Indian J.Sci.Res. 5(2) : 151-160, 2014

ISSN : 0976-2876 (Print)
ISSN : 2250-0138 (Online)

OCCURRENCE OF VIBRIO CHOLERAE IN SHRIMP CULTURE ENVIRONMENTS OF KERALA, INDIA

S. LEKSHMY[a1], A. NANSIMOLE[b], M. MINI[c], N. ATHIRA[d] AND TRESA RADHAKRISHNAN[e]

[abcde]Department of Aquatic Biology and Fisheries, University of Kerala, Karyavattom, Trivandrum, India

ABSTRACT

The present study was conducted in order to monitor the presence of *Vibrio cholerae* in extensive and modified extensive shrimp culture systems. In extensive system, *V. cholerae* count ranged from 180 to 380 cfu ml-1 and 187 to 668 ml^{-1} in modified extensive system. In the present study sediment registered highest *Vibrio* load in both systems (1725-2708 cfu g^{-1} and 2019 to 5699 cfu g^{-1}). In extensive system, percentage distribution of *V. cholerae* in water was 6.5, 7.2 and 16.6%, respectively, for premonsoon, monsoon and postmonsoon. In, modified extensive system, in water, percentage distribution of V. cholerae was the highest during postmonsoon (11.3%) and the lowest during monsoon (8.3%). Premonsoon registered 10.3% and annual mean, 10.3%. In the present study *V. cholerae* loads were found to increase with advancement of culture operation in both extensive and modified extensive culture systems. The presence of *V. cholerae* serve as an indicator of public health safety of water and food destined for human consumption. So an assessment of *V. cholerae* was done in order to evaluate the health status of the ponds.

KEYWORDS : *Vibrio cholerae*, extensive system, modified extensive system, *Penaeus monodon*, shrimp culture

Of the crustaceans suitable for aquaculture, shrimp is the most important and it is extensively farmed all over the world. Since shrimps grow rapidly within impoundments and are in great demand, they are ideal for intensive cultivation (Milne, 1972). The brackish water area available in India is esteemed to be about 1.2 million ha (Heran et al., 1992) of which 65000 ha area is now under shrimp farming. In Asia, the major cultivated species are *P. monodon* because of its high growth rate and better tolerance to many adverse environmental conditions (Otta et al., 1998).

To increase production, aquaculturists have been resorting to intensification with high stocking densities and heavy supplementary feeding. A natural consequence of this has been deterioration of the water quality and the outbreak of various diseases. The conducive physicochemical and ecobiological situations in confined and nearly stagnant aquatic environments, such as culture ponds, favour the development of heavy microbial loads leading to stress and precipitation of infections/diseases (Song Qingyun et al., 1991; Sharmila et al., 1996). Compared to intensive and semi-intensive culture systems, extensive/modified extensive farming is more eco-friendly and sustainable (Das and Saksena, 2001). However, the extensive/modified extensive culture systems are not free from such problems (Sengupta et al., 2003; Harish et al., 2003). In extensive and modified extensive systems, disease is observed where water quality management is poor (Ramaiah, 2006).

Among water and food borne pathogens in coastal ecosystems Vibrios contribute the major part. The members of the family Vibrionaceae contribute 60% of the total bacterial population (Simidu and Tsukamoto, 1985).

A few studies have investigated pathogenic and spoilage bacteria associated with shrimp rearing (Austin and Austin, 1989; Lightner, 1993; Thakur et al., 2004; Abraham et al., 2008). Shrimp processors have been facing problem of rejection of their produce due to the presence of human pathogenic *Vibrio* spp. (Karunasagar and Karunasagar, 2003). Contamination of hard skeleton of crustaceans and shells of bivalve molluscs with *Vibrio* and *Aeromonas* is also increasingly recognized as the cause of wound and blood infections following laceration of the skin sustained during handling of shellfish (Bonner et al., 1983; Flynn and Knepp, 1987). Incidence of *V. cholerae* in shrimp ponds were reported by Shubha et al. (2005), Ganesh et al. (2010), Rao & Surendran (2013).

The present study was undertaken to monitor the occurrence of *V. cholerae* of pond reared tiger prawn, *P. monodon*, pond water and sediment. In addition, the physicochemical characteristics of pond water and sediment were studied for checking whether there is any significant correlation between the changes in water/sediment quality and microbial load of shrimp and shrimp pond environment.

[1]**Corresponding author**

MATERIALS AND METHODS

For present study six ponds growing *P. monodon* were selected from Kollam district, Kerala state (9.28'45º N and 76.28'0º E). In two ponds, (0.7 ha each), located at Munrothuruthu, 30 km northwest of Kottiyam town and fed with water from the Ashtamudi lake, extensive type of culture (stocking density = 5 m^{-2}) having neither specific management practices nor supplementary feeding was practiced. In four ponds (0.4 to 0.6 ha each), two located at Mayyanadu about 12 km southwest and two at Pathayakkodi 5 km south of Kottiyam town, all fed with water from the Paravoor Kayal, modified extensive type of farming (stocking density =10 m^{-2}) was done. In these ponds pellet feed was given, thrice a day.

Shrimp specimens, water and sediment samples were collected aseptically from the six ponds for microbiological analyses. Shrimps were caught using cast net and packed in sterile polythene bags. Water samples were collected in sterile screw-capped tubes and sediment samples in sterile petri-dishes using a sterile spatula. The samples were brought to the lab in ice baskets. The samples were properly prepared for further detailed studies, as soon as possible and always within three hours since collection. The samples were analysed for *Vibrio cholerae*. Suspected colonies were purified and confirmatory tests were done. *Vibrio cholerae* were counted using TCBS, Thiosulphate Citrate Bilesalt Sucrose agar (Hi-Media, Mumbai). Plates were incubated at 36±1°C for 18-24 h. *V. cholerae* colonies appeared as large (2-3 mm) smooth, yellow, slightly flattened growths with opaque centers and translucent peripheries. In the lab, 100 µl of water sample was poured into pre-prepared media plates and spread using a sterile, L-shaped glass rod. For sediment analysis, 1 g of sediment sample was suspended in 100 ml distilled water and mixed. After the settlement of sediment, 100 µl of the supernatant was transferred to pre-prepared petri-plates and spread using a sterile L-rod. For detection of *V. cholerae* in specimens, sample was macerated with alkaline peptone water (APW) in the ratio 1:9 and incubated in a conical flask at 37°C. After 6-8 h and 16-24 h of incubation without shaking the flask, a loop full of surface growth was streaked on pre-set TCBS (Thiosulphate Citrate Bilesalt Sucrose) agar and incubated at 36±1°C for 18-24 h. Typical *V. cholerae* colonies appeared as large (2-3 mm) smooth, yellow, slightly flattened growths with opaque centers and translucent peripheries.

Gram staining and motility test (by hanging drop method) were carried out with suspected isolates. *V. cholerae* is Gram-ve and motile. Suspected isolates were inoculated to TSI (Triple Sugar Iron) agar and KIA (Klingler Iron Agar) agar slants by stabbing the butt and streaking slant and incubated at 37°C for 18-24 h. In TSI slants after incubation, *V. chlorae* showed acidic slant (yellow) and acidic butt (yellow) and no blackening. In KIA slants, *V. cholerae* showed alkaline slant (red) and acidic butt (yellow) and no blackening. Cultures showing typical reactions of V. chlorae were subjected to confirmation tests such as salt tolerance test (APHA, 1992), Hugh and Leifson (H & L) glucose Oxidation/Fermentation (O/F) Test (Collins and Lyne, 1970), Cytochrome oxidase or Oxidase test (*V. chlorae* is oxidase +ve) (Collins and Lyne, 1970) and fermentation of Carbohydrates, *V. cholerae* is glucose, sucrose and mannitol+ve and arabinose and inositol-ve (APHA, 1992; Johnson and Christine, 2001; Surendran et al., 2005).

Water temperature, pH and salinity (‰) were recorded at the site itself using a Celsius thermometer of + 0.5°C accuracy, portable pH meter of + 0.1 accuracy [Model No. ip (1-198107) RI, USA], and portable refractometer (Erma Inc., Tokyo), respectively. Dissolved oxygen content (DO, mg l^{-1}) was estimated employing the classical Winkler's (1888) method. Dissolved carbondioxide concentration (CO_2, mg l^{-1}) was estimated by following the procedure of APHA (1998). Hydrogen sulphide (H_2S, mg l^{-1}), total alkalinity (mg l^{-1}) , total solids (TS, mg l^{-1}), total dissolved solids (TDS, mg l^{-1}), total suspended solids (TSS, mg l^{-1}) and calcium hardness (mg l^{-1}) were estimated by the method of Trivedy et al. (1987), total hardness (mg l^{-1}) by the method of Golterman et al. (1978), ammonia concentration (NH3, mg l^{-1}) by the method of Koroleff (1983). The difference between total and Ca-hardness was reckoned as Mg-hardness. For spectrophotometric assays, a dual beam spectrophotometer (Model UV2-100, UNICAM, UK) was used.

Sediment samples were collected using a PVC corer of length 25 cm and diameter 7 cm. The sediment samples collected from four points were transferred into clean polythene bags and brought to the laboratory, pooled, air dried and sieved (sieve number-62 μm) before analysis. The temperature of the sediment was noted using a Celsius thermometer (calibrated before each collection) as soon as the corer was lifted out of water. Organic carbon content was estimated following Jhingran et al. (1988) and the results were expressed in percentage. Soil texture was determined by the sieve and pipette method (Krumblin & Petti John, 1938). pH was measured as per Trivedy et al. (1987) using a handheld pH meter (Model ip (1-198107) RI USA) calibrated before each set of measurement.

Statistical significance of associations (dependence) of estimated microbial variables among themselves and between them and hydrobiological variables were tested using correlation analysis. Relevant theoretical inputs for statistical analyses were adopted from Zar (1974) and analyses were done using "statistiXL 1.8" package.

RESULTS AND DISCUSSION

Percentage distribution of *V. cholerae* in extensive and modified extensive shrimp culture system are presented in Table 1; Figure. 1. The results of statistical analyses are included in Tables 2 to 5.

In extensive system, percentage distribution of *V. cholerae* in water was 6.5, 7.2 and 16.6%, respectively, for premonsoon, monsoon and postmonsoon. Annual mean *V. cholerae* in water was 9.6%. In sediment, it was the highest during monsoon (89.8%) and the lowest during postmonsoon (75.2%), whereas during premonsoon, it registered 82.5%. Annual mean *V. cholerae* in sediment was 82.7%. In shrimp, *V. cholerae* was the highest during premonsoon (11.1%), and the lowest during monsoon (3.0%); postmonsoon recorded 8.2% and annual mean, 7.8%. In, modified extensive system, in water, percentage distribution of *V. cholerae* was the highest during postmonsoon (11.3%) and the lowest during monsoon (8.3%). Premonsoon registered 10.3% and annual mean, 10.3%. In sediment, *V. cholerae* was the highest during monsoon (89.5%) and the lowest during postmonsoon (86.0%). Premonsoon recorded 87.7%. Annual mean *V. cholerae* in sediment was 87.4%. In shrimp, *V. cholerae* during the three seasons were 2.0, 2.2 and 2.7%, with an annual mean of 2.3%.

The results of correlation analyses of microbial loads in water, sediment and shrimp in extensive system and modified extensive system was significantly positively correlated among themselves ($P< 0.01$). Results of ANOVA showed that the observed differences in between compartments were statistically significant ($P < 0.01$) in both systems and between season ($P < 0.01$) in modified extensive system. In Table are presented the results of ANOVA comparing microbial loads of extensive and modified extensive shrimp culture systems between systems, between the three compartments, between seasons

Table 1. Mean Microbial Load (Given as Bacterial Count) and Percentage Distribution in Three Compartments, Water (Cfu ml^{-1}), Sediment (Cfu g^{-1}) and Shrimp (cfu g^{-1}) of Extensive and Modified Extensive Shrimp Culture Systems in Each Season of Shrimp Culture Operation.

Bacterial Counts	Compartments	Extensive System			Modified Extensive System		
		Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
	Water (cfu ml-1)	213.0	180.0	380.0	668.0	187.0	508.0
	Sediment (cfu g-1)	2708.0	2238.0	1725.0	5699.0	2019.0	3858.0
V. cholerae	Shrimp (cfu g-1)	363.0	75.0	188.0	131.0	49.0	121.0

Bacterial Counts	Compartments	Extensive System			Modified Extensive System		
		Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
	Water	6.5	7.2	16.6	10.3	8.3	11.3
V. cholerae	Sediment	82.5	89.8	75.2	87.7	89.5	86.0
(%)	Shrimp	11.1	3.0	8.2	2.0	2.2	2.7

Figure 1 : Seasonal Variation in *V. cholerae* Distribution in Extensive and Modified Extensive Penaeus monodon Culture Systems.

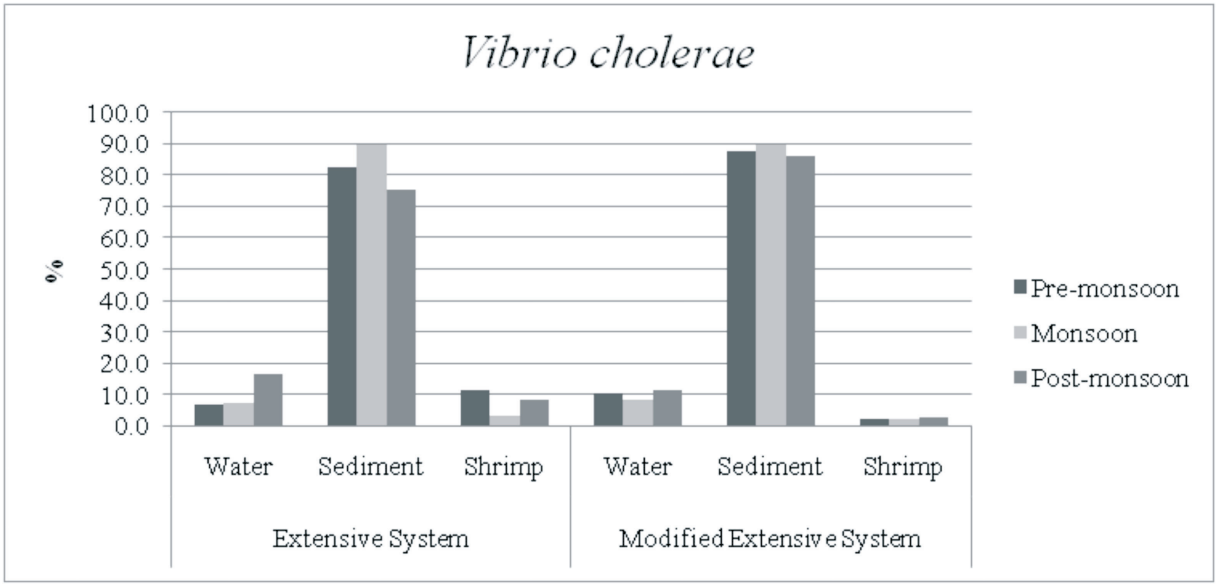

Table 2. Results of correlation Analysis Showing r Values Comparing *V. cholerae* Loads in Extensive (N = 20) and Modified Extensive (N=40) Shrimp Culture System.

	Extensive System		Modified Extensive System	
	VC_Wt	VC_Sh	VC_Wt	VC_Sh
VC_Sd	0.606#	0.524#	0.223#	0.576#
VC_Sh	0.539#		0.223#	

(VC_Sd, V. Cholerae in sediment; VC_Wt, V. Cholerae in water; VC_ Sh, V. Cholerae in shrimp)

* P < 0.05; # P < 0.01

Table 3. Results of ANOVA Comparing *V. cholerae* Loads of Extensive Shrimp Culture System Between the Three Compartments (Water, Sediment & Shrimp), Between Seasons and Showing Season*Compartment Interaction

Variable	Source	Type III SS	Df	Mean Sq.	F	
Extensive System						
V. cholerae	Season	606.692	2	303.346	1.741	
	Compartment	11788.6	2	5894.3	33.826	#
	Season*Compartment	339.974	4	84.993	0.488	
Modified Extensive System						
V. cholerae	Season	4164.233	2	2082.116	6.789	#
	Compartment	47906.168	2	23953.084	78.099	#
	Season*Compartment	2025.926	4	506.481	1.651	

Table 4. Results of Correlation Analysis Showing *r* Values Comparing *V.cholerae* Loads in Water, Sediment and Shrimp With Hydrographical Parameters of Extensive (N = 20) and Modified Extensive (N = 40) Shrimp Culture System

	Extensive system			Modified extensive system		
	Water	Sediment	Shrimp	Water	Sediment	Shrimp
Temp_W	-0.187	-0.026	0.071	0.16	0.049	0.196
pH	0.046	-0.118	-0.241	0.147	-0.299	0.002
Sal.	0.265	0.299	0.35	0.217	0.272	0.383*
DO	-0.133	-0.156	-0.033	0.167	-0.213	-0.019
CO_2	0.147	0.409	0.228	-0.146	0.356*	0.042
H_2S	0.329	0.113	0.483*	0.055	0.318*	0.268
NH_3	0.395	0.23	0.095	0.242	0.390*	0.441#
Alk.	0.324	0.274	0.163	0.298	-0.195	0.142
Har_T	0.146	0.213	0.436	0.182	0.344*	0.378*
Ca_H	0.14	0.309	0.551*	0.192	0.379*	0.387*
Mg_H	0.257	0.165	0.369	0.097	0.156	0.244
TS	0.167	0.311	0.478*	0.208	0.324*	0.273
TDS	0.173	0.459*	0.327	0.125	0.307	0.196
TSS	0.133	0.193	0.42	0.21	0.276	0.261
NO_3	0.379	0.282	0.035	-0.124	0.185	-0.113
NO_2	-0.055	-0.12	-0.209	-0.045	0.243	0.082
PO_4	0.000	-0.388	-0.092	0.235	-0.242	0.172
SiO_3	0.03	-0.086	0.116	-0.036	0.044	0.173
SO_4	0.142	0.167	0.235	0.241	0.082	0.296
***P* < 0.05; #*P* < 0.01**						

(**Alk.**, alkalinity; **Har_T**, total hardness; Ca_H, calcium hardness; Mg_H, magnesium hardness; **Sal.**, salinity; **Temp_W**, water temperature)

Table 5. Results of Correlation Analysis Showing r Values Comparing *V. cholerae* Loads With Sedimentological Parameters of Extensive (N = 20) and Modified Extensive (N=40) Shrimp Culture Systems

	Extensive system			Modified extensive system		
	Water	Sediment	Shrimp	Water	Sediment	Shrimp
Temp.	0.132	0.466*	0.168	0.167	0.151	0.178
pH	-0.232	-0.319	-0.263	-0.212	-0.075	-0.186
OC	0.153	0.344	0.159	-0.165	-0.07	-0.138
Sand	-0.302	-0.091	-0.452*	-0.443#	-0.346*	-0.453#
Clay	0.546*	0.3	0.548*	0.105	0.059	0.144
Silt	0.244	0.054	0.408	0.454#	0.358*	0.458#
***P* < 0.05; #*P* < 0.01**						
(Temp., temperature; OC, Organic Carbon)						

and showing various interactions.

An attempt was made to understand whether microbial loads were dependent on hydrographical and sedimentological properties of the shrimp culture systems. *V. cholerae* in shrimp in extensive system and in sediment in modified extensive system showed positive correlation with H_2S ($P < 0.05$). In modified extensive system *V. cholerae* in sediment ($P < 0.05$) and shrimp ($P < 0.01$) was positively correlated with NH_3. *V. cholerae* in shrimp in extensive system and in sediment in modified extensive system showed significant positive correlation with Total Solids ($P < 0.05$). *Vibrio cholerae* in shrimp ($P < 0.05$) in extensive system and in water ($P < 0.01$) and shrimp ($P < 0.01$) in modified extensive system were significantly negatively correlated with sand content. In modified extensive system *V. cholerae* in water ($P < 0.01$), sediment ($P < 0.05$) and shrimp ($P < 0.01$) were positively correlated with silt.

In the present study *V. cholerae* in water ranged from 180 to 668 cfu ml^{-1}, in sediment 1725 to 5699 cfu g^{-1} and in shrimp it was 49 to 363 cfu g^{-1}. In modified extensive *P. monodon* ponds, Thakur et al. (2004) reported *Vibrio* count ranging from 1.8 $x10^1$ to 7.8 $x10^4$ cfu ml^{-1} in pond water, 8.3 x106 cfu g^{-1} in the hepatopancreas of infected and 2.6 $x10^3$ cfu g^{-1} of healthy shrimp. Abou- Elela et al. (2009) obtained *Vibrio* count of 122 cfu ml^{-1} in water from North Delta, Egypt. Occurrence of *V. cholerae* in water, sediment and shrimp in *P. monodon* culture ponds has been reported by Sugumar et al. (2001). Otta et al. (1999), who isolated *V. cholerae* from water samples in ponds growing *P. monodon* in India, reported *Vibrio* count of 2.0-7.2 $x10^2$ cfu ml^{-1} in water samples collected from different farms on the east coast and 5.0 $x10^1$ to 2.8 $x10^4$ cfu ml^{-1} from farms on the west coast.

In the present study, both in extensive and modified extensive systems, *V. cholerae* registered the highest load in sediment throughout the three seasons. Similar observations by Ahmad et al. (2003), Harish et al. (2003), Daboor (2008) and Chandran et al. (2009). This might be because of the prolonged survival of bacteria in sediment, which offers more favourable chemical and biological environments. Pal and Chanchal (1992), Velammal (1993), Yongquan et al. (1994), Davies et al. (1995) and Michael et al. (2007) are of the view that sediment provides some sort of protection to bacteria to tide over unfavourable environmental conditions.

In the present study *V. cholerae* loads were found to increase with advancement of culture operation in both extensive and modified extensive culture systems. This may be due to the increase in bacterial count with increasing detritus organic load at the pond bottom (Sujatha, 2007; Kannapiran et al., 2009). According to Karunasagar et al. (1992), bacterial population in shrimp tissues, pond water and bottom mud increases because of accumulation of metabolites and unused feed. Mary (1977), Chen et al. (1989), Chen (1992) and Dalmin et al. (1997, 2002) also attest increase in bacterial load in water and sediment with increase in organic load. Thakur et al. (2004) reported increasing trend of *V. cholerae* up to the end of each culture season. Ganesh et al. (2010) found high *V. cholerae* population in the sediment towards end of culture (2.3 $x10^7$ cfu g^{-1} at 150th day of culture as against 1.9 $x10^7$ cfu g^{-1} at 25th day of culture).

In the present study in both systems, noted the maximum bacterial count during warmer months. Similar observations by, Chandrika and Nair (1994) in Cochin backwaters and Hassan (1995) in Abu Dhabi coastal waters, Harish et al. (2003) in extensive aquaculture ponds adjacent to Cochin backwaters, Gore et al. (1978) in Cochin backwaters, Abhay Kumar and Dube (1995) in old port, Bhavnagar coast and Daboor (2006, 2008) in El-Qanatar fish farm, Cairo. It is noteworthy here that according to Rheinheimer (1965, 1970), living conditions remaining favourable, microorganisms will quickly multiply during summer. The present results are in agreement with the foregoing.

In any aquatic system, environmental parameters such as temperature, salinity, pH and dissolved oxygen play a foremost part in the distribution of bacteria (Palaniappan, 1982). But, pond being a confined environment the optimum environmental parameters could be maintained throughout the culture period by proper pond management involving water exchange and lime application. Sharmila et al. (1996) suggested that environmental parameters did not

influence the distribution of bacterial load in the pond ecosystem because there was no dramatic change in the environmental parameters.

CONCLUSION

The overall results of the study indicate that, the presence of various microbial contaminants in substantial quantities in all three compartments (water, sediment and shrimp) of both systems, must be reckoned as a warning signal on the environmental deterioration of such ponds and on the high likelihood of precipitation of shrimp and human health hazards. The detectable, frequency of potentially human pathogenic, *V. cholerae* in shrimp ponds in Kerala suggests a probable risk for public health. Therefore, it is recommended to pay attention to postharvest handling and adequate cooking. Strict quality guidelines have been laid by the importing nations, for the food products that enter their markets. The mere presence of pathogenic *vibrios* is sufficient for rejection of the exported product. It is important to maintain the culture successfully through proper pond preparation, seeding quality larvae with moderate stocking density, maintaining stable phytoplankton bloom, good water quality, less feed waste and routine monitoring. It will reduce the bacterial load and ultimately reduce the chance of disease outbreak.

ACKNOWLEDGEMENTS

We are thankful to the Head, Department of Aquatic Biology and Fisheries, University of Kerala, Kariavattom, for providing necessary laboratory facilities. The first author acknowledges with thanks the financial support for this study from the University of Kerala, India.

REFERENCES

Abhay Kumar, V.K. and Dube, H.C., 1995. Occurrence and distribution of bacterial indicators of fecal pollution in the tidal waters of a muddy coast. J. mar. biol. Ass. India, 37: 98-101.

Abou-Elela G.M., El-Sersy, N.A., Abd-Elnaby, H. and Wefky, S.H., 2009. Distribution and biodiversity of fecal indicators and potentially harmful pathogens in North Delta (Egypt). Aust. J. Basic Appl. Sci., 3: 3374-3385.

Abraham T. J., Shanmugam S. A., Dhevendran K. and Palaniappan R., 2008. Luminous bacterial flora of penaeid shrimps and their environs in semi-intensive culture systems. Indian J. Fish., 55: 311-316.

Ahmad T., Tjaronge M. And Suryati E., 2003. Performances of tiger shrimp culture in environmentally friendly ponds. Indonesian J. Agri. Sci., 4: 48-55.

Anand Ganesh E., Sunita D., Chandrasekar K., Arun G. and Balamurugan S., 2010. Monitoring of Total Heterotrophic Bacteria and Vibrio Spp. in an Aquaculture Pond. Current Research Journal of Biological Sciences, 2: 48-52.

APHA 1992. Standard Methods for the Examination of Water and Waste Water. 18th ed., (A.E. Greenberg, L.S. Clesceri & A.D. Eaton eds.), Am. Public Hlth. Ass., Washington, DC.

APHA 1998. Standard Methods for the Examination of Water and Waste Water. 20th ed., Am. Public Hlth. Ass., Washington, USA, 1325 pp.

Austin B. and Austin D. A., 1989. General introduction. In, B. Austin and D.A. Austin, (eds.), Methods for the Microbiological Examination of Fish and Shellfish, Ellis Horwood Ltd., NY, pp.17.

Bonner J. R., Coker A. S., Berryman C. R. and Pollack, M.H., 1983. Spectrum of Vibrio infections in a Gulf coast community. Annl. Internal med., 99: 464-469.

Chandran A, Sheeja K. M., Hatha A. A. M. , Sherin V. and Thomas A. P., 2009. Role of biological factors on the survival of Escherichia coli, Salmonella paratyphi and Vibrio parahaemolyticus in a tropical estuary, India. Water, 1: 76-84.

Chandrika V. and Nair P. V. R., 1994. Seasonal variations of aerobic heterotrophic bacteria in Cochin backwater. J. mar. biol. Ass. India, 36: 81-95.

Chen D., 1992. An overview of the disease situation, diagnostic techniques, treatments and preventives used on shrimp farms in China. In, W. Fulks and K.L. Main, (eds.), Diseases of Cultured Penaeid Shrimp in Asia and the United States, The Oceanic Institute, Honolulu, pp.47-55.

Chen J. C., Tu C.C. and Yang W.S., 1989. Acute toxicity of ammonia to larval Penaeus japonicus. J. Fish. Soc. Taiwan, 16: 261-270.

Collins C. H. and Lyne P. M., 1970. Microbiological Methods. 3rd ed. Butterworth, London and Univ. Park Press, Baltimore, 454 pp.

Daboor S. M., 2006. Studies on Bacterial Flora and Its Role in the Clean-up of Hazardous Pollutants in the River Nile. Ph. D. Thesis (Botany), Faculty of Science, Zagazig Univ., Egypt.

Daboor S. M., 2008. Microbiological profiles of El-Qanater El-Khairia Fish Farm. Global Vet., 2: 51-55.

Dalmin G., Purushothaman A. and Kathiresan K., 2002. Distribution of total heterotrophic bacteria (THB) and Vibrio parahaemolyticus in shrimp culture ecosystem. Indian J. Fish., 49: 247-253.

Dalmin G., Purushothaman A., Kannapiran E., Sankar G. and Srinivasan, K., 1997. Microbiology of a shrimp pond with reference to total heterotrophic, coliform and beneficial bacteria. Proc. Natn. Sem. Water Quality Issues in Aquaculture Systems, 18-19 Dec., 1996, (R. Santanam, V. Ramadhas and P. Gopalakrishnan, eds.), Tamil Nadu Vet. and Anim. Sci. Univ., Tuticorin, pp. 9-14.

Das S. K. and Saksena D. N., 2001. Farm management and water quality in relation to growth of Penaeus monodon in modified extensive shrimp culture system. J. Inland Fish. Soc. India, 33: 55-61.

Davies C. M., Long J.A.H., Donald, M. and Ashbolt, N.J., 1995. Survival of faecal microorganisms in marine and freshwater sediments. Appl. Environ. Microbiol., 61: 1888-1896.

Flynn T. J. and Knepp I. G., 1987. Seafood shucking as an etiology for Aeromonas hydrophila infection. Arch. Internal med., 147: 1816-1817.

Golterman H. L., Clymo R.S. and Ohnstad M.A.M., 1978. Methods for Physical and Chemical Analysis of Freshwaters. IBP No. 8, Blackwell Sci. Publ., Oxford, 213 pp.

Gore P. S., Raveendran O. and Unnithan R.V., 1978. Pollution in Cochin backwaters with reference to indicator bacteria. Indian J. Mar. Sci., 8: 43-46.

Harish R., Nisha K.S. and Hatha A.A.M., 2003. Prevalence of opportunistic pathogens in paddy-cum-shrimp farms adjoining Vembanadu lake, Kerala, India. Asian Fish.Sci., 16: 185-194.

Harish R., Nisha K. S. and Hatha A. A. M. , 2003. Prevalence of opportunistic pathogens in paddy-cum-shrimp farms adjoining Vembanadu lake, Kerala, India. Asian Fish. Sci., 16: 185-194.

Hassan E.S., 1995. Monitoring of microbial water quality and saprophytic bacterial genera of Abu Dhabi coastal area, UAE. J. mar. biol. Ass. India, 37: 191-200.

Heran M. P., Surendran V., Madusudhan Reddy, K. and Subba Rao, V., 1992. A success story on scientific shrimp farming. Fishing Chimes, pp. 34.

Jhingran V. G., Natarajan A. V., Banerjee S. M. and David, A. A., 1988. Methodology on reservoir fisheries investigations in India. Bull. CICFRI, Barrackpore, 12: 81 pp.

Johnson T. R. and Christine L. C., 2001. Laboratory Experiments in Microbiology. 6th ed., Addison Wesley Longman, California, San Francisco, 440 pp.

Kannapiran E., Ravindran J., Chandrasekar R. and Kalaiarasi A., 2009. Studies on luminous, Vibrio harveyi associated with shrimp culture system rearing Penaeus monodon. J. Environ. Biol., 30: 791-795.

Karunasagar I. and Karunasagar I., 2003. Rapid detection of pathogenic Vibrio spp. in sea food using DNA probes. Fishing Chimes, 23: 13-14.

Karunasagar I., Ismail S. M., Amarnath H.V. and Karunasagar, I., 1992. Bacterial load of tropical shrimp and marine sediment. FAO Fish. Suppl. Rep., 470: 1-8.

Koroleff F., 1983. Determination of ammonia. In, K. Grasshoff and M. Ehrhardt, (eds.), Methods of Seawater Analysis, Verlag. Chemie. Gmb., Weinheim, pp. 150-157.

Krumbelin W. C. and Pettijohn, F. J., 1938. Manual of Sedimentary Petrography. Appleton Century Crafts, NY, 459 pp.

Lightner D. V., 1993. Diseases of penaeid shrimps. In, J.P. McVey, (ed.), CRC Handbook of Mariculture, 2nd ed. Vol. 1, Crustacean Aquaculture, CRC Press, Boca Raton, FL, pp. 393-486.

Madhusudana Rao B. and Surendran P. K., 2013. Pathogenic Vibrios in Penaeus monodon Shrimp Hatcheries and Aquaculture Farms. Fishery Technology, 50: 161 167.

Mary P. P., 1977. Studies on the Gastrointestinal Microflora of the Mullet, Liza dussumeri (Valenciennes) (Mugiliformes, Teleostei). Ph. D Thesis, Annamalai Univ., India.

Michael, A.M., Cahoon, L.B., Toothman, B.R., Parsons, D.C., McIver, M.R., Ortwine, M.L. and Harrington, R.N., 2007. Impact of a raw sewage spill on water and sediment quality in an urbanized estuary. Mar. Pollut. Bull., 54: 81-88.

Milne P.H., 1972. Fish and Shellfish Farming in Coastal Waters. Fishing News (Books), Ltd., London, pp. 208.

Otta S. K., Karunasagar I. and Karunasagar I., 1999. Bacterial flora associated with shrimp culture ponds growing Penaeus monodon in India. J. Aquacult. Trop., 14: 309-318.

Otta S. K., Karunasagar I., Tauro, P. and Karunasagar, I., 1998. Microbial diseases of shrimp. Indian J. Microbiol., 38: 113-125.

Pal D. and Chanchal D.G., 1992. Microbial pollution in water and its effect on fish. J. Aquat. Anim. Health, 4: 32-39.

Palaniappan R., 1982. Studies on the Microflora of the Prawn, Penaeus indicus Milne Edwards (Crustacea, Decapoda, Penaeidae) with Reference to its Digestive System. Ph. D. Thesis, Annamalai Univ., India.

Ramaiah N., 2006. A review on fungal diseases of algae, marine fishes, shrimps and corals. Indian J. Mar. Sci., 35: 380-387.

Rheinheimer G., 1965. Mikrobiologische unter Scuchungen in der Elbe ZwiSchen Schnakenburg and Cuxhaven. Arch. Hydrobiol., 29: 181-251.

Rheinheimer G., 1970. Chemische, mikrobiologische und Planktologische Untersuchungen in der Schlei im Hinblick auf deren Abwasser belastung. Kieler Meeresforsch., 26: 105-216.

Sengupta T., Sasmal D. and Abraham T. J., 2003. Antibiotic susceptibility of luminous bacteria from shrimp farm environs of West Bengal. Indian J. Mar. Sci.,32: 334- 336.

Sharmila R., Abraham T. J. and Sundararaj V., 1996. Bacterial flora of semi-intensive pond reared Penaeus indicus (H. Milne Edwards) and the environment. J. Aquacult. Trop., 11: 193-203.

Shubha G., Otta S. K., Sanath K., Indrani Karunasagar, Matsuaki Nishibuchi, Iddya Karunasagar,T., 2005. The occurrence of Vibrio species in tropical shrimp culture environments; implications for food safety. International Journal of Food Microbiology, 102: 151 159.

Simidu V. and Tsukamoto K., 1985. Habitat segregation and biochemical activities of marine members of the family Vibrionaceae. Appl. Environ. Microbiol., 105: 781-790.

Song Qingyun, Luying and Sunxiunqin, 1991. Experimental report on quantitative distribution of microbes in culturing environment of Penaeus orientalis in Shounguang country, Shandong Province, J. Oceanogr. Huunghai Bhoai Seas, 9: 64-68.

Sugumar G., Abraham T. J. and Shanmugham S., 2001. Human pathogenic bacteria in shrimp farming system. Indian J. Microbiol., 41: 269-274

Sujatha V., 2007. Studies on the Tetragenococcus halophilus and its Potential Probiotic Activity on Pathogenic Bacteria Associated with Aquaculture Systems. Ph. D. Thesis, Univ. Madras, India.

Surendran P. K., Nirmala, T., Nambiar N. and Lalitha, K.V., 2005. Laboratory Techniques for Microbiological Examination of Sea Foods. CIFT Training Manual, Cochin, 26pp.

Thakur A. B., Vaidya R. B. and Suryawanshi S. A., 2004. Vibrio abundance in modified extensive culture ponds of Penaeus monodon. Indian J. Fish., 51: 147-152.

Trivedy R. K., Goel P.K. and Trisal C.I., 1987. Practical Methods in Ecology and Environmental Sciences. Environ. Publ., Karad, India, 340 pp.

Velammal A., 1993. Studies on Vibrio cholerae and Vibrio parahaemolyticus from Pondicherry Coastal Environs (South India). Ph. D. Thesis, Annamalai Univ., India.

Winkler L. W., 1888 The determination of dissolved oxygen in waters. Berlin Duet.Chem. Ges., 21: 2843-2855.

Yongquan S., Xinyi C., Weihua W., Jun W. and Shuxin H., 1994. Relationship between Vibrio quantity in cultured penaeid and two shrimp diseases. J. Xiamen. Unive. Nat. Sci. Xiamen. Daxue. Xuebao., 33: 421-424.

Zar J.H., 1974. Biostatistical Analysis. Prentice-Hall Inc., N.J., 620 pp.