of NaCl, pH and temperature. No growth was observed at pH 2 and 4 and at $4^{o}C$.

Effect of NaCl concentrations, pH and temperature on the growth of various isolates obtained during the period of study are presented in Table 34. Among 644 isolates tested, 23.45 to 36.33% were showing maximum growth between 1 7% NaCl concentration, maximum (36.33%) at 7% NaCl. Absence of NaCl was the optimum condition for a very small percentage (0.47%) of isolates. Similarly 10% NaCl was ideal for 4.35% of isolates. A shift in the requirement of NaCl from 7 to 1% was seen in the culture pond from the date of stocking to

harvest. While in the later half of Apr. and beginning of May more than 42% of isolates preferred 7% NaCl, during the later half of May majority of them preferred 3% NaCl and during the beginning of Jun. 1% NaCl was preferred by 40.69% of isolates.

pH 7 was found to be optimum for majority of isolates (61.65%) and 4.81% preferred pH 11. A small percentage of isolates (6 to 19%) showing optimum growth at pH 11 appeared only in the beginning of Apr.

Nearly 73 to 95% of the isolates exhibited maximum growth at 30°C. While 16% preferred 40°C, only 4% preferred 10°C. The number of isolates capable of growing at 50°C was very low (0.15%) and none of them could grow at 4°C.

Effect of NaCl concentrations, pH and temperature on the growth of isolates obtained from different samples are presented in Table 35. It could be observed that only in juveniles from natural environment and water from happa, at least a small percentage of isolates showing optimum growth at all concentrations of NaCl could be observed. In most of the other samples maximum growth was mostly limited between 1 and 7% NaCl, except in gills, stomach and anterior intestine where a small percentage grew to maximum at 10% NaCl. While in body surface and gills about 42% of isolates were preferring

1% NaCl, in stomach, anterior intestine and posterior intestine 47 to 57% of the isolates preferred 7% NaCl.

In juveniles, water (from both happa and pond) and sediment the isolates showing maximum growth were differentiated between the ones requiring pH 7 and pH 11, and, in adults they were confined to pH 7 and 9. However, pH 7 was optimum for 49.06 to 81.82% of the isolates from all the samples.

In all the samples 49.35 to 96.88% of the isolates preferred 30°C for maximum growth. A small percentage (2.59 to 12.35%) of isolates from juveniles, water from happa and pond, sediment and posterior intestine were showing maximum growth at 10°C. Only 2% of the isolates from gill preferred 30°C for maximum growth.

The growth response of different genera to NaCl concentrations, pH and temperature are presented in Table 36. Except *Acinetobacter* all the other genera showed maximum growth between 1 and 10% NaCl concentrations. Majority of *Vibrio* (68.16%) preferred 7% NaCl for maximum growth and about 10% of them preferred 10% NaCl as optimum. About 53.60% of *Pseudomonas* showed maximum growth at 1% and the rest in 3% and 7% NaCl concentrations. *Acinetobacter* comprised at least a small percentage showing maximum growth in all the concentration of NaCl tested. However 45.45% of them showed maximum growth at 3% NaCl. The optimum range of *Moraxella* was confined within

1 and 3% NaCl, where 67% preferred the latter. About 54.23% of Micrococcus, and 51.81% of Coryneform group showed maximum growth at 3% NaCl. At the same time almost equal proportions of Bacillus (between 40.67 and 42.37%) showed optimum growth at 3 and 1% NaCl respectively.

More than 50% of the members of Vibrio, Coryneform group, Bacillus, Micrococcus, Enterobacteriaceae and Moraxella showed maximum growth at pH 7. Majority of Pseudomonas and Acinetobacter preferred pH 9. pH 11 was optimum for very small percentage of the isolates of Vibrio, Pseudomonas, Acinetobacter, Micrococcus, Bacillus and Coryneform group.

About 55 to 90% isolates of all the genera preferred 30°C for maximum growth. Except a very small percentage of Vibrio (1.1%) no other genera preferred 50°C as optimum. 10°C was suitable for a small percentage of many genera but 4°C was totally unacceptable.

3.3 DISCUSSION

In happa eventhough there were slight fluctuations in the physico-chemical parameters between collections, the magnitude of these differences was very small. However in one occasion (7.4.1982) temperature was very high (37°C). In pond the salinity was very low in June. In general all nutrients except organic nitrogen were at lower levels during June than the previous months. Mary (1977) observed during monsoon season large quantity of organic matter being carried into a fish culture pond by flood water. But in the present study the pond was well protected by bunds and the entry of water from the backwater was prevented by the closure of sluice gate. The salinity and the concentration of nutrients were brought down by the rainwater falling directly into the pond as well as on the slope of the bunds. The comparatively high temperature observed in April is characteristic of summer months.

Juveniles in happa harboured a lesser population than that of juveniles from natural environment. Also, the percentage of Gram-negative forms in the juveniles from happa was lesser than that of the juveniles from natural environment. The number of genera occurred in both these samples were same

except the family Enterobacteriaceae and Acinetobacter which were present additionally in the samples from natural environment. The common genera encountered were Vibrio, Pseudomonas, Moraxella, Micrococcus, Bacillus and Coryneform group. Juveniles from natural environment were found to harbour higher percentage of Vibrio than the juveniles from happa. At the same time other genera such as Moraxella, Micrococcus and Bacillus were more in juveniles from happa than in juveniles from natural environment. Thus, the bacterial density, percentage of Gram-negative bacteria and the percentage of Vibrio, were higher in the juveniles from natural environment than the juveniles from happa. The juveniles were collected from estuary and released into the happa. The change of the environment might have influenced the bacterial population associated with juveniles. The observed variations in generic composition indicate the definite influence of the environment. Similarly Vanderzant et al. (1970) observed a lesser population of bacteria in pond reared shrimp than those of most commercial samples from the Gulf of Mexico, and the difference could be attributed to differences in environment. Bacillus, Lactobacillus, Coryneform group and Flavobacterium were the important genera in pond reared shrimp. But, in the present observation, Vibrio was the dominant genus. In a tropical estuarine system, the dominance of Vibrio is well documented (Colwell and Kaper, 1977; Ivy Thomas, 1982; Lakshmanaperumalsamy, 1983 and Chandrasekaran et al. 1984).

THB of juveniles collected from happa showed a higher magnitude than that of the water in happa. Water contained more number of genera than that of juveniles from happa. Further, Gram-negative bacteria of water was very high in percentage than that of the animals. Acinetobacter and Enterobacteriaceae which were isolated from water in one or more occasions were absent in juveniles. A higher bacterial population associated with animals than in water has already been reported (Vanderzant et al. 1971; Mary, 1977 and Palaniappan, 1982). The dominant genera occurred in water were Vibrio, Pseudomonas, Coryneform group, Moraxella, Acinetobacter and Enterobacteriaceae.

An increasing order of THB was observed from body surface to gill and to alimentary canal of adult. Gram-negative bacteria of the alimentary canal was higher than that of the body surface and gill. The dominance of Pseudomonas on the body surface, Coryneform group in gills, Vibrio and Pseudomonas in stomach, Vibrio and Coryneform group in the anterior and posterior intestine was observed throughout the study period. Thus the important genera encountered were Vibrio, Pseudomonas and Coryneform group. The percentage of Vibrio increased from body surface to intestine and at the same time Pseudomonas was declining. The percentage occurrence of Micrococcus was almost double on surface, gill, stomach than in anterior and posterior intestine. Fluctuations of Coryneform group were irregular however the lowest level was recorded in stomach.

On the surface and gills of animals, Pseudomonas was the dominant flora. In water also same situation was recorded. This showed that the nature of the population existed in water reflected on the surface and gills of animals. But statistically a significant correlations between the percentage of Pseudomonas in water and that of the surface and gills was not observed. Roberts (1978) stated that the normal bacterial flora of fish is a direct reflection of the bacterial flora of water in which they swim. The genera encountered on the surface and gill in the decreasing order of dominance were Pseudomonas, Coryneform group, Vibrio and Micrococcus. Micrococcus in the gill showed a significant positive correlation with the Micrococcus of sediment, which showed that occurrence of Micrococcus in sediment was reflected in gills. In gills Moraxella was prominent than that of surface. Percentages of other genera were very small (<4%). In water also almost the same decreasing order of dominance in the percentage of various genera could be seen. Occurrence of Coryneform group in the gills throughout the study period suggested that gills might have served as a good habitat for attachment and growth of Coryneform group.

In all the collections THB of stomach was higher (1-2 magnitude) than that of the surface sediments from which the animal usually received the detritus. Higher bacterial

population in the stomach may be due to the selective feeding behaviour of the animals. This observation also conforms with that of Mary (1977), Ivy Thomas (1982), and Palaniappan (1982). Dall (1968) suggested that the prawns would seem to feed selectively upon epiflora and epifauna (bacteria, microalgae and protozoa) of the mud substrates. Moriarty (1976) revealed that prawns chose organic matter rich in bacteria from the sediment. Sriraman (1978) showed that the prawn P. indicus was found to feed mainly on organic detritus. It was found that most of the pelleted feed, given as supplementary diet to pond reared animals, was supporting good bacterial growth with an enhanced bacterial production in detritus (Moriarty, 1985). In sediment the genera occurred in the decreasing order of dominance, were Bacillus, Coryneform group, Pseudomonas and Moraxella. Vibrio was very small in percentage. But in stomach the genera in the decreasing order of dominance were Vibrio, Pseudomonas, Micrococcus, Coryneform group and Bacillus. The food of prawns contained remains of tiny animals and large portions of detritus matter (Ivy Thomas, 1982). In the present study apart from the decaying supplementary feed, the dead plankton and other animal and plant matter also might have formed the dietary components. This, in general, might have resulted in a higher bacterial population and floral difference in stomach than the sediment.

At the same time the increase of Pseudomonas observed in sediment from the day of stocking to that of harvest was significantly showing a positive relationship to the Pseudomonas in the stomach during the corresponding period.

In general, the THB of stomach was much lesser than that of the anterior and posterior intestine. At the same time the population of the posterior intestine was slightly lower than that of the anterior intestine. Similar observation was made by Palaniappan (1982) in the pond reared Penaeus indicus, where, he observed a higher bacterial counts in the midgut (intestine) than the foregut (stomach). The main digestive processes take place in the foregut itself and the midgut played a key role in the absorption of the digested food materials (Gopalakrishnan, 1957). The midgut provide a favourable environment to the tolerant strains and the active multiplication of these bacterial genera takes place in this region. Moriarty (1976) showed that microorganisms passing directly from the proventriculus to the midgut without passing through the digestive gland were poorly digested indicating that midgut was not a centre of digestion. Thus, a part of the bacteria which were consumed along with food might have entered directly into the intestine without passing through the digestive gland and yet another part might have

overcome the activity of the digestive enzymes and the low pH prevailing in the digestive tract. pH of the digestive tract during digestive processes was found to be 5 (Hood and Meyers, 1973). The cells which could achieve an entry into the intestine might have undergone a few cycles of division resulting in the higher population than that of the stomach.

In all the three regions of alimentary canal Vibrio was found to be the dominant flora. Moreover an increase of Vibrio could be seen from stomach region to intestine. The other genera such as Pseudomonas, Acinetobacter, Micrococcus and Bacillus were found to decline. Moraxella which was undetectably small in stomach increased in anterior and posterior intestine. Coryneform group also registered an increase in the anterior and posterior intestine. But the percentage of Enterobacteriaceae remained almost steady in all the three regions of alimentary canal. Thus the genera of bacteria encountered in the alimentary canal could be grouped into three, such as those multiplied when the food passed through intestine (Vibrio, Moraxella and Coryneform group), those which were lysed by the digestive enzymes (Pseudomonas, Acinetobacter, Micrococcus and Bacillus) and, those which were tolerant to the digestive juice but did not multiply (Enterobacteriaceae). Palaniappan (1982) also

reported Vibrio, Bacillus and Pseudomonas as the dominant flora in the foregut. In the midgut, Coryneform group, luminous Vibrio and Bacillus showed considerable increase. He has reported that the percentage of Vibrio and Pseudomonas which formed the resistant groups in foregut decreased slightly in their relative percentage, in the midgut. But in a study conducted with experimental diet he has noted a remarkable increase of Vibrio in the digestive tract of P. indicus. In the present observation the increase of Vibrio in the intestine is quite remarkable and the Pseudomonas declined considerably. Significantly, it was seen that the percentage of Vibrio in the alimentary canal is almost double the percentage seen in stomach. At the same time percentage of Vibrio in the posterior intestine was slightly lesser than that of the anterior intestine. Almost same was the changes happened in the case of Coryneform group in intestine. The posterior intestine received the remaining unutilized portion of the food which contained a lesser proportion of nutrients than the food found in anterior intestine. Possibly the competition for available nutrients might have adversely affected Vibrio population. Thus it becomes apparent that the alimentary canal forms a suitable microenvironment where Vibrio, Coryneform group and Moraxella can undergo a few cycles of division. Multiplication of Vibrio, Moraxella and Coryneform group can be cited as a reason for the higher

population (THB) observed in the intestine. Because, these genera totally constituted more than 75% compared to the situation in stomach where it was only about 45%. The bacterial cells which were able to tolerate the adverse conditions of the digestive process entered in the midgut (Palaniappan, 1982). There is no digestion taking place in this region and the absorption of digested food material takes place in the midgut region. Present investigation shows that the microenvironment of alimentary canal, in general, is highly suitable for Vibrio where it undergoes a few cycles of division. The percentage of Vibrio was lesser in water, sediment, body surface and gills compared to the alimentary canal. Prieur (1981) stated that Vibrio are apparently able to divide a number of times in the gut of bivalve molluscs. Further, if this phenomenon occurs, it would be an explanation of the presence of an important Vibrio-like population in the gut of bivalve compared to the bacterial population of sea water.

It was seen that Micrococcus suffered a remarkable decline along with Pseudomonas in the intestine, the percentage was less than half of what was seen in stomach. A similar observation was reported by Palaniappan (1982), in the digestive tract microflora fed with experimental diet. But contrary to his observation, in the present study, Pseudomonas was declining in the intestine. At the same time a slight

increase in the posterior intestine from what was seen in the anterior intestine could also be observed. The organisms must have suffered injury to varying extend in the stomach and digestive gland and afterwards the cells which escaped the process could have multiplied in the posterior intestine. Same is the case with Micrococcus which showed a slight increase in the posterior part of the intestine.

In the alimentary canal Vibrio was the dominant flora throughout the study period. At the same time in sediment, population of Bacillus was at higher level at most of the times and Pseudomonas during the other periods. It can be speculated that pond reared prawns harbour Vibrio in their intestine than in other body parts. Ivy Thomas (1982) observed high incidence of chitinoclastic vibrios in the intestine of marine and estuarine prawns. This situation deserves much concern, because higher percentage of Vibrio in intestine is not ideal in the health point of view when animals are put under stress, due to drastic fluctuations of the environmental factors which normally can happen in a culture pond. However such a situation was not met with in the present study. In such situations Vibrio may behave as an opportunistic pathogen invading the tissue and haemolymph through the intestinal wall as suggested by Davis and Sizemore (1982) in crab. Puzztai (1970) reported invasion of bacteria

from the intestine to the body fluid of the slaughter animals under stress. Herborg and Villadsen (1975) observed the same phenomenon in fishes (rainbow trout) under stress. They observed the infection in the fish muscle increasing with increasing physical stress and was higher for feeding fish than for starving trout. Eventhough the active invasion of vibrios from the gut to the haemolymph of prawns is not proved beyond doubt, the invasive nature of Vibrio in the larvae of bivalves is reported when the larvae became weak (Guillard, 1959; Tubiash et al. 1965 and Brown, 1973).

Genera such as Pseudomonas, Acinetobacter, Micrococcus and Bacillus were found to decline in the alimentary canal especially in the intestine. The lysed bacterial cells might be used as a direct source of food by the animal itself. Moriarty (1976) showed that the Metapenaeus bennettae can readily digest and assimilate bacteria. The high assimilation values obtained suggested that cell walls were not only lysed but digested and assimilated. He further stated that autolysis and enzyme activity in the digestive gland of the prawn would bring almost complete digestion of bacteria. The organisms which he used in his study were Escherichia coli, Pseudomonas fluorescens, Enterobacter aerogenes and Bacillus subtilis apart from blue green algae. This information agrees with the present study as far as Pseudomonas

and Bacillus are concerned. But the members of Enterobacteriaceae remained more or less the same in the different regions of the alimentary canal. Palaniappan (1982) noticed a drastic reduction of Micrococcus in the gut of prawn fed with an experimental diet, agreeing with the present observation. Thus bacteria formed an important part of diet, as stated by Moriarty (1976) when he observed 20 to 35% of the organic carbon in the proventriculus of the prawn M. bennettae feeding on muddy estuarine sediments is comprised of bacteria.

The gradual increase of the percentage of Pseudomonas in surface, gill and water was observed from the date of stocking to the day of harvest. During the experimental period no noticeable change of the environmental parameters was observed except a drop in salinity in June along with rains and hence no significant correlation could be observed between Pseudomonas and environmental variables. Christopher et al. (1978) also could not establish any relationship between the changes in the number and type of microorganisms in pond reared shrimp with physico-chemical parameters. Vanderzant et al. (1970) observed Bacillus and Lactobacillus, Coryneforms and Flavobacterium of greater significance in pond reared shrimp and further it harboured fewer Pseudomonas. The reason for this difference can be attributed to geographical variation. Similarly Vanderzant et al. (1971) observed Coryneform bacteria and to a

lesser extent Vibrio as the dominant isolates from pond reared shrimp. However his observation was based on the microflora present in the (beheaded) whole animal. Christopher et al. (1978) reported Corynebacteria and Vibrio spp. as the dominant microflora of the pond reared shrimp.

Proteolytic and ureolytic bacteria were found to be dominant in all samples followed by lipolytic, amylolytic and chitinoclastic bacteria. Juveniles and water from happa contained lesser percentage of various hydrolytic bacteria except lipolytic bacteria than that of juveniles from natural environment. This can be related with the lesser percentage of Vibrio observed in the juveniles from happa and the comparative higher percentage of Moraxella, Micrococcus and Bacillus. It could be noticed that 90 to 100% of vibrios in all the samples were able to elaborate all these hydrolytic enzymes. At the same time the comparative increase of the percentage of Moraxella, Micrococcus and Bacillus did not contribute significantly to the above hydrolytic groups.

It was quite remarkable to observe that all vibrios elaborated chitinase. Thus the increase of chitinoclastic vibrios in an aquatic system when the animals are crowded remains a major threat to the survival of the juveniles.

Lightner (1975) opined that holding of otherwise healthy shrimp occasionally results in the onset of bacterimia due in most cases to a Vibrio sp. In all cases some sort of physical or chemical stress or injury preceeded the onset of clinical disease. The capture and holding in tanks of wild penaeid shrimp often result in the disease syndromes. Cuticular injuries may provide a route of entry for potentially pathogenic bacteria which are a normal part of the microflora of pond reared or hatchery reared shrimp (Vanderzant et al. 1970 and 1971). In happa when the juveniles are crowded injuries can be frequently made by each other by the rostral spines. Through these injuries chitinoclastic vibrios can easily invade and cause shell necrosis. Further when the environmental factors fluctuate departing from the optimum, these invading vibrios can cause septicemia and death. Rosen (1970), Cook and Lofton (1973), Delves-Broughton and Poupard (1975) have related the shell disease of prawns with chitinoclastic Vibrio.

In pond also proteolytic and ureolytic bacteria were dominant in all the samples followed by lipolytic, amylolytic and chitinoclastic. The highest percentage of chitinoclastic bacteria were found in intestine, and a gradual increase in their percentage could be seen from surface to posterior intestine. Similar situation was seen with regard to lipolytic

bacteria also. Such an increase or decrease could not be seen in other hydrolytic groups. While 89.08% of Vibrio could elaborate chitinase, only 10% of other genera exhibited this property. Alimentary canal harboured the highest percentage of chitinoclastic vibrios than gill and body surface and chitinoclastic bacteria increased gradually from surface to intestine. Vibrios as the principal organisms elaborating chitinase have been observed by Okutani (1966), Chan (1970),and Ivy Thomas (1982). In the same pattern the increase of lipolytic bacteria from the surface to intestine was also recorded. Eventhough all the isolates of Enterobacteriaceae appeared to be lipolytic, their percentage occurrence was negligible and it could be seen that Vibrio was the main source of microbial lipase. The source of other hydrolytic enzymes such as protease and urease was not confined to certain genera alone. Vibrio and Pseudomonas formed the major source of amylase producers followed by other genera. It can be seen from the results that the majority of Vibrios (60%) and 50% of Pseudomonas were amylolytic. Contribution by other genera was comparatively less.

In water and sediment proteolytic and ureolytic bacteria occurred in similar proportion. Amylolytic bacteria were more in sediment. Lipolytic and chitinoclastic forms were less in sediment than in water. Pseudomonas was the

dominant flora in water and Bacillus in sediment. While more than 78% of Bacillus in sediment were amylolytic only 24.24% of Pseudomonas in water produced amylase.

Intestine of prawn appears to be an ideal environment for the chitinoclastic bacteria, than water and sediment. Hood and Meyers (1973) reported that the digestive tract of the white shrimp Penaeus setiferus harboured limited number of generic types, characterised by rapid growth, tolerance to low pH and elaboration of an array of extra cellular enzymes especially chitinase. A high incidence of chitinoclastic bacteria in the gastrointestinal tract of marine and estuarine prawns was observed by Ivy Thomas (1982). The gut content of prawns showed the chitinous material (Ivy Thomas, 1982). Chitinase activity within the digestive tract is correlated with ingestion of dietary chitin, concomitant with an increase in the chitinoclastic bacterial biomass. Bacteria may serve as a direct source of food for shrimp as well as in the elaboration of extra cellular enzymes in the animal digestive process (Hood and Meyers, 1973) Similar observations have already been made by Lear (1961), Seki and Taga (1963), Okutani (1966), Chan (1970), Goodrich and Morita (1977) and Okutani (1978). Hood and Meyers (1977) suggested that the enzyme produced by the predominant gut bacteria, Beneckea is a moderately active inducible chitinase,

while the shrimp has an indigenous constitutive chitinase and chitobiase system. This duel enzyme system suggests that metabolic chitin transformation may play a vital role in crustacean metabolism. In the present observation the high incidence of chitinoclastic vibrios in the intestine of pond reared prawns suggests its profound role in the digestion of chitin.

Thus Vibrio can play a duel role both beneficial as well as harmful in the pond reared shrimp. The vibrios are versatile groups capable of elaborating various hydrolytic enzymes including chitinase which may enhance the digestive process in the alimentary canal. At the same time when the environmental conditions become adverse mounting stress on the animal these bacteria may invade the tissue from the alimentary tract and if the stress factor persists for a longer duration, septicemia due to Vibrio may be resulted.

In general a shift in the requirement of NaCl from 7 to 1%, from the day of stocking to the time of harvest was observed. This indicated that moderately halophilic bacterial population was getting reduced from April to June. Salinity of pond water was low (10.25×10^{-3}) in June following the rains. This drop in salinity must have favoured the growth of lesser halophiles. Juveniles from natural environment and

water from happa contained a small percentage of bacteria showing maximum growth at all concentrations of NaCl. In alimentary canal 47 to 57% of the isolates showed maximum growth at 7% NaCl. At the same time about 42% of the isolates from surface the gills of adult grew to maximum at 1% NaCl. In water and sediment, 36 to 41% of the isolates preferred 1 and 3% NaCl for maximum growth. More than 68% of Vibrio preferred 7% NaCl as optimum for growth and around 10% behaved in the same way with 10% NaCl. In the alimentary canal Vibrio were the dominant flora. Ivy Thomas (1982) observed maximum growth of selected isolates of chitinoclastic Vibrio at 1% NaCl. Higher concentrations of NaCl were not found to be suitable for her isolates indicating that the organisms must have derived from freshwater environment. Surendran et al. (1983) reported that the strains of Vibrio required 2.5 to 3.0% NaCl for maximal growth. Chandrasekaran (1985) observed three strains of Vibrio (R 42, L 146 and F 10) showing maximal growth at 6% NaCl suggesting that the organisms are euryhaline. Pradeep (1986) noted 4% NaCl as the optimum for his isolates of Vibrio parahaemolyticus whereas approximately 3% NaCl was reported earlier to be optimum (Beuchat, 1974,1975). The above observations along with the present one explains that Vibrio occur in freshwater, brackish and marine environments, its occurrence and abundance being controlled by various environmental factors.

Pseudomonas (54%) showed maximum growth at 1% NaCl. It was found that on the body surface, gill and water, 42% of the total isolates were preferring the same NaCl concentratio Four strains of *Pseudomonas* studied by Surendran *et al*. (1983) showed an optimum range of NaCl from 2.5 to 3.5%. The maximum tolerable limit of NaCl was 10%. Out of the three strains of *Pseudomonas* studied by Chandrasekaran (1985), *Pseudomonas* R 8 and L 97 showed preference to 3% NaCl for maximal growth in flesh broth as well as in ZoBell's broth. *Pseudomonas* F 152 preferred 1% NaCl. In the present study 32% of *Pseudomonas* preferred 3% NaCl and 14.4% preferred 7% NaCl. All these show that there are strains of *Pseudomonas* non halophilic, slightly halophilic, and moderately halophilic, indicating their origin both from freshwater and marine environments.

Among Coryneform group 51.8% were showing maximum growth at 3% NaCl. In water of the pond in addition to *Pseudomonas*, Coryneform group also was an important genus. The reason for 34.41% of THB in this sample preferring 1 and 3% NaCl can be partly because of the higher percentage of Coryneform group.

The highest percentage of *Micrococcus* (54.23) was preferring 3% NaCl for maximum growth. The rest of the strains

preferred 1, 7 and 10% NaCl. These organisms were mainly from the juveniles from happa, surface, gills and stomach of adult. In surface and gill of the animal, occurrence of THB preferring NaCl concentrations ranging from 1 - 10% can be partly a result of the existence of strains of Micrococcus preferring a wide range of NaCl.

Bacillus showed maximum growth at 1 and 3% NaCl. In sediment it could be observed that 31 - 46% of the total isolates were preferring 1 - 3% NaCl for maximum growth.

Thus it becomes apparent that the extent of total halophilic bacteria in a sample is determined by variations in the genera. Low salinity due to rain might have favoured the growth of low salt requiring bacteria to dominate. At the same time it is seen that moderately halophilic forms are also not eliminated completely and they remain dormant. Stevenson (1978) stated that the physiological state of a significant portion of the bacterial community in most aquatic environment can be described as dormant.

Juveniles from natural environment and happa, and water from happa contained more bacteria showing maximum growth at pH 7. In the juvenile from natural environment 55% were behaving so, and the rest were requiring pH 9 and 11.

It was seen that in the latter case the percentage of vibrios were more than the former two cases. Among the total vibrios 34% were preferring alkaline pH (pH 9 and 11) and this might have lead to the situation of higher percentage occurrence of THB preferring alkaline pH in the juveniles from natural environment. Apart from Vibrio, 82% Moraxella and 80% Micrococcus also showed maximum growth at pH 7. Juveniles from happa harboured comparatively more Moraxella and Micrococcus. This could be attributed as the reason for the majority total isolates of juveniles from happa preferring pH 7 as the optimum.

On the body surface about 63% of the isolates were showing maximum growth at pH 7 and the rest at pH 9. It is seen that in this sample 41% of the flora is composed of Pseudomonas and the other important genera Vibrio, Micrococcus and Coryneform group were varying from 14 - 17%. It was observed that except Vibrio (58.4%), majority of the other genera listed above, preferred pH 7 as the optimum.

In gills, pH 7 and 9 were preferred by equal percentages of the isolates. In this sample in addition to Pseudomonas (34%), the other important genera such as Vibrio, Micrococcus and Coryneform group ranged from 15 - 20% each.

Eventhough 58% of Pseudomonas preferred pH 9 majority of the other genera were found to grow to maximum at pH 7 thus making 50% of the population preferring pH 7 and the other half pH 9.

In stomach, anterior and posterior intestine 57 to 62% of the isolates were showing maximum growth at pH 7. In all these three samples Vibrio were the prominent flora. In the present investigation 65% of the Vibrio were found to be preferring pH 7 as optimum. In water of the pond 54% of THB were growing to maximum at pH 7 and 41% at pH 9. Pseudomonas was the prominent flora, and the other important genera were Vibrio, Bacillus and Coryneform group. Eventhough,in general, a good percentage of Pseudomonas preferred pH 9, the major percentage of rest of the genera showed maximum growth at pH 7.

In sediment, while 58% of THB grew to maximum at pH 7, 38% preferred pH 9, Bacillus was the predominant flora and the other important genera were Pseudomonas and Coryneform group. Out of this, majority of Bacillus and Coryneform group preferred pH 7. Thus the change in the number of organisms in a sample preferring a particular pH may be attributed to the occurrence of strains requiring that pH.

Ivy Thomas (1982) recorded a pH range of 5 - 8 for Vibrio sp. and were found to be more sensitive towards acidic

conditions than alkaline. Chandrasekaran (1985) observed a pH range 6 - 10 for bacterial isolates.

Two strains of Vibrio parahaemolyticus studied by Pradeep (1986) showed that pH 8 was most favourable. Beuchat (1975) reported that pH 7 to 8.6 as the optimum for growth. Marine vibrios have an optimum pH in slightly alkaline conditions. Growth of Vibrio parahaemolyticus ranging from pH 5 - 11 (Twedt et al. 1969), 5 - 8 (Beuchat, 1974) and 5 - 10 (Ermolina and Shikatov, 1975) were already reported.

Thus in the culture environment the bacterial population in general is alkalophilic. pH of the environment was also alkaline showing that the situation is congenial for the different genera to exist together.

Majority of the isolates 73.95% were mesophilic preferring 30°C as the optimum. Psychrophilic forms were totally absent while 16% preferred 40°C for maximum growth. Very small percentage (0.5%) grew to maximum at 50°C, 10°C was the optimum for only 4%. In individual samples also except posterior intestine, majority of the isolates preferred 30°C. Invariably, in all the genera, majority of the cultures preferred 30°C. Ivy Thomas (1982) observed 30°C as the optimum

for a chitinoclastic Vibrio and Aeromonas. Chandrasekaran (1985) showed his isolates Pseudomonas, Vibrio and Acinetobacter preferring 30°C for maximum growth.

Vanderzant and Nickelson (1973) suggested that cultivation of marine species of prawns undercrowded conditions in a confined body of brackish water with temperature as high as 25 - 30°C is ideal for the proliferation of halophilic vibrios which can cause disease problems. In the present study the maximum temperature observed in happa was 37°C and in the pond 31°C. The highest salinity observed was 16.6×10^{-3}. Majority of vibrios were preferring 7% NaCl for optimal growth indicating that they were moderately halophilic. Large number of isolates of Vibrio were from the alimentary canal and from the juveniles, showing that the alimentary canal and juveniles generally might have provided these vibrios a favourable environment to proliferate. It can be speculated that the salt concentration of the intestinal contents of adults and the surface and intestine of juveniles may not be nearing the optimum NaCl required by the vibrios which thrive there. Availability of sufficient nutrients and growth factors in the semidigested and digested food along with some other unknown factors might be favouring these organisms to multiply even in the absence of optimum NaCl concentrations. In this context the suggestion putforth by Vanderzant and

Nickelson (1973) deserves much attention. These vibrios which form the normal flora in the intestinal tract of adult and the juveniles as a whole can behave as opportunistic pathogens when the animals are put under stress. Crowding of animals can be one of the stress factors which is normally encountered in happa as well as in culture pond.

Table 23. Physico-chemical parameters measured in happa and culture pond

Dates	Sample	Salinity (10^{-3})	Temperature (^{o}C)	pH	Dissolved oxygen ($ml.l^{-1}$)	Inorganic nitrogen ($\mu g.l^{-1}$)	Organic nitrogen ($\mu g.l^{-1}$)	Inorganic phosphorus ($\mu g.l^{-1}$)	Organic phosphorus ($\mu g.l^{-1}$)
3.4.1982	Water from happa	14.50	30	8.25	3.83	9.80	8.20	8.20	18.10
6.4.1982	Water from happa	13.80	29	7.90	3.95	10.00	9.50	9.30	16.10
7.4.1982	Water from happa (before introducing into pond)	15.50	37	8.60	4.10	9.00	8.90	8.90	9.0
7.4.1982	Water from pond at the time of releasing of juveniles	14.80	30	8.30	4.50	8.90	9.20	6.20	12.00
19.4.1982	Water from pond - 1st collection after introducing the juveniles	15.00	29	7.80	3.80	12.50	34.00	8.00	9.80
3.5.1982	Water from pond - 2nd collection	15.80	30	7.90	4.81	8.40	20.10	12.50	13.90
18.5.1982	Water from pond - 3rd collection	16.60	31	8.10	3.62	8.90	2.50	13.50	14.00
11.6.1982	Water from pond - 4th collection	10.25	28	7.50	4.20	4.50	2.80	5.60	5.80

Table 24. Quantitative estimation of Total Heterotrophic Bacteria (THB) in different samples collected from natural environment, happa and culture pond

Period	Juveniles from natural environment ($\times 10^5.g^{-1}$)	Juveniles from happa ($\times 10^5.g^{-1}$)	Water from happa ($\times 10^5.ml^{-1}$)	Adult from pond							
				Body surface ($\times 10^5.cm^2$)	Gills ($\times 10^5.g^{-1}$)	Stomach ($\times 10^5.g^{-1}$)	Anterior intestine ($\times 10^5.g^{-1}$)	Posterior intestine ($\times 10^5.g^{-1}$)	Alimentary canal ($\times 10^5.g^{-1}$)	Water ($\times 10^5.ml^{-1}$)	Sediment ($\times 10^5.g^{-1}$)
3.4.1982	22.21	7.23	33.65	ND	ND	ND	ND	ND	ND	ND	ND
6.4.1982	12.29	1.76	0.80	ND	ND	ND	ND	ND	ND	ND	ND
7.4.1982	ND	11.94	0.21	ND	ND	ND	ND	ND	ND	0.148	1.49
19.4.1982	ND	ND	ND	0.182	45.56	182.88	523.26	458.34	388.16*	0.92	14.89
3.5.1982	ND	ND	ND	0.018	367.87	1368.85	1025.86	1012.79	1135.83	2.68	24.45
18.5.1982	ND	ND	ND	0.010	95.65	134.51	1028.30	1014.75	725.85	25.16	54.35
11.6.1982	ND	ND	ND	0.014	29.18	96.40	180.00	173.17	149.85	3.92	23.10

ND : Not determined

*Average of stomach, anterior and posterior intestine

Table 25. Percentage distribution of THB in different regions of alimentary canal

Date of collection	Stomach	Anterior intestine	Posterior intestine	Alimentary canal
19.4.1982	15.70*	44.94	39.36	100
3.5.1982	40.17	30.11	29.72	100
18.5.1982	6.18	47.22	46.60	100
11.6.1982	21.44	40.04	38.52	100

*Percentage to the total of THB in alimentary canal

Table 26. Percentage occurrence of Gram negative bacteria in different samples from natural environment, happa and culture pond

Sample	3.4.1982		6.4.1982		7.4.1982		19.4.1982		3.5.1982		18.5.1982		11.6.1982		Total	
	T	G -ve	T	G -ve	T	G -ve	T	G -ve	T	G -ve	T	G -ve	T	G -ve	T	G -ve
Juveniles from natural environment	40	82.50	33	87.57	-	-	-	-	-	-	-	-	-	-	73	84.93
Juveniles from happa	17	52.94	30	80.00	34	64.71	-	-	-	-	-	-	-	-	81	67.90
Water from happa	14	71.42	14	78.57	16	93.75	-	-	-	-	-	-	-	-	44	81.82
Adults																
Surface	-	-	-	-	-	-	16	62.50	17	64.71	15	46.67	16	100.00	64	68.75
Gill	-	-	-	-	-	-	11	27.27	14	78.57	15	46.67	13	92.31	53	62.26
Stomach	-	-	-	-	-	-	17	64.71	11	54.55	17	70.58	8	87.50	53	67.93
Anterior intestine	-	-	-	-	-	-	19	73.68	18	83.33	13	76.92	14	78.57	64	78.13
Posterior intestine	-	-	-	-	-	-	21	80.95	22	77.27	17	70.59	17	82.35	77	77.92
*Alimentary canal	-	-	-	-	-	-	57	73.68	51	74.51	47	72.34	39	82.05	194	75.26
Water from pond	-	-	-	-	19	84.22	16	50.00	9	88.89	7	85.71	8	87.50	59	76.27
Sediment from pond	-	-	-	-	19	52.63	15	20.00	19	5.26	13	15.38	10	60.00	76	28.94
	71	73.23	77	83.12	88	71.59	115	57.39	110	62.72	97	57.73	86	84.88	644	68.79

*Total bacterial population of stomach, anterior and posterior intestine

T = Total G -ve = Gram-negative

Table 27. Temporal changes in percentage composition of different genera in various samples

	Juveniles from natural environment			Juveniles from happa			
	1 3.4.1982	2 6.4.1982	Total	1 3.4.1982	2 6.4.1982	3 7.4.1982	Total
Vibrio	77.50	51.52	65.75	41.18	56.67	44.12	48.15
Pseudomonas	5.00	12.12	8.22	11.76	10.00	2.94	7.41
Aeromonas	-	-	-	-	-	-	-
Acinetobacter	-	3.03	1.36	-	-	-	-
Flavobacterium	-	-	-	-	-	-	-
Alcaligenes	-	-	-	-	-	-	-
Moraxella	-	12.12	5.48	-	13.33	17.65	12.35
Enterobacteriaceae	-	9.09	4.10	-	-	-	-
Staphylococcus	-	-	-	-	-	-	-
Micrococcus	2.50	3.03	2.74	5.88	13.33	20.58	14.82
Bacillus	2.50	3.03	2.74	35.29	3.33	-	8.64
Coryneform group	12.50	6.06	9.59	5.88	3.33	14.71	8.64
	54.79	45.21	73.00	17.00	30.00	34.00	81.00

Table 27. Contd.

Genera	Water from happa				Adult - surface				
	1 3.4.1982	2 6.4.1982	3 7.4.1982	Total	1 19.4.1982	2 3.5.1982	3 18.5.1982	4 11.6.1982	Total
Vibrio	14.29	57.14	68.75	47.72	6.25	58.82	-	-	17.19
Pseudomonas	14.29	-	18.75	11.36	37.50	5.88	40.00	81.25	40.63
Aeromonas	-	-	-	-	-	-	-	-	-
Acinetobacter	14.29	7.14	-	6.82	-	-	-	6.25	1.56
Flavobacterium	-	-	-	-	12.50	-	-	-	3.13
Alcaligenes	-	-	-	-	-	-	-	-	-
Moraxella	14.29	14.29	-	9.09	-	-	6.67	6.25	3.13
Enterobacteriaceae	14.29	-	6.25	6.82	6.25	-	-	6.25	3.13
Staphylococcus	-	-	-	-	-	-	-	-	-
Micrococcus	7.14	7.14	-	4.55	25.00	17.64	13.33	-	14.07
Bacillus	-	7.14	-	2.27	-	-	-	-	-
Coryneform group	21.43	7.14	6.25	11.36	12.50	17.65	40.00	-	17.19
	31.82	31.82	36.36	44.00	25.00	26.56	23.44	25.00	64.00

Table 27. Contd.

Genera	Gills					Stomach				
	1 19.4.'82	2 3.5.'82	3 18.5.'82	4 11.6.'82	Total	1 19.4.'82	2 3.5.'82	3 18.5.'82	4 11.6.'82	Total
Vibrio	9.09	50.00	-	15.38	18.86	35.29	45.45	47.05	12.50	37.73
Pseudomonas	-	21.42	46.67	61.54	33.46	23.53	9.09	11.76	50.00	20.75
Aeromonas	-	-	-	-	-	-	-	-	-	-
Acinetobacter	-	-	-	7.69	1.89	-	-	-	12.50	1.88
Flavobacterium	-	-	-	-	-	-	-	-	-	-
Alcaligenes	-	-	-	-	-	-	-	-	-	-
Moraxella	18.18	7.14	-	7.69	7.55	-	-	-	-	-
Enterobacteriaceae	-	-	-	-	-	5.88	-	11.76	12.50	7.55
Staphylococcus	-	-	-	-	-	5.88	-	-	-	1.88
Micrococcus	45.45	7.14	13.33	-	15.09	23.52	36.36	11.76	-	18.87
Bacillus	-	-	6.67	-	1.88	-	-	5.88	12.50	3.77
Coryneform group	27.27	14.29	33.33	7.69	20.75	5.88	9.09	11.76	-	7.55
	20.75	26.42	28.30	24.53	53.00	32.08	20.75	32.08	15.09	53.00

Table 27. Contd.

Genera	Anterior intestine					Posterior intestine				
	1 19.4.'82	2 3.5.'82	3 18.5.'82	4 11.6.'82	Total	1 19.4.'82	2 3.5.'82	3 18.5.'82	4 11.6.'82	Total
Vibrio	68.42	66.67	61.53	57.14	64.06	71.43	59.09	52.94	52.94	59.76
Pseudomonas	-	-	15.38	14.28	6.25	-	-	17.64	17.64	7.79
Aeromonas	-	-	-	-	-	-	-	-	-	-
Acinetobacter	-	-	-	-	-	-	-	-	-	-
Flavobacterium	-	-	-	-	-	-	-	-	-	-
Alcaligenes	-	-	-	-	-	-	-	-	-	-
Moraxella	-	-	-	7.14	1.56	-	-	-	11.76	2.59
Enterobacteriaceae	5.26	16.67	-	-	6.25	9.52	18.18	-	-	7.79
Staphylococcus	-	-	-	-	-	-	-	-	-	-
Micrococcus	-	5.56	15.38	7.14	6.25	-	9.09	17.64	11.76	9.09
Bacillus	-	-	-	-	-	-	-	-	-	-
Coryneform group	26.32	11.11	7.69	14.28	15.63	17.04	13.63	11.76	5.88	12.98
	29.69	25.00	20.31	21.88	64.00	27.27	28.57	22.08	22.08	77.00

Table 27. Cohtd.

Genera	Alimentary canal				Total
	1 19.4.1982	2 3.5.1982	3 18.5.1982	4 11.6.1982	
Vibrio	31.78	28.07	23.36	16.82	107
Pseudomonas	19.05	4.76	33.33	42.86	21
Aeromonas	-	-	-	-	-
Acinetobacter	-	-	-	100.00	1
Flavobacterium	-	-	-	-	-
Alcaligenes	-	-	-	-	-
Moraxella	-	-	-	100.00	3
Enterobacteriaceae	28.57	50.00	14.29	7.14	14
Staphylococcus	100.00	-	-	-	1
Micrococcus	19.05	33.33	33.33	14.29	21
Bacillus	-	-	50.00	50.00	2
Coryneform group	41.67	25.00	20.83	12.50	24
	29.38	26.29	24.23	20.10	194

Table 27. Contd.

Genera	Water						Sediment					
	1 7.4.'82	2 19.4.'82	3 3.5.'82	4 18.5.'82	5 11.6.'82	Total	1 7.4.'82	2 19.4.'82	3 3.5.'82	4 18.5.'82	5 11.6.'82	Total
Vibrio	15.79	6.25	11.11	-	12.50	10.17	10.53	-	-	-	10.00	3.95
Pseudomonas	42.11	37.50	77.78	85.71	75.00	55.93	26.32	6.67	-	-	40.00	13.15
Aeromonas										7.69	-	1.32
Acinetobacter	5.26	-	-	-	-	1.69	5.26	6.67	5.26	-	-	3.95
Flavobacterium	-	-	-	-	-	-	-	-	-	-	-	-
Alcaligenes	-	-	-	-	-	-	-	-	-	-	-	-
Moraxella	10.52	-	-	-	-	3.38	10.53	-	-	7.69	10.00	5.26
Enterobacteriaceae	5.26	6.25	-	-	-	3.38	-	6.67	-	-	-	1.32
Staphylococcus	-	-	-	-	-	-	-	-	-	-	-	-
Micrococcus	-	12.50	11.11	-	-	5.08	-	13.33	-	-	-	2.63
Bacillus	5.26	18.75	-	14.28	-	8.47	31.58	60.00	73.68	69.23	30.00	53.95
Coryneform group	15.78	18.75	-	-	12.50	11.86	15.79	6.67	21.05	15.38	10.00	14.47
	32.20	27.12	15.25	11.86	13.55	59.00	25.00	19.74	25.00	17.11	13.16	76.00

Table 28. Percentage generic composition of bacterial strains occurred in juveniles, adults, water and sediment

Genera	Juveniles from natural environment	Juveniles from happa	Water from happa	Adult						Water	Sediment	Grand total
				Surface	Gills	Stomach	Anterior intestine	Posterior intestine	Alimentary canal			
Vibrio	65.75	48.15	47.72	17.19	18.36	37.73	64.06	59.76	55.15	10.17	3.95	38.04
Pseudomonas	8.22	7.41	11.36	40.63	33.96	20.78	6.25	7.79	10.82	55.93	13.15	19.41
Aeromonas	-	-	-	-	-	-	-	-	-	-	1.32	0.15
Acinetobacter	1.36	-	6.82	1.56	1.89	1.88	-	-	0.52	1.69	3.95	1.71
Flavobacterium	-	-	-	3.13	-	-	-	-	-	-	-	0.31
Alcaligenes	-	-	-	-	-	-	-	-	-	-	-	
Moraxella	5.48	12.35	9.09	3.13	7.55	-	1.56	2.59	1.55	3.38	5.26	5.12
Enterobacteriaceae	4.10	-	6.82	3.13	-	7.55	6.25	7.79	7.22	3.38	1.32	3.88
Staphylococcus	-	-	-	-	-	1.88	-	-	0.52	-	-	0.16
Micrococcus	2.74	14.82	4.55	14.07	15.09	18.87	6.25	9.09	10.82	5.08	2.63	9.16
Bacillus	2.74	8.64	2.27	-	1.88	3.77	-	-	1.03	8.47	53.95	9.16
Coryneform group	9.59	8.64	11.36	17.19	20.75	7.55	15.63	12.98	12.38	11.56	14.47	12.89
Total No. of isolates	73	81	44	64	53	53	64	77	194*	59	76	644

*Total bacterial population of stomach, anterior and posterior intestine together

Table 29. Correlation coefficients between percentage of bacterial genera of the animal and that of its environment.

Samples	Vibrio	Pseudomonas	Micrococcus	Bacillus	Coryneform group
		Surface			
Water	0.4	0	0.80	0	-0.70
Sediment	-0.4	-0.50	0.70	0	0.40
		Gills			
Water	-0.70	0.80	0.60	0.40	-0.10
Sediment	-0.10	0.60	1.00*	0.40	-0.10
		Stomach			
Water	-0.60	-0.10	0.90	0	-0.70
Sediment	-0.90	1.00*	0.20	0	-0.60
		Anterior intestine			
Water	-0.1	-0.60	-1.0*	0	0.90
Sediment	-0.80	0.50	-0.80	0	-0
		Posterior intestine			
Water	0	0.60	-0.80	0	0.1
Sediment	-0.50	0.50	-0.90	0	-0
		Alimentary canal			
Water	-0.20	0.20	0.10	-0.50	0.50
Sediment	-0.80	0.70	-0.40	-0.50	-0.30
		Water			
Sediment	0.60	0	0.60	0.30	-0.90

df: 2. *Significant level at $p<0.05$

Table 30. Percentage occurrence of different hydrolytic forms in various samples

Sample	Total no. of isolates	Amylolytic	Caseinolytic	Gelatinolytic	Lipolytic	Ureolytic	Chitinolytic
Juveniles from natural environment	73	71.23	97.26	100.00	35.61	100.00	63.01
Juveniles from happa	81	46.91	82.91	87.65	40.74	100.00	48.15
Water from happa	44	47.72	88.63	93.18	50.00	100.00	47.72
Adult body surface	64	15.63	87.50	96.87	32.81	98.43	12.50
Gill	53	30.18	100.00	94.33	37.73	100.00	16.98
Stomach	53	37.74	92.45	100.00	47.17	96.23	37.73
Anterior intestine	64	61.90	95.31	92.18	80.95	100.00	63.49
Posterior intestine	77	42.86	100.00	94.81	83.12	100.00	59.74
Alimentary canal	194*	47.42	96.39	95.36	73.20	98.96	54.64
Water from pond	59	33.89	96.61	91.53	74.58	98.30	35.59
Sediment from pond	76	59.21	96.05	97.36	42.10	100.00	23.68
Total	644	45.65	93.63	94.72	52.48	99.38	41.61

*Total bacterial population of stomach, anterior and posterior intestine

Table 31. Percentage distribution of various hydrolytic forms in different genera isolated from juveniles, adults, water and sediment

Organism	Total no. of isolates	Amylolytic	Caseinolytic	Gelatinolytic	Lipolytic	Ureolytic	Chitinolytic
Vibrio	245	75.92	98.37	100.00	84.49	100.00	97.96
Pseudomonas	125	22.40	88.00	100.00	36.50	100.00	8.80
Aeromonas	2	50.00	100.00	100.00	100.00	100.00	50.00
Acinetobacter	10	-	70.00	100.00	20.00	100.00	10.00
Flavobacterium	2	50.00	100.00	100.00	50.00	50.00	-
Alcaligenes	-	-	-	-	-	-	-
Moraxella	33	39.39	81.82	87.87	27.27	100.00	-
Enterobacteriaceae	25	48.00	92.00	100.00	60.00	100.00	4.00
Staphylococcus	1	-	100.00	100.00	-	100.00	-
Micrococcus	59	18.64	81.36	91.53	20.34	94.41	-
Bacillus	59	55.93	100.00	96.61	23.72	100.00	11.86
Coryneform group	83	9.63	100.00	72.29	36.14	100.00	8.43
	644	45.65	93.63	94.72	52.48	99.38	41.61

Table 32. Genericwise distribution of hydrolytic bacteria in various samples

Samples	Total no. of isolates	Amylolytic	Caseinolytic	Gelatinolytic	Lipolytic	Ureolytic	Chitinolytic	Total no. of isolates	Amylolytic	Caseinolytic	Gelatinolytic	Lipolytic	Ureolytic	Chitinolytic
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Vibrio							Pseudomonas					
Juveniles from natural environment	48	95.83	100	100	43.75	100	95.83	6	-	100	100	-	100	-
Juveniles from happa	39	94.87	100	100	82.05	100	100	6	-	83.33	100	-	100	-
Water from happa	21	90.48	100	100	100	100	100	5	-	20	100	20	100	-
Adults														
Body surface	11	-	100	100	100	100	72.73	26	15.38	76.92	100	3.85	100	-
Gills	10	60	100	100	100	100	80	18	38.89	100	100	16.67	100	-
Stomach	20	60	100	100	100	100	100	11	18.18	63	100	9.09	100	-
Anterior intestine	41	78.05	92.68	100	92.68	100	92.68	4	50	100	100	-	100	50
Posterior intestine	46	63.04	100	100	100	100	100	6	-	100	100	50	100	-
*Alimentary canal	107	68.22	97.20	100	97.20	100	97.20	21	19.05	90.95	100	19.05	100	9.50
Water	6	66.67	83.33	100	83.33	100	83.33	33	24.24	100	100	87.88	100	15.15
Sediment	3	33.33	100	100	100	100	100	10	50	100	100	80	100	40
Total	245	75.92	98.37	100	84.49	100	95.51	125	22.40	88.00	100	36.50	100	8.80**

*Total bacterial isolates of stomach, anterior and posterior intestine

Table 32. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
				Aeromonas							Acinetobacter			
Juveniles from natural environment	-	-	-	-	-	-	-	1	-	-	100	-	100	-
Juveniles from happa	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Water from happa	-	-	-	-	-	-	-	3	-	66.67	100	-	100	-
Adults														
Body surface	-	-	-	-	-	-	-	1	-	100	100	-	100	-
Gills	-	-	-	-	-	-	-	1	-	100	100	-	100	100
Stomach	-	-	-	-	-	-	-	1	-	100	100	-	100	-
Anterior intestine	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Posterior intestine	-	-	-	-	-	-	-	-	-	-	-	-	-	-
*Alimentary canal	-	-	-	-	-	-	-	1	-	100	100	-	100	-
Water	1	-	100	100	100	100	-	-	-	-	-	-	-	-
Sediment	1	100	100	100	100	100	100	3	-	66.67	100	66.67	100	-
Total	2	50	100	100	100	100	50	10	-	70	100	20	100	10 **

Table 32. Contd.

1	2	3	4	5	6	7	8
				Flavobacterium			
Juveniles from natural environment	-	-	-	-	-	-	-
Juveniles from happa	-	-	-	-	-	-	-
Water from happa	-	-	-	-	-	-	-
Adult							
Body surface	2	50	100	100	50	50	-
Gills	-	-	-	-	-	-	-
Stomach	-	-	-	-	-	-	-
Anterior intestine	-	-	-	-	-	-	-
Posterior intestine	-	-	-	-	-	-	-
*Alimentary canal	-	-	-	-	-	-	-
Water	-	-	-	-	-	-	-
Sediment	-	-	-	-	-	-	-
Total	2	50	100	100	50	50	- **

Table 32. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Moraxella						Enterobacteriaceae						
Juveniles from natural environment	4	100	100	100	-	100	-	3	-	100	100	-	100	-
Juveniles from happa	10	-	40	100	-	100	-	-	-	-	-	-	-	-
Water from happa	4	50	100	100	-	100	-	3	-	100	100	-	100	-
Adults														
Body surface	2	50	100	100	100	100	-	2	50	100	100	100	100	-
Gills	4	25	100	100	50	100	-	-	-	-	-	-	-	-
Stomach	-	-	-	-	-	-	-	4	75	100	100	100	100	-
Anterior intestine	1	100	100	100	100	100	-	4	75	100	100	100	100	-
Posterior intestine	2	-	100	100	100	100	-	6	66.67	100	100	100	100	-
*Alimentary canal	3	33.33	100	100	100	100	-	14	71.43	100	100	100	100	-
Water	2	100	100	-	-	100	-	2	50	100	100	100	100	50
Sediment	4	50	100	50	50	100	-	1	-	100	100	-	100	-
Total	33	39.39	81.82	87.87	27.27	100	-	25	48	100	100	72	100	4**

Table 32. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
			Staphylococcus								Micrococcus			
Juveniles from natural environment	-	-	-	-	-	-	-	2	100	50	100	-	100	-
Juveniles from happa	-	-	-	-	-	-	-	12	-	41.67	66.67	8.33	100	-
Water from happa	-	-	-	-	-	-	-	2	-	100	50	-	100	-
Adults														
Body surface	-	-	-	-	-	-	-	9	33.33	100	100	22.22	100	-
Gills	-	-	-	-	-	-	-	8	25	100	100	25	100	-
Stomach	1	-	100	100	-	100	-	10	30	100	100	20	80	-
Anterior intestine	-	-	-	-	-	-	-	4	-	100	100	50	100	-
Posterior intestine	-	-	-	-	-	-	-	7	-	100	100	-	100	-
*Alimentary canal	1	-	100	100	-	100	-	21	14.29	100	100	19.05	90.48	-
Water	-	-	-	-	-	-	-	3	33.33	66.67	100	33.33	66.67	-
Sediment	-	-	-	-	-	-	-	2	-	-	100	100	100	-
Total	1	-	100	100	-	100	-	59	18.64	81.36	91.53	20.34	94.91	-**

Table 32. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
			Bacillus							Coryneform group				
Juveniles from natural environment	2	-	100	100	-	100	-	7	-	100	100	71.43	100	-
Juveniles from happa	7	-	100	85.71	-	100	-	7	14.29	100	28.57	-	100	-
Water from happa	1	-	100	-	-	100	-	5	-	100	80	-	100	-
Adult														
Body surface	-	-	-	-	-	-	-	11	-	100	81.81	18.18	100	-
Gills	1	-	100	100	-	100	-	11	-	100	72.72	27.27	100	-
Stomach	2	-	100	100	-	100	-	4	-	100	100	25	100	-
Anterior intestine	-	-	-	-	-	-	-	10	-	100	50	60	100	-
Posterior intestine	-	-	-	-	-	-	-	10	-	100	60	70	100	-
*Alimentary canal	2	-	100	100	-	100	-	24	-	100	62.50	58.33	100	-
Water	5	20	100	100	40	100	20	7	42.86	100	57.14	57.14	100	42.86
Sediment	41	78.04	100	100	29.26	100	14.63	11	36.36	100	100	18.18	100	36.36
Total	59	55.93	100	96.61	23.72	100	11.86	83	9.63	100	72.29	36.14	100	8.43**

Table 33. Percentage contribution of different genera for particular hydrolytic enzyme in a sample

Hydrolytic form	Total no. of isolates	Vibrio	Pseudomonas	Aeromonas	Acinetobacter	Flavobacterium	Alcaligenes	Moraxella	Enterobacteriaceae	Staphylococcus	Micrococcus	Bacillus	Coryneform group
1	2	3	4	5	6	7	8	9	10	11	12	13	14
						Juveniles from natural environment							
Amylolytic	52	88.46	-	-	-	-	-	7.69	-	-	3.85	-	-
Caseinolytic	71	67.60	8.45	-	-	-	-	5.63	4.23	-	1.41	2.82	9.86
Gelatinolytic	73	65.75	8.22	-	1.37	-	-	5.47	4.11	-	2.74	2.74	9.)
Lipolytic	26	80.77	-	-	-	-	-	-	-	-	-	-	19.23
Ureolytic	73	65.73	8.22	-	1.37	-	-	5.47	4.10	-	2.73	2.73	9.59
Chitinolytic	46	100	-	-	-	-	-	-	-	-	-	-	-

Table 33. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
						Juveniles from happa							
Amylolytic	38	97.37	-	-	-	-	-	2.63	-	-	-	-	-
Caseinolytic	67	58.21	7.46	-	-	-	-	5.97	-	-	7.46	10.45	10.45
Gelatinolytic	71	54.93	8.45	-	-	-	-	14.08	-	-	11.26	8.45	2.81
Lipolytic	33	96.97	-	-	-	-	-	-	-	-	8.03	-	-
Ureolytic	81	48.15	7.41	-	-	-	-	12.35	-	-	14.81	8.64	8.64
Chitinolytic	39	100	-	-	-	-	-	-	-	-	-	-	-
						Water from happa							
Amylolytic	21	90.48	-	-	-	-	-	9.52	-	-	-	-	-
Caseinolytic	39	53.85	2.56	-	5.13	-	-	10.26	7.69	-	5.13	2.56	12.82
Gelatinolytic	41	51.21	12.19	-	7.32	-	-	9.76	7.32	-	2.44	-	9.76
Lipolytic	22	95.45	4.55	-	-	-	-	-	-	-	-	-	-
Ureolytic	44	47.73	11.36	-	6.82	-	-	9.09	6.82	-	4.54	2.27	11.36
Chitinolytic	21	100	-	-	-	-	-	-	-	-	-	-	-

Table 33. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Adults								
					Body surface								
Amylolytic	10	-	40	-	-	10	-	10	10	-	30	-	-
Caseinolytic	58	18.97	34.48	-	1.73	3.45	-	3.45	3.45	-	15.51	-	18.97
Gelatinolytic	62	17.74	41.94	-	1.61	3.23	-	3.23	3.23	-	14.52	-	14.52
Lipolytic	21	52.38	4.76	-	-	4.76	-	9.52	9.52	-	9.52	-	9.52
Ureolytic	63	17.46	41.26	-	1.59	1.59	-	3.17	3.17	-	14.28	-	17.46
Chitinolytic	8	100	-	-	-	-	-	-	-	-	-	-	-
					Gills								
Amylolytic	16	37.50	43.75	-	-	-	-	6.25	-	-	12.50	-	-
Caseinolytic	53	18.87	33.96	-	1.89	-	-	7.55	-	-	15.09	1.89	20.75
Gelatinolytic	47	21.28	38.29	-	2.13	-	-	8.51	-	-	17.02	2.12	17.02
Lipolytic	20	50	15	-	-	-	-	10	-	-	10	-	15
Ureolytic	53	18.87	33.96	-	1.89	-	-	7.55	-	-	15.09	1.89	20.75
Chitinolytic	9	88.88	-	-	11.11	-	-	-	-	-	-	-	-

Table 33. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
						Stomach							
Amylolytic	20	60	10	-	-	-	-	-	15	-	15	-	-
Caseinolytic	49	40.81	14.28	-	2.04	-	-	-	8.16	2.04	20.40	4.08	8.16
Gelatinolytic	53	37.73	20.75	-	1.89	-	-	-	7.54	1.89	18.86	3.78	7.54
Lipolytic	28	71.42	3.57	-	-	-	-	-	14.28	-	7.14	-	3.57
Ureolytic	51	39.22	21.56	-	1.96	-	-	-	7.84	1.96	15.68	3.92	7.84
Chitinolytic	20	100	-	-	-	-	-	-	-	-	-	-	-
						Anterior intestine							
Amylolytic	38	84.20	5.26	-	-	-	-	2.63	7.89	-	-	-	-
Caseinolytic	61	62.29	6.56	-	-	-	-	1.64	6.56	-	6.56	-	16.39
Gelatinolytic	59	69.49	6.78	-	-	-	-	1.69	6.78	-	6.78	-	8.47
Lipolytic	51	74.50	-	-	-	-	-	1.96	7.84	-	3.92	-	11.76
Ureolytic	64	64.06	6.25	-	-	-	-	1.56	6.25	-	6.25	-	15.63
Chitinolytic	40	95	5	-	-	-	-	-	-	-	-	-	-

Table 33. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
						Posterior intestine							
Amylolytic	33	87.88	-	-	-	-	-	-	12.12	-	-	-	-
Caseinolytic	77	59.74	7.79	-	-	-	-	2.59	7.79	-	9.09	-	12.98
Gelatinolytic	73	63.01	8.21	-	-	-	-	2.73	8.22	-	9.59	-	6.22
Lipolytic	64	71.87	4.69	-	-	-	-	3.13	9.38	-	-	-	10.94
Ureolytic	77	59.74	7.79	-	-	-	-	2.59	7.79	-	9.09	-	12.99
Chitinolytic	46	100	-	-	-	-	-	-	-	-	-	-	-
						Alimentary canal							
Amylolytic	91	80	4.39	-	-	-	-	1.10	10.99	-	3.50	-	-
Caseinolytic	187	55.61	9.09	-	0.53	-	-	1.60	7.49	0.53	11.23	1.07	12.83
Gelatinolytic	185	57.84	11.35	-	0.54	-	-	1.62	7.57	0.54	11.35	1.08	8.11
Lipolytic	143	72.73	2.80	-	-	-	-	2.10	9.79	-	2.79	-	9.79
Ureolytic	192	55.73	10.94	-	0.52	-	-	1.56	7.29	0.52	9.90	1.04	12.50
Chitinolytic	106	98.11	1.89	-	-	-	-	-	-	-	-	-	-

Table 33. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
						Water							
Amylolytic	20	20	40	-	-	-	-	10	5	-	5	5	15
Caseinolytic	57	8.77	57.89	1.75	-	-	-	3.51	3.51	-	3.51	8.77	12.28
Gelatinolytic	54	11.11	61.11	1.85	-	-	-	-	3.70	-	5.56	9.25	7.40
Lipolytic	44	11.36	65.90	2.27	-	-	-	-	4.55	-	2.27	4.54	9.09
Ureolytic	58	10.34	56.89	1.73	-	-	-	3.45	3.45	-	3.45	8.62	12.07
Chitinolytic	15	33.33	33.33	-	-	-	-	-	6.67	-	-	6.67	20
						Sediment							
Amylolytic	45	2.22	11.11	2.22	-	-	-	4.49	-	-	-	71.11	8.89
Caseinolytic	73	4.11	13.69	1.37	2.73	-	-	5.47	1.36	-	-	56.16	15.06
Gelatinolytic	74	4.05	13.51	1.35	4.05	-	-	2.70	1.35	-	2.70	55.41	14.86
Lipolytic	32	9.37	25	3.13	6.25	-	-	6.25	-	-	6.25	37.50	6.25
Ureolytic	76	3.95	13.15	1.32	3.95	-	-	5.26	1.31	-	2.63	53.94	14.47
Chitinolytic	18	16.67	22.22	5.55	-	-	-	-	-	-	-	33.33	22.22

Table 34. Percentage of isolates showing maximum growth at various NaCl concentration, pH and temperature

Periods	Total no. of isolates	NaCl concentration					pH					Temperature (^{o}C)				
		0	1	3	7	10	2	4	7	9	11	4	10	30	40	50
3.4.1982	71	2.82	12.68	47.89	11.27	25.35	-	-	47.88	32.39	19.71	-	7.04	84.50	8.45	-
6.4.1982	77	1.29	9.09	42.85	46.75	-	-	-	85.71	7.79	6.49	-	5.19	94.81	-	-
7.4.1982	88	-	18.18	36.36	45.45	-	-	-	67.05	19.32	13.63	-	15.91	84.09	-	-
19.4.1982	115	-	23.48	33.91	42.61	-	-	-	63.47	31.30	-	-	-	73.91	26.08	-
3.5.1982	110	-	25.45	17.27	55.45	1.82	-	-	60	40	--	-	0.90	77.27	21.81	-
18.5.1982	97	-	29.89	46.39	23.71	-	-	-	60.82	39.18	-	-	-	74.22	25.78	-
11.6.1982	86	-	40.69	30.23	19.76	9.30	-	-	39.53	60.46	-	-	3.46	73.25	22.09	1.16
Total	644	0.47	23.45	35.40	36.33	4.35	-	-	61.65	33.59	4.81	-	4.19	79.50	16.15	0.15*

*Percentage calculated from the total isolates showing maximum growth

Table 35. Percentage of isolates showing maximum growth at varying NaCl concentrations, pH and temperature

Sample	Total no. of isolates	NaCl concentration					pH					Temperature (°C)				
		0	1	3	7	10	2	4	7	9	11	4	10	30	40	50
Juveniles from natural environment	73	1.37	9.59	4.79	17.80	23.28	-	-	54.79	34.24	10.96	-	5.48	93.15	1.37	-
Juveniles from happa	81	-	9.88	46.91	43.21	-	-	-	81.48	3.70	14.81	-	12.35	83.95	3.70	-
Water from happa	44	4.55	13.64	29.54	50	2.23	-	-	81.82	6.82	11.36	-	6.82	88.63	4.55	-
Adult																
Body surface	64	-	42.19	26.56	31.25	-	-	-	62.50	37.50	-	-	-	96.88	3.13	-
Gills	53	-	41.51	28.30	22.64	7.55	-	-	49.06	50.94	-	-	-	92.45	5.66	1.89
Stomach	53	-	32.08	16.98	47.17	3.78	-	-	62.26	37.74	-	-	-	83.02	16.98	-
Anterior intestine	64	-	6.25	29.69	57.81	6.25	-	-	56.25	43.75	-	-	-	81.25	18.75	-
Posterior intestine	77	-	11.68	40.25	48.05	-	-	-	57.14	42.85	-	-	2.59	49.35	48.05	-
Water	59	-	40.67	35.50	23.72	-	-	-	54.24	40.68	5.08	-	5.08	71.10	23.72	-
Sediment	76	-	35.52	39.47	25	-	-	-	57.89	38.15	3.95	-	6.58	65.78	27.63	-
Total	644	0.47	23.45	35.40	36.33	4.35	-	-	61.65	33.59	4.81	-	4.19	79.50	16.15	0.15*

*Percentage calculated from the total isolates showing maximum growth

Table 36. Growth response of different genera to NaCl concentration, pH and temperature

Genera	Total no. of isolates	NaCl concentrations (%)					pH					Temperature (°C)				
		0	1	3	7	10	2	4	7	9	11	4	10	30	40	50
Vibrio	245	-	1.22	20.82	68.16	9.79	-	-	64.89	33.88	1.22	-	0.81	80.63	17.55	1.10
Pseudomonas	125	-	53.60	32.00	14.40	-	-	-	39.20	58.40	2.40	-	4.80	88.80	6.40	-
Aeromonas	1	-	-	-	100	-	-	-	-	100	-	-	-	-	100	-
Acinetobacter	11	27.27	9.09	45.45	9.09	9.09	-	-	27.27	54.54	18.18	-	9.09	72.72	18.18	-
Flavobacterium	2	-	50	50	-	-	-	-	100	-	-	-	-	100	-	-
Alcaligenes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Moraxella	33	-	33.33	66.67	-	-	-	-	81.81	18.18	-	-	9.09	90.90	-	-
Enterobacteriaceae	25	-	60	36	4	-	-	-	84	16	-	-	-	100	-	-
Staphylococcus	1	-	100	-	-	-	-	-	100	-	-	-	-	100	-	-
Micrococcus	59	-	23.72	54.23	20.34	1.69	-	-	79.66	18.64	1.69	-	1.69	98.10	-	-
Bacillus	59	-	42.37	40.67	15.25	1.69	-	-	62.71	28.81	8.47	-	3.38	67.79	28.81	-
Coryneform group	83	-	13.25	51.80	32.53	2.41	-	-	50.60	34.94	14.45	-	10.84	55.42	33.73	-
Total	644	0.47	23.45	35.40	36.33	4.35	-	-	61.65	33.59	4.81	-	4.19	79.50	16.15	0.15*

*Percentage calculated from the total isolates showing maximum growth

Fig. 7. Culture pond area

p - Location of the pond

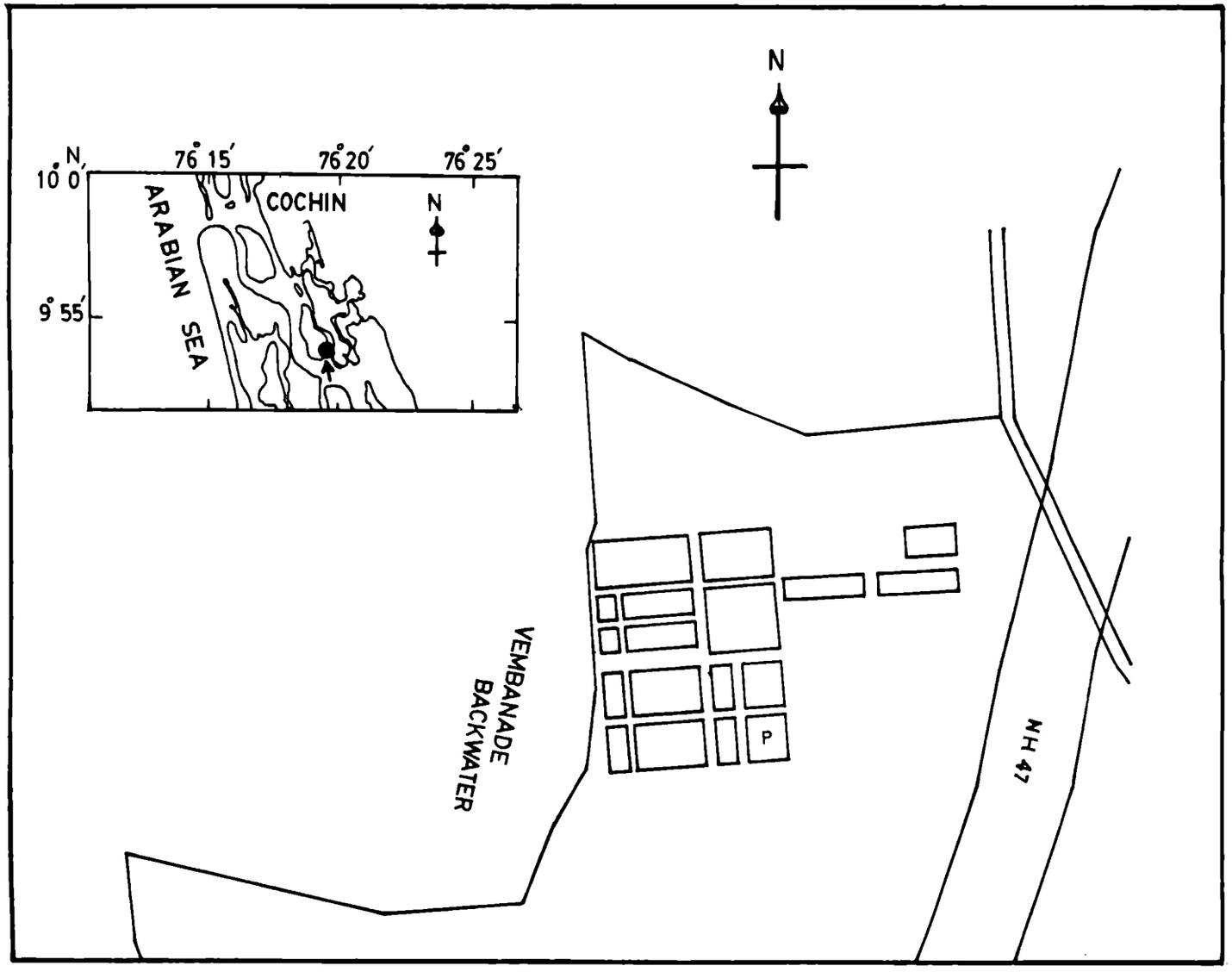

N
10° 0′ N
76° 15′
76° 20′
76° 25′
9° 55′
ARABIAN SEA
COCHIN
N
VEMBANADE BACKWATER
P
NH 47

FIG. 7

Fig. 8. Culture pond showing the sluice gate (a) and feeder canal (b)

a

b

Fig. 9. The Indian white prawn *P. indicus*

A. Showing the external morphology of the animal

B. Showing the digestive system

ST. Stomach (Foregut)

AI. Anterior intestine (Anterior half of the midgut)

PI. Posterior intestine (Posterior half of the midgut and the entire hindgut

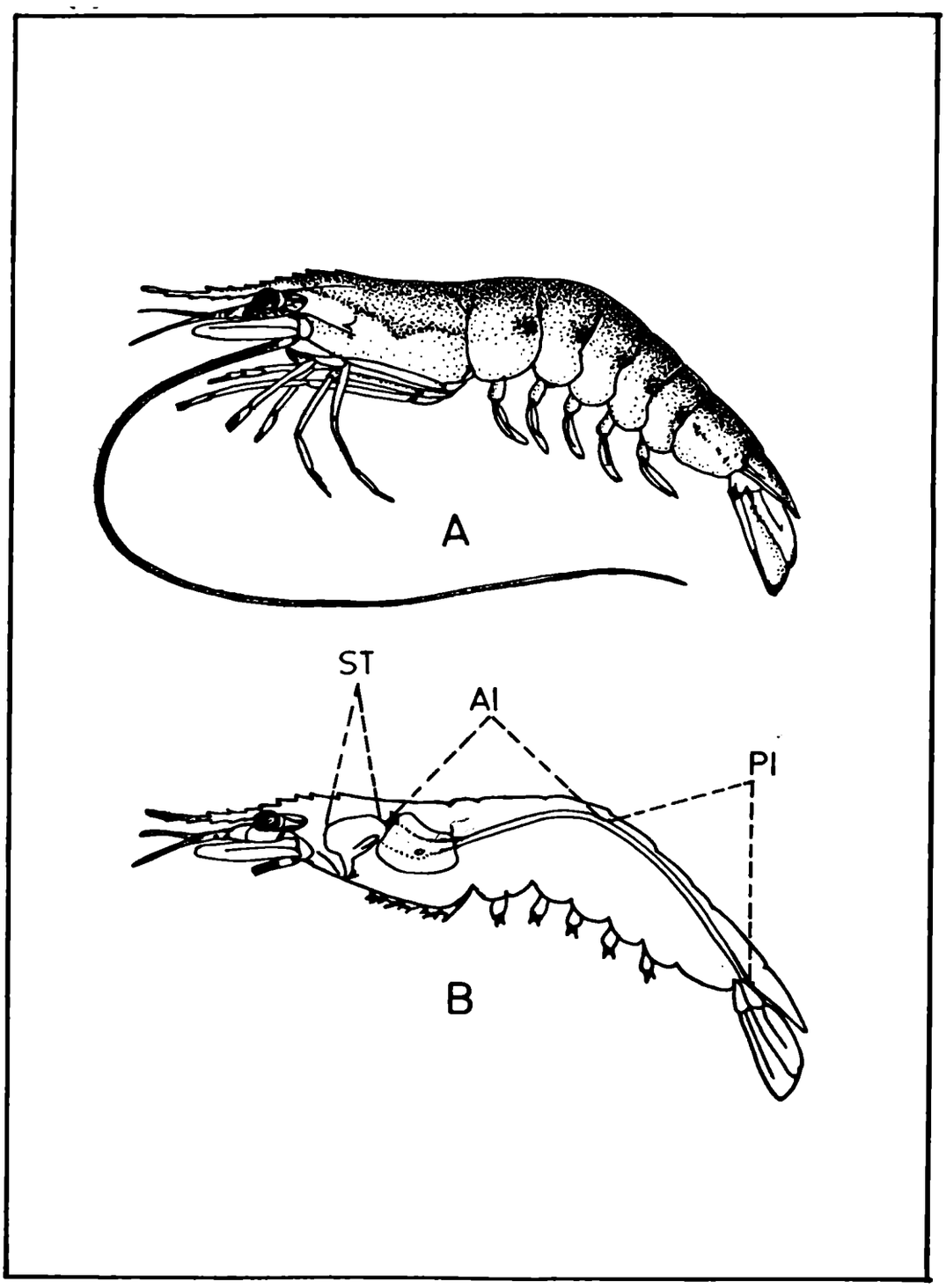


FIG. 9.

Fig. 10a. Temporal changes in the percentage occurrence of hydrolytic bacteria isolated from the juveniles collected from natural environment

S - Amylolytic

C - Caseinolytic

G - Gelatinolytic

L - Lipolytic

U - Ureolytic

Ch - Chitinolytic

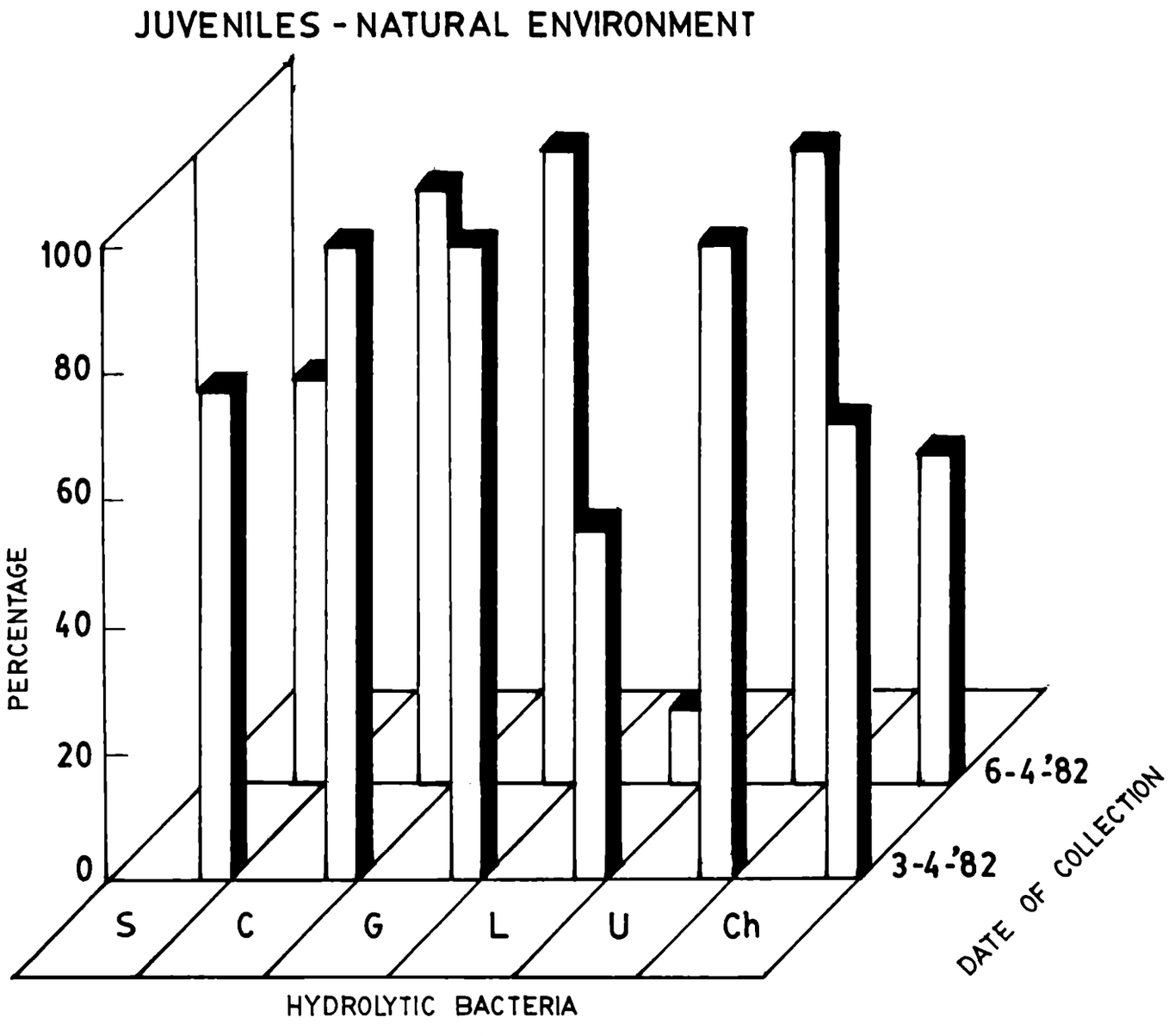

JUVENILES - NATURAL ENVIRONMENT
100
80
60
40
20
0
PERCENTAGE
S
C
G
L
U
Ch
HYDROLYTIC BACTERIA
6-4-'82
3-4-'82
DATE OF COLLECTION

FIG.10 a.

Fig. 10b. Temporal changes in the percentage occurrence of hydrolytic bacteria isolated from the juveniles stocked in happa

S - Amylolytic

C - Caseinolytic

G - Gelatinolytic

L - Lipolytic

U - Ureolytic

Ch - Chitinolytic

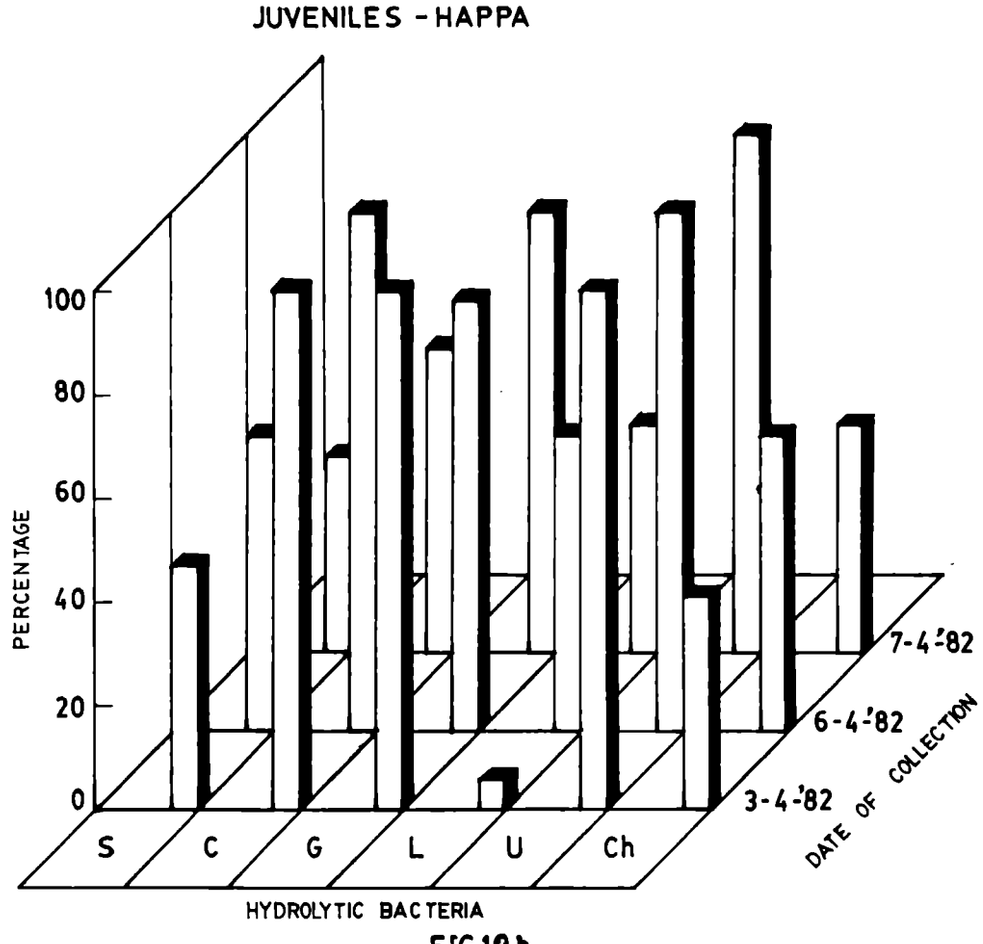

JUVENILES - HAPPA
100
80
60
40
20
0
PERCENTAGE
S
C
G
L
U
Ch
HYDROLYTIC BACTERIA
7-4-'82
6-4-'82
3-4-'82
DATE OF COLLECTION

FIG.10 b.

Fig. 10c. Temporal changes in the percentage occurrence of hydrolytic bacteria isolated from the water collected from happa

S - Amylolytic

C - Caseinolytic

G - Gelatinolytic

L - Lipolytic

U - Ureolytic

Ch - Chitinolytic

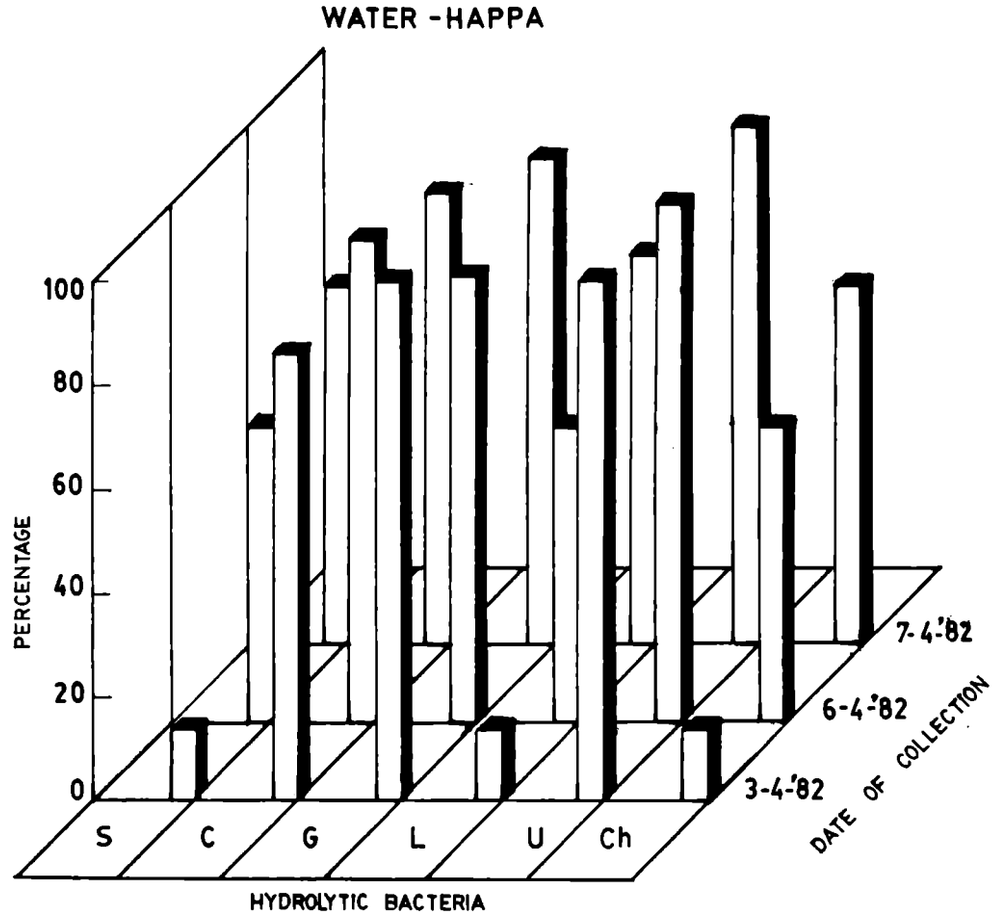

WATER -HAPPA
100
80
60
40
20
0
PERCENTAGE
S
C
G
L
U
Ch
HYDROLYTIC BACTERIA
7-4-'82
6-4-'82
3-4-'82
DATE OF COLLECTION

FIG.10c.

Fig. 10d. Temporal changes in the percentage occurrence of hydrolytic bacteria isolated from the body surface of adults cultured in pond

S - Amylolytic

C - Caseinolytic

G - Gelatinolytic

L - Lipolytic

U - Ureolytic

Ch - Chitinolytic

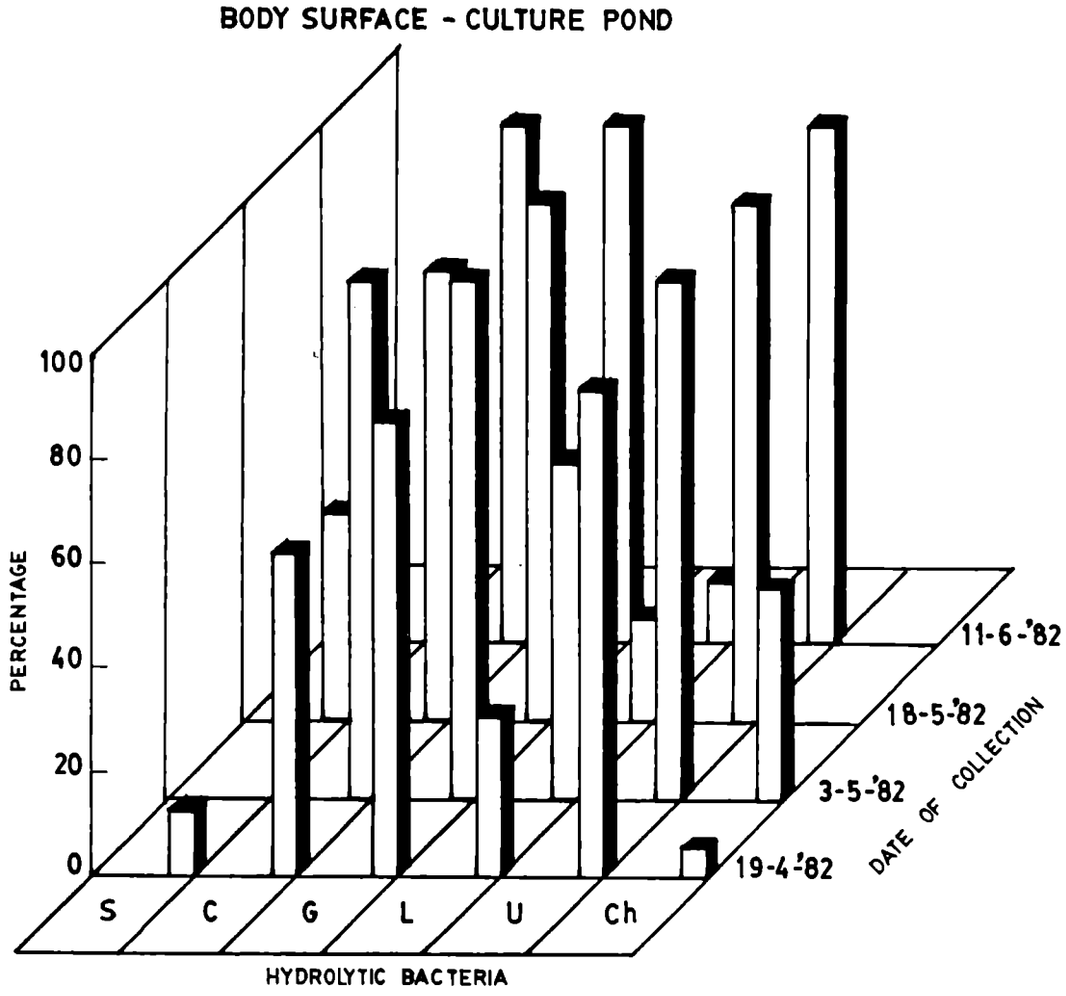

BODY SURFACE - CULTURE POND
PERCENTAGE
100
80
60
40
20
0
S
C
G
L
U
Ch
HYDROLYTIC BACTERIA
19-4-'82
3-5-'82
18-5-'82
11-6-'82
DATE OF COLLECTION

FIG.10 d.

Fig. 10e. Temporal changes in the percentage occurrence of hydrolytic bacteria isolated from the gills of adults cultured in pond

S - Amylolytic

C - Caseinolytic

G - Gelatinolytic

L - Lipolytic

U - Ureolytic

Ch - Chitinolytic

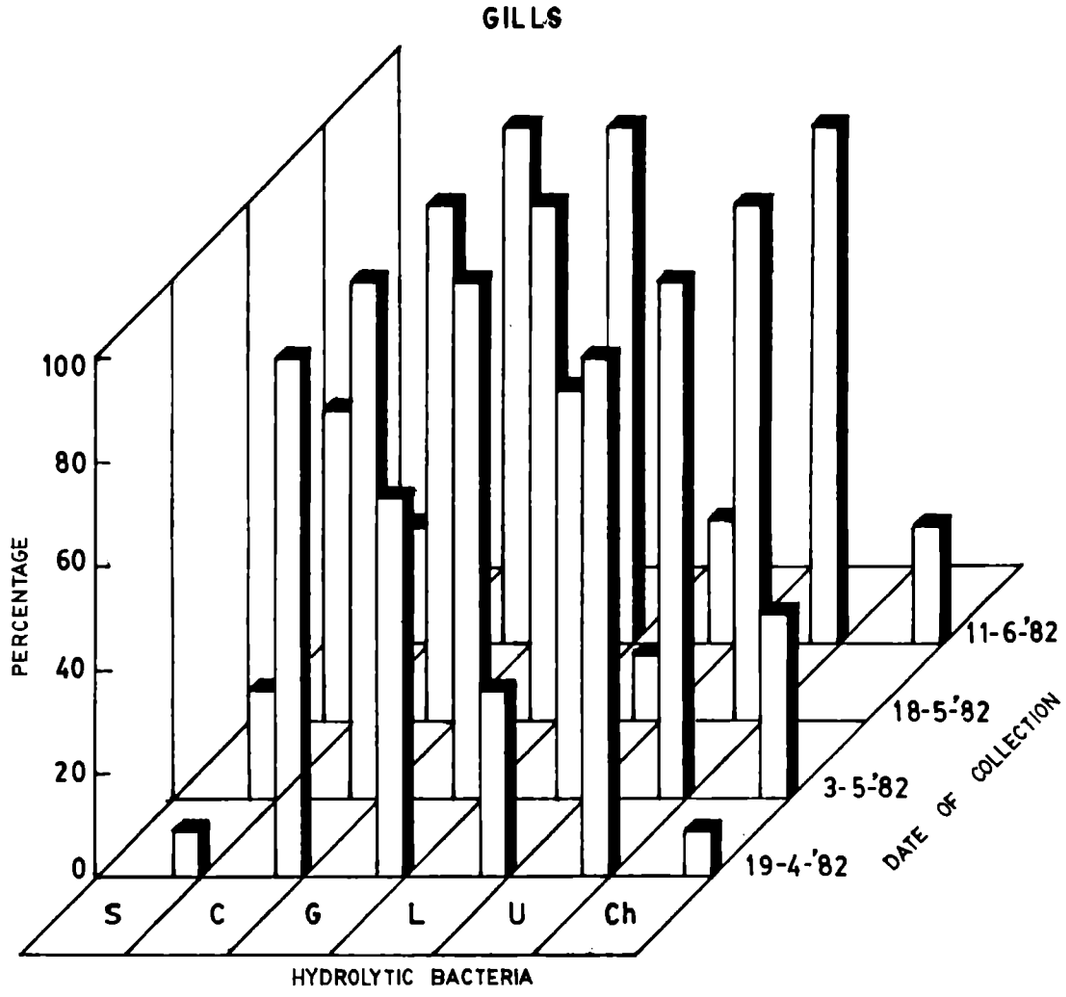

GILLS
100
80
60
40
20
0
PERCENTAGE
S
C
G
L
U
Ch
HYDROLYTIC BACTERIA
19-4-'82
3-5-'82
18-5-'82
11-6-'82
DATE OF COLLECTION

FIG.10e.

Fig. 10f. Temporal changes in the percentage occurrence of hydrolytic bacteria isolated from the stomach contents of adults cultured in pond

S - Amylolytic

C - Caseinolytic

G - Gelatinolytic

L - Lipolytic

U - Ureolytic

Ch - Chitinolytic

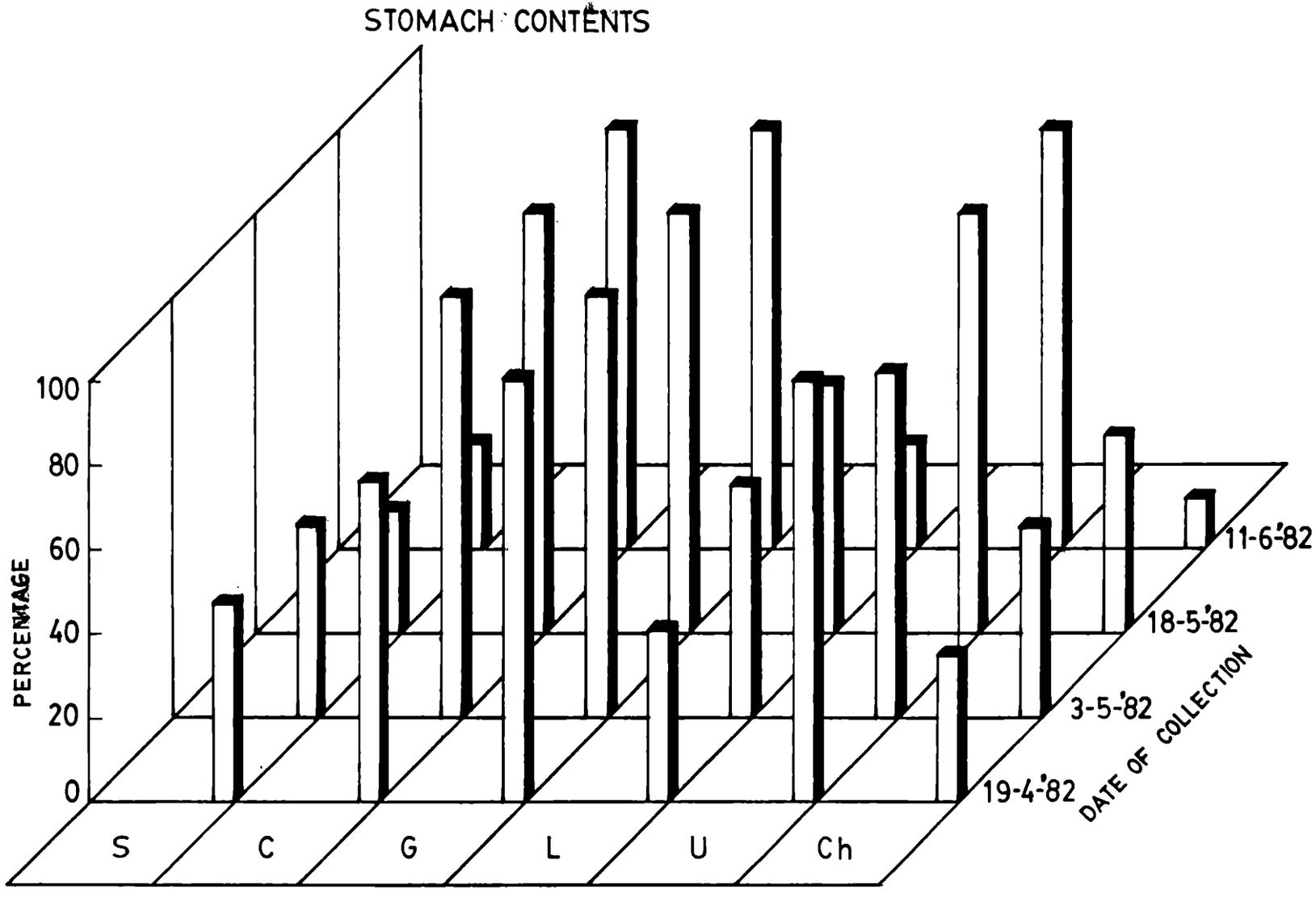

STOMACH CONTENTS
100
80
60
40
20
0
PERCENTAGE
S
C
G
L
U
Ch
HYDROLYTIC BACTERIA
19-4-'82
3-5-'82
18-5-'82
11-6-'82
DATE OF COLLECTION

FIG.10 f.

Fig. 10g. Temporal changes in the percentage occurrence of hydrolytic bacteria isolated from the anterior intestinal contents of adults cultured in pond

S - Amylolytic

C - Caseinolytic

G - Gelatinolytic

L - Lipolytic

U - Ureolytic

Ch - Chitinolytic

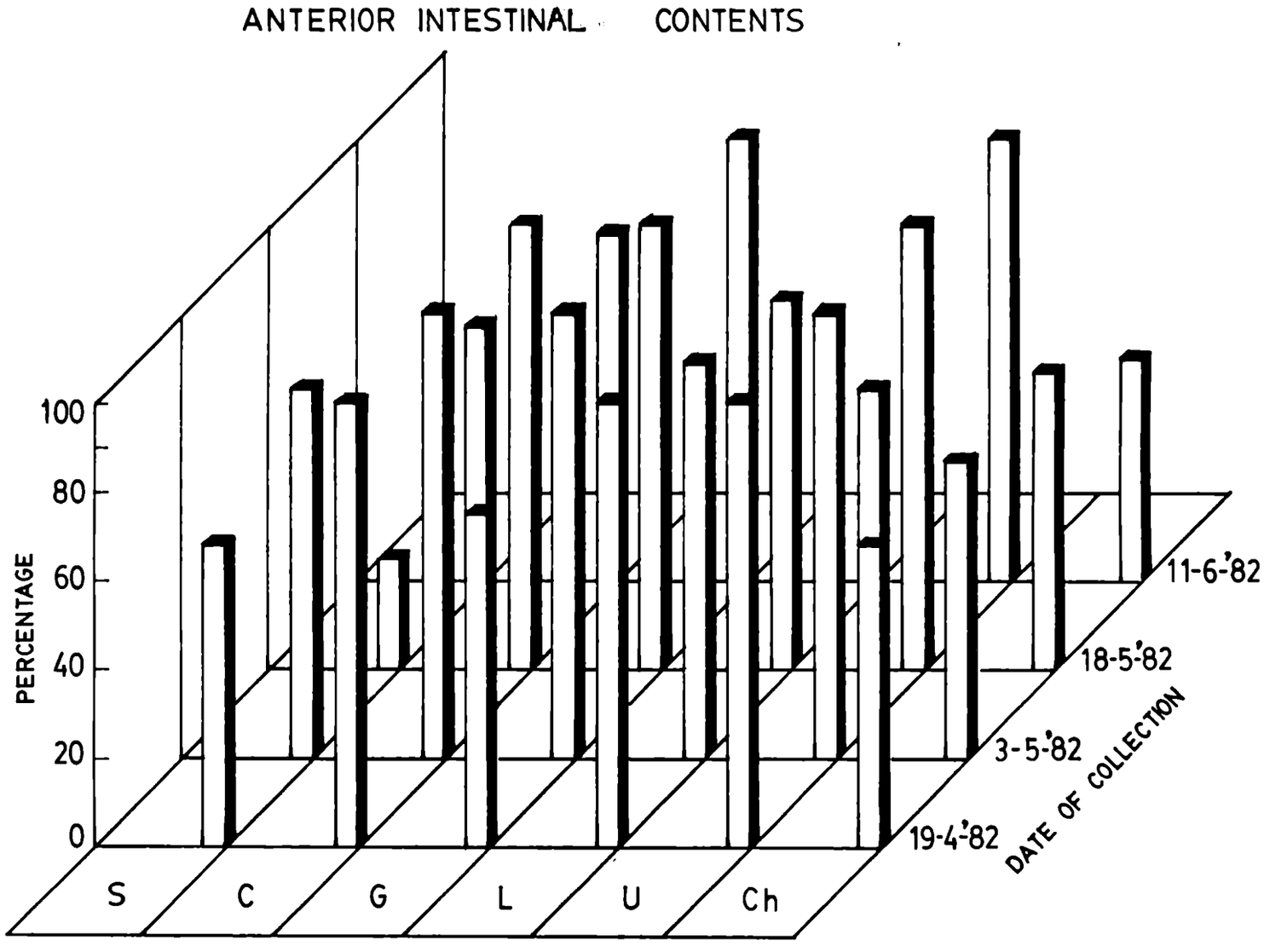

ANTERIOR INTESTINAL CONTENTS
PERCENTAGE
100
80
60
40
20
0
S
C
G
L
U
Ch
HYDROLYTIC BACTERIA
19-4-'82
3-5-'82
18-5-'82
11-6-'82
DATE OF COLLECTION

FIG.10 g.

Fig. 10h. Temporal changes in the percentage occurrence of hydrolytic bacteria isolated from the posteri intestinal contents of adults cultured in pond

S - Amylolytic

C - Caseinolytic

G - Gelatinolytic

L - Lipolytic

U - Ureolytic

Ch - Chitinolytic

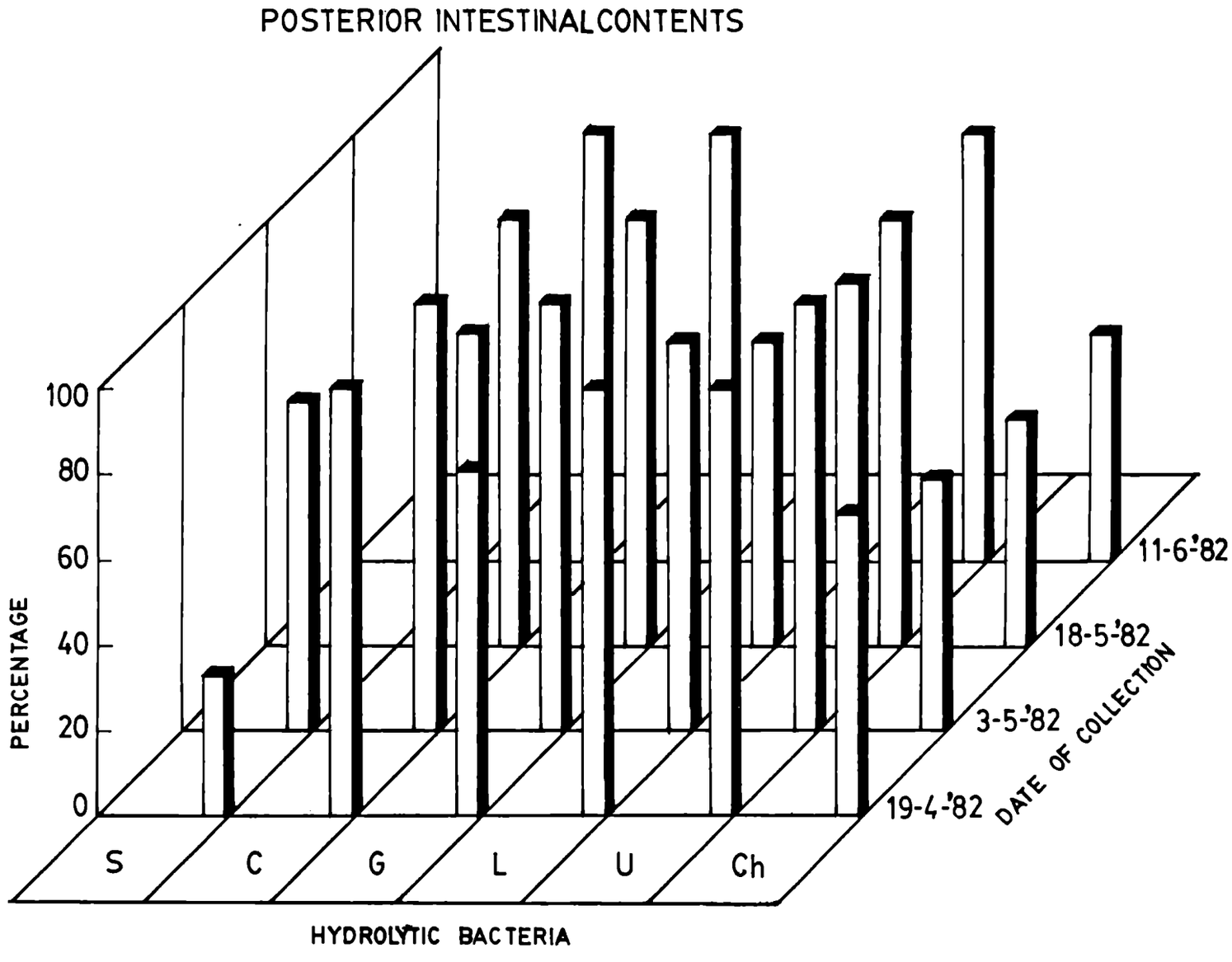

POSTERIOR INTESTINAL CONTENTS
PERCENTAGE
100
80
60
40
20
0
S
C
G
L
U
Ch
HYDROLYTIC BACTERIA
19-4-'82
3-5-'82
18-5-'82
11-6-'82
DATE OF COLLECTION

Fig. 10i. Temporal changes in the percentage occurrence of hydrolytic bacteria isolated from the alimentary canal contents of adults cultured in pond

S - Amylolytic
C - Caseinolytic
G - Gelatinolytic
L - Lipolytic
U - Ureolytic
Ch - Chitinolytic

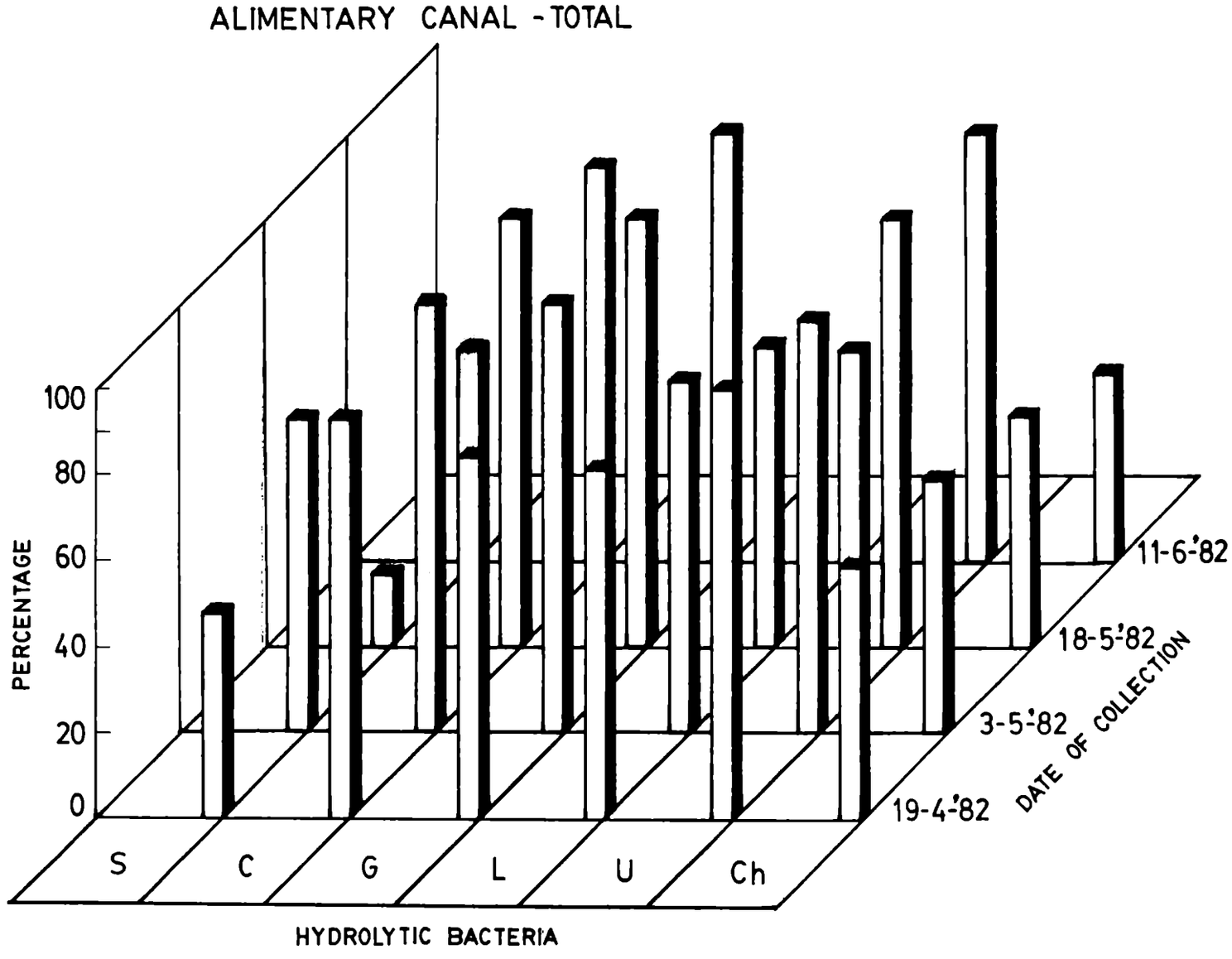

ALIMENTARY CANAL - TOTAL
PERCENTAGE
100
80
60
40
20
0
S
C
G
L
U
Ch
HYDROLYTIC BACTERIA
19-4-'82
3-5-'82
18-5-'82
11-6-'82
DATE OF COLLECTION

FIG.10 i.

Fig. 10j. Temporal changes in the percentage occurrence of hydrolytic bacteria isolated from the water of the pond where the prawns were cultured

S - Amylolytic

C - Caseinolytic

G - Gelatinolytic

L - Lipolytic

U - Ureolytic

Ch - Chitinolytic

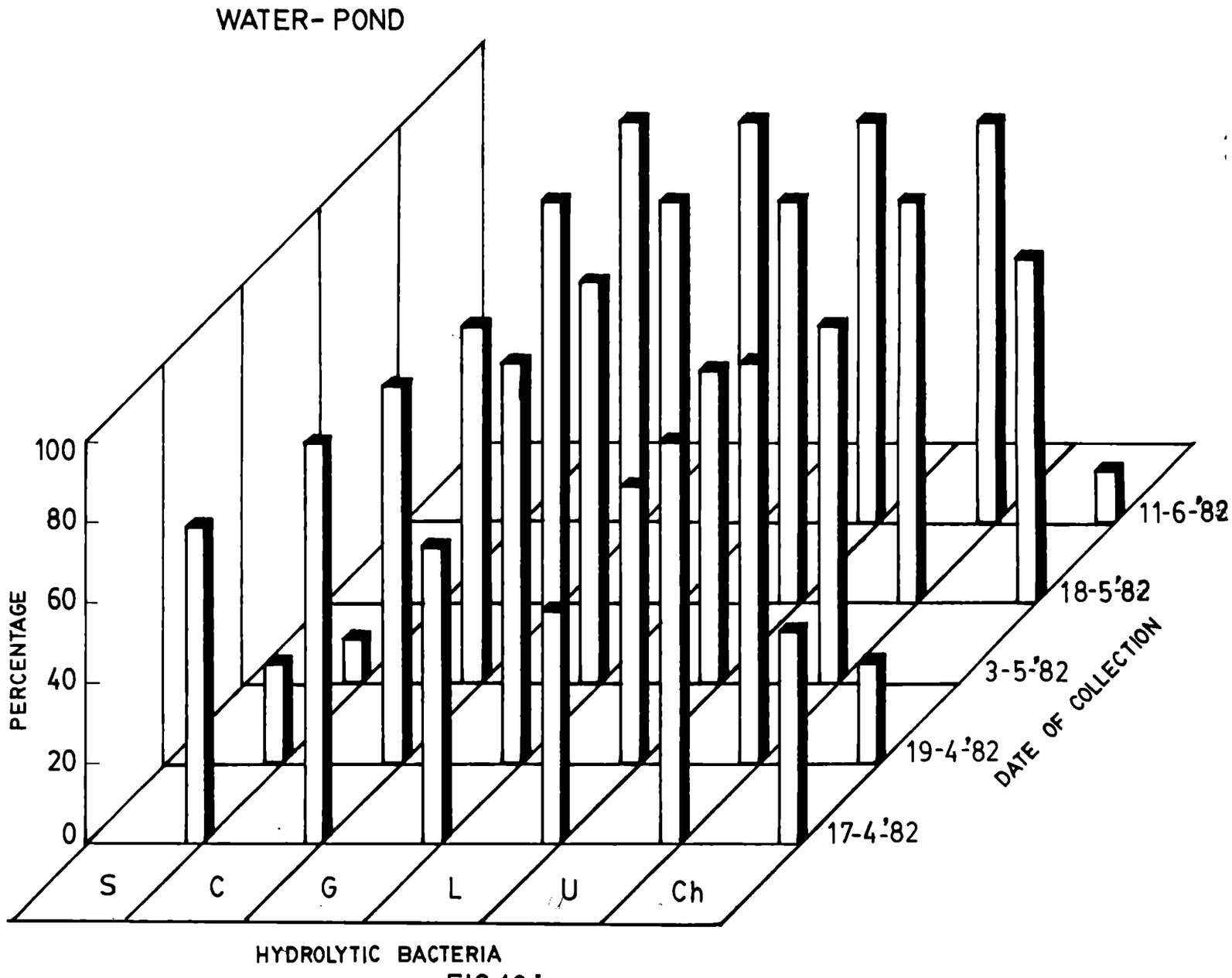

WATER- POND
100
80
60
40
20
0
PERCENTAGE
S
C
G
L
U
Ch
HYDROLYTIC BACTERIA
17-4-'82
19-4-'82
3-5-'82
18-5-'82
11-6-'82
DATE OF COLLECTION

FIG.10j.

Fig. 10k. Temporal changes in the percentage occurrence of hydrolytic bacteria isolated from the sediment of the pond where the prawns were cultured

S - Amylolytic

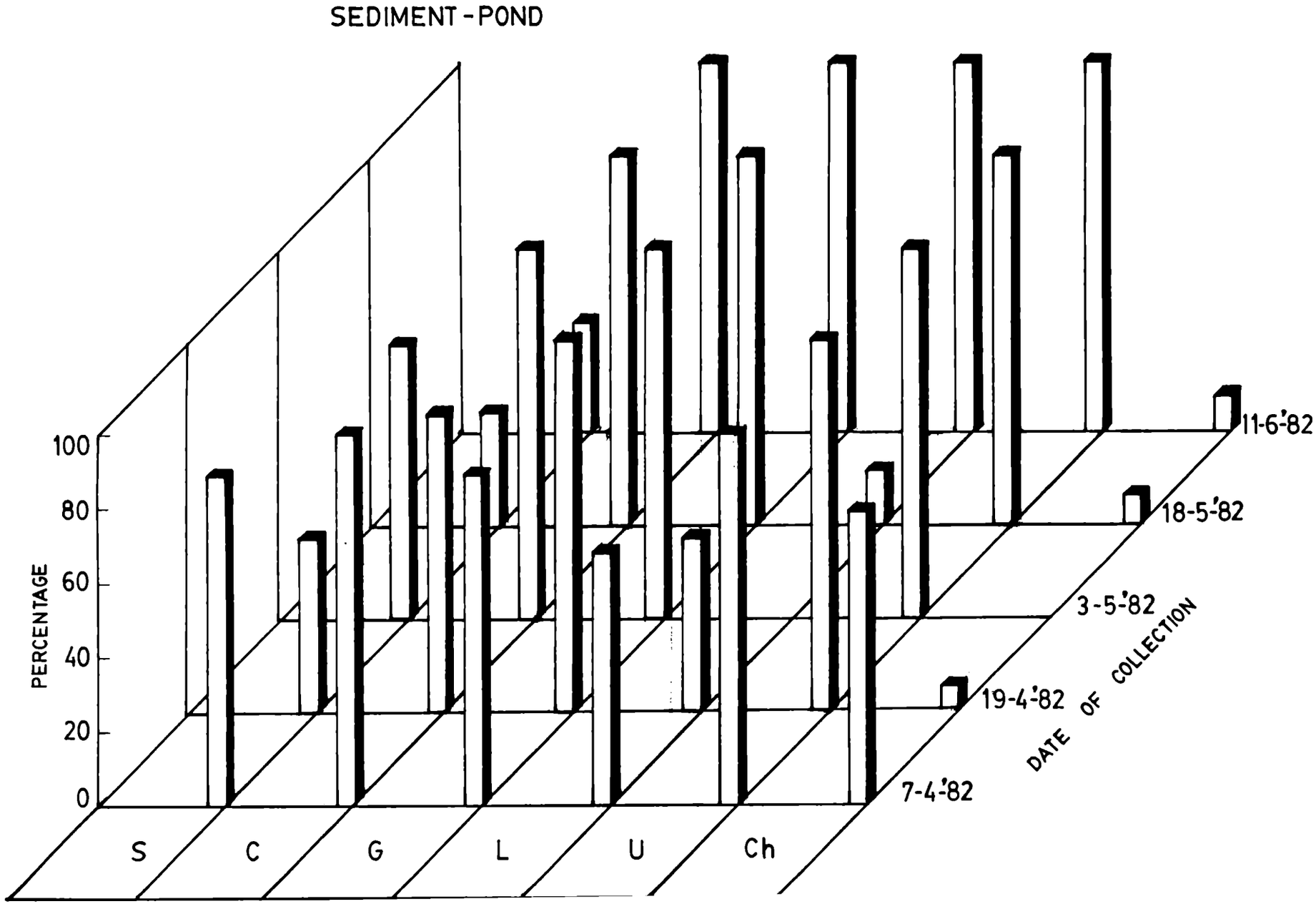
C - Caseinolytic

G - Gelatinolytic

L - Lipolytic

U - Ureolytic

Ch - Chitinolytic


SEDIMENT - POND
PERCENTAGE
100
80
60
40
20
0
S
C
G
L
U
Ch
7-4-'82
19-4-'82
3-5-'82
18-5-'82
11-6-'82
DATE OF COLLECTION

NATURAL ENVIRONMENT

4. BACTERIA ASSOCIATED WITH POST LARVAE, JUVENILES AND ADULTS OF P. INDICUS

4.1. MATERIALS AND METHODS

4.1.1 Study area

Vembanad lake is situated between lat. $9^{o}28'$ and $10^{o}10'N$ and long. $76^{o}13'$ and $76^{o}31'E$ and forms the largest brackish water system of south west coast of India. It has a length of about 90 km extending from Alleppey in the south to Thuruthipuram (east of Azhicode) in the north. The total area is about 256 sq.km (Shetty, 1965). The depth at the Cochin navigation channel varies from 8-12 m and the depth of other parts of the lake is 1-5 m. The width of the lake varies from a few 100 m to about 8 km. There are two permanent openings to the Arabian sea, one at Cochin, a 450 m wide channel which forms the main entrance to the Cochin harbour and the other at Azhicode. Being subjected to regular tidal influences, the entire lake system has all the characteristics of a tropical estuary (Qasim et al. 1969). Tides of this area are semidiurnal type with substantial differences in range and time. The main source of freshwater for the lake is two large rivers, Periyar in the north and Pamba, in the south. Four other small rivers, viz.

Achancoil, Manimala, Meenachil and Moovattupuzha also empty into the lake. During the southwest monsoon, the lake receives an average rainfall of 3300 mm and is virtually converted into a freshwater lake (Pillai, 1978). The freshwater discharge from the rivers make the lake a tropical estuary as per Pritchard's classification (Pritchard, 1967). The run off plus precipitation exceed evaporation and it is a positive type estuary (Balakrishnan, 1957).

The heavy river discharge and an abundant reciprocal inflow of upwelled nutrient rich seawater through the deep and wide barmouth at Cochin make the backwater very rich in nutrients. The primary production is also very high almost comparable to the inshore waters of the seas around India (Nair et al. 1970). The magnitude of primary production of Vembanad lake (annual average rate of 272-293 gc/m^2) could sustain a very rich biota of organisms feeding at different trophic levels (Jhingran, 1982). Average per hectare fish catch from Vembanad lake works out to be more than 50 kg as compared to 35 kg from Chilka and 30 kg from Pulicat lake. Prawns constitute the major item in the catches contributing to about 80% of the total (Raman, 1980). The prawn catch is formed of Metapenaeus dobsoni, M. monoceros and P. indicus. P. indicus was more during Mar. - Apr. when the salinity was found high (Menon, 195). Small quantities of P. monodon,

comprising mostly small sizes and M. affinis are also found among the catch. Sizeable quantities of Macrobrachium rosenberg are caught from the backwaters during the monsoon months.

Planktonic post larvae of prawns enter the estuary and settle to the benthic mode of life of the juveniles. George (1962) observed that the post larvae of P. indicus enter Cochin backwater in all the months except from Jun. to Sep. Kuttyamma (1975) observed that the post larvae of P. indicus were predominant in the collection during Apr. - May and Oct. - Nov.

The estuaries present congenial conditions for the feeding and growth of the juveniles of prawns. Their favourite food such as phytoplankton, small animals and detritus are abundantly available in this ecosystem in addition to protection from predators. All these factors besides their favourable physico-chemical conditions might be attracting them to the estuaries which thus form their nursery area (Raman, 1980). The absence of smaller sizes of these prawns in the inshore and offshore catches is a strong evidence to suggest that the estuarine phase is a necessity in their life cycle (Mohamed and Rao, 1971).

For the present study, P. indicus was collected from two stations of Cochin backwater, the locations of the sampling

stations 1 and 2 are given in Fig. 1 (long. 76°17'E and lat. 9°59'N). From the station 1 post larvae and juveniles were obtained from Nov. to May/Jun. and from the station 2 the adults were collected from Dec. to May. Station 1 situating in the nearshore area of Ramanthuruthu island was found to be an ideal one for the collection of post larvae and juveniles. The area was much shallow and free from fishing activities and other disturbances. These might be the reasons for the aggregation of post larvae and juveniles in this area. Station 2 a fairly deeper area (3-5 m)was found to be an area where operation of country boats for prawns used to be carried out throughout the season. This location also was not much disturbed by navigation compared to the other areas.

4.1.2 Sample

Penaeus indicus H Milne - Edwards, 1837

Biology and identifying characters of post larvae, juveniles and adults are presented in section 2.1.7.5 and 3.1.5.1.

4.1.3 Food and feeding

These prawns feed on whatever suitable food material they come across. Laboratory observations suggested that in nature the species is partly predatory in habit and chase

smaller creatures which can be seized between the appendages. Larger creatures are devoured only in dead conditions. They usually prefer small particles of food, which are grasped by the setae of the peraeopods and passed onto the mouth.

Food of young penaeids consists of detritus matter formed on the surface of mud from extremely small organisms and algal materials. They include Coscinodiscus, Pleurosigma, Rhizosolenia, Trichodesmium and cuttings of seaweeds. The crustaceans include copepods, ostracods, amphipods, tiny decapods and their larval stages.

4.1.4 Growth

When the animals are in backwaters and estuaries the rate of growth is relatively high. Normally the animals which are caught from the estuaries do not exceed 120 mm. Under the prevailing conditions in their brackish water habitat most of the prawns move out into the sea or caught before they are about 100 mm in length, although the adults may reach double that size. The size frequency distribution at 126-130 mm for male and 141-145 mm for female represent the first year class, those at 161-165 mm for male and 171-175 mm for female represent the second year class and those above 195 mm represent the third year.

4.1.5 Movements

The life cycle of the species is completed after passing through two distinct environments - the sea and the estuary. Larval development takes place in the sea and the migration into the estuaries, lakes and backwaters commences when they are in late mysis or early post larval stages, before they are 10 mm long. This process of migration is continuous throughout the breeding period. The seaward migration begins after they attain 120 mm size. Further growth, attainment of sexual maturity and other life processes take place in the sea.

4.1.6 Reproduction

The two sexes are distinguishable by the petasma in males and thelycum in females. In males, the endopodites of the second pair of abdominal appendages bear out-growths known as appendix masculina. The pired genital apertures are in the exopodite of the fifth pair of walking legs (peraeopods) in the males and in the coxae of the third pair of walking legs in the females. The petasma helps in transfering the spermatophores to the thelycum of females. Fertilization is external, the eggs being discharged into the surrounding water, when spermatozoa liberated from the seminal reciptacles of the thelycim meet them.

4.1.7 Life cycle

Eggs hatchout into nauplii which metamorphosed into post larvae through zoeae (protozoea) and mysis.

4.1.8 Fishing season

Fishing of this species is carried out through out the year in the parts of backwater where the tidal influence is prominent. In other parts Jun./Jul. to Oct./Nov. appear to be the off seasons.

4.1.9 Depth range in which fishing is carried out

The estuarine and backwater fishing for the juveniles and adults of P. indicus is carried out in very shallow waters not exceeding 10 m in depth. Commercial fishing for adults is generally carried out in the coastal waters upto a depth of 50 m along the Indian coasts.

4.1.10 Fishing equipment

In the backwaters P. indicus is caught in large quantities in stake nets, cast nets, drag nets, dip nets and small scoop nets. In the inshore marine fishing the principal types of gear employed in the capture of prawn are boat seine and the shore seines. Along the Kerala coast and on the southern end of the west coast of India,

cast nets of various dimensions form an important gear for the capture of prawns. From the deeper regions prawns are caught in trawls and stake nets only.

Small dug out canoes (4-6 m long) are the principal craft in use in the backwater. Larger dug-out canoes (6-10 m long), and catamarans are used in the inshore fishery, in the west coast of India. On the east coast, plank built canoes and catamarans are in use. The shrimp trawls are operated from 7 to 11 m. Pablo type wooden hull boats are also in operation powered by 10 to 30 hp diesel engines. A few larger steel built boats are also operated as shrimp trawls.

4.1.11 Commercial significance

P. indicus locally called as 'Naran Chemmeen' and as 'White Prawn' in trade circles enjoy an overriding commercial significance. By virtue of their high protein content they form a good source of animal protein and become one of the major commodities for export. They are exported as frozen, canned and dried prawns. Of these frozen prawns are reported to dominate the trade (MPEDA, 1985). Both Japan and USA are reported to be the main markets for the frozen shrimps. The rest is shared by as many as 24 countries including France, West Germany, UK, Netherlands, Belgium, Australia etc.

4.1.12 Collection of samples

Monthly collections were made for water and sediment samples from Nov. 1981 to Oct. 1982 from station 1 and 2. Water samples were collected from station 1 at 0.5 m depth using sterile glass bottle. At station 2, a Casella type water sampler was used for collecting water at a depth of about 3 m and was transferred to sterile glass bottles. Sediment was collected using a hand correr at station 1 and at station 2 using Peterson grab. About 100 g of the sediment was transferred to sterile polyethylene bags.

Post larvae (upto 12 mm in total length) and juveniles (12-14 mm in total length) were collected using a drag net from station 1. From station 2 adults (40-120 mm in total length) were collected using a cast net . They were transporte to the laboratory, in sterile polyethylene bags kept in ice box

4.1.13 Estimation of Physico-chemical parameters

Estimation of temperature, pH, salinity, dissolved oxyge and nutrients of water were carried out as detailed in 2.1.10.

4.1.14 Bacteriological analysis

Processing of samples, plating procedure, enumeration, isolation, identification, test for hydrolytic activity and growth studies of the isolates were performed as given in 2.1.11 to 2.1.13 and 3.1.13.

4.1.15 Statistical analysis

Trend lines of monthwise variation of THB, and Gram-negative bacteria were found out using the model $Y = a+bx$ where 'x' stands for month and 'y' the variables. The constants 'a' and 'b' were estimated using least square method (Snedecor and Cochran, 1967).

Simple correlation between environmental parameters and bacterial population and between the genera encountered in the environment and the animal were computed as described in 2.1.14.2.

4.2 RESULTS

4.2.1 Physico-chemical parameters

Physico-chemical parameters of water collected from two stations in Cochin backwater during one year period (Nov. 1981 to Oct. 1982) are given in Fig. 11 and App. Table 4.

Temperature did not show much fluctuations during the period of study. In both the stations minimum temperature (25^{o} and $24^{o}C$ for station 1 and 2 respectively) was recorded in Jul. Station 1 recorded a maximum temperature ($30.5^{o}C$) in Dec., Jan. and Feb. Whereas in station 2 maximum temperature ($30^{o}C$) was recorded during the months of Nov., Mar. and Apr.

pH, similar to temperature, showed fluctuations at a lesser magnitude (7 to 8.43 in station 1 and 7 to 8.3 in station 2). In both the stations lowest pH was recorded in Oct. The highest pH was observed during Nov. in station 1 and during Aug. in station 2.

Salinity of water varied between 0.39 to 28.91 x 10^{-3} during the period of study. During Jan. to May the salinity was moderately high (ranging from 21.10 to 28.91 x 10^{-3}) in the water, recording a maximum in Feb. With the onset of mon

a sudden drop in salinity was observed which built upto 16.12×10^{-3} in station 1 and 18.47×10^{-3} in station 2.

During the period of study dissolved oxygen varied widely (2.46 to 7.8 ml^{-1}). In both the stations (1 and 2) the peaks in oxygen level were observed during Jun. (7.8 and 7.4 $ml.l^{-1}$ respectively). In station 1 the lowest oxygen level (4.29 $ml.l^{-1}$) was recorded during Feb. and in station 2 (2.46 $ml.l^{-1}$) during Dec. In general the dissolved oxygen content in station 2 was lesser than that of station 1.

In both the stations inorganic nitrogen showed considerable fluctuations in their level. At station 1 after recording a maximum (21.0$\mu g.l^{-1}$) in Jan. the concentration of inorganic nitrogen declined gradually till Aug. and later shot up to significant level. At station 2, on the other hand, maximum level of inorganic nitrogen was observed during Dec. (21.0$\mu g.l^{-1}$) and in Feb. (40.0$\mu g.l^{-1}$).

Organic nitrogen in water samples of station 1 and 2 was high during Jan. (189.0 and 93.0$\mu g.l^{-1}$) respectively and low during Mar. (1.2 and 1.3$\mu g.l^{-1}$ respectively). During the rest of the months it varied slightly. The concentration of inorganic phosphorus was high in Feb. (90.0$\mu g.l^{-1}$) at station 1. During rest of the months the level remained without much variation (1.4 to 11.3$\mu g.l^{-1}$).

In station 2, the highest and the lowest concentrations of the inorganic phosphorus were recorded during Dec. and Jun. respectively. Both in stations 1 and 2 organic phosphorus showed the highest peaks in Apr. and Aug. respectively. However, the primary and secondary peaks in station 1 were observed in Apr. and Aug. Whereas in station 2 they were in Aug. and Apr. In general its concentration varied between 1.8 and 16.6µg.l^{-1} in station 1, and 1.0 and 20.9µg.l^{-1} in station 2.

4.2.2 Quantitative distribution of heterotrophic bacteria

The quantitative distribution of heterotrophic bacteria in prawn, water and sediment is presented in Fig. 12 and App. Table 5.

Station 1

THB of post larvae varied between 11.03 x 10^8 and 2.6 x $10^5.g^{-1}$ showing a steady decline from Nov. to May except for an increase in Apr. In juveniles THB showed a declining trend from Dec. to Jun. recording a maximum (38.40 x $10^7.g^{-1}$) in Dec. and a minimum (3.9 x $10^5.g^{-1}$) during May. The number showed a slight increase in Jun. THB of water fluctuated widely during the study period and recorded the lowest (0.44 x $10^5.ml^{-1}$) in Jul. A decline in population was seen from Nov. (23.0 x $10^5.g^{-1}$) to Mar. (1.5 x $10^5.g^{-1}$) in sediment.

During the later period an increasing trend in THB was observed, except in Jul. The highest population (150 x $10^5.g^{-1}$) was recorded in Oct.

Station 2

THB associated with the body surface of adult prawns ranged between 3.5 and 207 x $10^5.cm^2$. The THB present in gills, also showed two peaks, one in Dec. (17.90 x $10^7.g^{-1}$) and the other in Jan. (70.8 x $10^6.g^{-1}$). The range of variation between the highest and the lowest population recorded was of one magnitude. Stomach contents harboured bacteria which varied between 34 x $10^5.g^{-1}$ and 31.60 x $10^7.g^{-1}$ during Feb. and Jan. Two peaks, one during Jan. and the other in May (29.60 x $10^5.g^{-1}$) were prominent. The population showe fluctuations during various months. THB of the anterior intestinal content ranged between 63 x $10^5.g^{-1}$ (Jan.) and 12.6 x $10^7.g^{-1}$ (Dec.). In general the population fluctuated during the study period. Posterior intestinal contents, on the other hand, showed an increasing trend in population from Dec. (10.0 x $10^7.g^{-1}$) to May (26.80 x $10^8.g^{-1}$). Lowest population was recorded in Apr. (540 x $10^5.g^{-1}$).

The bacterial population in the alimentary canal, in general, exhibited an increasing trend from Dec. (9.0 x 10^7. to May (10.34 x $10^8.g^{-1}$) with a slight decrease in Feb. (3.86 x $10^7.g^{-1}$) and Apr.(6.30 x $10^7.g^{-1}$).

THB of water in station 2 varied between 1.22 x 10^5.ml^{-1} (Sep.) and 125 x 10^5.ml^{-1} (Nov.). Three peaks could be seen, one primary and two secondary. The former was in Nov. (125 x 10^5.ml^{-1}) and the latter during Mar. (9.8 x 10^5.ml^{-1}). THB of sediment in station 2 varied between 0.26 x 10^5.g^{-1} (Feb.) and 27.0 x 10^7.g^{-1} (Oct.). Two peaks were prominent, one in Oct. and the other in May.

THB in different regions of alimentary canal is presented in Table 37. In general, THB increased from stomach to posterior intestine. When stomach harboured 18.39% of the total population of the alimentary canal, the anterior and posterior intestine contained 21.75 and 59.86% respectively. Similar trend of increase was seen in various months. Generally the bacterial population was very high in the intestine than in stomach.

The trend of monthly variation of THB in all the above mentioned samples are expressed statistically as $Y = a+bx$ where 'Y' stands for THB and 'x' for months. Table 38 and Fig. 12 show the trend lines and correlation coefficients with their significance between months and THB of various samples. A significant linear trend of declining in THB was observed in post larvae and juveniles. In all the other samples although a low linear increase or decrease in trend line was observed, it was not found significant ($\underline{P}<0.05$ level).

4.2.3 Qualitative analysis of THB

4.2.3.1 Gram-negative bacteria:

The percentage of Gram-negative bacteria present in various samples collected from station 1 and 2 are presented in Fig. 13a,b and App. Table 6.

Among the total (1669) isolates, 56.14% were Gram-negative and the rest were Gram-positive. Gram variables were not encountered. Maximum percentage of Gram-negative bacteria was found on body surface (68.43%) and the least (47.69%) was recorded in post larvae.

Station 1

In post larvae an increase of Gram-negative rods (6.38 to 97.5%) from Nov. to May could be seen. But, in juveniles eventhough an increasing trend could be seen from Nov. (29.41%) to May (90%), during Jun. it declined to 28.28%.

Station 2

The body surface of the adult prawn showed an increase in percentage of Gram-negative forms from Dec. (6.45%) to May (100%) with a slight decline in Mar. (66.66%). In gills also they increased from 13.89% (Dec.) to 100% (May) with a decline in Mar. (46.67%).

In stomach content the lowest percentage was recorded in Mar. (10%) and the highest (94.73%) in Apr. The anterior intestinal contents on the other hand, showed an increase in percentage from Dec. (0%) to May (100%). However a maximum (100%) in Feb. and a sudden drop in Mar. was also noticed. Posterior intestine registered an increase of Gram-negative forms from Dec. (33.34%) to May (100%), with a decline in Feb. (14.29%). Alimentary canal in general showed an increase from 18.25% (Dec.) to 77.78% (May). But the highest percentage was recorded in Apr. (91.16%)

Water samples showed an increasing trend from Nov. (33.33%) to Oct. (80%). But the highest percentage was recorded in Apr. (87.50%) and the lowest in Dec. (26.68%). Sediment samples on the other hand showed fluctuations in the percentage with three peaks, Nov. (90%), Apr. (100%) and Jul. (92.31%) and a minimum in Sep. (9.09%).

The trend of monthly variations of the percentage of Gram-negative bacteria in all the samples are expressed statistically as $Y = a+bx$ where 'Y' stands for the percentage of Gram-negative forms and 'x' for months. Fig. 13a,b and Table 39 present the trend lines and correlation coefficients with their significance, between months and the percentage of Gram-negative bacteria. A significant increase of Gram-negative bacteria was observed in post larvae, water of station 1,

body surface, gill and the contents of alimentary canal of prawn, and water of station 2. In all the other cases, an insignificant low level of linear increasing or decreasing trend was observed.

4.2.3.2 Percentage generic composition of bacterial strains:

The bacterial strains were identified to various genera and the results are presented in Fig. 14a,b and App. Table 7. Altogether twelve genera were encountered, four were Gram-positive and the rests were Gram-negative. Gram-negative bacteria of the genera Vibrio and Pseudomonas, Gram-positive bacteria of the genera Micrococcus, Bacillus and Coryneform group were encountered as the prominent microflora in animal as well as in water and sediment. The percentage of all the other genera occurred were negligible. Micrococcus (22.59%) and Vibrio (21.81%) showed the maximum percentage of occurrence among Gram-positive and Gram-negative bacteria. The order of dominance of other genera was Bacillus (17.80%), Pseudomonas (15.99%), Coryneform group (7.97% Acinetobacter (3.59%), Moraxella (3.54%), Flavobacterium (3.24%) Aeromonas (1.50%), Enterobacteriaceae (1.38%), Staphylococcus (0.36%) and Alcaligenes (0.18%). In general, this order of dominance was reflected in individual samples also.

Station 1

The percentage of Vibrio was high (31.54%) in post larvae. The Gram-positive bacteria Micrococcus covered 26.83% followed by Bacillus (17.89%) and Coryneform group (11.79%). Other genera which were encountered were in lesser percentages ranging from 0.41% to 8.94%. In juveniles Vibrio (26.09%) dominated the flora followed by Micrococcus (21.07%), Bacillus (16.72%), Pseudomonas (16.39%), and Coryneform group (9.70%).

The predominant genera in water were Pseudomonas (25%), Bacillus (23.08%), Micrococcus (16.67%) and Vibrio (16.03%). Percentage of other genera ranged between 0.64 and 9.62%. In sediment, Bacillus was found to be dominant (28.48%) followed by Pseudomonas (27.15%) and Vibrio (14.57%). The percentage of other genera varied from 1.99 to 8.61%. Thus, it could be observed that while Pseudomonas and Bacillus in water and Bacillus and Pseudomonas in sediment were the prominent flora in the above order, Vibrio was the dominant flora in post larvae.

Station 2

Micrococcus recorded high percentage (38.0%) followed by Pseudomonas (21.0%) in body surface. Moraxella and Vibrio were found to be the other dominant genera. The percentage of all other genera were much less. In gill, the prominent genera

were Micrococcus (42.62%) and Vibrio (20.49%). Pseudomonas was only 9.02%. The percentagesof other genera were negligible.

Stomach content contained Micrococcus (29.87%) and Vibrio (28.57%) as prominent flora. Pseudomonas, Bacillus, Acinetobacter and Coryneform group ranged from 3.89 to 11.69% only. Anterior intestine harboured Vibrio (34.31%) and Micrococcus (31.37%). In posterior intestine Vibrio was dominant (34.88%). Pseudomonas, Aeromonas, and Flavobacterium and Bacillus showed an increase in the percentage in posterior intestine than in anterior intestine. It could be seen that there was a clear increase in percentage of Vibrio from stomach (28.57%) to posterior intestine (34.88%). Similarly Pseudomonas also showed an increase in percentage from 3.89 in stomach to 11.63% in posterior intestine. Acinetobacter, Moraxella, Enterobacteriaceae and Coryneform group showed a trend of decline. However, Micrococcus recorded a slight increase at the anterior intestine. The various genera of heterotrophic bacteria encountered in the alimentary canal could be divided into two groups on the basis of their level of occurrence from stomach to posterior intestine. The genera which showed increasing trend from stomach to posterior intestine were Vibrio, Pseudomonas, Aeromonas and Bacillus and the genera which recorded a declining trend were Acinetobacter, Moraxella, Enterobacteriaceae, Micrococcus and

Coryneform group. Alimentary canal as a whole showed the dominance of only two genera, Vibrio (32.83%) and Micrococcus (28.30%). The level of other genera were falling between 0.38 and 9.06%.

The prominent genera in water were Vibrio and Pseudomonas (19.28% each) and, Micrococcus and Bacillus (18.67% each). Percentage of Coryneform group was 9.64. The other genera ranged from 0.6 to 4.82%. In sediment Bacillus was dominant (36.59%) followed by Vibrio (18.9%) and Pseudomonas (17.07%). It could be seen that when the percentage of Vibrio and Pseudomonas were equal in water, Vibrio was dominant over Pseudomonas in sediment. While Micrococcus and Bacillus were in same level in water, Bacillus dominated (36.59%) considerably in sediment. It was observed that the percentage of all the genera in the alimentary canal were not in par with either that of water or sediment. While in alimentary canal, Vibrio and Micrococcu were the dominant flora, in water the dominant forms were Vibrio, Pseudomonas, Micrococcus and Bacillus and in sediment they were Bacillus, Vibrio and Pseudomonas in the order of dominance. The principal difference between the percentage microflora of the environment and that of the animal was that, in the former Pseudomonas was high in Gram-negative bacteria and Bacillus in Gram-positive forms, in the latter it was

Vibrio and Micrococcus except on body surface which harboured more Pseudomonas.

4.2.3.3 Monthwise distribution of bacterial genera in various samples:

The generic composition of bacteria in various samples observed in various months are presented in Fig. 15a-l and App. Table 8.

Station 1

A shift in the generic composition from more Gram-positive forms such as Coryneform group, Bacillus, Micrococcus, Staphylococcus to more Gram-negative bacteria was seen in post larvae from Nov. 1981 to Mar. 1982. During Nov. the Gram-positive bacteria Micrococcus, Bacillus and Coryneform group occurred at similar level (30%). Pseudomonas was recovered in all the months. Vibrio which occurred very low during Nov. to Mar. raised to the maximum in Apr. and May. Occurrence of Acinetobacter, Flavobacterium, Moraxella, Enterobacteriaceae and Staphylococcus was generally limited, which did not appear in May.

In Juveniles also, similar to post larvae, high percentage of Vibrio was found (45 and 85%) during Apr. and May respectively. However during Jun. the Gram-positive forms were dominating and the percentage of Vibrio was highly reduced.

In Apr. Vibrio, Pseudomonas and Acinetobacter together constituted 90%.

In water, Gram-negative forms Vibrio, Pseudomonas and Moraxella together constituted 92.29% in Apr. In Jun. Vibrio, Pseudomonas, Aeromonas and Enterobacteriaceae together constituted 72.72%. Further in Sep. the Gram-negative forms (87.50%) were composed of Vibrio and Pseudomonas. In water samples from Nov. to Mar. the Gram-positive bacteria such as Micrococcus, Bacillus and Coryneform group were dominant. But from Apr. onwards Vibrio and Pseudomonas increased.

Generally Gram-positive bacteria were dominant in sediment. Among them, Bacillus was present in considerable percentage 18.18% (Sep.) to 82.35% (Jun.). During other months (Nov., Apr., May, Jul. and Oct.) Gram-negative forms were dominant (Acinetobacter 57.15% in Nov., Vibrio 66.67% in Apr., Pseudomonas 91.67% in Jul. and Vibrio 60% in Oct.). During May, no single genus was dominant.

On the adult body surface, Pseudomonas (68.42%) and Vibrio (100%) dominated during Apr. and May, respectively. Vibrio which was recorded at a very low level (3.24%) during Dec. was found to attain 100% during May. However no Vibrio could be observed in Apr. Similarly Pseudomonas also reached to 68% (Apr.) from 3.23% (Dec.). Interestingly Moraxella was found increasing from 0% (Dec.) to 37.5% (Feb.). Once again

it declined and none could be recorded in May. During Dec. and Jan. Micrococcus was high (87.10% and 37.50% respectively). In general Micrococcus declined from Dec. (87%) to Mar. (16.67%).

In gills, Vibrio recorded a maximum (94.44%) in May. As on the body surface both Pseudomonas and Moraxella were found to be dominant in Apr. However both genera attained 80%. During Feb. and Mar. none of the groups showed any remarkable dominance. In Dec. Micrococcus was recorded as high as 86.11%. This declined in the following months and was not recovered in May.

When the data of bacterial population recovered from different regions of the alimentary canal were considered as a whole, it was seen that there remained a very clear shift in the bacterial flora from Gram-positive to Gram-negative especially from Micrococcus (78.26%) in Dec. to Vibrio (78.39%) during May.

The generic composition of bacteria in the stomach, anterior intestine and posterior intestinal contents when compared monthwise following observations could be made. Micrococcus dominated in stomach, anterior intestine and posterior intestine (78.57%, 94.12% and 60% respectively) in Dec. During Jan. Micrococcus declined to 15.38% in stomach, 44.0% in the anterior intestine and 37.5% in the posterior

intestine. At the same time Bacillus was high (23.08%) in stomach and less in the anterior intestine (8.0%) and in the posterior intestine (6.67%). Similarly Acinetobacter also showed a maximum (38.46%) in stomach and minimum in (16.0%) anterior intestine and in the posterior intestine (13.33%).

During Feb. Micrococcus which was 25.0% in stomach increased to 28.57% in the posterior intestine and was absent in anterior intestine. Bacillus increased from 16.66% (stomach) to 57.14% (posterior intestine) but could not be recovered from the anterior intestine. Pseudomonas, which was also recorded at a very low level (8.33%) in the stomach reached 36.36% in the anterior intestine. Vibrio (25%) in stomach exhibited an increase in percentage (27%) in the anterior intestine, which once again reduced to 14.29% in posterior intestine.

Bacillus which was revovered from the stomach (10%) increased to 10.52% in the anterior intestine and 20% in the posterior intestine in Mar. Micrococcus on the other hand got reduced from 50% (stomach) to 26.32% (anterior intestine) and could not be recovered from the posterior intestine. Moraxella which was 10% in stomach got reduced to 5.26% in the anterior intestine and completely disappeared in the posterior intestine. Coryneform group reduced from 30% (stomach) to 10.52% (anterior intestine) and disappeared completely in the posterior intestine.

During Apr. stomach harboured 89.47% of Vibrio. A gradual reduction in Vibrio was seen in anterior intestine (88.24%) and in posterior intestine (80.0%). Moraxella in the stomach was 5.26% and was absent in anterior and posterior intestine. Coryneform group showed a gradual increase from 5.26% in stomach to 5.88% in the anterior intestine and to 13.33% in posterior intestine.

In May, the stomach contents showed the presence of Vibrio, Pseudomonas, Acinetobacter, Micrococcus and Coryneform group, where the former three genera were 11.11% each and later were 22.22%, 44.44% respectively. But in the anterior and posterior intestine Vibrio alone could be recovered.

In the water samples collected from station 2 Gram-positive forms such as Micrococcus, Bacillus and Coryneform group dominated during Nov. to Mar. and May. In the rest of the months Gram-negative forms such as Vibrio and Pseudomonas showed dominance.

In the sediment samples also,Gram-positive forms such as Micrococcus, Bacillus and Coryneform group were dominant in Nov. to Mar., Jun. and Sep. Gram-negative bacteria such as Vibrio and Pseudomonas were found to be dominant during other period.

4.2.4 Statistical analysis

4.2.4.1 Correlation coefficient between environmental and microbiological parameters:

Station 1

Post larvae

A highly significant negative correlation between salinity and THB, temperature and the percentage of Vibrio and a positive correlation between pH and THB were observed (Table 40).

Juveniles

No significant relationship was obtained between the environmental factors and microbiological parameters.

Water

A significant negative correlation existed between pH and the percentage of Gram-negative bacteria, pH and percentage of Pseudomonas, oxygen and percentage of Micrococcus.

Station 2

Surface

A significant negative correlation between salinity and Micrococcus, oxygen and Micrococcus, and, a significant

positive correlation between oxygen and Gram-negative bacteria were seen.

Gills

There existed a significant negative correlation between salinity and THB, oxygen and THB, oxygen and Microco and, organic phosphorus and Micrococcus. At the same time a significant positive correlation between oxygen and Gram-negative bacteria, inorganic phosphorus and THB and inorganic phosphorus and Micrococcus was recorded.

Water

A significant negative correlation was obtained between oxygen and THB, oxygen and Micrococcus, inorganic phosphorus and Pseudomonas, and, organic phosphorus and the percentage of Micrococcus. At the same time a significant positive correlation existed between oxygen and Pseudomonas, inorganic phosphorus and THB, inorganic phosphorus and Micrococcus.

4.2.4.2 Correlation coefficients between the percentage bacterial genera of the animal and that of its environment:

Table 41 presents the correlation coefficients between the percentages of various bacterial genera of the

animals and that of its environment (water and sediment). It also gives the correlation coefficients between the various genera of sediment and that of the overlying water.

No significant relationship was observed between any of the genera of the post larvae and their habitat and genera of stomach and that of water and sediment. Whereas significant positive correlations were observed for Bacillus of juveniles and that of water, Micrococcus of the body surface and that of the environment, Pseudomonas of the body surface and that of th sediment, Micrococcus of the gill and that of water, Vibrio and Pseudomonas of anterior intestine and that of the water, Vibrio of posterior intestine and that of water, Vibrio and Micrococcus in the posterior intestine with that of sediment, and Vibrio and Micrococcus of the alimentary canal and that of water.

Both in station 1 and 2, no significant relationship was found between the percentage of all the five genera of water with that of sediment for the period during which the animals were available.

4.2.5 Hydrolytic properties of bacteria

4.2.5.1 Distribution of hydrolytic bacteria in various samples

There was no uniform pattern of distribution of various hydrolytic groups in the samples (Table 42). Proteolytic and

ureolytic bacteria were found to be the most dominant. Amount 1669 isolates tested for the ability to produce various hydrolytic enzymes, 97.72% were ureolytic followed by gelatinolytic (97.66%), caseinolytic (88.80%), lipolytic (52.79%), amylolytic (44.43%) and chitinolytic (26.06%). Chitinoclastic forms were least in all the samples. The maximum and minimum percentage of various enzymatic groups occurred in different samples were as follows:

Enzyme	Maximum %	Sample	Minimum %	Sample
Amylase	53.00	Body surface	31.15	Gill
Caseinase	97.66	Juveniles	76.74	Posterior intestine
Gelatinase	100.00	Post larvae, gills	87.25	Anterior intestine
Lipase	72.09	Posterior intestine	44.23	Water 1
Urease	100.00	Post larvae, body surface, anterior intestine, sediment 2	87.18	Water 1
Chitinase	41.86	Posterior intestine	13.00	Body surface

The data (Table 43) show that in general most of the isolates were proteolytic (gelatinolytic and caseinolytic) and ureolytic, during various months in all the samples. Also, an increase in percentage of proteolytic bacteria

could be observed from Nov. to May. At the same time a slight variation could be observed in a few samples in certain months. However the above enzymatic groups dominated in all the samples and occupied the highest level throughout the period. The maximum and minimum of amylolytic bacteria did not show any common pattern in their distribution in all samples throughout the period of study. In lipolytic enzymatic group also no uniform pattern in the maximum minimum distribution in the samples among various months was observed.

Maximum number of chitinoclastic bacteria were observed during Apr. - May in all the samples. But the minimum of the above group was not uniformly distributed in all the samples for any particular month. Proteolytic and ureolytic bacteria were dominant in all the months. Amylolytic bacteria showed highest percentage during Apr. and May, except in sediment from station 1, stomach, anterior intestine and water from station 2, where the highest percentage was observed during the earlier months (Feb. and Mar.). Monthwise variation of lipolytic bacteria was not uniform.

4.2.5.2 Generic composition of various hydrolytic bacteria:

Generic wise distribution of hydrolytic enzyme producing bacteria in all the samples is presented in

Table 44 and 45. Among the genera isolated and tested for their potential hydrolytic activity Vibrio, Pseudomonas, Micrococcus, Bacillus and Coryneform group were found to be the prominent flora. All the isolates of vibrios were proteolytic and ureolytic in prawn, water and sediment. From stomach to posterior intestine of the animal, an increase in the amylolytic vibrios was seen (54.09 to 66.67%). In the other samples, it was above 72.0%. Lipolytic vibrios varied widely in the samples (28.34 to 100%). At the same time, above 80.65% of vibrios were chitinoclastic.

More than 90% of Pseudomonas were proteolytic and ureolytic except for the 67% of ureolytic forms encountered in water from station 1. Amylolytic and lipolytic Pseudomonas ranged from 13.64 to 62.50% and 28.13 to 90.90% respectively. Chitinoclastic forms were absent in many samples and when present they ranged from 3.57 to 33.33%.

Proteolytic Micrococcus ranged from 50 to 100% in various samples. At the same time 77 to 100% of them were ureolytic. Amylolytic and lipolytic Micrococcus were comparatively smaller (7.69 to 71.05% and 15.38 to 78.79% respectively). Chitinase producers were absent except for a negligible percentage (7.94) observed in juveniles.

Among Bacillus gelatinolytic and ureolytic forms were higher (75 to 100%) than the caseinolytic forms (50 to 100%). Amylolytic Bacillus ranged from 9.09 to 60.47% and were absent on the surface and gill of the animal. Lipolytic Bacillus ranged from 12.50 to 83.33% and the chitinas producers were absent among the group, except for a very small number encountered in sediment and stomach.

There was a reduction in the proteolytic Coryneform grou from the stomach to posterior intestine, whereas it was totally absent in the latter. In other samples it ranged from 66.67 tc 100%. Amylolytic and lipolytic Coryneform group were absent ir many samples. When they were present they varied from 6.25 to 60% and 6.67 to 100% respectively. Chitinoclastic Coryneform group were absent in all the samples except in water where it ranged from 6.25 - 6.67%.

The results of the percentage contribution of different genera for a particular hydrolytic enzyme are presented in Table 46.

Station 1

Post larvae

Amylolytic, caseinolytic, gelatinolytic, lipolytic and ureolytic forms were mainly contributed by Vibrio, Pseudomonas, Flavobacterium, Micrococcus, Bacillus and

Coryneform group. Contribution by other genera ranged from 0.4 to 4.5% only. Vibrio, Pseudomonas, Aeromonas, Acinetobacter and Flavobacterium were the sources of chitinase in this sample. However, 74.63% of chitinoclasts were Vibrio followed by Flavobacterium (17.91%).

Juveniles

The chitinoclastic bacteria were mainly the members of Vibrio and Pseudomonas. Flavobacterium was the dominant flora among gelatinolytic group and Vibrio followed by Pseudomonas and Bacillus were the major forms producing hydrolytic enzymes other than chitinase.

Water

Sources of hydrolytic enzymes other than chitinase were mainly five genera such as Vibrio, Pseudomonas, Micrococcus, Bacillus and Coryneform group. Source of chitinase was mainly Vibrio.

Sediment

The major hydrolytic enzyme (amylase, protease, lipase and urease) producers were Vibrio, Pseudomonas, Micrococcus, Bacillus and Coryneform group. The contribution by other genera ranged from 0 to 8.33%. Chitinase was mainly elaborated by Vibrio (50%), followed by Pseudomonas (15.79%, Bacillus (21.05%), Aeromonas (7.89%) and Flavobacterium (5.26%).

Station 2

Body surface

The major contributors of chitinase, caseinase, gelatinase, lipase and urease were Vibrio, Pseudomonas, Moraxella, Micrococcus and Bacillus. Amylase was mainly from Vibrio, Pseudomonas and Micrococcus. Contribution by other genera towards the above mentioned hydrolytic enzymes ranged from 0 to 4.92%. Chitinase was mainly from Vibrio (84.62%) followed by Aeromonas and Acinetobacter.

Gill

Caseinase, gelatinase, lipase and urease production was shown mainly by Vibrio, Pseudomonas, Acinetobacter, Moraxella, Micrococcus and Bacillus. The other genera contributed 0 to 7.89% only. Amylase was mainly elaborated by Vibrio and Pseudomonas. Chitinase was produced mainly by Vibrio (96.15%) and Aeromonas (3.85%).

Stomach

Vibrio, Acinetobacter, Micrococcus, Bacillus and Coryneform group were the major sources of caseinase, gelatinase, lipase and urease. Pseudomonas was not found to be a prominent member in this case. Contribution by other genera ranged from 0 to 5%. Elaboration of amylase

was shown by majority of the members of Vibrio, Aeromonas, Micrococcus and Bacillus and that by other genera ranged from 0 to 3.70%. Chitinoclastic activity was mainly by Vibrio (83.33%) followed by Bacillus (8.33%), Pseudomonas and Enterobacteriaceae (4.17%) each.

Anterior intestine

Hydrolytic enzymes other than chitinase were mainly contributed by Vibrio, Pseudomonas and Micrococcus. 87.5% of vibrios were chitinoclastic.

Posterior intestine

Majority of Vibrio, Pseudomonas, Aeromonas, Micrococcus and Bacillus showed caseinase, gelatinase, urease and lipase activity. Chitinoclastic activity was shown mainly by Vibrio (83.33%).

Alimentary canal

Gelatinase, lipase and urease were mainly contributed by Vibrio, Micrococcus, Pseudomonas, Acinetobacter, Bacillus and Coryneform group. Chitinase enzyme was elaborated mainly by Vibrio (85%).

Water

All the potential hydrolytic enzyme activity except that of chitinase was mainly contributed by Vibrio, Pseudomona

Micrococcus, Bacillus and Coryneform group. However, the contribution of Coryneform group to amylase activity was just 1.5%. Chitinase was mainly elaborated by Vibrio (78.05%).

Sediment

Protease, lipase and urease were mainly produced by members of Vibrio, Pseudomonas, Micrococcus, Bacillus and Coryneform group. Contribution by other genera varied from 0 to 8.77%. The major source of chitinase was Vibrio (86.21%).

Gram-negative bacteria Vibrio and Pseudomonas and Gram-positive bacteria Micrococcus, Bacillus and Coryneform group were the major source of amylase, lipase, protease and urease. This had happened by way of being the five genera dominant among the total heterotrophic bacteria. At the same time the only dominant group which produced chitinase was Vibrio.

4.2.6 Effect of NaCl concentrations, pH and temperature on the growth of bacteria

The bacterial isolates were grown at various environmental conditions such as varying temperatures, pH and sodium chloride concentrations and the maximum growth was found out. The results showed that maximum growth of all the

isolates was not confined to one concentration of NaCl, pH and temperature. No growth was observed at pH 2 and 4 and at 4°C.

Table 47 presents the effect of NaCl concentrations, pH and temperature on the growth of bacteria isolated during various months. Among the isolates (1669) tested, about 75% of them showed optimum growth between 1 and 3% NaCl concentrations, maximum being at 3%. Zero and 10% supported only 5.15 and 6.96% of isolates to grow maximum. From Nov. 1981 to Jun. 1982 some of the isolates recorded optimum growth at higher sodium chloride concentrations (7 and 10%). pH 7 was the optimum for 58.89% of the cultures and pH 9 for 37.22%. Only 3.78% grew well at pH 11. pH 2 and 4 were unfavourable. For majority of the cultures pH range of 7 - 9 was found optimum. Majority of the isolates irrespective of the period of collection preferred pH 7 for maximum growth. All the isolates were found to be mesophilic since 82.80% grew to maximum at 30°C. Only 12.4% recorded preference to 40°C for optimum growth. None of them showed maximum growth at 4°C.

Table 48 gives the effect of NaCl concentrations, pH and temperature on the growth of isolates obtained from different samples. It could be observed that in all the samples at least a small percentage exhibited maximum growth,

at all concentrations of NaCl tested. Majority of the cultures preferred a range of 1 and 3% NaCl as optimum for growth. But majority of the isolates of post larvae and juveniles preferred 3 and 7% NaCl concentrations. Sodium chloride concentration of 3% was favourable to a maximum number of isolates irrespective of their source except for the isolates from juveniles. In post larvae pH 9 was preferred by majority of the isolates. In all other samples 51.95 to 69.98% of cultures showed maximum growth at pH 7.

About 69 to 100% of the isolates showed maximum growth at 30°C. 40°C was favourable for 3.49 - 21.0% of isolates None recorded maximum growth at 4°C and very small percentages, 4.19 and 0.66, showed same pattern at 10°C and 50°C respectively.

Effect of NaCl concentrations, pH and temperature on the growth of different genera are presented in Table 49. At least a small percentage of the members of Vibrio, Pseudomonas, Bacillus and Coryneform group showed maximum growth at all concentrations of NaCl. However 44.44to 45.90% of the above genera showed 3% NaCl as the optimum. At the same time 19.78% of Vibrio and 21.88% of Bacillus grew to maximum at 7% NaCl. 11.98% of Pseudomonas and 1.37% of Vibrio did not require NaCl for maximum growth. While 16.42% of Vibrio preferred 10% NaCl

concentration as the optimum, only 4.71 to 9.73% of other genera behaved in the similar pattern.

About 36% of Vibrio were moderately halophic having an optimum range of NaCl, 7-10% followed by Pseudomonas (13.47%), Aeromonas (16%), Moraxella (18.64%), Bacillus (26.59%) and Coryneform group (12.78%). All the other genera were slightly halophilic, favouring a range of NaCl concentration from 1 - 3%.

The preference for pH 7 was shown by 48.15 to 100% of the isolates belonging to various genera. More than 50% of the members of Vibrio, Pseudomonas, Aeromonas, Acinetobacter, Moraxella, Micrococcus, Bacillus and Coryneform group showed a requirement of pH 7 for optimum growth. pH 9 was the optimum for 8 to 66.67% of various genera. At the same time pH 11 was least preferred. Representatives of Vibrio (8.24%), Pseudomonas (5.24%), Flavobacterium (1.85%), Micrococcus (4.51%) and Coryneform group (0.75%) showed the requirement of pH 11 for maximum growth. Considering pH 9 and 11 in the alkaline range, 46.97% of Vibrio, 31.46% of Pseudomonas, 50.13% of Micrococcus, 34.68% of Bacillus and 43.60% of Coryneform group preferred it as optimum.

Ranging from 67 to 100% of all genera, preferred 30°C as optimum for growth. Among the major genera 21.21%

of Bacillus, 21.34% Pseudomonas, 12.78% of Coryneform group and about 6% of Vibrio and Micrococcus each preferred 40°C as the optimum growth. 12% of Aeromonas, 18.03% of Acinetobacter also preferred 40°C. Among the other genera, members preferring 40°C as the optimum were either very low in percentage or absent completely. Vibrio, Pseudomonas, Acinetobacter, Flavobacterium, Enterobacteriaceae, Micrococcus, Bacillus and Coryneform group showed a very small percentage preferring 10°C as the optimum. At the same time Pseudomonas and Coryneform group only contained at least a few isolates favouring 50°C as the optimum.

4.3 DISCUSSION

Total heterotrophic bacteria (THB) of post larvae declined from November 1981 to May 1982 and this was found to be statistically significant. Significant negative correlation existed between THB and salinity and there was a significant increase in the percentage of Gram-negative bacteria from November 1981 to May 1982, with the concomitant reduction in Gram-positive forms. *Vibrio* increased from November to May while Coryneform group, *Bacillus* and *Micrococcus* declined. Higher salinity in estuarine waters is an indirect indication of tidal influx and mixing of seawater with the freshwater (Mohankumar, 1979). The higher saline water might have favoured the growth of vibrios which are mostly halophilic (Beuchat, 1974,1975 and Pradeep, 1986). At the same time the higher salinity might have adversely affected the lesser halophilic forms. Simultaneously the body surface and intestine of post larvae might have provided the vibrios a suitable environment to attach and colonise and multiply under favourable environmental conditions. Colwell *et al*. (1980) observed attachment of *Vibrio* on copepods and Sochard *et al*. (1979) showed that a significant number of marine vibrios were associated with the gut of copepods.

No significant correlation existed between the percentage genera of water and that of the post larvae indicating that the associated flora of animal in all the cases may not be a strict reflection of its environment. Selectivity can exist as shown by Sochard et al. (1979) in copepods and Prieur (1981) in bivalves, where, vibrios were specifically found growing in the intestine of these animals. The positive significant correlation existed between THB of post larvae and the pH of water showed that higher bacterial population in post larvae is seen at high pH (alkalir It is seen that most of the isolates obtained from post larvae showed maximum growth at pH ranging from 7-9.

In juveniles THB showed a significantly linear decrease from November 1981 to May 1982. Simultaneously an increasing trend of Gram-negative bacteria was observed from November to May. Similar to post larvae in juveniles also increase of Vibrio was found during this period. However during June the Gram-positive forms dominated and the populatio of Vibrio was highly reduced. This can be attributed to the onset of monsoon and river discharge which lead to the sharp drop in salinity (2.30×10^{-3}). Lesser salinity resulted during monsoon might have eliminated much of the moderately halophilic strains of vibrios and favoured the lesser halophilic ones to survive. A significant positive correlation

observed between Bacillus of juveniles and that of water showed that the changes in the number of Bacillus in water was reflected on the juveniles.

THB of water did not show a statistically significant linear decrease or increase from November 1981 to October 1982 Environmental factors monitored were not found to exert a statistically significant influence on the THB of water. Mohankumar et al. (1979) observed wide fluctuations of bacterial population in the surface samples from two station, in Arabian sea, one at the open ocean and the other in an estuary. The bacterial population fluctuated more widely at the estuarine region than at the open ocean. In the open ocean the bacterial population of surface waters increased with increase of temperature. But such pattern was not seen in estuarine samples. He attributed this to the considerable mixing taking place because of tidal effect and river flow. A significant increasing trend of Gram-negative bacteria was found from November 1981 to October 1982 with the highest percentage in April. From November 1981 to March 1982 the Gram-positive forms such as Micrococcus, Bacillus and Coryneform group were dominating. But from April onwards the dominance of the above genera became less pronounced and Gram-negative forms such as Vibrio and Pseudomonas started

dominating. The important genera were Pseudomonas, Bacillus, Micrococcus and Vibrio in the decreasing order of dominance.

In sediment both THB and Gram-negative bacteria were not showing any statistically significant variations. The decreasing order of dominance of various genera was Bacillus, Vibrio, Pseudomonas, Micrococcus and Coryneform group Monthwise variation in the percentage genera in sediment did no follow a regular pattern.

Thus at station 1, a significant increase of THB existed in both post larvae as well as in juveniles from November 1981 to May 1982. While in post larvae the increase of Gram-negative forms were found to be significantly linear, with respect to months, in juveniles it was not seen eventhough an increase was recorded. In both post larvae and juveniles a generic shift from Gram-positive to Gram-negative bacteria leading to the dominance of Vibrio in May was seen. In post larvae alone a meaningful interpretation of the role of salinity on the bacterial population and the dominance of Vibrio could be given. In juveniles at the same time during June following monsoon an increase in the Gram-positive bacteri and decrease of Gram-negative forms could be explained as a result of fall, which lead to the influx of river run off

bringing the terrestrial bacteria especially Bacillus and Micrococcus and allowing them to thrive there by keeping the salinity of the system low (2.39×10^{-3}). This change in the water column due to monsoon was clearly reflected on the animal by a significant positive correlation between Bacillus of water and that of the animal.

Both in water and sediment the variations of THB with respect to months was not statistically significant. In the present study, the station 1 being a shallow area, was subjected to changes due to land run off as well as by the river influx. During the monsoon season large quantity of Salivenia sp. was getting washed into the backwater from the less saline areas by river run off. This weed got decayed and settled at the bottom enriching the sediment and the overlying water (Raman, 1980). This may be one of the reasons for the increase of THB seen in sediment during the postmonsoon period. However, the bacterial population of water was more or less similar throughout the period. This can be a result of mixing of freshwater with tidal influx (Mohankumar et al. 1979). Both in water and sediment the highest percentage of Vibrio was recorded during April. During May also Vibrio was in good percentage along with Pseudomonas. This can be attributed to summer and the influence of tidal influx and the consequent increase of salinity.

On the body surface of adults THB did not show any statistically significant change from December 1981 to May 1982. At the same time between salinity of water and THB of the animal surface there existed a significant negative correlation showing that increase of salinity of water can decrease the THB of the body surface of animal. Increase of Gram-negative bacteria observed from December 1981 to May 1982 was found to be significant with respect to months. Dissolved oxygen of water and Gram-negative bacteria on the body surface showed a significant positive correlation, indicating that the increase of dissolved oxygen in the water column may be promoting the growth of Gram-negative bacteria on the animal surface. Highest percentage of Vibrio and Pseudomonas were recorded in May and April respectively on the animal surface. A highly significant positive correlation existed between the Micrococcus of water and that of the animal suggested that the changes in the percentage of Micrococcus occurred on the body surface were directly influenced by the population of Micrococcus in water. The significant negative correlation observed between salinity of water and the percentage Micrococcus of the animal surface suggested that the comparatively higher salinity prevailed during the premonsoon period and its gradual increase from November 1981 to May 1982 might have adversely affected the

survival of Micrococcus in water and this might have influenced the percentage occurrence of them on the animal surface; or, the higher salinity might have directly acted upon adversely on the Micrococcus of the animal surface. Likewise, the increase in the percentage of Pseudomonas in sediment from December 1981 to May 1982 was reflected on the body surface. A significant negative correlation existed between the dissolved oxygen of water and the percentage of Micrococcus on the animal surface. From December 1981 to May 1982 dissolved oxygen was increasing (2.46 to 6.48 $ml.l^{-1}$). Micrococcus eventhough being a strict aerobe (Buchanan and Gibbon, 1974) was not influenced favourably by the increase of dissolved oxygen in water. This may be because of two reasons; firstly the minimum quantity of oxygen observed may not be a limiting factor for Micrococcus and secondly, the adverse effect of higher salinity on the organism might not have been compensated by the increase of oxygen level.

On gills of animals eventhough THB showed two peaks, one in December 1981 and the other in January 1982, no significant linear increase or decrease of THB could be seen from December 1981 to May 1982. At the same time there existed a significant negative correlation between salinity of water and THB of the gills. There existed a significant negative correlation between the dissolved oxygen and THB also.

At the same time a significant positive correlation between oxygen level in water and the Gram-negative bacteria of gills was observed. Thus it becomes apparent that increased salinity during the pre-monsoon period lead to the declining of THB, while, Gram-negative forms showed an increase. Vibrio, Pseudomonas and Moraxella were the prominent Gram-negative forms in May and April respectively. Salinity was more decisive in the change of flora in gills than the increase of oxygen observed in the water column. The significant negative correlation existed between salinity and Micrococcus further showed that variation of oxygen level in the water within the range observed might not have exerted any influence on the organisms especially on Micrococcus in the higher saline conditions. The significant positive correlation seen between the percentage of Micrococcus in water and that of gills showed that the variations in the percentage of Micrococcus taken place in water was reflected in the gills. A significant negative correlation existed between organic phosphorus and Micrococcus. At the same time, a significant positive correlation was seen between organic phorphorus of water and the percentage of Micrococcus in gills, and between inorganic phosphorus of water and THB of gills. The exact relationship between these parameters requires detailed study.

In the stomach THB did not show any significant linear trend of increase or decrease from December 1981 to May 1982, while two peaks one in January 1982 and the other in April 1982 were prominent. The increasing trend of Gram-negative bacteria seen in stomach from December to May was not statistically significant. Simultaneously percentage of Micrococcus declined and Vibrio increased. At the same time the sediment contained a lower population than that of the stomach content of animals, the difference being of one to three magnitude. Moreover no significant correlation was recorded between the genera existed in water and sediment during that period and that of the stomach showing that bacterial population of stomach was not a reflection of that of the water and sediment. This may be due to the selective feeding behaviour of the animals. This observation also agrees with that of Mary (1977), Palaniappan (1982) and Ivy Thomas (1982). Dall (1968) suggested that prawns would selectively feed upon epiflora and epifauna (bacteria, microalgae and protozoa) of the mud substrates. Kurien and Sebastian (1976) have concluded that prawns have food preference for crustaceans, molluscs or algae. Moriarty (1976) revealed that prawns chose organic matter rich in bacteria from the sediment. Sriraman (1978) showed that the prawn P. indicus was found to feed mainly on organic

detritus. All these suggest that it is quite natural to observe higher bacterial load in the stomach content than that of the sediment. Dead and decaying matter of plant and animal origin may contain more bacteria than the surrounding environment. This may also be one of the reasons for getting a different flora in stomach than that of the sediment.

In general, the THB of stomach was much lesser than that of the anterior and posterior intestine. The population of the posterior intestine was much higher than that of the anterior intestine. Palaniappan (1982) made a similar observation in the pond reared P. indicus where a high bacterial count in the midgut (intestine) than the foregut (stomach) was seen. The main digestive processes take place in the foregut itself and the midgut plays a key role in the absorption of the digested food materials (Gopalakrishnan, 1957). The midgut provide a favourable environment to the strains which tolerate the digestive processes, and, the active multiplication of these bacterial genera takes place in the midgut region. Moriarty (1976) showed that microorganisms passing directly from the proventriculus to the midgut without passing through the digestive gland were poorly digested indicating that midgut is not a centre of digestion. Thus two processes might have happened, i) a part of the bacteria

consumed along with food might have entered directly into the intestine without passing through the digestive gland and ii) another part might have overcome the digestive activity of the digestive enzymes and low pH prevailing in the digestive tract. pH of the digestive tract during digestive processes was found to be 5 (Hood and Meyers, 1973). The organisms which could achieve an entry into the intestine by any one of the above means might have undergone a few cycles of division resulting in the higher population than that of the stomach.

It could be seen that there was a vivid increase of Vibrio from stomach to posterior intestine. Similarly Pseudomonas, Aeromonas and Bacillus also showed such an increase. Aeromonas and Bacillus showed a decline in anterior intestine and then increased in the posterior intestine. The genera which showed a reduction in the intestine from the stomach were Acinetobacter, Moraxella, Enterobacteriaceae, Micrococcus and Coryneform group. Palaniappan (1982) reported Vibrio, Bacillus and Pseudomonas as the dominant flora in the foregut. In the midgut Coryneform group, luminous Vibrio and Bacillus showed considerable increase. He reported that the percentage of Vibrio and Pseudomonas which formed the resistant group in foregut decreased slightly in their relative percentage in the midgut. But in a study conducted with experimental diet

he has noted remarkable increase of Vibrio in the digestive tract of P. indicus. The present investigation agrees with his findings to a large extent as far as the increase of Vibrio, Pseudomonas and Bacillus were concerned. Further in the present investigation it was seen that the percentage of Vibrio and Pseudomonas observed in the anterior intestine was maintained in the posterior intestine also. It becomes apparent that the alimentary canal of prawns in the natural environment forms a suitable microenvironment where Vibrio, Pseudomonas, Aeromonas and Bacillus which can undergo a few cycles of division. The organisms which were able to tolerate the adverse effects of the digestive processes entered in the midgut (Palaniappan, 1982). There is no digestion taking plac in this region and the absorption of digested food material alone takes place in the midgut region. The present investig shows that the microenvironment of alimentary canal in gener is highly suitable for Vibrio, the percentage of which was lesser in water, sediment, body surface and gills compared t the alimentary canal. Prieur (1981) stated that vibrios we apparently able to divide a number of times in the gut of bivalve molluscs. Further if this phenomenon occurs, it w be an explanation for the presence of an important Vibrio population in the gut of bivalves compared to the bacteria population of sea water. Davis and Sizemore (1982) suppo

the hypothesis that Vibrio spp. selectively colonized the stomach of crab and he considered them as the predominant enteric organisms in blue crab. Ivy Thomas (1982) observed high incidence of chitinoclastic vibrios in the intestine of marine and estuarine prawns.

Among the prominent genera which showed reduction in the intestine from the stomach, Micrococcus and Coryneform group deserves much attention. Palaniappan (1982) observed reduction of Micrococcus in the alimentary canal of P. indicus fed with an experimental diet. In the present observation Micrococcus was found to increase slightly in the anterior intestine and decrease in the posterior intestine. The other genera which showed reduction in the intestine were Acinetobacter, Enterobacteriaceae and Moraxella. This reduction might be due to the lysis of cells by the digestive enzymes. The lysed bacterial cells might be used as a direct source of food by the animal itself. Moriarty (1976) showed that M. bennettae can readily digest and assimilate bacteria. The high assimilation values obtained suggested that cell walls were not only lysed but digested and assimilated. He further stated that autolysis and enzyme activity in the digestive gland of the prawn bring almost complete digestion of bacteria. The organisms which he used in his study were E. coli, P. florescens, E. aerogenus and B. subtilis. But in the

present study Bacillus and Pseudomonas were not found to be declining in the intestine. This may be because of the fact that the organisms can directly escape into the midgut without passing through the digestive gland (Moriarty, 1976). This leads to another speculation that certain organisms (e.g. vibrios) even if they pass through the digestive gland can withstand the digestive processes. Palaniappan (1982) showed that hepatopancreas extract did not show any lethal effect on the genus Vibrio at any stage during his study. At the same time few other forms such as Bacillus and Pseudomonas were susceptible to the digestive processes in the digestive gland, and in case they escape the proventriculus they may be able to multiply in the intestine. However bacteria form an important dietary component of prawn as stated by Moriarty (1976) when he observed 20 to 35% of the organic carbon in the proventiculus of the prawn M. bennettae feeding on muddy estuarine sediment was composed of bacteria.

Majority of isolates were ureolytic followed by proteolytic, lipolytic, amylolytic and chitinolytic. Chitinoclastic forms were least in all the samples. An increase of proteolytic and chitinoclastic bacteria could be seen from November 1981 to May 1982 with slight variations. Ureolytic bacteria were higher in all the months. Amylolytic

and lipolytic bacteria did not show a common pattern in their monthwise distribution in various samples.

Uniformly among all the genera encountered in various samples proteolytic and ureolytic forms were very high in percentage. Among Vibrio and Aeromonas, compared to other genera, amylolytic and lipolytic strains were very high. Similarly chitinolytic strains were also very high among the vibrios isolated, followed by Aeromonas and Flavobacterium.

At station 1 and 2 the source of amylase, protease and lipase were mainly Vibrio, Pseudomonas, Micrococcus, Bacillus and Coryneform group. Vibrio was the major source of microbial chitinase. Members of all the genera were able to produce the above mentioned hydrolytic enzymes, and the major source of these enzymes was attributed to the above mentioned five genera by virtue of them being the dominant ones. At the same time the only dominant group which produced chitinase was Vibrio. Ivy Thomas (1982) reported a positive correlation between the chitinoclastic bacterial population and environmental temperature. Higher percentage of chitinoclasts was recorded during the month of May when the temperature was found to be comparatively higher. In the present observation it was seen that the chitinoclastic bacteria increased in water, sediment and prawn from November 1981 to May 1982. In the present study no environmental factors could

be identified as the cause(s) of the increase of Vibrio during the premonsoon period. Probably tidal influx may be one of the reasons along with availability of suitable microenvironment for the Vibrio to grow in the alimentary tract of animals, whereas the tidal water might have brought halophilic vibrios into the backwater, before getting colonized in a suitable environment. The influence of tidal influx was much evident from the high salinity prevailed during the premonsoon period.

Higher incidence of chitinoclastic bacteria could be seen in the alimentary tract of the animal. On the surface and gill they were comparatively lesser in percentage. Further, an increase of chitinase producers could be seen from stomach to posterior intestine. Moreover an increase of the above mentioned group from November to May also could be seen. It has already been explained that intestine of P. indicus is a suitable microenvironment for the vibrios to survive and undergo division. Hood and Meyers (1973) found that the bacteria of the digestive tract of the wild shrimp P. setiferus comprise a limited number of generic types, characterised by rapid growth, tolerance to low pH and an elaboration of an array of extracellular enzymes, especially chitinase. Chitinase is being elaborated by specific bacteria within the microenvironment of the shrimp digestive tract.

Chitinase activity within the shrimp digestive tract is correlated with ingestion of dietary chitin, concomitant with an increase of chitinoclastic bacterial biomass. Bacteria may serve as a direct source of nutrients for shrimp if they are susceptible to the digestive enzymes in the proventriculus or if not, they elaborate extracellular enzymes and take part in the digestion of food in the alimentary canal. Hood and Meyer (1977) further explained that the enzyme produced by the predominant gut bacteria, Beneckea is a moderately active inducible chitinase, while the shrimp has an indigeneous constituative chitinase and chitobiase system. This duel enzyme system suggests that metabolic chitin transfermations may play a vital role in crustacean metabolism. Goodrich and Morita (1977) indicated that a major portion of chitin decomposition may occur in the digestive tracts of the sediment dwelling animals including both fish and invertebrates. Present investigation supports this view by showing that higher percentage of chitinoclastic bacteria especially vibrios were seen in the alimentary tract of prawns, than in sediment and water. Ivy Thomas (1982) observed that Vibrio was the most predominant genus among chitinoclasts in water, sediment and prawns followed by Aeromonas, Micrococcus and Alcaligenes. The present observation of Vibrio as the dominant group to produce chitinase is supported by her findings also.

The dominance of vibrios in an ecosystem has profound importance as far as the potential hydrolytic activity prevailing in that system is concerned. Because, majority of the isolates of Vibrio were capable of elaborating all the six hydrolytic enzymes which were tested here. Among the other genera majority of the strains were capable of producing protease and urease, but the percentage of isolates able to elaborate amylase, lipase and chitinase were considerably smaller compared to that of vibrios. This was highly evident as far as the chitinase is concerned, which was mainly elaborated by Vibrio. Thus Vibrio can be designated as a versatile group taking part in the digestion of food as well in the mineralization of organic matter in water and sediment. Simultaneously many species of Vibrio are designated as pathogens to prawns at varying levels (Cook and Lofton, 1973; Vanderzant and Nickelson, 1973; Delves-Broughton and Poupared, 1975; Lightner, 1975; Lightner and Lewis, 1975 and Johnson, 1978). But Lightner (1985) concluded that vibrios are one of the normal flora of prawns which assume the role of an opportunistic pathogen at situation when the animals are undergoing stress. In open waters unless chemically polluted, the emergence of such stress factors are uncommon. Hence the role of Vibrio in the open waters as a pathogen of prawn cannot be recognised beyond doubt.

Among the isolates tested about 75% showed optimum growth at 1 and 3% NaCl, of which 3% was preferred by the highest percentage of isolates. The percentage of isolates preferring 7 and 10% NaCl were more during November 1981 to January 1982 and absent during July to October. The absence of moderately halophilic forms during the monsoon period may be explained as a result of river discharge which might have eliminated the moderately halophilic flora. During monsoon the salinity of both the stations were considerably lower due to influx of freshwater and this might have affected adversely on halophilic bacteria. At the same time during postmonsoon and premonsoon season, the salinity was built up which might have favoured the moderately halophilic forms.

About 36% of Vibrio were moderately halophilic showing an optimum range of NaCl from 7 to 10% followed by Bacillus, Aeromonas, Moraxella, Pseudomonas and Coryneform group. However large number of the isolates preferred NaCl ranging from 1-3%. Ivy Thomas (1982) observed maximum growth of selected isolates of chitinoclastic vibrios at 1% NaCl. Surendran et al. (1983) showed that the optimum salt requirement of selected isolates of Vibrio, Moraxella and Acinetobacter ranged from 2.5 to 3.5%. The present investigation showed that there were at least few isolates each in most of the genera preferring 0-10% NaCl for maximum growth. This indicated that members of

various genera existed in the backwater were comprised of strain originated from both freshwater brackish and marine environments Rheinheimer (1980) stated that brackish water carry not only the genera of marine bacteria and salt tolerant freshwater forms, but also halophilic bacteria whose specific habitat is brackish water. Ivy Thomas (1982) reported that vibrios may even be originated from freshwater environments. Co-existence of organisms exhibiting maximum growth at all concentrations of NaCl suggested that the animal and its habitat harbour physiologically diverse groups of organisms.

pH ranging from 7 to 9 was found to be optimum for a large portion of isolates. More than 50% of the members of Vibrio, Pseudomonas, Aeromonas, Acinetobacter, Moraxella, Micrococcus, Bacillus and Coryneform group showed pH 7 as the optimum. Among the genera which preferred pH 11, Vibrio ranked first (7.69%). Ivy Thomas (1982) recorded a pH range of 5-8 for Vibrio and were found to be more sensitive to acidic conditions, than alkaline. Chandrasekaran (1985) observed a pH range of 6-10 for his bacterial isolates such as Pseudomonas, Vibrio and Acinetobacter. Strains of V. parahaemolyticus studied by Pradeep (1986) showed that pH 8 was most favourable. Beuchat (1975) reported that pH 7.6 to 8.6 are the optimum for growth of marine vibrios having an

optimum pH in slightly alkaline conditions. Growth of *V. parahaeomolyticus* ranging from pH 5-11 (Twedt *et al.* 1969) 5 to 8 (Beuchat, 1975), 5-10 (Ermolina and Shikatov, 1975) were already reported. In the alimentary canal of *P. indicus* vibrios were found to increase in number indicating that they were not affected by the acidic pH prevailing in the gut. This may be because the vibrios are either able to grow at a wide range of pH (5-11) or they may be protected from the acidic pH by physical, chemical and biological factors prevailing in the gut which are not understood so far.

Highest percentage of isolates preferred 30°C as optimum. Some isolates of *Bacillus*, *Pseudomonas*, Coryneform group, *Vibrio* and *Micrococcus* in the decreasing order of importance, also preferred 40°C. Preference to 50°C was restricted to a few isolates of *Pseudomonas* and Coryneform group. Isolates preferring 10 to 40°C as optimum was seen in most of the genera. Ivy Thomas (1982) observed 30°C as the optimum for chitinoclasti *Vibrio* and *Pseudomonas*. Chandrasekaran (1985) showed his isolates (*Pseudomonas*, *Vibrio* and *Acinetobacter*) preferring 30°C as optimum.

Thus it was noted that while majority of the isolates fall under the group preferring NaCl concentrations ranging from 1-3%, a considerable percentage preferred to grow at 7 and 10% NaCl. The maximum salinity attained in the

backwater during the study period was only 28.91×10^{-3}. Organisms preferring a wide range of pH(7-11)existed together, where the pH of the environment varied from 7 to 8.43 only. While bacteria which could grow to maximum at a temperature ranging from 10 to 50 could be isolated when the temperature at both the stations were 20 to 30.5°C only. Thus it can be concluded that diverse physiological groups of bacteria with various optimal conditions for maximum growth may exist together in a system where the available physico-chemical factors are not satisfying for all the bacteria to attain maximum growth. When an organism is introduced into a new environment, they may die or adjust to the new environment or enter into a state of neither death or activity - dormancy. Stevenson (1978) postulated that the physiological state of a significant portion of the bacterial community in most aquatic environments can be described as dormant. Stevenson and Erkenbrecher (1976) stated that it would be fruitless to propose that a single property would be responsible for the fitness of all the organisms to inhabit their respective ecosyst Several properties (fitness traits) may contribute to the contin presence of bacteria in aquatic systems; the important traits probably include tolerance to or dependence on saline conditions competitative advantage in the utilization of organic nutrients, capacity for attachment to particulate materials and subsequent

colonization and most important of all, dormancy, which was defined by Sussman and Halvorson (1966) as the rest period which is exogenous. This can be attributed as one of the reasons for the coexistence of bacteria having diverse physiological traits in an ecosystem. Further the micro-ecosystem provided by the different regions of the animal (P. indicus) may be affording a protective environment, where the protection is rendered by the essential growth factors, vitamins etc., with which the lack of certain required physical and chemical factors are nullified.

Table 37. Percentage distribution of THB in different regions of alimentary canal

Months and year	Stomach	Anterior intestine	Posterior intestine	Alimentary canal
1981 December	16.30	46.67	37.04	100
1982 January	54.74	1.09	44.17	100
February	2.93	9.92	87.15	100
March	12.01	12.17	75.82	100
April	14.82	56.61	28.57	100
May	9.54	4.03	86.43	100
Average	18.39	21.75	59.86	100

Table 38. The trend lines and correlation coefficients with their significance between months and THB of various samples

Sample	Sample size	a	b	r	p
Station 1					
Post larvae	7	9.59	-0.5250	-0.8850	<0.01*
Juvenile	8	8.85	-0.4050	-0.8410	<0.01*
Water (along with the animal)	8	6.72	-0.0640	-0.1897	>0.10
Water (around the year)	12	6.57	-0.0200	-0.0959	>0.10
Sediment (along with the animal)	8	6.01	-0.0165	-0.0836	>0.10
Sediment (around the year)	12	5.61	0.0844	0.5076	<0.10 >0.05
Station 2					
Adult					
Surface	6	6.31	-0.0051	-0.0132	>0.10
Gill	6	8.32	-0.1437	-0.7492	>0.05 <0.10
Stomach	6	7.44	0.0680	0.1759	>0.10
Anterior intestine	6	7.08	0.1290	-0.4165	>0.10
Posterior intestine	6	7.64	0.1650	0.5000	>0.10
Alimentary canal	6	8.05	0.1310	0.4836	>0.10
Water (along with the animal)	6	5.45	0.1950	0.3801	>0.10
Water (around the year)	12	6.53	-0.0134	-0.0656	>0.10
Sediment (along with the animal)	6	5.21	0.1670	0.3378	>0.10
Sediment (around the year)	12	5.36	0.1421	0.5015	<0.10 >0.05

Table 39. The trend lines and correlation coefficients with their significance between mouths and the percentage occurrence of gram negative bacteria of various samples

Sample	Sample size	a	b	r	p
Station 1					
Post larvae	7	-8.6400	14.1200	0.8703	<0.02* >0.01
Juveniles	8	43.0432	2.6760	0.2548	>0.10
Water (around the year)	12	16.8642	5.4483	0.7599	<0.01* >0.01
Water (along with the animal)	8	2.7664	7.2464	0.5763	>0.10
Sediment (around the year)	12	52.8894	0.4107	0.0523	>0.10
Sediment (along with the animal)	6	38.5979	1.5564	0.1196	>0.10
Station 2					
Adult					
Surface	6	-9.1633	17.1800	0.8863	<0.02*
Gills	6	-17.7168	16.9874	0.9054	<0.01
Stomach	6	28.0800	3.6780	0.2270	>0.10
Anterior intestine	6	-15.3750	17.6900	0.8089	<0.10 >0.05
Posterior intestine	6	-0.5029	13.5400	0.7847	<0.10 >0.05
Alimentary canal	6	-4.0550	11.6290	0.8423	<0.05* >0.02
Water (around the year)	12	26.1450	4.1900	0.5985	<0.05 >0.02
Water (along with the animal)	6	-1.0026	10.8306	0.7583	<0.10 >0.05
Sediment (around the year)	12	64.0029	-1.5049	-0.1796	>0.10
Sediment (along with the animal)	6	9.0090	10.4243	0.6947	>0.10

Table 40. Contd.

1	2	3	4	5	6	7	8	9
				b) Juveniles				
THB	0.1100	0.5537	0.0053	-0.3236	0.6605	0.7034	-0.1761	-0.1973
Gram negative	0.5374	0.0460	-0.0291	-0.0880	-0.3272	-0.2476	0.0495	-0.2142
Vibrio	0.2286	-0.1544	-0.0684	0.1186	-0.4638	-0.3385	-0.1845	-0.1315
Pseudomonas	0.1453	-0.0049	-0.1322	0.0147	0.0494	0.0249	0.3639	-0.3050
Micrococcus	-0.3924	0.2403	0.5041	-0.3110	0.6078	0.4877	-0.2834	0.0509
Bacillus	-0.5094	-0.3387	-0.4620	0.4829	-0.0085	0.0488	0.1231	0.0703
Coryneform group	0.4434	0.3345	0.1342	-0.3705	-0.0951	-0.3632	0.3943	0.6134
df : 6								
				c) Water				
THB	-0.0847	-0.1857	0.0775	0.1268	-0.3151	-0.2427	-0.0248	-0.0850
Gram negative	-0.4632	-0.5586	-0.5752*	0.5286	-0.0984	-0.4787	-0.1538	-0.1021
Vibrio	-0.1429	-0.1527	0.0260	0.1995	-0.2172	-0.4004	-0.3568	0.3033
Pseudomonas	-0.3921	-0.4132	-0.7112**	0.4754	0.1349	-0.2909	0.0476	-0.3898
Micrococcus	0.3696	0.5304	0.6153	-0.6273*	0.2653	0.4114	0.2739	0.1349
Bacillus	0.1543	0.2086	0.1925	-0.1282	-0.0386	0.2036	0.1056	-0.2557
Coryneform group	0.2932	0.1854	0.1153	-0.0855	-0.1228	0.1887	-0.2267	0.4289

df : 10

Table 40. Contd.

Station 2

1	2	3	4	5	6	7	8	9
			d) Adult surface					
THB	-0.2682	-0.0498	-0.1040	0.0770	-0.4359	0.7693	-0.2948	-0.2090
Gram negative	0.7482	-0.0134	0.5346	0.8803*	-0.1438	-0.1270	-0.8030	0.8380*
Vibrio	0.3834	-0.4737	-0.3755	0.6424	-0.1702	0.0108	-0.1533	0.0854
Pseudomonas	-0.2701	0.5287	0.4821	0.2464	-0.5659	0.0763	-0.3597	0.3225
Micrococcus	-0.8184*	0.0160	-0.5852	-0.8472*	0.0683	0.1069	0.8675*	-0.8665*
Bacillus	0.1469	-0.6533	-0.4099	-0.5625	0.6947	0.6532	0.2621	-0.5144
Coryneform group	0.0372	0.5813	0.4948	0.1195	-0.2099	-0.4574	-0.3644	0.4005
			e) gill					
THB	-0.8614*	0.1231	-0.4603	-0.8231*	0.0343	0.0093	0.8997*	-0.7676
Gram negative	0.6596	-0.0321	0.4264	0.8271*	-0.1163	-0.2784	-0.5775	0.7598
Vibrio	0.5568	-0.5760	-0.2958	0.6506	0.0064	0.0430	-0.2011	0.1769
Pseudomonas	0.1766	0.2864	0.5828	0.1206	0.1067	-0.2904	-0.1375	0.5056
Micrococcus	-0.7015	-0.0599	-0.5242	-0.9521**	0.2861	0.1542	0.8693*	-0.8446*
Bacillus	-0.0998	0.7443	0.0377	0.0153	-0.2929	0.3692	-0.4015	-0.0723
Coryneform group	-0.0755	0.2516	0.1427	0.0396	-0.2931	0.2075	-0.4190	0.0290

Table 40. Contd.

				f) Water				
THB	-0.0376	0.3130	0.3051	-0.6960*	0.0172	-0.3305	0.7840**	-0.5692
Gram negative	-0.2782	-0.5601	0.0470	0.5417	-0.4951	0.0027	-0.3527	0.5852*
Vibrio	-0.0448	-0.1118	0.2998	0.2992	-0.5672	-0.3902	-0.1367	0.2925
Pseudomonas	-0.4974	-0.6550	-0.2657	0.5971*	0.0564	0.3303	-0.6488*	0.6005*
Micrococcus	0.4105	0.5159	0.1467	-0.8464**	0.4598	0.0334	0.8833**	-0.7355**
Bacillus	-0.1198	0.1462	0.0339	0.1950	0.1725	-0.1054	-0.4201	0.0592
Coryneform group	0.0063	-0.1640	0.4543	0.2817	-0.3016	0.2201	-0.3956	0.1681

df : 10

Table 41. Correlation coefficients between the percentage bacterial genera of the animal and that of its environment

Sample/ genera	Vibrio	Pseudomonas	Micrococcus	Bacillus	Coryneform group
1	2	3	4	5	6
Station-1					
		Postlarvae			
Water	0.40	0.70	0.50	0.40	0.60
Sediment	0.60	-0.10	0.20	0.10	0.10
		Juvenile			
Water	0.20	0.60	0.60	0.80*	0.60
Sediment	0.30	-0.20	0	0.60	0.50
		Water			
Sediment	0.60	- 0	0.70	0.80	0.80

df : 5

Table 41 (Contd.)

1	2	3	4	5	6
Station-2					
		Adult			
		Surface			
Water	0.40	-0.10	0.90**	0	0.60
Sediment	0.40	0.90**	0.10	0.70	0.40
		Gill			
Water	0.40	0.40	0.90**	0.50	0.80
Sediment	0.80	0.60	0.30	-0.20	0.10
		Stomach			
Water	0.70	0.60	0.60	0.40	0.40
Sediment	0.40	-0.50	0.30	0.40	-0.10
		Anterior intestine			
Water	0.90**	0.90**	0.80	0.60	0.70
Sediment	0.80	0.40	0.10	-0.20	0
		Posterior intestine			
Water	0.90**	-0.40	0.60	0.70	-0.30
Sediment	0.90**	0.10	0.50	0.90**	-0.30
		Alimentary canal			
Water	0.90**	0.70	0.90**	0.80	0.50
Sediment	0.80	-0.40	0.30	0.80	-0.20
		Water			
Sediment	0.60	-0.30	0.50	0.50	0.10

df : 4

Table 42. Percentage occurrence of different hydrolytic forms in various samples

Sample	Total no. of isolates	Amylolytic	Caseinolytic	Gelatinolytic	Lipolytic	Ureolytic	Chitinolytic
Station 1							
Post larvae	246	43.90**	83.33	100	54.87	100	27.23
Juveniles	299	46.82	97.66	98.67	49.83	97.99	30.10
Water	156	50	82.05	99.35	44.23	87.18	17.31
Sediment	151	50.99	92.05	98.68	47.68	98.01	25.17
Station 2							
Adult							
Surface	100	53.00	97.00	97.00	61.00	100	13.00
Gill	122	31.15	94.26	100	48.36	99.18	21.31
Stomach	77	35.06	77.92	89.61	54.55	98.70	31.16
Anterior intestine	102	35.29	77.45	87.25	58.82	100	39.21
Posterior intestine	86	44.19	76.75	93.02	72.09	96.51	41.86
Alimentary canal	265	38.11	77.36	88.68	61.13	98.49	37.73
Water	166	52.40	86.75	99.39	45.18	96.99	25.30
Sediment	164	84.75	95.73	99.39	59.18	100	18.90
Total	1669	44.43	88.80	97.66	52.79	97.72	26.06*

*Total percentage calculated from the total isolates under each hydrolytic group

Table 43. Monthwise percentage distribution of different hydrolytic forms in various samples

Months	Total no. of isolates	Amylolytic	Caseinolytic	Gelatinolytic	Lipolytic	Ureolytic	Chitinolytic	Total no. of isolates	Amylolytic	Caseinolytic	Gelatinolytic	Lipolytic	Ureolytic	Chitinolytic
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Station 1														
		Post larvae							Juveniles					
Nov.	47	27.66	70.21	100	63.83	100	6.38	51	13.73	100	100	78.43	100	11.76
Dec.	31	41.94	74.19	100	45.16	100	-	51	76.47	94.11	100	54.90	100	64.70
Jan.	44	25	56.82	100	100	100	25	40	20	100	100	42.50	100	20
Feb.	35	22.86	100	100	34.29	100	5.71	47	61.70	91.49	91.49	12.77	97.87	10.63
Mar.	34	50	100	100	76.47	100	17.65	50	24	100	100	52	100	18
Apr.	15	60	100	100	60	100	46.67	20	90	100	100	60	100	45
May	40	92.50	100	100	-	100	95	20	85	100	100	85	100	85
Jun.								20	50	100	100	15	75	15
Total	246	43.90	83.33	100	54.88	100	27.24	299	46.82	97.66	98.66	49.83	97.99	30.10*

*Percentage calculated from the total isolates

Table 43. Contd. (Station 1)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
				Water							Sediment			
Nov.	13	53.85	61.54	100	61.54	53.85	30.77	7	57.14	100	100	42.86	71.43	-
Dec.	21	61.91	85.71	100	33.33	100	-	16	68.75	87.50	100	50	87.50	6.28
Jan.	14	-	14.28	100	85.71	100	-	15	60	80	100	100	100	53.33
Feb.	10	30	100	100	-	100	-	15	33.33	100	100	33.33	100	20
Mar.	17	41.18	76.47	100	52.94	100	17.64	6	100	33.33	100	33.33	100	16.67
Apr.	13	76.92	100	100	15.38	100	46.15	9	66.66	100	100	33.33	100	66.67
May	9	33.33	66.67	100	-	100	22.22	11	18.18	81.82	81.82	27.27	100	36.36
Jun.	12	50	100	100	8.33	100	16.67	17	35.29	94.12	100	35.29	100	11.76
Jul.	11	63.64	100	100	36.36	100	27.27	24	8.33	100	100	8.33	100	8.33
Aug.	9	55.56	88.89	88.89	88.89	88.89	22.22	10	80	100	100	70	100	-
Sep.	16	87.50	100	100	87.50	18.75	-	11	100	100	100	100	100	36.36
Oct.	11	27.27	100	100	36.36	90.91	45.45	10	70	100	100	70	100	60
Total	156	50	82.05	99.36	44.23	86.54	17.31	151	50.99	92.05	98.68	47.68	98.68	25.17*

Table 43. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Station 2														
							Adult							
			Surface								Gills			
Dec.	31	90.32	100	100	32.26	100	3.23	36	8.33	86.11	100	63.89	100	-
Jan.	24	12.50	100	100	75	100	8.33	31	22.58	100	100	41.94	100	9.68
Feb.	8	50	62.50	62.50	62.50	100	37.50	12	50	100	100	25	91.67	41.67
Mar.	12	25	100	100	75	100	8.33	15	13.33	100	100	40	100	-
Apr.	19	47.40	100	100	68.40	100	-	10	30	80	100	40	100	10
May	6	100	100	100	100	100	100	18	94.44	100	100	55.55	100	94.40
Total	100	53	97	97	61	100	13	122	31.15	94.26	100	48.36	99.18	21.31 *

Table 43. Contd. (Station 2)

1	2	3	4	5	6	7	8
			Stomach				
Dec.	14	50	14.29	57.14	14.29	100	-
Jan.	13	23.08	92.31	100	100	92.31	30.77
Feb.	12	58.33	100	100	66.67	100	25
Mar.	10	-	80	90	10	100	-
Apr.	19	47.37	94.74	94.74	94.74	100	89.47
May	9	11.11	88.89	100	-	100	-
Total	77	35.06	77.92	89.61	54.55	98.70	31.17 *

Table 43. Contd. (Station 2)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
			Anterior intestine								Posterior intestine			
Dec.	17	11.76	-	47.06	11.76	100	-	15	46.67	13.33	86.67	13.33	100	-
Jan.	25	16	100	100	100	100	-	24	33.33	95.83	100	83.33	100	8.33
Feb.	11	81.81	81.81	81.81	100	27.27	-	7	14.29	71.42	71.42	42.86	71.42	14.28
Mar.	19	36.84	84.21	94.73	47.37	100	36.84	10	50	80	100	8	90	50
Apr.	17	29.41	94.12	94.12	94.12	100	94.12	15	13.33	86.67	86.67	93.33	100	86.67
May	13	69.23	100	100	-	100	100	15	100	100	100	100	100	100
Total	102	35.29	77.45	87.25	58.82	100	39.22	86	44.19	76.74	93.02	72.09	96.51	41.86 *

Table 43. Contd. (Station 2)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
				Water						Sediment				
Nov.	15	100	100	100	33.33	100	33.33	10	50	100	100	20	100	-
Dec.	15	26.67	93.33	100	40	100	20	19	5.26	78.95	100	52.63	100	5.26
Jan.	18	-	38.89	94.44	66.67	94.44	-	24	20.83	91.67	100	79.17	100	20.83
Feb.	6	33.33	100	100	33.33	100	-	11	27.27	100	100	27.27	100	18.18
Mar.	16	75	50	100	18.75	100	18.75	8	25	100	100	50	100	12.50
Apr.	16	62.50	100	100	68.75	100	62.50	7	28.57	100	100	100	100	28.57
May	8	50	100	100	25	100	37.50	10	20	90	90	40	100	50
Jun.	16	25	93.75	100	12.50	100	6.25	22	22.27	100	100	31.82	100	4.50
Jul.	19	57.89	100	100	31.58	100	31.58	13	11.54	100	100	38.46	100	38.46
Aug.	14	42.86	92.86	100	50	100	35.71	9	77.78	100	100	77.78	100	11.11
Sep.	13	92.30	100	100	100	100	7.69	11	100	100	100	100	100	9.10
Oct.	10	70	100	100	60	70	40	20	30	100	100	90	100	25
Total	166	52.41	86.75	99.40	45.18	97.59	24.70	164	34.76	95.73	99.39	59.15	100	17.68

Table 44. Percentage distribution of various hydrolytic enzyme producers in different genera isolated from various samples

Genera	Total no. of isolates	Amylolytic	Caseinolytic	Gelatinolytic	Lipolytic	Ureolytic	Chitinolytic
Vibrio	364	83.24	100	100	71.70	99.18	95.88
Pseudomonas	267	38.58	94.38	98.87	50.94	93.26	9.74
Aeromonas	25	92.00	100	100	92.00	96.00	68.00
Acinetobacter	60	41.67	86.67	98.33	53.33	95.00	6.67
Flavobacterium	54	44.44	94.44	98.15	53.70	98.15	29.63
Alcaligenes	3	-	100	100	33.33	100	-
Moraxella	59	15.25	69.49	83.05	52.54	98.31	3.39
Enterobacteriaceae	23	56.52	95.65	100	26.09	95.65	13.04
Staphylococcus	6	50	83.33	100	50	100	-
Micrococcus	377	27.58	77.72	95.49	47.48	98.14	0.53
Bacillus	297	36.70	85.52	99.33	42.76	99.33	3.38
Coryneform group	134	18.66	89.55	96.26	39.55	99.25	1.49
Total	1669	739	1482	1630	881	1631	431

Table 45. Generic wise distribution of hydrolytic bacteria in various samples

Sample	Total no. of isolates	Amylolytic	Caseinolytic	Gelatinolytic	Lipolytic	Ureolytic	Chitinolytic	Total no. of isolates	Amylolytic	Caseinolytic	Gelatinolytic	Lipolytic	Ureolytic	Chitinolytic
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
			Vibrio							Pseudomonas				
Station 1														
Post larvae	53	98.11	100	100	28.34	100	94.34	22	13.64	100	100	59.09	100	13.64
Juveniles	78	85.90	100	100	79.49	100	100	49	51.02	97.95	100	42.86	89.79	20.41
Water	25	72	100	100	48	100	96	39	61.54	97.43	100	48.72	66.67	-
Sediment	22	90.90	100	100	86.36	100	86.36	41	17.07	92.68	95.12	26.83	100	14.63
Station 2														
Adult														
Surface	11	100	100	100	100	100	100	21	42.86	100	100	66.67	100	-
Gill	25	100	100	100	52	100	100	11	45.45	54.55	100	90.90	100	-
Stomach	22	54.09	100	100	95.45	100	95.45	3	33.33	100	100	66.67	100	33.33
Anterior intestine	35	60	100	100	62.85	100	100	11	36.36	100	100	81.82	100	18.18
Posterior intestine	30	66.67	100	100	100	100	100	10	-	90	100	90	100	10
Water	32	96.88	100	100	96.88	90.63	100	32	62.50	90.63	100	28.13	100	6.25
Sediment	31	80.65	100	100	80.65	100	80.65	28	17.85	96.42	96.42	67.86	100	3.57

Table 45. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Aeromonas							Acinetobacter					
Station 1														
Post larvae	1	100	100	100	100	100	100	7	71.43	100	100	71.43	100	14.28
Juveniles	2	50	100	100	100	100	-	7	57.14	71.43	85.71	57.14	85.71	-
Water	3	100	100	100	100	66.67	33.33	1	100	100	100	100	100	100
Sediment	3	100	100	100	100	100	100	6	66.67	100	100	16.67	83.33	-
Station 2														
Adult														
Surface	1	-	100	100	-	100	100	4	25	100	100	75	100	25
Gill	1	100	100	100	-	100	100	8	12.50	75	100	50	87.50	-
Stomach	2	100	100	100	100	100	-	7	14.29	71.42	100	71.42	100	-
Anterior intestine	2	100	100	100	100	100	100	6	33.33	100	100	66.66	100	-
Posterior intestine	4	100	100	100	100	100	100	5	-	60	100	60	100	-
Water	4	100	100	100	100	100	50	4	25	100	100	50	100	25
Sediment	2	100	100	100	100	100	100	5	100	100	100	-	100	-

Table 45. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
			Flavobacterium						Alcaligenes					
Station 1														
Post larvae	16	75	100	100	62.50	100	75	-	-	-	-	-	-	-
Juveniles	11	18.18	100	100	45.45	100	-	1	-	100	100	100	100	100
Water	4	-	75	100	-	100	-	-	-	-	-	-	-	-
Sediment	6	83.33	83.33	100	100	100	33.33	-	-	-	-	-	-	-
Station 2														
Adult														
Surface	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gill	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stomach	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anterior intestine	2	-	100	100	100	100	-	-	-	-	-	-	-	-
Posterior intestine	5	-	100	100	-	100	-	-	-	-	-	-	-	-
Water	4	25	75	75	50	75	50	1	-	100	100	-	100	-
Sediment	6	16.67	100	100	66.67	100	-	1	-	100	100	-	100	-

Table 45. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
			Moraxella						Enterobacteriaceae					
Station 1														
Post larvae	5	-	80	100	40	100	-	1	100	100	100	-	100	-
Juveniles	4	-	25	25	75	100	-	3	100	66.67	100	-	100	-
Water	6	50	50	100	16.67	100	-	1	-	100	100	-	100	-
Sediment	4	50	50	100	50	100	-	2	50	100	100	50	100	-
Station 2														
Adult														
Surface	14	14.29	78.57	78.57	57.14	100	-	3	66.67	100	100	33.33	100	-
Gill	8	-	100	100	50	100	-	3	100	100	100	66.67	100	-
Stomach	3	-	33.33	66.67	66.67	66.67	-	2	50	100	100	50	100	50
Anterior intestine	3	-	-	-	100	100	-	1	100	100	100	-	100	100
Posterior intestine	1	-	-	100	100	100	-	1	100	100	100	100	-	100
Water	8	12.50	100	100	25	100	12.50	3	-	100	100	-	100	-
Sediment	3	33.33	100	100	100	100	33.33	3	-	100	100	-	100	-

Table 45. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
			Staphylococcus							Micrococcus				
Station 1														
Post larvae	2	100	100	100	100	100	-	66	36.36	60.60	100	78.79	100	-
Juveniles	2	-	100	100	-	100	-	63	11.11	100	100	49.21	95.23	7.94
Water	-	-	-	-	-	-	-	26	38.46	61.54	100	57.69	76.92	-
Sediment	-	-	-	-	-	-	-	13	23.07	84.61	100	30.77	92.30	-
Station 2														
Adult														
Surface	1	100	100	100	100	100	-	38	71.05	100	100	47.37	100	-
Gill	-	-	-	-	-	-	-	52	-	100	100	38.46	100	-
Stomach	-	-	-	-	-	-	-	23	21.74	52.17	73.91	21.74	100	-
Anterior intestine	1	-	-	100	-	100	-	32	15.63	50	71.88	40.63	100	-
Posterior intestine	-	-	-	-	-	-	-	20	60	50	90	55	100	-
Water	-	-	-	-	-	-	-	31	32.26	77.42	100	35.43	100	-
Sediment	-	-	-	-	-	-	-	13	7.69	84.62	100	15.38	100	-

Table 45. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
			Bacillus						Coryneform group					
Station 1														
Post larvae	44	9.09	68.18	100	25	100	-	29	13.79	100	100	82.76	100	-
Juveniles	50	50	100	100	38	100	-	29	20.69	100	100	13.79	100	-
Water	36	52.77	69.44	100	47.22	100	-	15	-	73.33	93.33	6.67	93.33	6.67
Sediment	43	60.47	90.69	100	37.20	100	18.60	11	54.55	100	100	81.71	90.90	-
Station 2														
Adult														
Surface	6	-	100	100	83.33	100	-	1	-	100	100	-	100	-
Gill	9	-	100	100	38.46	100	-	5	60	100	100	-	100	-
Stomach	6	50	83.33	100	50	100	33.33	9	11.11	88.88	88.88	11.11	100	-
Anterior intestine	4	25	50	100	50	100	-	5	-	80	80	60	100	-
Posterior intestine	8	12.50	50	75	12.50	75	-	2	-	-	-	100	100	-
Water	31	54.83	70.96	100	32.26	100	-	16	6.25	87.50	100	25	100	6.25
Sediment	60	21.67	100	100	61.67	100	-	12	33.33	66.67	100	41.67	100	-

Table 46. Percentage contribution of different genera for a particular hydrolytic enzyme

Hydrolytic forms	Total no. of isolates	Vibrio	Pseudomonas	Aeromonas	Acinetobacter	Flavobacterium	Alcaligenes	Moraxella	Enterobacteriaceae	Staphylococcus	Micrococcus	Bacillus	Coryneform group
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Station 1													
				Post larvae									
Amylolytic	108	48.15	2.78	0.93	4.63	11.11	-	-	0.93	1.85	22.22	3.70	3.70
Caseinolytic	205	25.85	10.73	0.49	3.41	7.80	-	1.95	0.49	0.98	19.51	14.63	14.15
Gelatinolytic	246	21.54	8.94	0.41	2.84	6.50	-	2.03	0.41	0.81	26.83	17.88	11.78
Lipolytic	135	11.11	9.62	0.74	3.70	7.41	-	1.48	-	1.48	38.52	8.14	17.78
Ureolytic	246	21.54	8.94	0.41	2.85	6.50	-	2.03	0.41	0.81	26.82	17.89	11.79
Chitinolytic	67	74.63	4.48	1.49	1.49	17.91	-	-	-	-	-	-	-

Table 46. Contd.(Station 1)

1	2	3	4	5	6	7	8	9	10	11	12	13	14
						Juveniles							
Amylolytic	140	47.86	17.86	0.71	2.85	1.43	-	-	2.14	-	5.00	17.86	4.29
Caseinolytic	292	26.71	16.44	0.69	1.71	3.77	0.34	0.34	0.69	0.69	21.57	17.12	9.93
Gelatinolytic	295	26.44	16.60	0.68	2.03	44.00	0.34	0.34	1.02	0.68	21.36	16.95	9.83
Lipolytic	149	41.61	14.09	1.34	2.68	3.35	0.67	2.01	-	-	18.79	12.75	2.68
Ureolytic	293	26.62	15.01	0.68	2.05	3.75	0.34	1.37	1.02	0.68	21.50	17.06	9.89
Chitinolytic	90	86.67	11.11	-	-	-	-	-	-	-	2.22	-	-
						Water							
Amylolytic	78	23.08	30.76	3.85	1.28	-	-	2.34	3.84	-	12.82	24.35	-
Caseinolytic	128	19.53	29.69	2.34	0.78	2.34	-	2.34	0.78	-	12.50	21.09	8.59
Gelatinolytic	155	16.13	25.16	1.94	0.65	2.58	-	3.87	0.65	-	16.78	23.26	9.03
Lipolytic	69	17.39	27.53	4.34	1.45	-	-	1.45	-	-	21.74	24.63	1.45
Ureolytic	135	18.52	19.26	1.48	0.74	2.96	-	4.44	0.74	-	14.81	26.67	13.37
Chitinolytic	27	88.89	-	3.70	3.70	-	-	-	-	-	-	-	3.70

Table 46. Contd. (Station 1)

1	2	3	4	5	6	7	8	9	10	11	12	13	14
						Sediment							
Amylolytic	77	25.97	9.09	3.89	5.19	6.49	-	2.59	1.29	-	3.90	33.77	7.80
Caseinolytic	139	15.83	27.34	2.16	4.32	3.59	-	1.44	1.44	-	7.91	28.05	7.91
Gelatinolytic	149	14.77	26.17	2.01	4.03	4.03	-	2.68	1.34	-	8.72	28.85	12.50
Lipolytic	72	26.39	15.28	4.17	1.39	8.33	-	2.78	1.39	-	5.56	22.22	12.50
Ureolytic	149	14.77	27.52	2.01	3.36	4.03	-	2.68	1.34	-	8.05	28.85	7.38
Chitinolytic	38	50.00	15.79	7.89	-	5.26	-	-	-	-	-	21.05	-
Station 2													
Adult													
						Surface							
Amylolytic	53	20.75	16.98	-	1.89	-	-	3.77	3.77	1.89	50.94	-	-
Caseinolytic	97	11.34	21.65	1.03	4.18	-	-	11.34	3.09	1.03	39.18	6.19	1.03
Gelatinolytic	97	11.34	21.65	1.03	4.12	-	-	11.34	3.09	1.03	39.18	6.19	1.03
Lipolytic	61	18.03	22.95	-	4.92	-	-	13.11	1.64	1.64	29.51	8.19	-
Ureolytic	100	11.00	21.00	1.00	4.00	-	-	14.00	3.00	1.00	38.00	6.00	1.00
Chitinolytic	13	84.62	-	7.69	7.69	-	-	-	-	-	-	-	-

Table 46. Contd. (Station 2)

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Gill								
Amylolytic	38	65.79	13.16	2.63	2.63	-	-	-	7.89	-	-	-	7.89
Caseinolytic	115	21.74	5.21	0.87	5.21	-	-	6.96	2.61	-	45.22	7.83	4.35
Gelatinolytic	122	20.49	9.02	0.82	6.56	-	-	6.56	2.46	-	42.62	7.38	4.09
Ureolytic	121	20.66	9.09	0.83	5.79	-	-	6.61	2.48	-	42.97	7.44	4.13
Lipolytic	59	22.03	16.95	-	6.78	-	-	6.78	3.38	-	33.89	10.17	-
Chitinolytic	26	96.15	-	3.85	-	-	-	-	-	-	-	-	-
					Stomach								
Amylolytic	27	48.15	3.70	7.40	3.70	-	-	-	3.70	-	18.52	11.11	3.70
Caseinolytic	60	36.67	5.00	3.33	8.33	-	-	1.67	3.33	-	20.00	8.33	13.33
Gelatinolytic	69	31.88	4.35	2.89	10.14	-	-	2.89	2.89	-	24.63	10.00	11.59
Lipolytic	42	50.00	4.76	4.76	11.90	-	-	4.76	2.38	-	11.90	7.14	2.38
Ureolytic	76	28.95	3.95	2.63	9.21	-	-	2.63	2.63	-	30.26	7.89	11.84
Chitinolytic	24	83.33	4.17	-	-	-	-	-	4.17	-	-	8.33	-

Table 46. Contd. (Station 2)

1	2	3	4	5	6	7	8	9	10	11	12	13	14
						Anterior intestine							
Amylolytic	36	58.33	11.11	5.56	5.56	-	-	-	2.78	-	13.59	2.78	-
Caseinolytic	79	44.30	13.92	2.53	7.59	2.53	-	-	1.27	-	20.25	2.53	5.06
Gelatinolytic	89	39.33	12.36	2.25	6.74	2.25	-	-	1.12	1.12	25.84	4.49	4.49
Lipolytic	60	36.67	15.00	3.33	6.66	3.33	-	5.00	-	-	21.67	3.33	5.00
Ureolytic	102	34.31	10.78	1.96	5.88	1.96	-	2.94	0.98	0.98	31.37	3.92	4.90
Chitinolytic	40	87.50	5.00	5.00	-	-	-	-	2.50	-	-	-	-
						Posterior intestine							
Amylolytic	38	52.63	-	10.53	-	-	-	-	2.63	-	31.58	2.63	-
Caseinolytic	66	45.45	13.69	6.06	4.55	7.58	-	-	1.52	-	15.15	6.06	-
Gelatinolytic	80	37.50	12.50	5.00	6.25	6.25	-	1.25	1.25	-	22.50	7.50	-
Lipolytic	62	48.38	14.52	6.45	4.84	-	-	1.61	1.61	-	17.74	1.61	3.23
Ureolytic	83	36.14	12.05	4.82	6.02	6.02	-	1.20	-	-	24.10	7.23	2.41
Chitinolytic	36	83.33	2.78	11.11	-	-	-	-	2.78	-	-	-	-

Table 46. Contd. (Station 2)

1	2	3	4	5	6	7	8	9	10	11	12	13	14
						Alimentary canal							
Amylolytic	101	53.47	4.95	7.92	2.97	-	-	-	2.97	-	21.78	4.95	0.99
Caseinolytic	205	42.43	11.22	3.90	6.83	3.41	-	0.49	1.95	-	18.54	5.37	5.85
Gelatinolytic	238	36.55	10.08	3.36	7.56	2.94	-	1.26	1.68	0.42	24.37	6.72	5.04
Lipolytic	164	44.51	12.26	4.88	7.32	1.22	-	3.66	1.22	-	17.68	3.66	3.66
Ureolytic	261	3.33	10.72	3.06	6.90	2.68	-	2.90	1.15	0.38	28.74	6.13	6.13
Chitinolytic	100	85.00	4.00	6.00	-	-	-	-	3.00	-	-	2.00	-
						Water							
Amylolytic	87	35.63	22.98	4.59	1.15	2.29	-	1.15	-	-	11.49	19.54	1.15
Caseinolytic	144	22.22	20.14	2.78	2.78	2.08	0.69	5.56	2.08	-	16.67	15.28	9.72
Gelatinolytic	165	19.39	19.39	2.42	2.42	1.82	0.61	4.85	1.82	-	18.79	18.79	9.70
Lipolytic	75	41.33	12.00	5.33	2.67	2.67	-	2.67	-	-	14.67	13.33	5.33
Ureolytic	162	17.90	19.75	1.47	2.47	1.85	0.62	4.94	1.85	-	19.13	19.14	9.88
Chitinolytic	41	78.05	4.88	4.88	2.44	4.88	-	2.44	-	-	-	-	2.44

Table 46. Contd. (Station 2)

1	2	3	4	5	6	7	8	9	10	11	12	13	14
						Sediment							
Amylolytic	57	43.86	8.77	3.51	8.77	1.75	-	1.75	-	-	1.75	22.81	7.02
Caseinolytic	157	19.75	17.20	1.27	3.18	3.82	0.64	1.91	1.91	-	7.01	38.21	5.09
Gelatinolytic	163	19.02	16.56	1.23	3.07	3.68	0.61	1.84	1.84	-	7.98	36.81	7.36
Lipolytic	97	25.77	19.59	2.06	-	4.12	-	3.09	-	-	2.06	38.14	5.15
Ureolytic	164	18.90	17.07	1.21	3.05	3.66	0.61	1.83	1.83	-	7.93	36.59	7.32
Chitinolytic	29	86.21	3.45	6.89	-	-	-	3.45	-	-	-	-	-

Table 47. Percentage of isolates showing maximum growth in varying NaCl concentrations, pH and temperature

Months and year	Total no. of isolates	NaCl concentrations (%)					pH					Temperature (°C)				
		0	1	3	7	10	2	4	7	9	11	4	10	30	40	50
Nov. '81	143	2.09	69.93	23.78	1.39	2.79	-	-	25.17	74.83	-	-	-	78.32	21.68	-
Dec.	266	3.91	34.37	47.36	13.28	3.13	-	-	63.28	30.46	9.77	-	-	85.76	12.11	2.73
Jan. '82	272	8.82	33.82	25.37	23.16	8.82	-	-	65.07	34.93	-	-	19.12	73.16	6.25	1.47
Feb.	174	1.15	17.24	74.14	6.32	1.15	-	-	43.68	56.32	-	-	1.15	80.46	18.39	-
Mar.	197	-	35.53	32.49	22.84	9.14	-	-	76.65	21.32	2.03	-	-	82.74	17.26	-
Apr.	160	6.88	11.88	58.13	19.38	3.75	-	-	77.50	22.50	-	-	-	89.38	10.63	-
May	159	2.52	15.09	38.99	10.69	32.70	-	-	27.67	55.32	16.98	-	-	100	-	-
Jun.	87	4.59	27.58	47.12	8.39	2.29	-	-	68.96	29.89	1.15	-	5.75	77.01	17.24	-
Jul.	67	20.89	38.81	40.29	-	-	-	-	67.16	31.34	1.49	-	10.74	85.08	4.48	-
Aug.	42	21.42	40.48	38.09	-	-	-	-	73.81	23.81	2.38	-	-	97.62	2.38	-
Sep.	51	1.96	68.63	29.41	-	-	-	-	68.27	7.84	5.88	-	-	68.63	31.37	-
Oct.	51	7.84	35.29	56.86	-	-	-	-	64.71	35.29	-	-	7.84	74.50	17.64	-
Total	1669	5.15	32.77	42.24	12.88	6.95	-	-	58.89	37.22	3.78	-	4.19	82.80	12.34	0.66*

*Percentage calculated from the total isolates

Table 48. Percentage of isolates showing maximum growth in varying NaCl concentrations, pH and temperature

Sample	Total no. of isolates	NaCl concentrations (%)					pH					Temperature (°C)				
		0	1	3	7	10	2	4	7	9	11	4	10	30	40	50
Station 1																
Post larvae	246	0.41	21.14	55.69	19.38	2.03	-		36.18	63.82	-	-	12.19	69.11	18.69	-
Juveniles	299	0.67	39.46	30.10	20.40	9.36	-		63.21	34.45	2.34	-	2.34	82.27	13.04	2.34
Water	156	5.13	32.69	40.38	13.46	8.33	-	-	67.94	30.12	0.92	-	2.56	77.56	19.87	-
Sediment	151	11.92	30.46	40.39	7.95	9.27	-	-	59.61	39.73	0.66	-	7.94	82.78	9.27	-
Station 2																
Adult																
Surface	100	7.00	36.00	37.00	5.00	15.00	-	-	64.00	36.00	-	-	1.00	78.00	21.00	-
Gill	122	5.74	20.49	54.09	4.92	14.75	-	-	60.66	39.34	-	-	0.82	87.70	9.02	2.46
Stomach	77	7.79	23.38	58.44	9.09	1.29	-	-	51.95	38.96	9.09	-	-	100	-	-
Anterior intestine	102	6.86	23.53	49.02	14.71	5.88	-	-	61.76	14.70	23.53	-	2.94	88.24	8.82	-
Posterior intestine	86	9.30	34.88	37.20	15.12	3.49	-	-	59.30	25.58	15.12	-	1.16	94.14	3.49	1.16
Water	166	9.64	50.60	24.69	10.84	4.21	-	-	69.98	27.71	2.40	-	6.63	86.74	6.63	-
Sediment	164	3.66	38.42	50.61	3.66	3.66	-	-	61.58	35.98	2.44	-	-	87.19	12.80	-
Total	1669	5.15	32.77	42.12	12.88	6.95	-	-	58.89	37.32	3.77	-	4.19	32.80	12.34	0.66*

*Percentage calculated from the total isolates

Table 49. Growth response of different genera to NaCl concentrations, pH and temperature

Genera	Total no. of isolates	Percentage showing maximum growth in varying NaCl concentration					pH					Temperature (°C)				
		0	1	3	7	10	2	4	7	9	11	4	10	30	40	50
Vibrio	364	1.37	16.48	45.87	19.78	16.48	-	-	53.02	38.73	8.24	-	1.65	92.30	6.04	-
Pseudomonas	267	11.98	28.83	45.31	3.74	9.73	-	-	68.54	26.22	5.24	-	5.24	73.03	21.34	0.37
Aeromonas	25	-	48.00	36.00	8.00	8.00	-	-	92.00	8.00	-	-	-	88.00	12.00	-
Acinetobacter	61	39.34	31.15	29.51	-	-	-	-	62.29	37.70	-	-	1.64	80.32	18.03	-
Flavobacterium	54	1.90	55.56	20.37	22.22	-	-	-	48.15	50	1.85	-	3.70	90.74	5.56	-
Alcaligenes	3	33.33	33.33	33.33	-	-	-	-	100	-	-	-	-	100	-	-
Moraxella	59	-	30.51	50.85	10.17	8.47	-	-	64.40	35.59	-	-	-	98.30	1.69	-
Enterobacteriaceae	23	-	69.57	21.74	-	8.69	-	-	86.96	13.04	-	-	4.35	91.30	4.34	-
Staphylococcus	6	-	33.33	-	66.67	-	-	-	33.33	66.67	-	-	-	66.67	33.33	-
Micrococcus	377	2.91	48.28	39.79	9.01	-	-	-	49.86	45.62	4.51	-	3.71	89.38	6.89	-
Bacillus	297	2.36	26.59	44.44	21.88	4.71	-	-	65.31	34.68	-	-	10.43	68.35	21.21	-
Coryneform group	133	3.76	37.59	45.86	7.52	5.26	-	-	56.39	42.85	0.75	-	0.75	78.95	12.78	7.52
Total	1669	5.15	32.77	42.24	12.88	6.95	-	-	58.90	37.33	3.77	-	4.19	82.80	12.34	0.66*

*Percentage calculated from the total isolates

Fig. 11. Physico-chemical parameters measured in the natural environment at Station 1 and 2 during the study period

Fig. 12. Quantitative estimation of Total Heterotrophic Bacteria (THB) and the trend lines with respect to to months in P. indicus collected from the natural environment

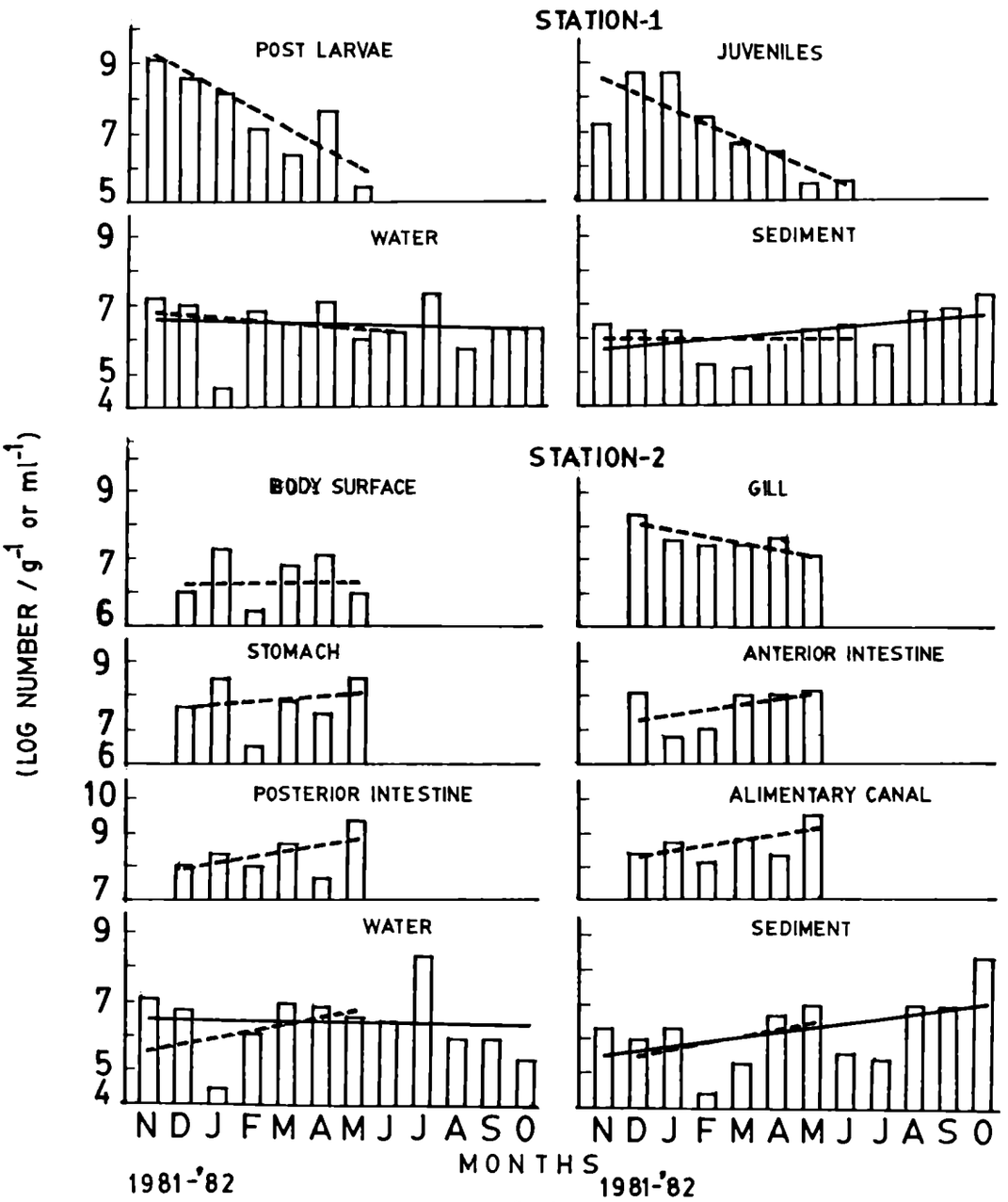

STATION-1
POST LARVAE
JUVENILES
WATER
SEDIMENT
STATION-2
BODY SURFACE
GILL
STOMACH
ANTERIOR INTESTINE
POSTERIOR INTESTINE
ALIMENTARY CANAL
WATER
SEDIMENT
(LOG NUMBER / g⁻¹ or ml⁻¹)
N D J F M A M J J A S O N D J F M A M J J A S O
MONTHS
1981-'82
1981-'82

FIG.12.

Fig. 13a. Percentage occurrence of Gram-negative bacteria and the trend lines in various samples of Station 1

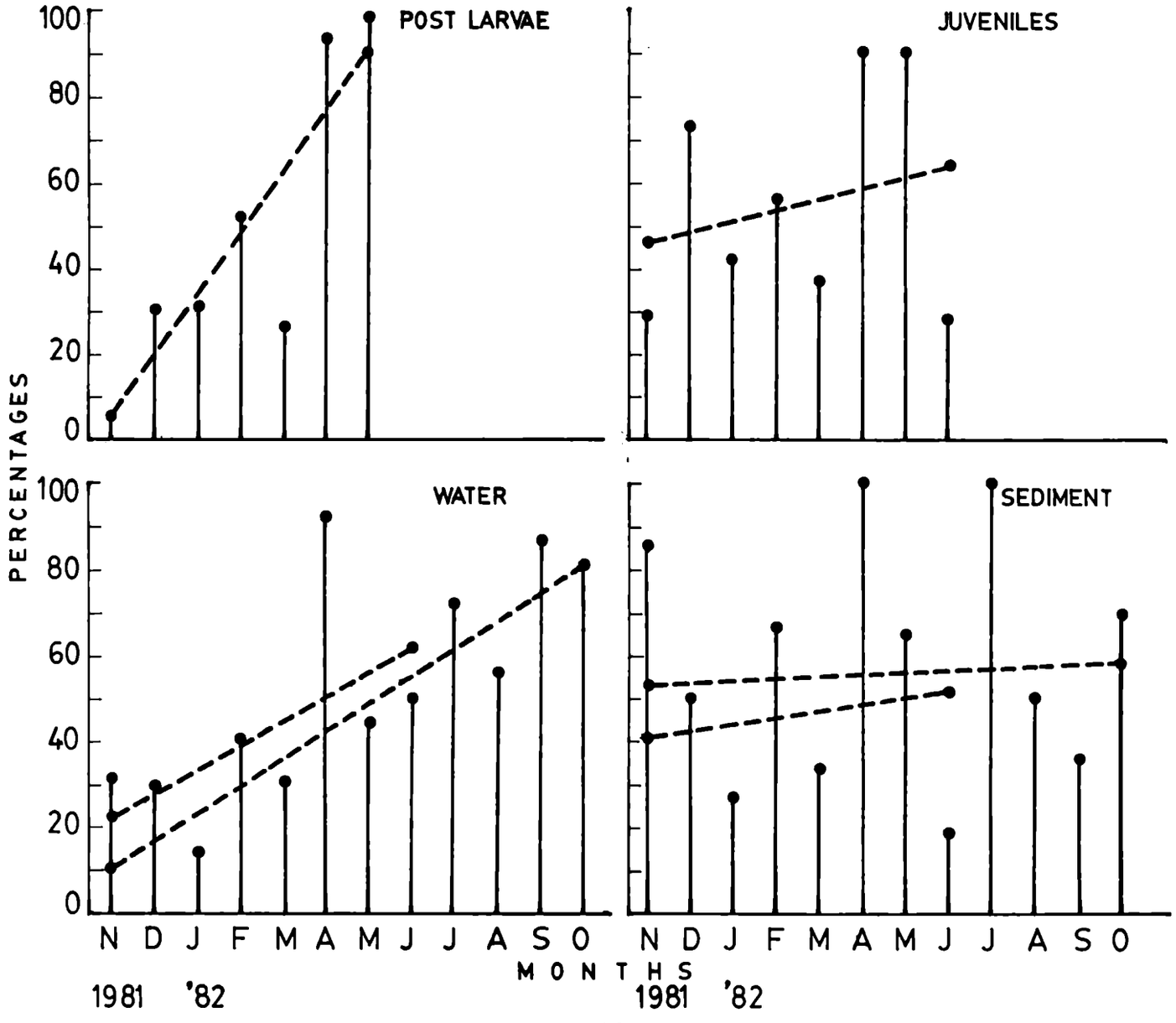

POST LARVAE
JUVENILES
WATER
SEDIMENT
PERCENTAGES
100
80
60
40
20
0
N D J F M A M J J A S O
MONTHS
1981 '82

FIG.13 a.

Fig. 13b. Percentage occurrence of Gram-negative bacteria and the trend lines in various samples of Station 2

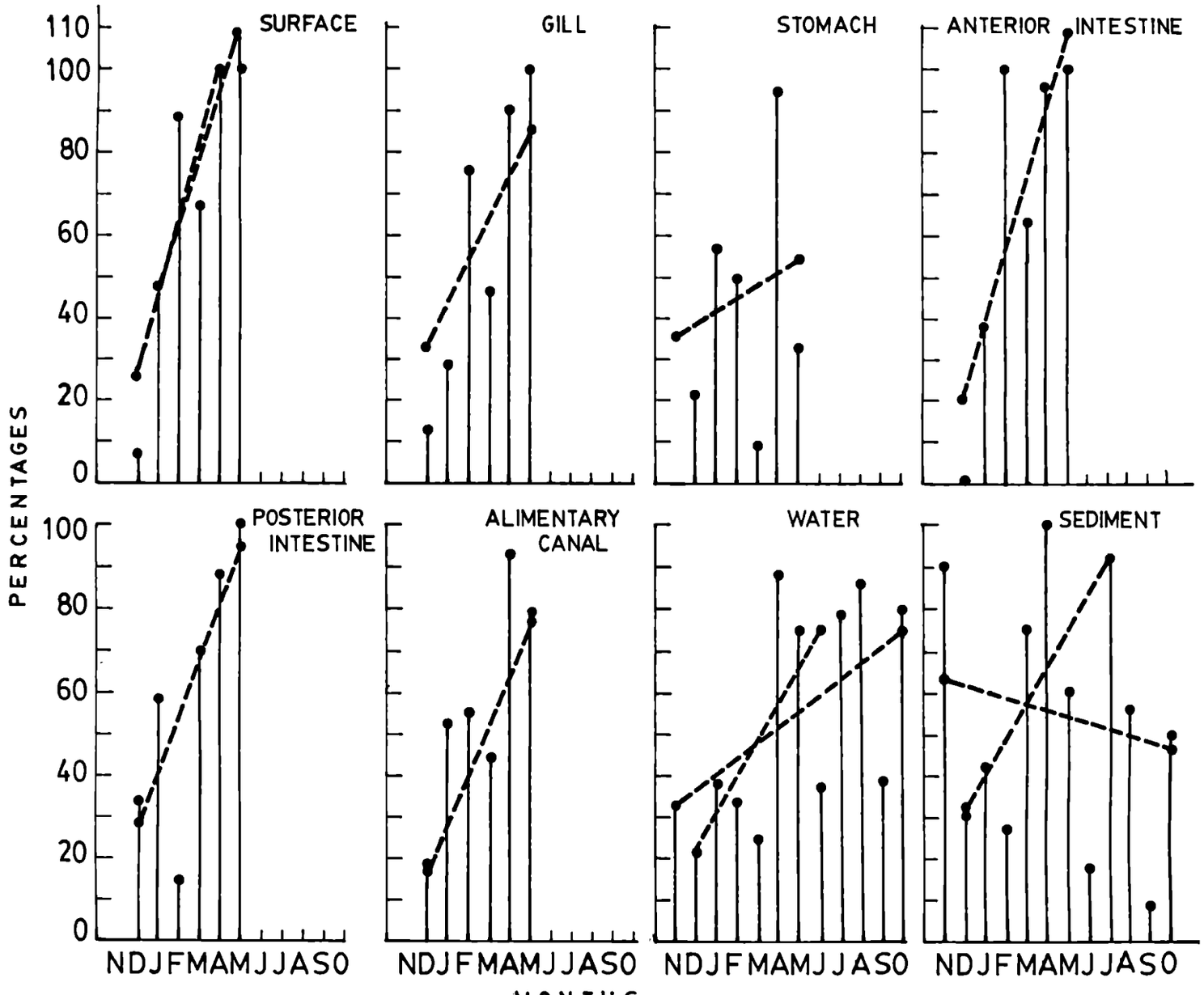

SURFACE
GILL
STOMACH
ANTERIOR INTESTINE
POSTERIOR INTESTINE
ALIMENTARY CANAL
WATER
SEDIMENT
PERCENTAGES
110
100
80
60
40
20
0
N D J F M A M J J A S O
MONTHS
1981-'82

FIG.13b.

Fig. 14b. Percentage generic composition of bacterial strains isolated from prawn, water and sediment in Station 2

V - *Vibrio*

P - *Pseudomonas*

Ae - *Aeromonas*

A - *Acinetobacter*

F - *Flavobacterium*

Al - *Alcaligenes*

M - *Moraxella*

E - Enterobacteriaceae

S - *Staphylococcus*

Mi - *Micrococcus*

B - *Bacillus*

C - Coryneform group

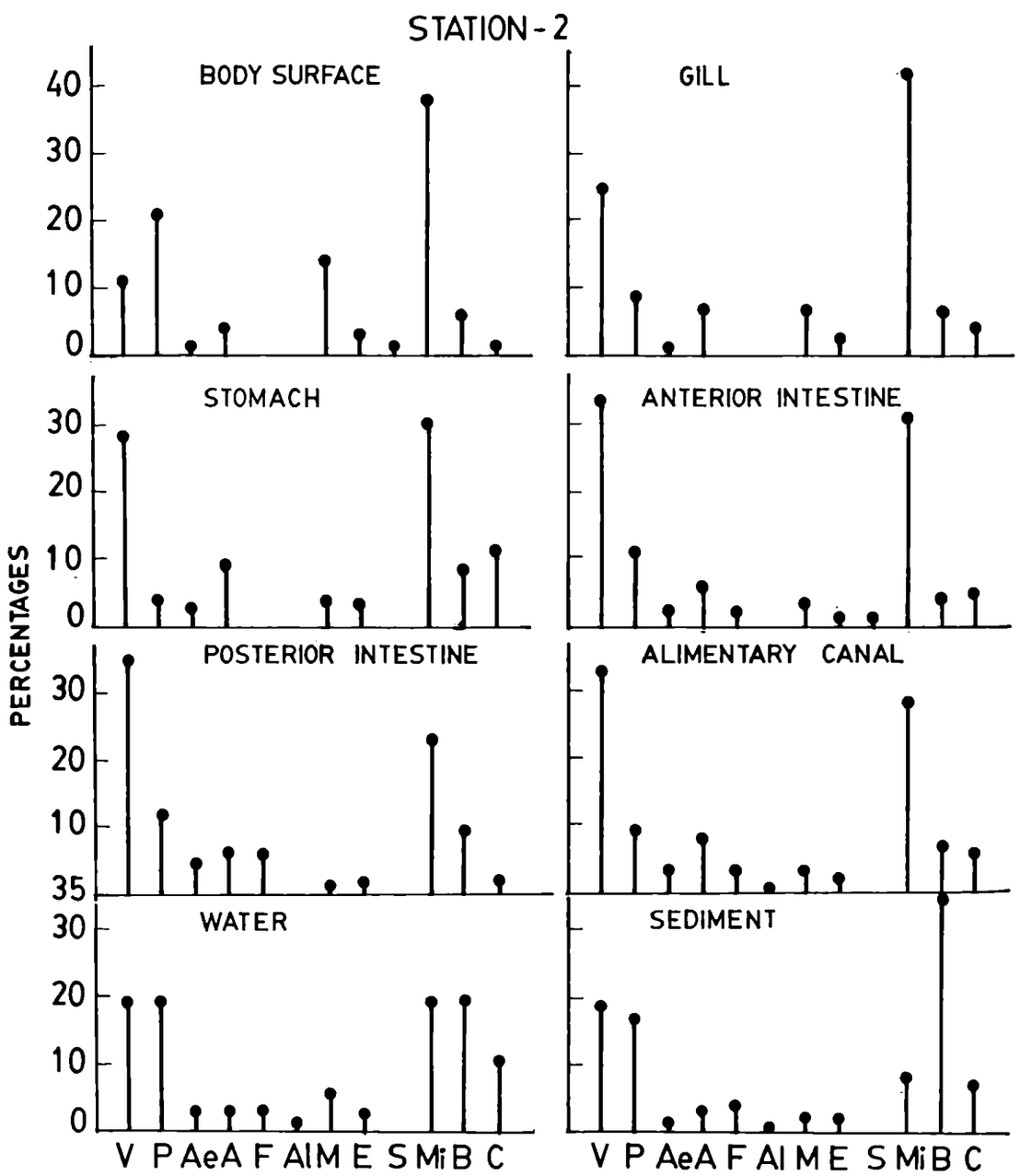

STATION - 2
BODY SURFACE
GILL
STOMACH
ANTERIOR INTESTINE
POSTERIOR INTESTINE
ALIMENTARY CANAL
WATER
SEDIMENT
PERCENTAGES
V P AeA F AlM E S MiB C

FIG. 14 b.

Fig. 15a. Monthwise percentage distribution of different genera of bacteria isolated from postlarvae

V - *Vibrio*

P - *Pseudomonas*

Ae - *Aeromonas*

A - *Acinetobacter*

F - *Flavobacterium*

Al - *Alcaligenes*

Mo - *Moraxella*

E - Enterobacteriaceae

S - *Staphylococcus*

Mi - *Micrococcus*

B - *Bacillus*

C - Coryneform group

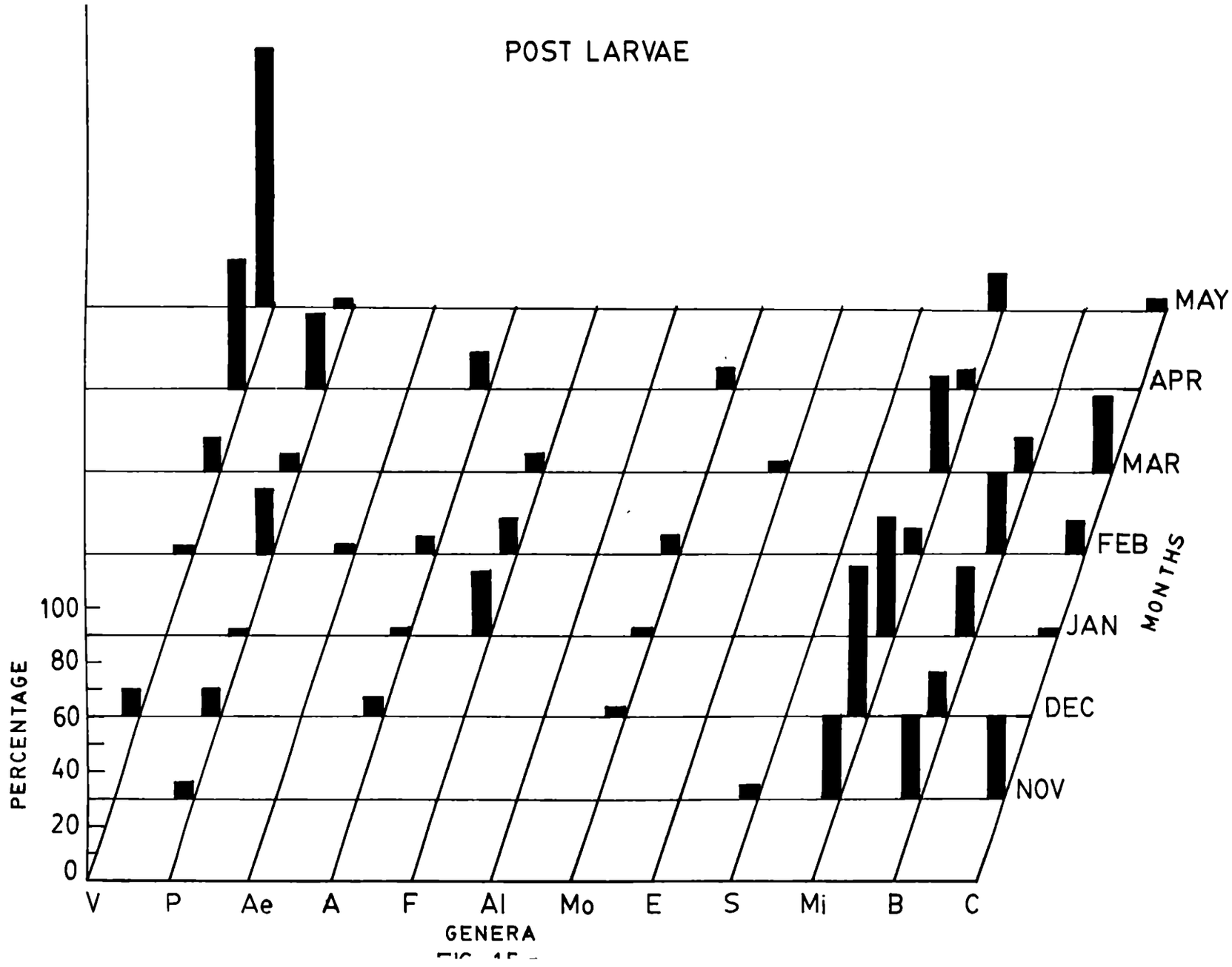

POST LARVAE
MAY
APR
MAR
FEB
JAN
DEC
NOV
MONTHS
PERCENTAGE
100
80
60
40
20
0
V
P
Ae
A
F
Al
Mo
E
S
Mi
B
C
GENERA

Fig. 15b. Monthwise percentage distribution of different genera of bacteria isolated from juveniles

V - *Vibrio*

P - *Pseudomonas*

Ae - *Aeromonas*

A - *Acinetobacter*

F - *Flavobacterium*

Al - *Alcaligenes*

Mo - *Moraxella*

E - Enterobacteriaceae

S - *Staphylococcus*

Mi - *Micrococcus*

B - *Bacillus*

C - Coryneform group

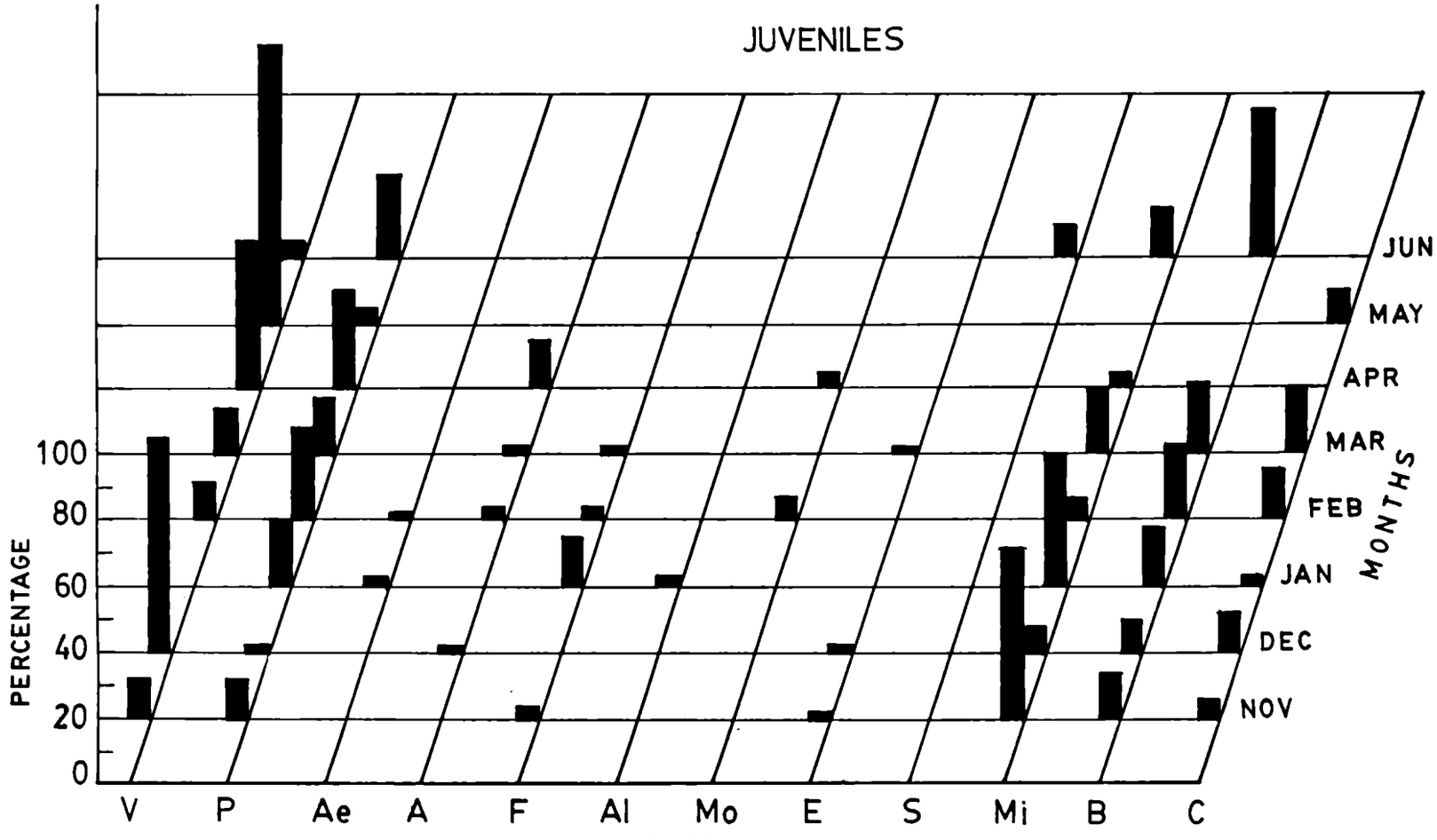

JUVENILES
PERCENTAGE
100
80
60
40
20
0
V
P
Ae
A
F
Al
Mo
E
S
Mi
B
C
JUN
MAY
APR
MAR
FEB
JAN
DEC
NOV
MONTHS

FIG.15b.

Fig. 15c. Monthwise percentage distribution of different genera of bacteria isolated from water of Station 1

V - *Vibrio*

P - *Pseudomonas*

Ae - *Aeromonas*

A - *Acinetobacter*

F - *Flavobacterium*

Al - *Alcaligenes*

Mo - *Moraxella*

E - Enterobacteriaceae

S - *Staphylococcus*

Mi - *Micrococcus*

B - *Bacillus*

C - Coryneform group

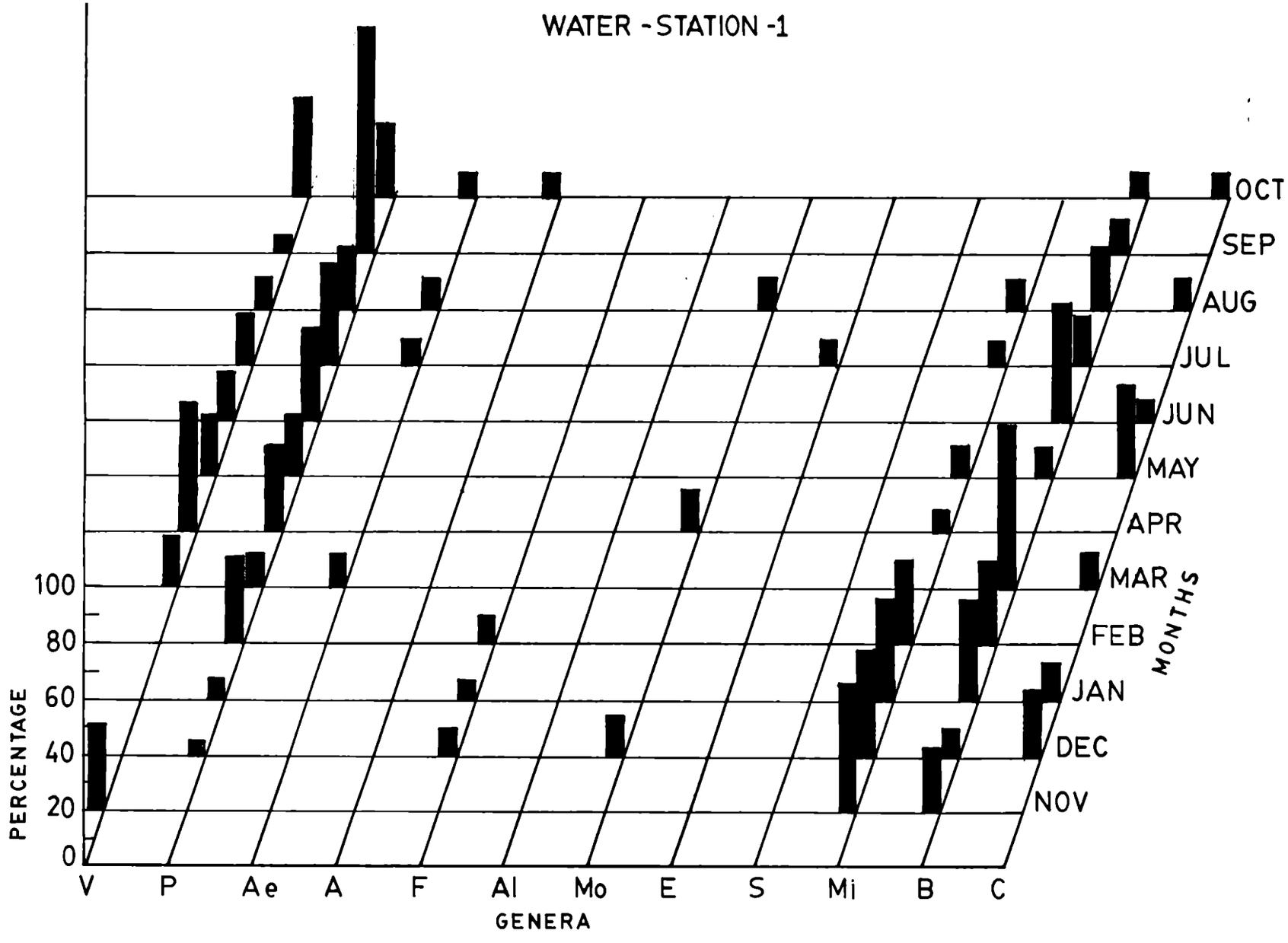

WATER - STATION -1
PERCENTAGE
0
20
40
60
80
100
V
P
Ae
A
F
Al
Mo
E
S
Mi
B
C
GENERA
OCT
SEP
AUG
JUL
JUN
MAY
APR
MAR
FEB
JAN
DEC
NOV
MONTHS

Fig. 15e. Monthwise percentage distribution of different genera of bacteria isolated from body surface of adults

V - *Vibrio*

P - *Pseudomonas*

Ae - *Aeromonas*

A - *Acinetobacter*

F - *Flavobacterium*

Al - *Alcaligenes*

Mo - *Moraxella*

E - Enterobacteriaceae

S - *Staphylococcus*

Mi - *Micrococcus*

B - *Bacillus*

C - Coryneform group

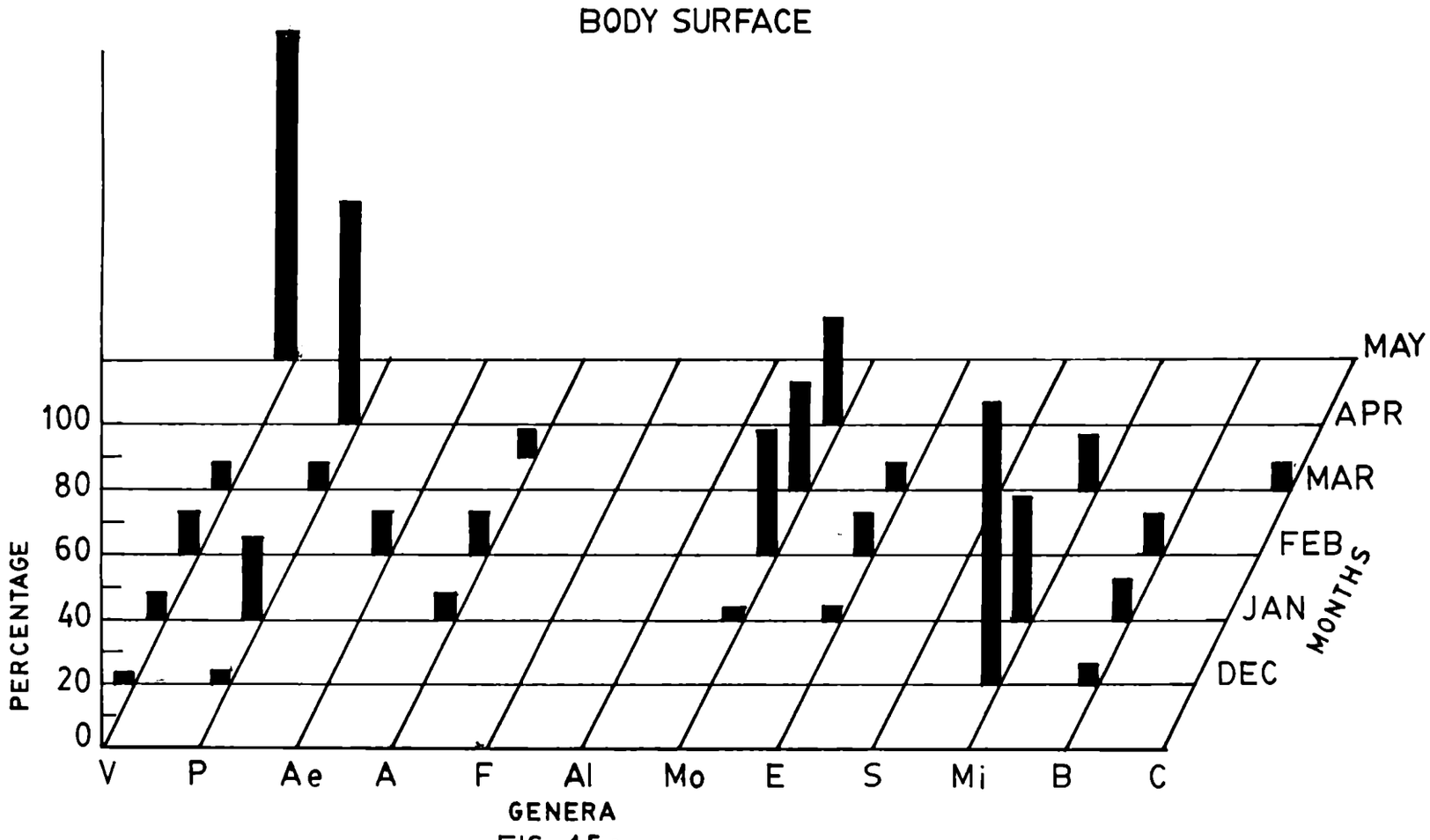

BODY SURFACE
PERCENTAGE
100
80
60
40
20
0
V
P
Ae
A
F
Al
Mo
E
S
Mi
B
C
GENERA
MAY
APR
MAR
FEB
JAN
DEC
MONTHS

FIG. 15e

Fig. 15f. Monthwise percentage distribution of different genera of bacteria isolated from gill of adults

V - *Vibrio*

P - *Pseudomonas*

Ae - *Aeromonas*

A - *Acinetobacter*

F - *Flavobacterium*

Al - *Alcaligenes*

Mo - *Moraxella*

E - Enterobacteriaceae

S - *Staphylococcus*

Mi - *Micrococcus*

B - *Bacillus*

C - Coryneform group

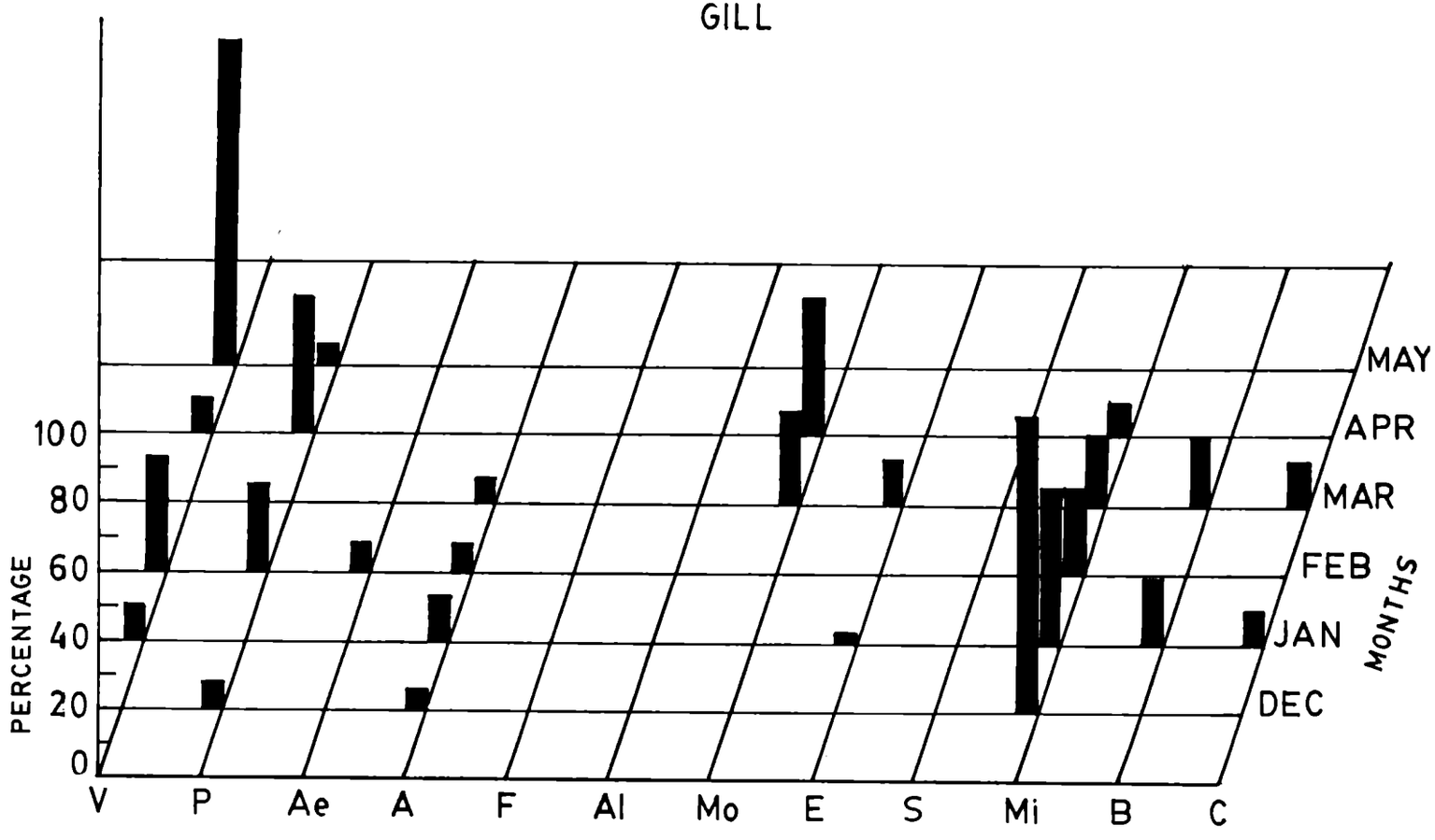

GILL
PERCENTAGE
100
80
60
40
20
0
V
P
Ae
A
F
Al
Mo
E
S
Mi
B
C
GENERA
MAY
APR
MAR
FEB
JAN
DEC
MONTHS
FIG. 15 F.

Fig. 15g. Monthwise percentage distribution of different genera of bacteria isolated from stomach content of adults

V - *Vibrio*

P - *Pseudomonas*

Ae - *Aeromonas*

A - *Acinetobacter*

F - *Flavobacterium*

Al - *Alcaligenes*

Mo - *Moraxella*

E - Enterobacteriaceae

S - *Staphylococcus*

Mi - *Micrococcus*

B - *Bacillus*

C - Coryneform group

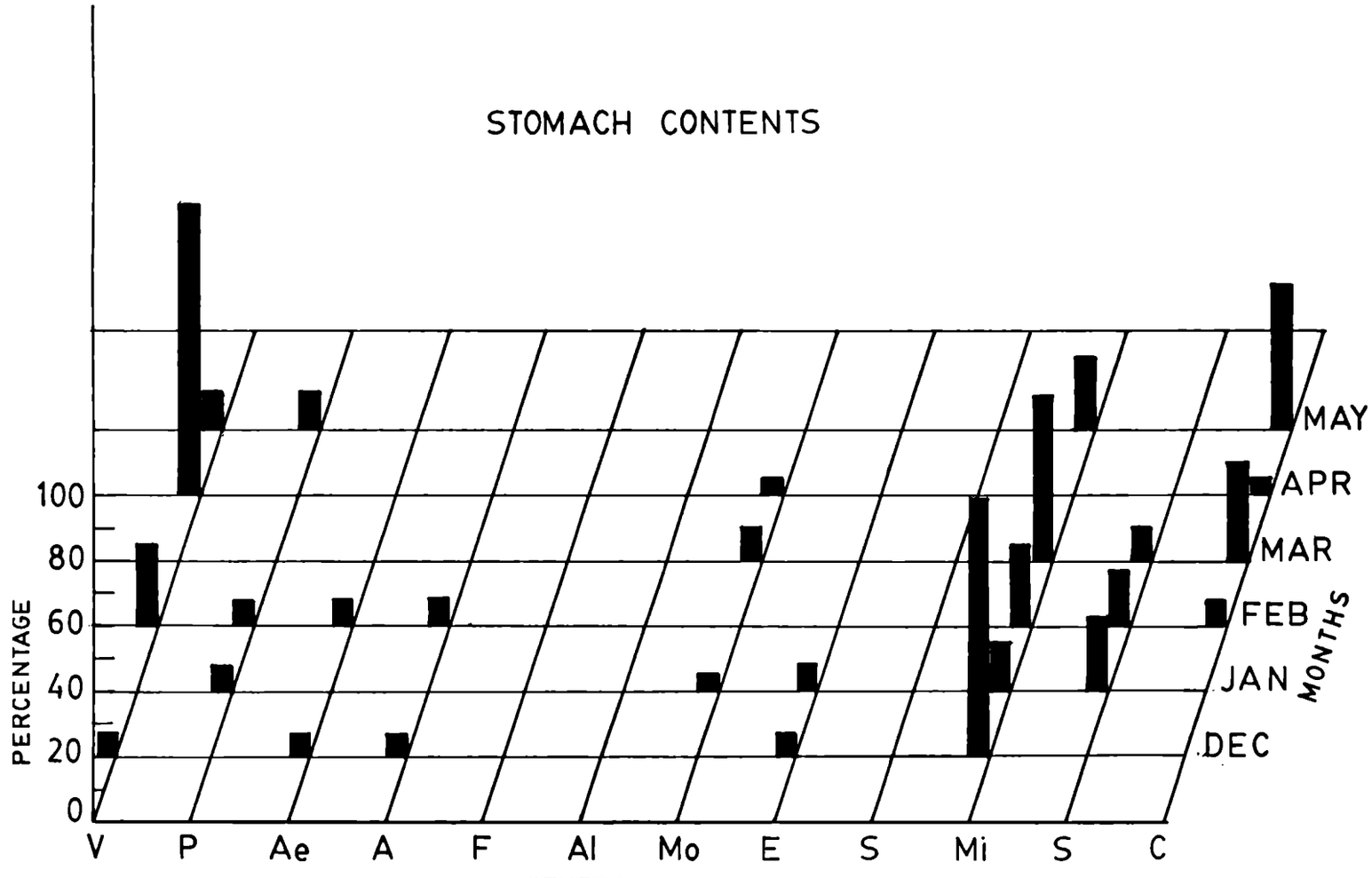

STOMACH CONTENTS
PERCENTAGE
100
80
60
40
20
0
V
P
Ae
A
F
Al
Mo
E
S
Mi
S
C
GENERA
MAY
APR
MAR
FEB
JAN
DEC
MONTHS

FIG. 15g.

Fig. 15h. Monthwise percentage distribution of different genera of bacteria isolated from anterior intestinal content of adults

V - *Vibrio*

P - *Pseudomonas*

Ae - *Aeromonas*

A - *Acinetobacter*

F - *Flavobacterium*

Al - *Alcaligenes*

Mo - *Moraxella*

E - Enterobacteriaceae

S - *Staphylococcus*

Mi - *Micrococcus*

B - *Bacillus*

C - Coryneform group

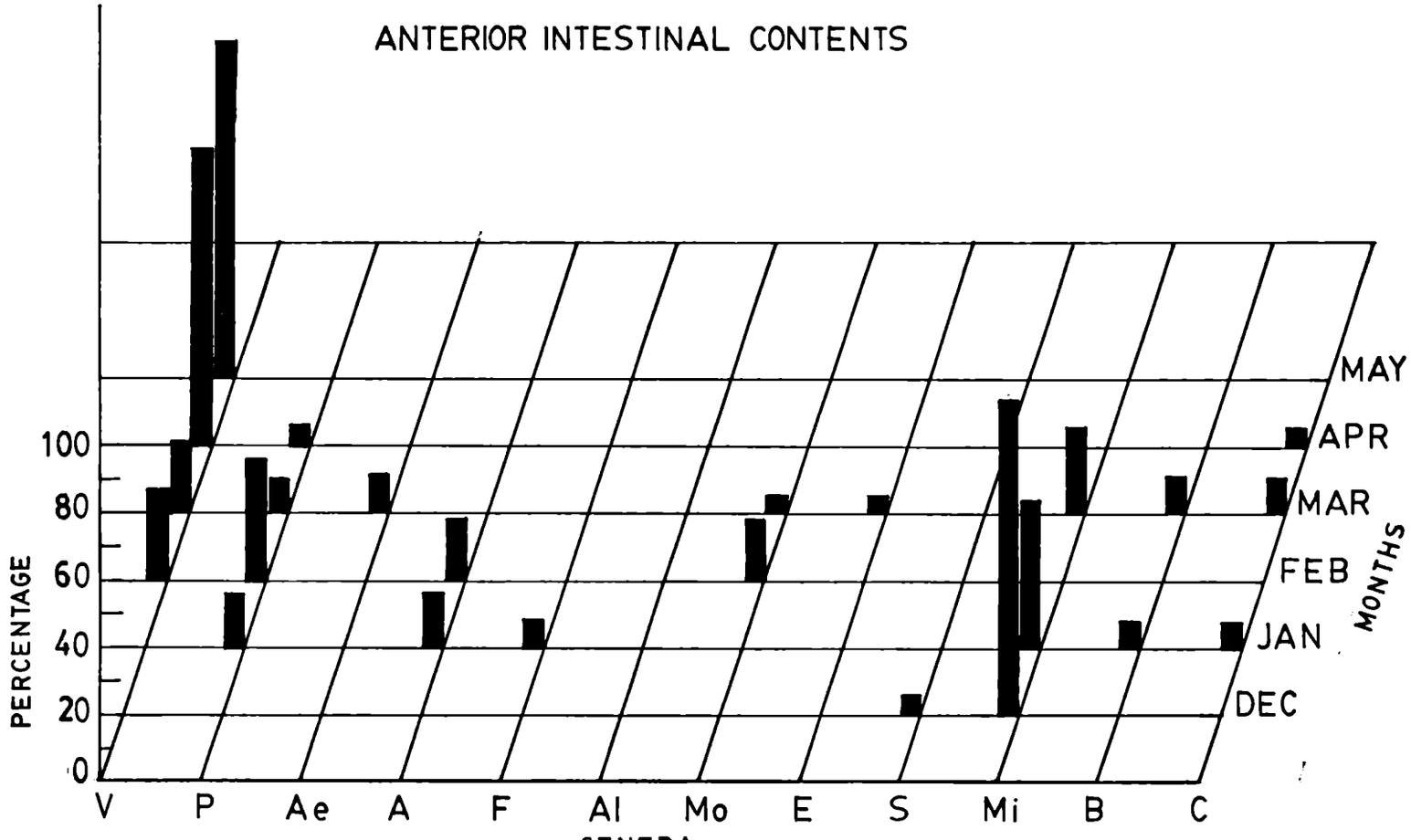

ANTERIOR INTESTINAL CONTENTS
PERCENTAGE
100
80
60
40
20
0
V
P
Ae
A
F
Al
Mo
E
S
Mi
B
C
GENERA
MAY
APR
MAR
FEB
JAN
DEC
MONTHS

FIG. 15h.

Fig. 15i. Monthwise percentage distribution of different genera of bacteria isolated from posterior intestinal content of adults

V - *Vibrio*

P - *Pseudomonas*

Ae - *Aeromonas*

A - *Acinetobacter*

F - *Flavobacterium*

Al - *Alcaligenes*

Mo - *Moraxella*

E - Enterobacteriaceae

S - *Staphylococcus*

Mi - *Micrococcus*

B - *Bacillus*

C - Coryneform group

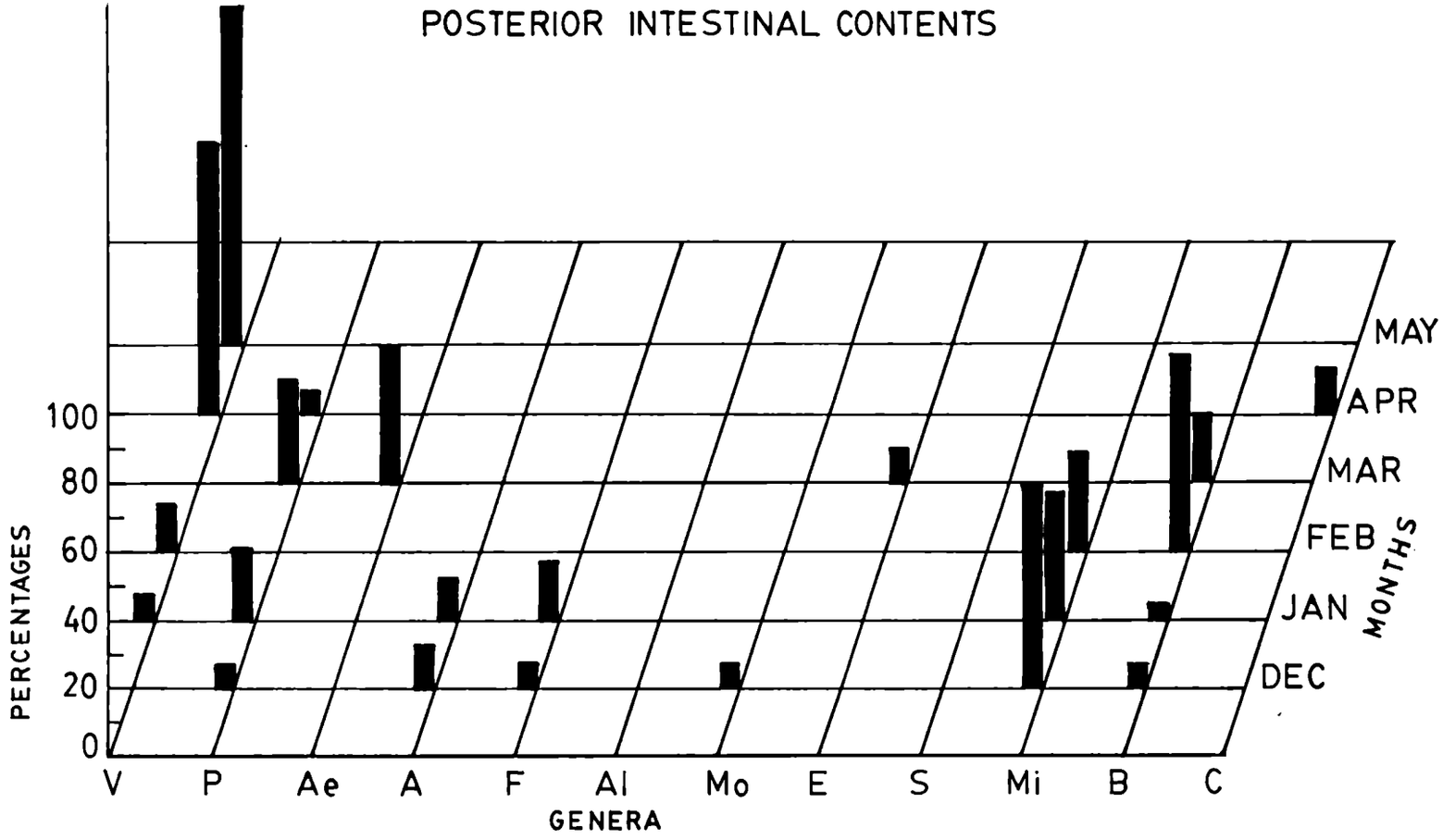

POSTERIOR INTESTINAL CONTENTS
PERCENTAGES
100
80
60
40
20
0
V
P
Ae
A
F
Al
Mo
E
S
Mi
B
C
GENERA
MAY
APR
MAR
FEB
JAN
DEC
MONTHS

FIG. 15i.

Fig. 15j. Monthwise percentage distribution of different genera of bacteria isolated from alimentary canal content of adults

V - *Vibrio*

P - *Pseudomonas*

Ae - *Aeromonas*

A - *Acinetobacter*

F - *Flavobacterium*

Al - *Alcaligenes*

Mo - *Moraxella*

E - Enterobacteriaceae

S - *Staphylococcus*

Mi - *Micrococcus*

B - *Bacillus*

C - Coryneform group

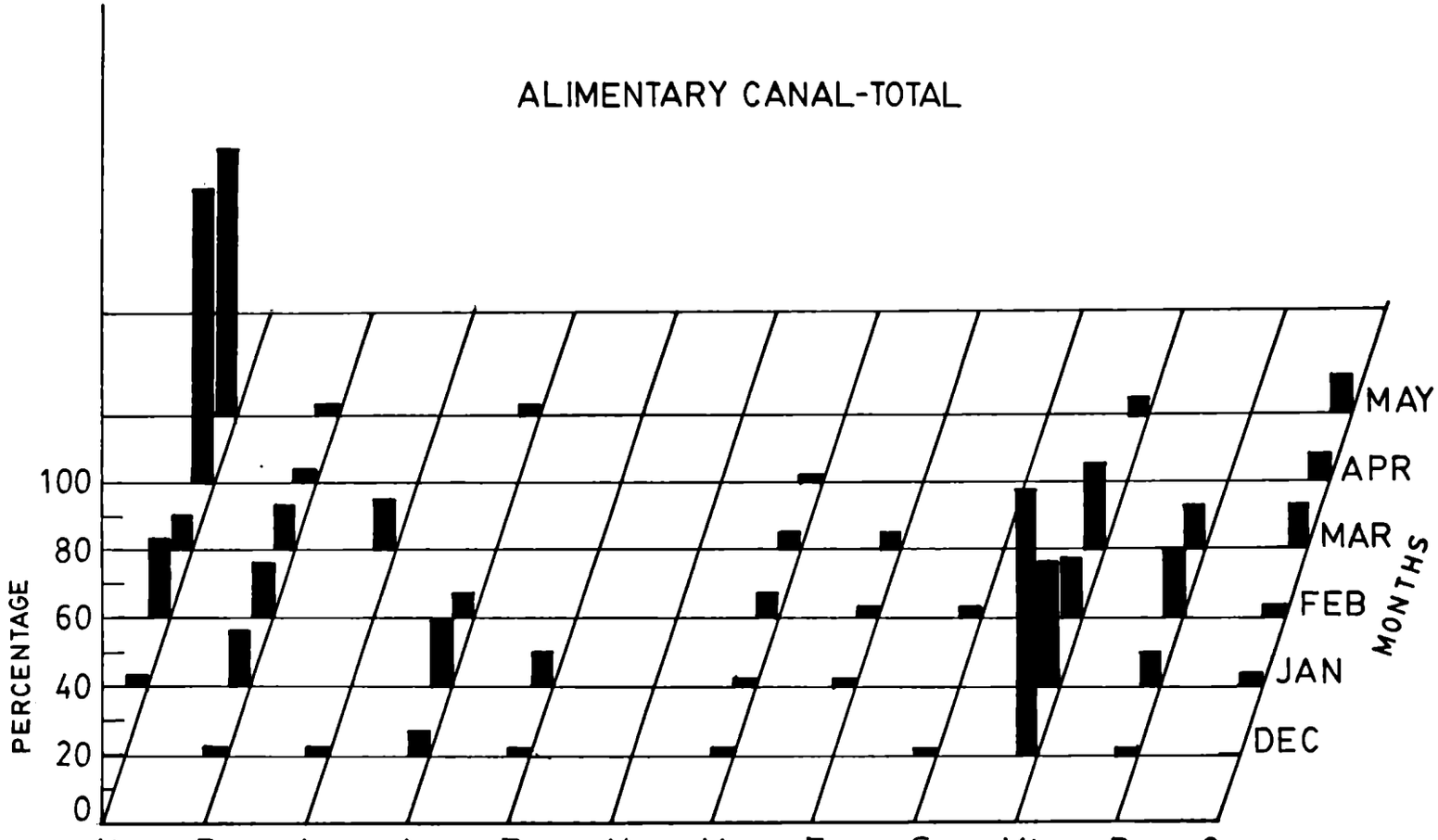

ALIMENTARY CANAL-TOTAL
PERCENTAGE
100
80
60
40
20
0
V
P
Ae
A
F
Al
Mo
E
S
Mi
B
C
GENERA
MAY
APR
MAR
FEB
JAN
DEC
MONTHS

FIG. 15j

Fig. 15k. Monthwise percentage distribution of different genera of bacteria isolated from water of Station 2

V - Vibrio

P - Pseudomonas

Ae - Aeromonas

A - Acinetobacter

F - Flavobacterium

Al - Alcaligenes

Mo - Moraxella

E - Enterobacteriaceae

S - Staphylococcus

Mi - Micrococcus

B - Bacillus

C - Coryneform group

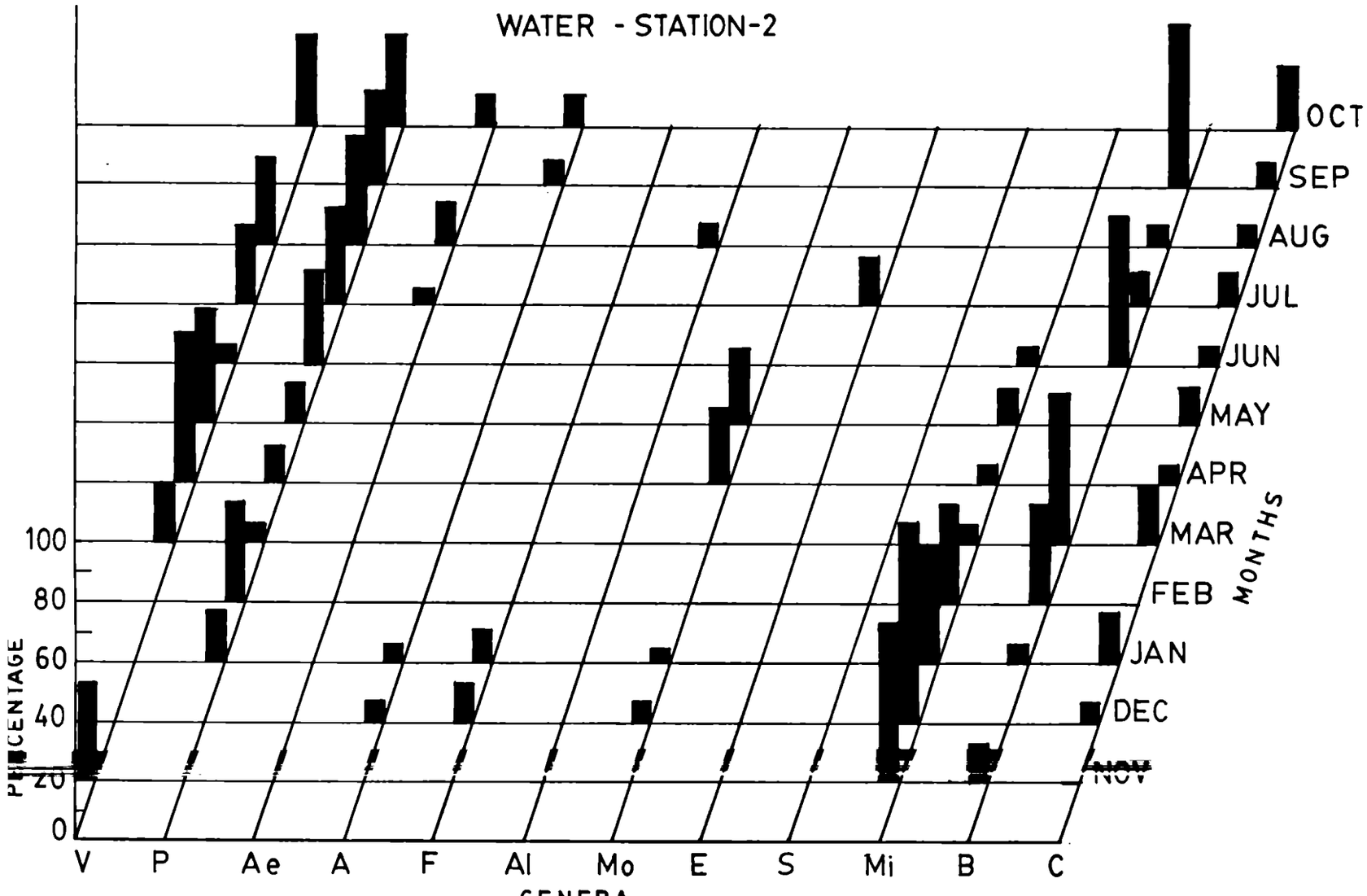

WATER - STATION-2
OCT
SEP
AUG
JUL
JUN
MAY
APR
MAR
FEB
JAN
DEC
NOV
MONTHS
100
80
60
40
20
0
PERCENTAGE
V
P
Ae
A
F
Al
Mo
E
S
Mi
B
C
GENERA

FIG.15k.

Fig. 151. Monthwise percentage distribution of different genera of bacteria isolated from sediment of Station 2

V - *Vibrio*

P - *Pseudomonas*

Ae - *Aeromonas*

A - *Acinetobacter*

F - *Flavobacterium*

Al - *Alcaligenes*

Mo - *Moraxella*

E - Enterobacteriaceae

S - *Staphylococcus*

Mi - *Micrococcus*

B - *Bacillus*

C - Coryneform group

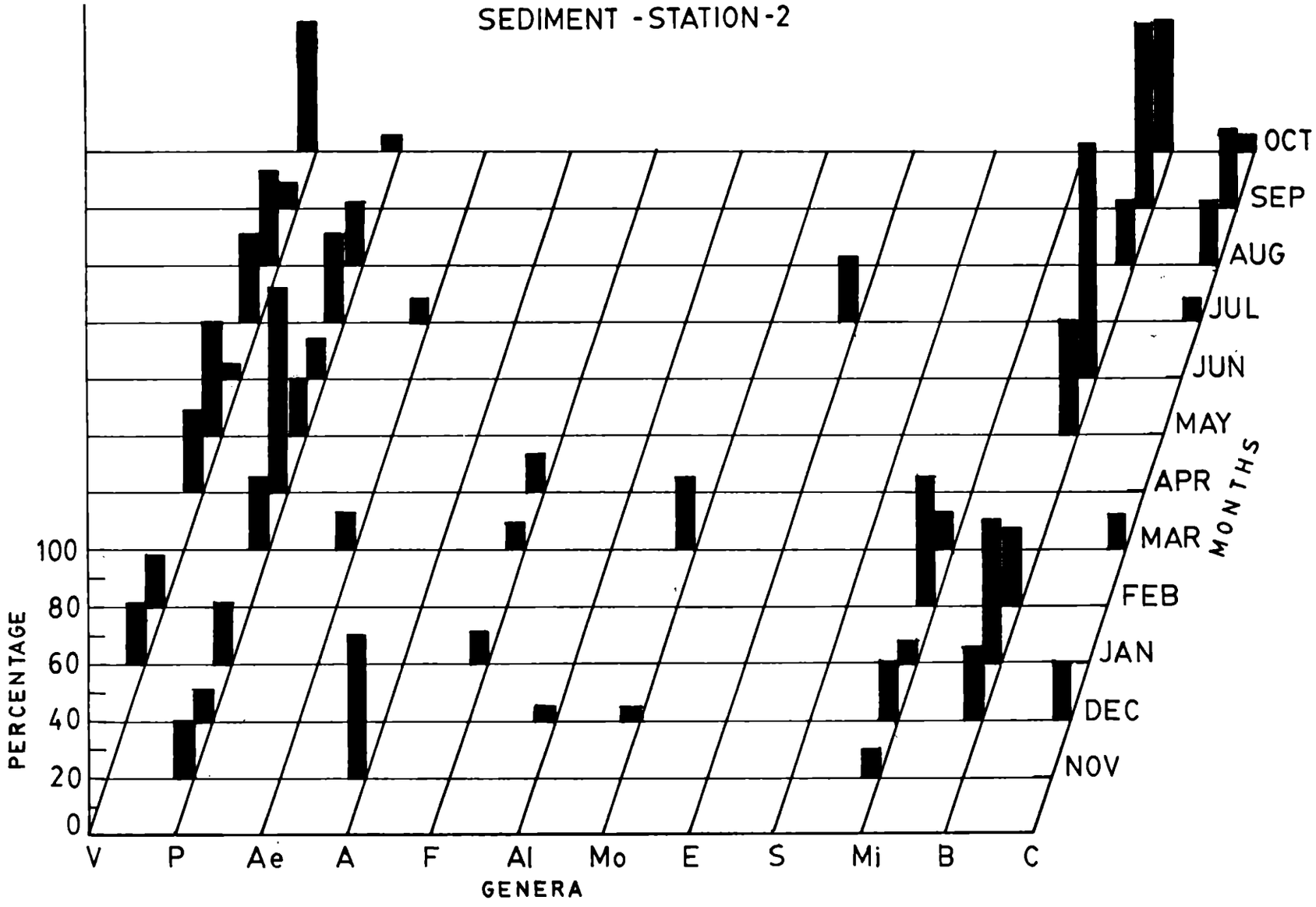

SEDIMENT - STATION - 2
OCT
SEP
AUG
JUL
JUN
MAY
APR
MAR
FEB
JAN
DEC
NOV
MONTHS
PERCENTAGE
100
80
60
40
20
0
V
P
Ae
A
F
Al
Mo
E
S
Mi
B
C
GENERA

FIG. 151

COMPARATIVE STUDY

5 BACTERIOLOGY OF P. INDICUS IN HATCHERY, CULTURE POND AND NATURAL ENVIRONMENT - A COMPARATIVE STUDY

Bacteria play a vital role in the life of P. indicus at various stages, as food, as agents for the elaboration of digestive enzymes and as pathogens causing mass mortality. Management of hatchery and culture pond system can well be improved if adequate awareness is created regarding the type of microflora associated with various stages, it's life history and their behaviour at different conditions. Hence in the present investigation heterotrophic bacterial population associated with P. indicus at various stages of it's life history in a culture system, eggs to postlarvae in hatchery and juvenile to adults (marketable size) in a culture pond were quantitatively and qualitatively analysed, tested for their ability to elaborate various hydrolytic enzymes, and checked their growth response to varying temperature, pH and NaCl concentrations. Investigation was also made on the heterotrophs associated with postlarvae, juveniles and adults from natural habitat viz. backwater. The influence of various physico-chemical parameters on the bacterial population, in association with the animal as well as it's habitat, influence of bacterial genera on the survival and metamorphosis of eggs and larvae, the relationship between

various genera occurred in the environment and on the animal were statistically analysed.

The ensuing pages give an account of the bacteriology of eggs and larvae of P. indicus in a hatchery system, and compare the bacterial population associated with a) postlarvae in hatchery and postlarvae of natural environment b) juveniles in happa and juveniles of natural environment, and c) adults of culture pond and adults of natural environment, assessing critically the features which demark a culture system from the natural environment in its microbiological perspective.

The larval history of P. indicus in hatchery was completed within a short span of twelve days during which the eggs hatched out into nauplii and metamorphosed to postlarvae through zoea and mysis by a series of moultings. During this crucial period in the life of the animal the environment was maintained semiclosed with a partial replacement of water with the fresh filtered one from the estuary. Eventhough the period of larval rearing was found to be very short, quite remarkable bacteriological changes took place in the hatchery system. THB declined from eggs to postlarvae and correspondingly the percentage of Gram-negative bacteria increased. Simultaneous Vibrio was found to increase and at the same time all the other genera declined. Stricking feature of these changes was the

sharp reduction of the generic diversity index from eggs to postlarvae. Factors such as short generation time of Vibrio compared to other bacteria, ability for the uptake of substrates at low concentrations, confinement of water for a longer time resulting in the loss of interaction of bacteria and the environment, presence of moulted exoskeleton in the water column, ability of Vibrio to attach to larvae than the other genera have been suggested as the possible reasons for the significant proliferation of vibrios. Application of multiple regression models suggested that in the first three stages (eggs, nauplii and zoeae) the survival and metamorphosis, were depended on the different genera of bacteria existed and the physico-chemical parameters encountered in the hatchery pools. However, in the succeeding stages (mysis and postlarvae) the survival and metamorphosis could be explained as a direct simple function of Vibrio, as shown by the simple correlation coefficient.

Occurrence of more chitinoclastic vibrios towards the later part of the larval history deserves much concern. Normally, lysis of the shell of healthy larvae may not be taking place because of the continuous moulting. However, the organism may be able to get an entry into the larval body through the injuries formed when they are reared in large numbers.

Occurrence of the higher percentage of moderately halophilic and higher temperature preferring vibrios for maximum growth, towards postlarval stage of the larval history were the two other stricking features of the hatchery system. These occurred when the ambient conditions of the pools were much different from the optimal requirements of vibrios. Possibly the larval body (surface as well as the intestinal tract) might have been providing a protective environment to these bacteria. Can this requirement of higher NaCl concentrations and temperature be one of the manifestations of pathogenecity of Vibrio? Much work is needed in this line.

Vibrio alone increased in the hatchery system when the eggs hatched out and nauplii metamorphosed into postlarvae through various stages. In the pond reared P. indicus as well as the animals from the natural environment there was seen an increase of Vibrio in the alimentary canal when food passed from stomach to posterior intestine. It was interpreted as a result of the few cycles of division of Vibrio occurring in the intestine where practically no digestion was taking place, but only the absorption of digest food materials. This may result in the occurrence of higher number of vibrios than any other group in the faecal matter. In the zoea, mysis and postlarva of the animal also a similar phenomenon may be taking place. The larvae start feeding

from the zoea stage onwards and the increase of Vibrio in the whole larval body was much pronounced in the mysis and postlarval stages. In a confined body of water where larvae are reared in higher density the possible discharge of Vibrio through the faecal matter can contribute significantly to the accumulation of vibrios in the water column. However experimental studies are warranted to find out the release of Vibrio through the faecal pellets of prawn.

a) Environmental factors monitored in the hatchery, where the postlarvae have been reared, did not show wide differences from their natural habitat. At the same time THB associated with animals and their rearing medium in hatchery were considerably lesser than that was observed in postlarvae and water in natural environment. The percentage of Gram-negative bacteria and Vibrio were higher in hatchery samples (larvae and water). On the contrary the postlarvae from the natural habitat harboured comparatively more Pseudomonas, Micrococcus, Bacillus and Coryneform group than in the postlarvae of hatchery. The proteolytic, ureolytic and lipolytic bacteria were recorded more or less at similar proportions in both the environments. At the same time amylolytic and chitinoclastic forms were much higher in hatchery. The potential hydrolytic enzyme producers in hatchery reared postlarvae were mainly members of Vibrio. But in the natural environment various genera elaborated hydrolytic enzymes

with an exception of chitinase, which was mainly produced by Vibrio. Majority of isolates of postlarvae and water in hatchery preferred higher (40°C) temperature as optimum while in natural environment almost the same proportion preferred 30°C. When pH 7 was found to be the optimum for majority of the isolates from the postlarvae and water in hatchery, comparatively higher population in the animals from natural environment was preferring pH 9. At the same time corresponding water samples contained more bacteria preferring pH 7. While higher percentages of the isolates from the hatchery preferred to grow at 7 and 10% NaCl, higher percentage of isolates from the natural environment recorded maximum growth at 1 and 3%, showing that moderately halophilic bacteria were more in hatchery than the natural environment. Thus lower THB, higher Gram-negative forms, higher percentage of Vibrio more amylolytic and chitinoclastic forms, higher percentage of isolates preferring 40°C and 7 and 10% NaCl as optimum in the hatchery reared postlarvae than that of the postlarvae obtained from the natural environment, were the characteristic differences recorded. This implies that the proliferation of moderately halophilic and higher temperature preferring organisms might have occurred more due to the confinement of water for longer periods and crowding of animals. Apart from this the other possible reasons for the increase of vibrios in the hatchery system have already been explained. Further, absence of other

genera such as Pseudomonas, Micrococcus, Bacillus and Coryneform group in considerable percentages in the postlarvae of hatchery and their presence in the postlarvae from natural environment gives the indication that Vibrio in hatchery are not exposed to the sort of competition and antagonism by other organisms which is generally prevailing in the natural environment. If the salinity and temperature of water go up, the strains of Vibrio preferring higher temperature and NaCl may proliferate faster and may lead to enhanced invasion of the larvae and the consequent death. The situation becomes worse when environmental parameters mount stress on the larvae.

b) Concentration of nutrients was comparatively at higher level in natural environment where from the juveniles were collected than the happa where they were maintained for a short while before introducing into ponds. Juveniles from natural environment harboured higher load of THB and more number of genera than the juveniles from happa. While the percentage of Gram-negative bacteria in both these habitates and the corresponding animals were more or less the same, juveniles from happa harboured more Vibrio and lesser Pseudomonas, Micrococcus and Bacillus than the animals from backwater. Consequently all the potential hydrolytic enzymes are linked mostly to vibrios in the juveniles and water from happa, and in the other samples the sourcesof different enzymes except the chitinase were distributed in other genera also.

Chitinase was elaborated mainly by vibrios in both the systems. In happa, while most of the isolates preferred to grow at 3 and 7% NaCl, in the natural environment almost the same proportion of isolates preferred 1 and 3% NaCl, showing that the isolates from happa were more halophilic than that of the natural environment. While majority of the isolates from happa preferred pH 7 as optimum, in the natural environment eventhough pH 7 was preferred mostly for larger group of organisms pH 9 was the optimum. In both the systems 30°C was preferred by most of the isolates.

Thus, comparatively lesser nutrients in water, low THB population in both juveniles and water, more Vibrio and the higher percentage of organisms preferring 3-7% NaCl for maximum growth were the characteristics of the environment of happa and the juveniles, making it different from the natural habitat. Higher number of vibrios may be an after effect of crowding of animals. In pond reared prawns it was observed that the intestine harboured more percentage of Vibrio than that of the animals from the natural environment. In the postlarval stages also vibrios were found more in hatchery than in the natural environment. Likewise in juveniles from happa also intestine may be harbouring more Vibrio. The disadvantage of having Vibrio in larger percentages when the animals are crowded has already been discussed.

c) In the natural habitat of P. indicus, the water was more saline and contained comparatively more inorganic and organic nitrogen compounds than the culture pond. The natural habitat (both water and sediment) harboured more THB than the culture pond, where Vibrio, Pseudomonas, Micrococcus and Bacillus were the predominant flora. However, Pseudomonas was the predominant flora in the water of culture pond. But, sediment from both the environments harboured mainly Bacillus followed by Pseudomonas and Vibrio. The percentage of various hydrolytic groups were almost similar in both culture pond and natural environment. While Pseudomonas was the main source of all the hydrolytic enzymes other than chitinase in the water of culture pond, members of Vibrio, Pseudomonas and Micrococcus elaborated these enzymes in the water of natural habitat. But chitinase was mainly produced by Vibrio and Pseudomonas in the water of culture pond. But at the same time Vibrio was the main source of this enzyme in the water of natural environment. Generally Bacillus was the main source of all the hydrolytic enzymes in the sediment of culture pond and natural habitat. However chitinase was mainly extended by Vibrio. The organisms generally preferred 30°C, pH 7 and 1 and 3% NaCl for maximum growth in both the environments.

Shell surface and gill of pond reared P. indicus harboured lesser population than that of the animals from

backwater. Pseudomonas was the prominent flora on the shell surface and gill of pond reared prawns. At the same time Micrococcus was the predominant flora on the surface and gills of adults from backwater, eventhough Vibrio and Pseudomonas were in considerable percentages. The percentage of different hydrolytic groups were almost similar in the surface and gills of the animals in both the environments. Generally, Vibrio, Pseudomonas, Micrococcus, Bacillus and Coryneform group were the major source of all the hydrolytic enzymes except chitinase which was mainly elaborated by Vibrio. Optimum temperature for the majority of cultures was 30°C. pH 7 was widely preferred followed by pH 9. Majority of the isolates from both gill and surface of animals in both the environments preferred to grow at 1 and 3% NaCl. The surface and gill of P. indicus in pond, differed from that of the animals in natural environment by having lower population comprising more of Pseudomonas.

Total bacterial population, in the three regions of alimentary canal, was higher in P. indicus collected from natural habitat than that of the pond reared ones. At the same time percentage of Gram-negative forms were more or less similar. But, the percentage of Vibrio in the alimentary canal of pond reared prawn was higher than that of the animals from their natural habitat. In the alimentary canal of both

pond reared prawn as well as the ones from natural environment, Vibrio was increasing from stomach to posterior intestine. Pseudomonas, Aeromonas and Bacillus also showed an increase from stomach to posterior intestine in the animals collected from backwater and such an increase was observed only for Moraxella, and Coryneform group in the pond reared prawns. The genera which showed a reduction from stomach to posterior intestine in the prawns from natural habitat were Acinetobacter, Moraxella, Enterobacteriaceae, Micrococcus and Coryneform group. However in the pond reared prawns such a reduction was experienced for Pseudomonas, Acinetobacter, Micrococcus and Bacillus. Total percentage of various hydrolytic groups occurred in the three regions of alimentary canal was more or less similar in both pond reared as well as the wild animal. In the elaboration of all the hydrolytic enzymes, other than chitinase, along with Vibrio other genera also were taking prominent role. But in the case of chitinase, Vibrio was the main source. Majority of isolates in the alimentary canal of both the pond reared and the ones from backwater were preferring 3 and 7% NaCl for optimum growth. pH 7 was the optimum for majority of isolates followed by pH 9. However 30°C was preferred by most of the isolates.

Thus uniformly in all the samples of culture pond the total population was low than that of natural environment.

This may be an after effect of the confinement of aquatic systems with very little contact with the open waters. Because, in hatchery also the total population at the postlarval stage was much lesser than that of the natural environment. Apart from that, physiologically, the cultures behaved almost similarly in both system. Same pattern was seen with regard to the bacterial flora of surface and gills of animals. Uniformly in both pond reared as well as in the animals from natural environment, Vibrio was increasing from stomach to posterior intestine and Acinetobacter and Micrococcus were decreasing in their percentages. It had already been explained that vibrios were best suited for the microenvironment of alimentary canal where it could undergo a few cycles of division, and, Acinetobacter and Micrococcus being lysed by the digestive juice of the animal. However no uniform pattern in the decrease or increase of other genera was observed in the alimentary canal of animals from both culture pond and natural environment.

SUMMARY

6 SUMMARY

This thesis presents a detailed account on: a) THB associated with eggs, nauplii, zoeae, mysis and postlarvae of Penaeus indicus collected from Azhicode hatchery (long. 76°12'E and lat. 10°12'N) during March 1981 to March 1984, juveniles and adults of P. indicus from a culture pond (long. 76°19'E and lat. 9°54'N) during April 1982 to June 1982 and postlarvae, juveniles and adults of P. indicus from the natural environment (long. 76°17'E and lat. 9°59'N) during December 1981 to May 1982 besides various physico-chemical parameters, b) distribution of Gram-negative bacteria and various genera associated with all the above developmental stages of P. indicus, c) the influence of physico-chemical parameters on the distribution of THB, Gram-negative bacteria and various genera and survival and metamorphosis of eggs and larvae of P. indicus (statistical significance - simple correlation, multiple regression, diversity index and fitting of trend lines) d) ability of isolates to produce various extracellular hydrolytic enzymes such as amylase, protease (caseinase and gelatinase) lipase, urease and chitinase and e) the effect of environmental

parameters such as temperature, (4,10,30,40 and 50°C), pH (2,4,7,9 and 11) and sodium chloride concentration (0,1,3,7 and 10%) on the maximum growth of selected isolates.

1. THB declined as the eggs hatched out and nauplii metamorphosed to postlarvae through zoeae and mysis.

2. Gram-negative bacteria and Vibrio increased and the generic diversity index declined from eggs to postlarvae as well as the corresponding water samples. During the early stages (eggs and nauplii) Pseudomonas and Acinetobacter were the dominant ones and in the later stages Vibrio took over.

3. A significant relationship was not seen between the THB of water and that of the different larval stages suggesting that theywere independent. No significant relationships were found between the THB of eggs and larvae and their percentage survival and metamorphosis suggesting that the survival and metamorphosis of eggs and larvae were not dependent on the THB.

4. A significant positive correlation existed between the pH and the percentage of Vibrio in mysis as well as in water collected along with it suggesting that alkaline pH

favoured the proliferation of Vibrio. Factors such as short generation time of Vibrio compared to others, ability for the uptake of substrate at low concentrations, confinement of water for a longer period resulting in the loss of interaction of bacteria and the environment, ability of vibrios to attach on the larval body are suggested as the possible reasons for the increase of Vibrio in larvae.

5. A significant negative correlation existed between the percentage of Vibrio and the percentage survival and metamorphosis of mysis and the postlarvae. Application of multiple regression models showed that the survival and metamorphosis of eggs, nauplii and zoeae were depended on the percentage of various genera associated with and the physico-chemical factors prevailing in the culture pool. At the same time the survival and metamorphosis of mysis and postlarvae were not a linear function of all the genera together and the various physico-chemical parameters, but a simple direct function of Vibrio, which was shown by a significant negative correlation coefficient.

6. The significant negative correlation existed between pH and the survival and metamorphosis of mysis, and the significant positive correlation existed between

pH and the percentage of Vibrio in mysis suggested that the comparatively higher pH existed in the pools with mysis might have accelerated the multiplication of Vibrio and resulted in the larval mortality.

7. The significant negative correlation existed between temperature and the survival of postlarvae and the absence of a significant correlation between temperature and the percentage of Vibrio in postlarvae suggested that the higher temperature prevailed in the pools with postlarvae must have weakened the larvae so that they could easily become the prey for Vibrio invasion.

8. It could be seen that Vibrio was the only genus comprising the highest percentage of hydrolytic enzyme producers. Majority of Vibrio were chitinoclastic. When the larvae moult and metamorphose, large quantity of exoskeleton may be shed into the water which might be forming a good substrate for the chitinoclastic vibrios to attach and grow. When the chitinoclastic vibrios proliferate and dominate the microbial population both in water and in larvae, they can cause serious health hazards to the later, provided the organisms can get an entry into the larval body through some serious injuries which normally occur while the larvae are grown in higher densities.

9. The shift in the requirement of NaCl concentration from 3 to 10% while the eggs transformed into postlarvae could be seen.

10. In postlarval stages most of the isolates preferred 40°C and pH of about 7.

11. The presence of moderately halophilic and higher temperature preferring vibrios in the hatchery suggested that the larval body (surface as well as intestine) might have provided these organisms a 'protective environment'.

12. THB, percentage of Gram-negative bacteria and the percentage of Vibrio declined when juveniles collected from the natural environment were maintained in happa.

13. An increase of THB was observed from body surface to gill and to alimentary canal of adult, in culture pond. Gram-negative bacteria of the alimentary canal was higher than that of the body surface and gill.

14. The surface and gills of the pond reared prawns and pond water contained Pseudomonas as the dominant flora. This showed that the nature of the population in water reflected on the surface and gills. But statistically a significant correlation was not obtained. Micrococcus present in gill showed a significant positive correlation

with the Micrococcus of the pond sediment, showing that the population of Micrococcus in gills was directly influenced by the population of Micrococcus in the sediment.

15. THB of stomach was higher than that of the THB in sediment of the pond and was containing a different generic composition showing that the animal feed selectively upon the detritus and the decaying supplementary feed which harbour a different bacterial population than that of sediment and water.

16. In general THB of stomach was much lesser than that of the anterior and posterior intestine, which showed that the strains tolerant to the digestive processes in stomach passed into the alimentary canal and multiplied, where only absorption of the digested food materials take place. In all the three regions of alimentary canal Vibrio was found to be the dominant flora. Moreover an increase of Vibrio could be seen from stomach to intestine.

17. The genera encountered in the alimentary canal could be grouped into three, such as those multiplied when the food passed through the intestine (Vibrio, Moraxella and Coryneform group), those which were lysed by the digestive enzymes (Pseudomonas, Acinetobacter,

Micrococcus and *Bacillus*) and, those which were tolerant to the digestive juice but did not multiply (Enterobacteriaceae). It became apparent that the alimentary canal formed a suitable microenvironment where *Vibrio* underwent a few cycles of division. Higher percentage of *Vibrio* in intestine is not ideal in the health point of view when animals are put under stress. In such situations, *Vibrio* may behave as an opportunistic pathogen invading the tissue and haemolymph through the intestinal wall.

18. Genera such as *Pseudomonas*, *Acinetobacter*, *Micrococcus* and *Bacillus* were found to decline in the alimentary canal especially in the intestine. The lysed bacterial cells might be used as a direct source of food by the animal itself.

19. All vibrios in happa elaborated chitinase. Highest percentage of chitinoclastic bacteria were found in intestine, and a gradual increase in their percentage could be seen from surface to posterior intestine of adults. Similar situation was seen with regard to lipolytic bacteria also. *Vibrio* may play a dual role both beneficial as well as harmful in the pond reared shrimp. The vibrios are versatile groups capable of elaborating various hydrolytic enzymes including chitinase which may enhance the digestive processes in the alimentary canal. At the same time when the environmental conditions become adverse mounting stress on the animal, the bacteria from alimentary canal also may invade the tissue. If the stress factor persists for a

longer duration, septicemia due to Vibrio may be resulted.

20. In general, a shift in the requirement of NaCl from 7 to 1% from the day of stocking to the time of harvest was observed for THB in pond. This indicated that moderately halophilic bacterial population was getting reduced from April to June. Drop in salinity following rains must have favoured the growth of lesser halophiles. It was apparent from the present investigation that in a culture environment Vibrio, Pseudomonas and Micrococcus might have come from fresh, brackish and marine environments.

21. In the culture environment the bacterial population in general is alkalophilic. pH of the environment was also alkaline showing that the situation was congenial for the different genera.

22. Majority of the isolates were mesophilic preferring 30°C.

23. In culture pond environment majority of Vibrio from the alimentary canal of prawns preferred 7% NaCl for optimum growth showing that the alimentary canal provided a protective environment for organisms preferring higher salt concentration.

24. In natural environment THB of postlarvae declined

significantly from November 1982 to May 1982. Vibrio increased from November to May while Coryneform group, Bacillus and Micrococcus declined. The higher saline water during this period might have favoured the growth of vibrios which were mostly halophilic. At the same time higher salinity might have adversely affected the lesser halophilic forms.

25. A positive significant correlation existed between THB of postlarvae and the pH of water. It was seen that most of the isolates obtained from postlarvae showed maximum growth at pH 7-9.

26. In juveniles THB declined and a generic shift from Gram-positive to Gram-negative bacteria leading to the dominance of Vibrio from November 1981 to May 1982 was seen. Later an increase of the Gram-positive bacteria and decrease of Gram-negative forms could be explained as a result of rainfall, which lead to the influx of river runoff bringing the terrestrial bacteria especially Bacillus and Micrococcus and allowing them to thrive by keeping the salinity of the system low.

27. The significant negative correlation observed between salinity of water and the percentage Micrococcus of the anima surface and gill suggested that the comparatively higher

salinity prevailed during the premonsoon period and its gradual increase from November 1981 to May 1982 might have already affected the survival of Micrococcus in water and the animal surface and gill in natural environment.

28. THB of stomach content was higher than that of the sediment in natural environment and the stomach contained a different bacterial composition showing that animal was selective in it's feeding behaviour in the natural environment. THB of stomach was much lower than that of the alimentary canal of the animal in natural environment showing that the strains which could tolerate the digestive process in the proventriculus multiplied in the alimentary canal.

29. The organisms which showed an increase from stomach to posterior intestine were Vibrio, Pseudomonas, Aeromonas and Bacillus and the organisms which showed a reduction in the intestine were Acinetobacter, Moraxella, Enterobacteriaceae, Micrococcus and Coryneform group.

30. At both stations the amylolytic, proteolytic and lipolytic bacteria were mainly the members of Vibrio, Pseudomonas, Micrococcus, Bacillus and Coryneform group. But large number of vibrios were chitinolytic. Chitinoclastic

bacteria increased in water, sediment and prawn from November 1981 to May 1982. Tidal influx during the premonsoon period might have favoured the growth of chitinoclastic vibrios during this period.

31. It was suggested that in natural environment diverse physiological groups of bacteria with various optimal conditions for maximum growth may exist together where the available physico-chemical factors are not favourable for maximum growth for all the bacteria. It was observed that higher temperature ($40^{o}C$) and sodium chloride concentration (7% and 10%) were found to be highly suitable for the proliferation of many of the Vibrio strains. Further, vibrios are known to multiply faster than other genera. The alkaline pH, increase of temperature and NaCl concentration in the confined system (hatchery and pond) will lead to the increase of vibrios and may cause mortality when the larvae become weak. The observations made in the present investigation show that care should be taken to maintain pH around 7.5, temperature $<25^{o}C$ and salinity $<20 \times 10^{-3}$ in a tropical climate so that the number of vibrios could be checked in any confined system and thus mass mortality due to vibrios could be avoided.

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*Not referred in original

APPENDIX

Table -2. Pool wise distribution of different genera of bacteria isolated from various stages

Stages	Pool No.	No. of isolates	Vibrio	Pseudomonas	Aeromonas	Acinetobacter	Flavobacterium	Alcaligenes	Moraxella	Enterobacteriaceae	Staphylococcus	Micrococcus	Bacillus	Coryneform group	Percentage survival and metamorphosis to next stage
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Eggs	1	26*	11.53	34.50	-	30.76	-	-	3.85	-	-	3.85	11.54	3.85	97.14
		26**	15.38	26.92	-	34.62	-	-	-	-	-	3.84	15.38	-	-
	2	22	9.09	36.36	-	31.82	-	-	9.09	-	-	4.55	4.55	4.55	93.75
		20	10.00	40.00	-	30.00	-	-	5.00	-	-	5.00	5.00	5.00	
	3	20	10.00	30.00	-	30.00	-	-	10.00	-	-	20.00	-	-	92.85
		20	15.00	10.00	-	30.00	-	-	10.00			10.00	15.0	15.00	
	4	10	-	30.00	-	30.00	-	-	10.00	-	-	20.00	-	-	93.75
		16	10.00	31.25	-	18.75	-	-	31.25	-	-	18.75	-	-	
	5	9	11.11	33.33	-	33.33	-	-	22.22	-	-	-	-	-	88.88
		5	20.00	40.00	-	20.00	-	-	20.00	-	-	-	-	-	
	6	9	11.11	-	-	-	-	-	22.22	55.55	-	-	11.11	-	80.00
		11	18.18	-	-	-	-	-	18.18	45.00			18.18		
		96	10.42	30.21	-	28.13	-	-	10.42	5.21	-	8.33	5.21	2.08	91.06
			12.24	24.49	-	25.51	-	-	18.22	5.10	-	7.14	10.20	4.08***	

*From eggs and larvae **From water of the corresponding stage

Table - 2. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Nauplii	1	33*	30.30	21.21	-	27.27	-	-	3.03	-	-	-	12.12	6.06	80.18
		21**	19.05	23.80	-	28.57	-	-	14.29	-	-	-	9.52	4.76	
	2	21	19.05	23.81	-	42.86	-	-	9.52	-	-	-	4.76	-	73.33
		21	23.81	28.57	-	23.81	-	-	9.52	-	-	4.76	4.76	4.76	
	3	19	15.78	15.78	-	26.32	-	-	15.78	-	-	5.26	10.53	10.53	60.00
		17	5.88	5.88	5.88	52.94	-	-	29.41	-	-				
	4	10	-	20.00	-	30.00	-	-	40.00	-	-	10.00	-	-	97.33
		9	-		-	11.11	-	-	77.78	-	-	11.11	-	-	
	5	5	20.00	40.00	-	20.00	-	-	20.00	-	-	-	-	-	87.50
		16	-	62.50	-	-	-	-	12.50	-	-	12.50	6.25	6.25	
	6	7	14.29	-	-	-	-	-	14.29	57.14	-	-	14.29	-	83.33
		7	28.57	-	-	-	-	-	14.28	42.86	-	-	14.28	-	
		95	20.00	20.00	-	28.42	-	-	12.63	4.21	-	2.11	8.42	4.21	80.27
		91	13.19	24.18	1.09	23.08	-	-	21.98	3.29	-	4.39	5.49	3.29***	

Table - 2. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Zoeae	1	25*	56.00	16.00	-	20.00	-	-	4.00	-	-	-	-	4.00	26.85
		21**	47.62	14.29	-	23.81	-	-	4.76	-	-	-	4.76	4.76	
	2	21	61.90	9.52	-	19.05	-	-	4.76	-	-	4.76	-	-	48.19
		28	67.86	10.71	-	10.71	-	-	3.57	-	-	3.57	3.57	-	
	3	18	72.22	5.56	-	16.67	-	-	5.56	-	-	-	-	-	25.64
		15	53.33	6.66	-	13.33	-	-	13.33	-	-	-	-	13.33	
	4	5	80.00	-	-	20.00	-	-	-	-	-	-	-	-	89.04
		9	-	22.22	-	22.22	-	-	11.11	-	-	44.47	-	-	
	5	11	9.09	63.64	-	9.09	-	-	-	-	-	9.09	9.09	-	94.44
		14	-	64.28	-	14.29	-	-	-	-	-	21.42	-	-	
	6	3	-	-	-	66.67	-	-	-	-	-	33.33	-	-	66.66
		5	20.00	20.00	-	-	-	-	-	20.00	-	20.00	20.00	-	
		83	54.22	16.87	-	19.28	-	-	3.61	-	-	3.61	1.20	1.20	58.47
		92	41.30	20.65	-	15.21	-	-	5.43	1.09	-	3.78	3.26	3.26***	

Table - 2. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Mysis	1	17*	76.00	5.88	-	17.65	-	-	-	-	-	-	-	-	14.28
		20**	75.00	5.00	-	5.00	-	-	5.00	-	-	-	5.00	5.00	
	2	23	78.26	4.35	-	8.70	-	-	4.35	-	-	4.35	-	-	9.43
		22	72.72	4.55	-	13.67	-	-	4.55	-	-	4.55	-	-	
	3	21	85.71	9.52	-	4.76	-	-	-	-	-	-	-	-	10.00
		21	66.67	9.52	4.76	4.76	-	-	9.52	-	-	-	-	-	
	4	10	50.00	10.00	-	20.00	-	-	-	10.00	-	10.00	-	-	53.84
		6	-	16.67	-	16.67	-	-	66.67	-	-	4.76	-	-	
	5	10	50.00	30.00	-	-	-	-	10.00	-	-	-	-	10.00	45.00
		8	25.00	37.50	-	-	-	-	37.50	-	-	-	-		
	6	5	20.00	40.00	-	-	-	-	20.00	-	-	-	-	20.00	80.00
		8	25.00	37.50	-	-	-	-	25.00	-	-	-	-	12.50	
		86	69.77	11.63	-	9.30	-	-	3.49	1.16	-	2.33	-	2.33	35.45
		85	57.65	12.94	1.18	7.06	-	-	15.29	-	-	2.35	1.18	2.35***	

Table - 2. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Post larvae	1	19*	89.47	5.26	-	5.26	-	-	-	-	-	-	-	-	14.00
		22**	59.09	19.05	-	14.28	-	-	4.54	-	-	-	4.54	-	
	2	20	90.00	5.00	-	5.00	-	-	-	-	-	-	-	-	16.00
		21	76.19	4.76	-	14.28	-	-	4.76	-	-	-	-	-	
	3	20	90.00	-	-	-	-	-	5.00	-	-	-	5.00	-	17.00
		17	94.11	-	-	5.88	-	-	-	-	-	-	-	-	
	4	6	85.33	-	-	16.67	-	-	-	-	-	-	-	-	42.85
		5	100.00			-	-	-	-	-	-	-	-	-	
	5	3	66.67	-	-	-	-	-	-	-	-	-	33.33	-	50.00
		3	33.33	-	-	-	-	-	-	-	-	33.33	33.33	-	
	6	21	76.19	4.76	-	14.29	-	-	4.76	-	-	-	-	-	40.00
		19	31.58	15.79	-	21.05	-	-	15.79	-	-	-	15.79	-	
		89	85.39	3.37	-	6.74	-	-	2.24	-	-	-	2.25	-	29.98
		87	67.06	9.41	-	12.94	-	-	5.88	-	-	-	1.18	-	***

Table 3. Temporal changes in the percentage occurrence of hydrolytic forms in various samples

Period	Total no. of isolates	Amylolytic	Caseinolytic	Gelatinolytic	Lipolytic	Ureolytic	Chitinolytic
1	2	3	4	5	6	7	8
			Juveniles from natural environment				
3.4.1982	40	77.50	100.00	100.00	55.00	100.00	72.50
6.4.1982	33	63.69	93.93	100.00	12.12	100.00	51.52
Total	73	71.23	97.26	100.00	35.61	100.00	63.01*
			Juveniles from happa				
3.4.1982	17	47.06	100.00	100.00	5.88	100.00	41.18
6.4.1982	30	56.66	100.00	83.33	56.67	100.00	56.67
7.4.1982	34	38.23	58.82	85.29	44.12	100.00	44.12
Total	81	46.91	82.21	87.65	40.74	100.00	48.15*

*The total percentage calculated from the total No. of isolates

Table - 3. Contd.

1	2	3	4	5	6	7	8
			Water from happa				
3.4.1982	14	14.28	85.72	100.00	14.28	100.00	14.28
6.4.1982	14	57.14	92.86	85.71	57.14	100.00	57.14
7.4.1982	16	68.75	87.50	93.75	75.00	100.00	68.75
Total	44	47.72	88.63	93.18	50.00	100.00	47.72*
			Adult from culture pond				
19.4.1982	16	12.50	62.50	87.50	31.25	93.75	-
3.5.1982	17	-	100.00	100.00	64.70	100.00	41.17
18.5.1982	15	40.00	86.67	100.00	20.00	100.00	-
11.6.1982	16	12.50	100.00	100.00	12.50	100.00	-
Total	64	15.63	87.50	96.87	32.81	98.43	12.50*

Table 3 Contd.

1	2	3	4	5	6	7	8
			Gills				
19.4.1982	11	9.09	100.00	72.72	36.36	100.00	9.09
3.5.1982	14	21.43	100.00	100.00	78.57	100.00	35.71
18.5.1982	15	60.00	100.00	100.00	13.33	100.00	-
11.6.1982	13	23.07	100.00	100.00	23.07	100.00	23.07
Total	53	30.18	100.00	94.33	37.73	100.00	16.98*
			Stomach				
19.4.1982	17	47.06	76.47	100.00	41.18	100.00	35.29
3.5.1982	11	45.45	100.00	100.00	54.55	81.82	45.45
18.5.1982	17	29.42	100.00	100.00	58.82	100.00	47.06
11.6.1982	8	25.00	100.00	100.00	25.00	100.00	12.50
Total	53	37.74	92.45	100.00	47.17	96.23	37.73*

Table - 3. Contd.

1	2	3	4	5	6	7	8
			Anterior intestine				
19.4.1982	20	68.42	100.00	75.00	100.00	100.00	68.42
3.5.1982	18	83.33	100.00	100.00	88.88	100.00	66.66
18.5.1982	12	25.00	100.00	100.00	83.33	100.00	66.67
11.6.1982	14	57.14	78.52	100.00	42.86	100.00	50.00
Total	64	61.90	95.31	92.18	80.95	100.00	63.49*
			Posterior intestine				
19.4.1982	21	33.33	100.00	80.95	100.00	100.00	71.42
3.5.1982	22	77.27	100.00	100.00	90.91	100.00	59.09
18.5.1982	17	-	100.00	100.00	70.58	100.00	52.94
11.6.1982	17	52.94	100.00	100.00	64.70	100.00	52.94
Total	77	42.86	100.00	94.81	83.12	100.00	59.74 *

Table - 3. Contd.

1	2	3	4	5	6	7	8
			Alimentary canal				
19.4.1982	58	48.27	93.10	84.48	81.03	100.00	58.62
3.5.1982	51	72.55	100.00	100.00	82.35	96.08	58.82
18.5.1982	46	17.39	100.00	100.00	69.56	100.00	54.35
11.6.1982	39	48.72	92.31	100.00	48.72	100.00	43.59
Total	194	46.73	96.35	96.12	70.41	99.02	53.85*
			Water				
7.4.1982	19	78.94	100.00	73.68	57.89	100.00	52.63
19.4.1982	16	25.00	93.73	100.00	68.75	100.00	25.00
3.5.1982	9	11.11	88.88	100.00	77.78	88.88	-
18.5.1982	7	-	100.00	100.00	100.00	100.00	85.71
11.6.1982	8	-	100.00	100.00	100.00	100.00	12.50
Total	59	33.89	96.61	91.53	74.58	98.30	35.59*

Table - 3. Contd.

1	2	3	4	5	6	7	8
. .			Sediment				
7.4.1982	19	89.47	100.00	89.47	68.42	100.00	78.94
19.4.1982	15	46.66	80.00	100.00	46.66	100.00	6.67
3.5.1982	19	73.68	100.00	100.00	-	100.00	-
18.5.1982	13	30.76	100.00	100.00	15.38	100.00	7.69
11.6.1982	10	30.00	100.00	100.00	100.00	100.00	10.00
Total	76	59.21	96.05	97.36	42.10	100.00	23.68*

Table - 8. Contd. (Station 1)

Genera	Total	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.
						Water							
Vibrio	25	30.77	-	-	-	17.65	46.15	22.22	16.67	18.18	11.11	6.25	36.36
Pseudomonas	39	-	4.76	7.14	30.00	11.76	30.76	22.22	33.33	36.36	22.22	81.25	27.27
Aeromonas	3	-	-	-	-	-	-	-	-	9.09	11.11	-	9.09
Acinetobacter	1	-	-	-	-	-	-	-	-	-	-	-	9.09
Flavobacterium	4	-	9.52	7.14	10.00	-	-	-	-	-	-	-	-
Alcaligenes	0	-	-	-	-	-	-	-	-	-	-	-	-
Moraxella	6	-	14.29	-	-	-	15.38	-	-	-	11.11	-	-
Enterobacteriaceae	1	-	-	-	-	-	-	-	-	9.09	-	-	-
Staphylococcus	0	-	-	-	-	-	-	-	-	-	-	-	-
Micrococcus	26	46.15	38.09	35.71	30.00	-	7.69	11.11	-	9.09	11.11	-	-
Bacillus	36	23.08	9.52	35.71	30.00	58.82	-	11.11	41.67	18.18	22.22	12.50	9.09
Coryneform group	15	-	23.81	14.28	-	11.76	-	33.33	8.33	-	11.11	-	9.09
Total	156	13	21	14	10	17	13	9	12	11	9	16	11

Table - 8. Contd. (Station 1)

Genera	Total	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.
						Sediment							
Vibrio	22	-	-	6.67	20.00	-	66.67	27.27	-	-	20	9.09	60
Pseudomonas	41	28.57	6.25	6.67	-	-	22.22	27.27	17.65	91.67	30	27.27	10
Aeromonas	3	-	-	-	-	16.67	-	-	-	8.33	-	-	-
Acinetobacter	6	57.15	-	6.67	-	-	11.11	-	-	-	-	-	-
Flavobacterium	6	-	18.75	6.67	13.33	-	-	-	-	-	-	-	-
Alcaligenes	-	-	-	-	-	-	-	-	-	-	-	-	-
Moraxella	4	-	25.00	-	-	-	-	-	-	-	-	-	-
Enterobacteriaceae	2	-	-	-	-	16.67	-	9.09	-	-	-	-	-
Staphylococcus	-	-	-	-	-	-	-	-	-	-	-	-	-
Micrococcus	13	14.29	18.75	20.00	40.00	-	-	-	-	-	-	-	-
Bacillus	43	-	18.75	53.33	26.67	66.67	-	27.27	82.35	-	30	18.18	20
Coryneform group	11	-	12.50	-	-	-	-	9.09	-	-	20	45.45	10
Total	151	7	16	15	15	6	9	11	17	24	10	11	10

Table - 8. Contd. (Station 2)

Genera	Total	Dec.	Jan.	Feb.	Mar.	Apr.	May	Total	Dec.	Jan.	Feb.	Mar.	Apr.	May
Station 2					Adult									
		Surface							Gill					
Vibrio	11	3.23	8.33	12.50	8.33		100	25	-	9.68	33.33	-	10	94.44
Pseudomonas	21	3.23	25.00	-	8.33	68.42	-	11	8.34	-	25.00	-	40	5.55
Aeromonas	1	-	-	12.50	-	-	-	1	-	-	8.33	-	-	-
Acinetobacter	4	-	8.33	12.50	8.33	-	-	8	5.56	12.90	8.33	6.67	-	-
Flavobacterium	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alcaligenes	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Moraxella	14	-	4.17	37.50	33.33	31.58	-	8	-	-	-	26.67	40	-
Enterobacteriaceae	3	-	4.17	12.50	8.33	-	-	3	-	3.23	-	13.33	-	-
Staphylococcus	1	-	-	-	8.33	-	-	-	-	-	-	-	-	-
Micrococcus	38	87.10	37.50	-	16.67	-	-	52	86.11	45.16	25.00	20.00	10	-
Bacillus	6	6.45	12.50	12.50	-	-	-	9	-	19.35	-	20.00	-	-
Coryneform group	1	-	-	-	8.33	-	-	5	-	9.68	-	13.33	-	-
Total	100	31	24	8	12	19	6	122	36	31	12	15	10	18

Table - 8. Contd. (Station 2)

Genera	Total	Dec.	Jan.	Feb.	Mar.	Apr.	May	Total	Dec.	Jan.	Feb.	Mar.	Apr.	May
			Stomach							Anterior intestine				
Vibrio	22	7.14	-	25.00		89.47	11.11	35	-	-	27.27	21.05	88.24	100
Pseudomonas	3	-	7.69	8.33	-	-	11.11	11	-	16	36.36	10.52	5.88	-
Aeromonas	2	7.14	-	8.33	-	-	-	2	-	-	-	10.52	-	-
Acinetobacter	7	7.14	38.46	-	-	-	11.11	6	-	16	18.18	-	-	-
Flavobacterium	-	-	-	-	-	-	-	-	-	8	-	-	-	-
Alcaligenes	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Moraxella	3	-	5.26	-	10	5.26	-	-	-	-	18.18	5.26	-	-
Enterobacteriaceae	2		7.69	8.33	-	-	-	1	-	-	-	5.25	-	-
Staphylococcus	-	-	-	-	-	-	-	1	5.88	-	-	-	-	-
Micrococcus	23	78.57	15.38	25.00	50	-	22.22	32	94.12	44	-	26.32	-	-
Bacillus	1	-	23.08	16.66	10	-	-	4	-	8	-	10.52	-	-
Coryneform group	9	-	-	8.33	30	5.26	44.44	5	-	8	-	10.52	5.88	-
Total	77	14	13	12	10	19	9	102	17	25	11	19	17	13

Table - 8. Contd. (Station 2)

Genera	Total	Dec.	Jan.	Feb.	Mar.	Apr.	May	Total	Dec.	Jan.	Feb.	Mar.	Apr.	May
		Posterior intestine							Alimentary canal					
Vibrio	30	-	8.34	14.29		80.00	100	87	2.17	3.23	23.33	10.26	86.27	78.38
Pseudomonas	10	6.67	20.83	-	30	6.67	-	24	2.17	16.13	16.67	12.82	3.92	2.70
Aeromonas	4	-	-	-	40	-	-	7	2.17	-	-	15.38	-	-
Acinetobacter	5	13.33	12.50	-	-	-	-	18	6.52	19.35	6.67	-	-	2.70
Flavobacterium	5	6.67	16.67	-	-	-	-	7	2.17	9.68	-	-	-	-
Alcaligenes	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Moraxella	1	6.67	-	-	-	-	-	7	2.17	1.61	6.67	5.13	1.96	-
Enterobacteriaceae	1	-	-	-	10	-	-	4	-	1.61	3.33	5.13	-	-
Staphylococcus	-	-	-	-	-	-	-	2	2.17	-	3.33	-	-	-
Micrococcus	20	60.00	37.50	28.57	-	-	-	75	78.26	35.48	16.67	25.64	-	5.41
Bacillus	8	6.67	4.17	57.14	20	-	-	18	2.17	9.68	20.00	12.82	-	-
Coryneform group	2	-	-	-	-	13.33	-	16	-	3.23	3.33	12.82	7.84	10.81
Total	86	15	24	7	10	15	15	265	46	62	30	39	51	37

Table - 8. Contd. (Station 2)

Genera	Total	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.
						Water							
Vibrio	32	33.33	-	-	-	18.75	50.00	37.50	6.25	26.32	28.57	-	30
Pseudomonas	32	-	-	16.67	33.33	6.25	12.00	12.50	31.25	31.58	35.71	30.77	30
Aeromonas	4	-	-	-	-	-	-	-	-	5.26	14.29	-	10
Acinetobacter	4	-	6.67	5.56	-	-	-	-	-	-	-	7.69	10
Flavobacterium	4	-	13.34	11.11	-	-	-	-	-	-	-	-	-
Alcaligenes	1	-	-	-	-	-	-	-	-	-	7.14	-	-
Moraxella	8	-	6.67	5.56	-	-	25.00	25.00	-	-	-	-	-
Enterobacteriaceae	3	-	-	-	-	-	-	-	-	15.79	-	-	-
Staphylococcus	-	-	-	-	-	-	-	-	-	-	-	-	-
Micrococcus	31	53.33	66.67	38.89	33.33	6.25	6.25	12.50	6.25	-	-	-	-
Bacillus	31	13.33	-	5.56	33.33	50.00	-	-	50.00	10.53	7.14	53.85	-
Coryneform group	16	-	6.67	16.67	-	18.75	6.25	12.50	6.25	10.53	7.14	7.69	20
Total	166	15	15	18	6	16	16	8	16	19	14	13	10

Table - 8. Contd. (Station 2)

Genera	Total	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.
						Sediment							
Vibrio	31	-	-	20.83	18.18	-	28.57	40	4.55	30.76	33.33	9.09	45
Pseudomonas	28	20.00	10.53	20.83	-	25.00	71.42	20	13.64	30.76	22.22	-	5
Aeromonas	2	-	-	-	-	12.50	-	-	-	7.69	-	-	-
Acinetobacter	5	50.00	-	-	-	-	-	-	-	-	-	-	-
Flavobacterium	6	20.00	10.53	-	9.09	12.50	-	-	-	-	-	-	-
Alcaligenes	1	-	5.26	-	-	-	-	-	-	-	-	-	-
Moraxella	3	-	5.26	-	-	25.00	-	-	-	-	-	-	-
Enterobacteriaceae	3	-	-	-	-	-	-	-	-	23.06	-	-	-
Staphylococcus	-	-	-	-	-	-	-	-	-	-	-	-	-
Micrococcus	13	10.00	21.05	8.33	45.45	12.50	-	-	-	-	-	-	-
Bacillus	60	-	26.32	50.00	27.27	-	-	40	81.82	-	22.22	63.63	45
Coryneform group	12	-	21.05	-	-	12.50	-	-	-	7.69	22.22	27.27	5
Total	164	10	19	24	11	8	7	10	22	13	9	11	20

PUBLICATIONS

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