Seafood Safety - Proceedings of the Symposium on Seafood Safety - Status and Strategies, 28-30 May 2002, Cochin, India,370-378.

Ecology and Distribution of *Vibrio parahaemolyticus* in Fish and Fishery Environments

K. SUDHA*, NIRMALA THAMPURAN and P.K. SURENDRAN
Central Institute of Fisheries Technology
P.O. Matsyapuri, Cochin - 682029, India

Vibrio parahaemolyticus is a major etiological agent in the seafood borne gastroenteritis. It is naturally present in aquatic systems, particularly saline waters. A study has been conducted to determine the ecology and distribution of *V. parahaemolyticus* in fish and fishery environments in the Arabian Sea, off Cochin. Water, sediment, plankton, fish and shellfish samples were analyzed for total halophilic vibrios, total vibrios and *V. parahaemolyticus*. The percentage of *V. parahaemolyticus* to the total vibrios was estimated as 5.6 to 50, 12.5 to 90 and 5.3 to 40 in water, sediment and plankton samples, respectively. Out of the 17 different fish species comprising 46 samples from pelagic and demersal sources, the organism was detected in skin and muscle in 35% of the samples, in gills in 53% and in the intestine of 71% samples. In 25 samples of shrimp comprising 6 different species, the prevalence was 83% both in intestine and muscle with shell on. The values of the Kanagawa positives were in the range of 5 to 22% (mean: 14%) of the total *V. parahaemolyticus* present in water, sediment, plankton and fish/shellfish.

Key words: Ecology, distribution, *Vibrio parahaemolyticus*, fish, fishery environment

Vibrio parahaemolyticus is a major etiological agent in seafood borne gastroenteritis and is autochthonus to aquatic system where salinity and temperature are on the higher side. Reports show that food poisoning due to consumption of seafood contaminated with *Vibrio* species, particularly *V. parahaemolyticus* has increased considerably during the recent years in US, Japan and Korea (Daniels *et al.*, 2000; Wong *et al.*, 2000; Lee *et al.*, 2001). The outbreaks in Korea and Japan have been associated with climatic conditions, festival season, vacations and above all the food culture of the regions (Lee *et al.*, 2001). It has been postulated that the high incidence of infection due to *V. parahaemolyticus* can be correlated to the emergence of a new serotype O3: K6, which posses a high infection frequency and capacity

Present address: Dept of Biotechnology, St. Peter's College, Kolencherry, Cochin - 682 311, India

to spread globally (Wong *et al.*, 2000; Matsumoto *et al.*, 2000; Daniels *et al.*, 2000) and this necessitates intensive monitoring of this organism.

V. parahaemolyticus is considered as an inherent pathogen of aquatic origin and it can tolerate lower temperatures better than the other common mesophiles of terrestrial origin. The generation time reported for this pathogen is very short, ranging from 8-18 min depending on temperature and food material (Twedt, 1989). This short generation time coupled with the frequent prevalence in seafood of tropical regions renders it a food safety hazard in tropical fish products.

India ranks fourth in the export of seafood to other countries and along with increase in export new restrictions have been imposed on the exported material and presence of *V. parahaemolyticus* in seafood is one among them. The EU regulations stipulate that this organism should not be present in the processed seafood. A limit of $100.g^{-1}$ of *V. parahaemolyticus* is allowed for the raw seafood exported to Japan. Being a part of the natural flora of the marine and brackish waters, the present limit of total absence of this organism in processed seafood needs further scrutiny.

This necessitates in-depth studies and collection and compilation of data derived from several countries regarding the existence of these bacteria in warm aquatic environments. It is envisaged that the present work may give useful information on the occurrence of *V. parahaemolyticus* in fish and fishery environments in the Arabian Sea off Cochin (India).

Materials and Methods

Samples analyzed were seawater, sediment, plankton, finfishes and shellfishes. Water, sediment and plankton samples were collected from different locations in the open sea off Cochin (9°25'-10°10'N lat; 76°13'-76°30' E long.). Twelve samples each of water, sediment and plankton were used in the study over a period of 3 years. 17 commercially available finfishes and 6 prawn species were also analyzed. Fish/shellfish samples were collected from vessels, fish landing places or local markets, immediately kept under ice and brought to the laboratory for analysis. Microbiological analysis included total halophilic count (THC), total vibrio count (TVC) and *V. parahaemolyticus* count.

The total halophilic count was determined in trypticase soy agar (Oxoid) with 3% added sodium chloride (TSSA) and total vibrio count (TVC) in TCBS (thiosulphate citrate bile salt) agar (Oxoid). Counts were determined

by spread plating appropriate dilution of skin with muscle, gill and intestine of the samples. All green colonies developing on TCBS were isolated and purified in TSSA and identified to species level as per Elliott *et al.* (1995). Percentage prevalence of *V. parahaemolyticus* in each of these samples was determined after identification of the constituent *Vibrio* species in the sample. A reference strain of *V. parahaemolyticus* ATCC 17802 was used in identification along with the isolates to check the authenticity of the results. All the isolates confirmed as *V. parahaemolyticus* was subjected to Kanagawa test on Wagatsuma medium using fresh human blood (Elliott *et al.*, 1995).

Results and Discussion

The physicochemical parameters of the water samples collected from various locations are presented in Table 1. As seen from the data, wide variations were observed in dissolved oxygen and salinity. Statistical analysis of the data showed that *Vibrio* population was not significantly ($p < 0.05$) related to the tested parameters like temperature, salinity, pH and dissolved oxygen (unpublished). This finding is contrary to the reports from temperate regions wherein striking correlation could be observed between water temperature and *Vibrio* populations (Wright *et al.*, 1996). Such correlation can be related to conspicuous variations in temperature due to seasonal fluctuations in temperate zones. O'neil *et al.* (1992) have reported a temperature variation from -1 to 29°C in US coastal waters and claimed that seasonal variation in *V. vulnificus* was due to this temperature variation. However, in tropical climate where variations in temperature are within limit, this phenomenon may not be significant for *V. parahaemolyticus*.

Table 1. Physicochemical parameters of water samples from different stations off Cochin

Parameter	Range
Depth, m	6 - 15
Temperature, °C	26 - 29.5
pH	7.4 - 8.2
Salinity, ppt*	19.50 - 35.20
Dissolved oxygen, mg. l^{-1}	2.8 - 8.8

* Parts per thousand

The prevalence of *V. parahaemolyticus* in water, sediment and plankton are given in Table 2. Total *Vibrio* population in water, sediment and zooplankton were in the range of $6.0x10^2$ - $8.2x10^3$, $9.8x10^5$ - $7.3x10^7$ and

$1.8x10^7$ - 1.85 $x10^8.g^{-1}$, respectively. *V. parahaemolyticus* constituted 12.8% of *Vibrio* population in the water. In sediment it was the most predominant species among vibrios and constituted 18.1% of the total flora. The corresponding value in the plankton was 15.0%. The results indicate that highest density of this organism is in sediment samples and least in the water. The THC and TVC counts were also comparatively higher for sediment than the other two sources.

Table 2. Prevalence of *Vibrio parahaemolyticus* in water, sediment and plankton samples collected from different stations off Cochin

Water*		Sediment*		Plankton*	
Total isolates per sample	(%) *V. parahaemolyticus*	Total isolates per sample	(%) *V. parahaemolyticus*	Total isolates per sample	(%) *V. parahaemolyticus*
9	-	11	9.1	12	25.0
15	13.3	24	20.8	8	-
12	50.0	8	12.5	11	18.2
11	18.2	10	20.0	22	-
26	-	19	15.8	10	10.0
14	21.4	20	20.0	17	11.8
18	5.6	8	25.0	10	40.0
12	-	8	-	19	5.3
7	-	5	20.0	10	20.0
19	-	12	16.7	9	11.1
18	2.22	9	22.2	12	33.3
18	27.8	10	30.0	7	28.6
179	12.8	144	18.1	147	15.0

* 12 samples each over a period of 3 years

Distribution of vibrios can be attributed to the suspended particulate matter of the water column (Monticelli & Crisafi, 1995) and is further influenced by various limiting factors like salinity, pH, depth of water column, tidal cycle and/or unidentified biological factors (Oliver, 1989; Kasper & Tamplin, 1993; Wright *et al.*, 1996). Williams & Lecock (1985) reported that density of vibrios in sediment is nearly 3 times more than the overlying water. Association with sediment has been considered to be a tool for overcoming unfavorable environmental conditions for this bacterium (El-Sahn *et al.*, 1982). Association of *V. parahaemolyticus* with plankton is reported by several authors. The plankton carries high numbers of vibrios and prolongs their existence by association with chitinous component of plankton (Huq *et al.*, 1984).

V. parahaemolyticus in various body parts of the commercially important fishes are given in Table 3. The total *Vibrio* count was in the range of $1.0x10^4$ - $2.1x107.g^{-1}$ in skin and muscle, $1.0x10^5$ - $2.1x10^7.g^{-1}$ in gills and 2.3x105 - $1.35x10^8.g^{-1}$ in intestines. Out of the 17 fish species comprising 46 samples from pelagic and demersal sources, the organism was detected in skin and muscle of 35% of the samples, in gills of 53% and in the intestine of 71% samples. Percentage of *V. parahaemolyticus* exhibited a fluctuating trend within the sample itself. Gill and intestine are frequent reservoirs of *V. parahaemolyticus*. Gutting and deheading can thus reduce the initial level considerably. Table 4 presents the distribution of *V. parahaemolyticus* in meat portion including the shell and intestine of shellfishes collected from this area. In 25 samples of shrimp comprising 6 species, the prevalence was 83% both in intestine and muscle with shell on. The percentage of this organism varied among the prawn species, but there was no striking difference between body and intestines of these shellfish samples.

Table 3. Prevalence of *Vibrio parahaemolyticus* in skin and muscle, gill, and intestine of commercially important fishes collected from various sources in Cochin

Name of the fish	No. of Samples tested	Skin and muscle		Gill		Intestine	
		Total isolates	%	Total isolate	%	Total isolate	%
Arius dussumieri	3	17	47.1	7	-	16	-
Decapterus russelli	3	3	-	8	-	6	-
Euthynnus affinis	3	3	-	5	40.0	9	22.2
Gerres filamentoses	3	4	-	9	11.2	8	-
Himantura bleekeri	3	15	-	6	-	7	28.6
Lates calcarifer	2	7	-	9	22.2	10	30.0
Lutjanus malabaricus	3	10	-	14	28.6	12	8.3
Mugil cephalus	3	4	-	8	25.0	12	-
Nemipterus japonicus	3	4	100.0	5	-	8	-
Selar crumenophthalmus	2	9	22.2	10	-	8	25.0
Sardinella sp.	2	9	-	5	-	9	-
Johnius dussumieri	3	14	-	7	42.9	9	11.1
Rastrelliger kanagurta	3	9	-	6	-	11	9.1
Sardinella longiceps	3	11	-	9	22.2	14	21.4
Scomberomorus commerson	3	15	26.7	10	-	17	5.9
Nemipterus japonicus	3	8	-	14	21.4	13	7.7
Strongylura strongylura	3	13	23.1	9	-	12	25.0
Total	46	155	13.5	141	13.5	181	11.0

Table 4. Prevalence of *Vibrio parahaemolyticus* in body parts of commercially important shrimps collected from Cochin

Shrimp	No. of samples	Muscle		Intestine	
		Total isolates	%	Total isolates	%
Parapenaeopsis stylifera	6	12	16.7	10	-
Penaeus indicus	4	14	21.4	17	17.6
Penaeus monodon	4	9	11.1	22	36.4
Metapenaeus dobsoni	4	12	16.7	23	17.4
Metapenaeus affinis	4	8	37.5	29	17.2
Metapenaeus monoceros	3	10	-	21	4.8
Total	25	65	16.9	122	17.2

Inconsistency in the distribution of *V. parahaemolyticus* was quite evident in the present study. Maximum vibrio count of $10^7.g^{-1}$ was observed in some instances. This could be attributed to the eco-physical factors as reported for other regions (Williams & Lecock, 1985). Incidence of *V. parahaemolyticus* in various seafood are generally quite low (Twedt, 1989). *V. parahaemolyticus* counts reported for majority of the cases of freshly caught finfish and shellfish are $<100.g^{-1}$ in India (Karunasagar *et al.*, 1984., Nair, 1985; Sanjeev & Stephen, 1993) and elsewhere (Asakawa *et al.*, 1974., Sakazaki *et al.*, 1979). For freshly caught shellfish, slightly higher counts of *V. parahaemolyticus* have been reported (Sanjeev & Stephen, 1993). But values greater than $1100.g^{-1}$ of *V. parahaemolyticus* has also been reported for freshly caught shellfish (El-Sahn *et al.*, 1982). Fish from markets, on the other hand, are found to carry higher loads of *V. parahaemolyticus* (Nair, 1985; Sanjeev & Stephen, 1993).

The Kanagawa reaction of the isolates is presented in Table 5. As seen from the data, the values of Kanagawa positives were in the range of 5-22%, lowest being for isolates from water and highest for fish isolates. An average value of 14% has been recorded in this study and it is in agreement with the values reported earlier by Karunasagar & Kumar (1980) Bandekar *et al.*, (1982) and Sanjeev & Stephen (1995). The values reported for Kanagawa positives from India are slightly higher than those reported from elsewhere. In Japan, 0.35 to 3.56% Kanagawa positive strains were isolated from seafood and marine environments (Sakazaki *et al.*, 1968; Wagatsuma, 1974). In USA, only 4 out of 2218 confirmed *V.*

Table 5. Prevalence of Kanagawa positive *Vibrio parahaemolyticus* in water, sediment, fish and shellfish

Source	Total isolates	% of Kanagawa positives
Water	179	5
Sediment	144	11
Plankton	147	13
Fish	470	22
Shellfish	187	19
Total	1117	14

parahaemolyticus isolates from water, sediment and oyster in Galveston Bay were Kanagawa positive (Thompson *et al.*, 1976). The proportion of Kanagawa positive strains in the environment is much lower when compared to that of clinical isolates in which case almost 96% isolates are Kanagawa positive (Sakazaki *et al.*, 1968). The infectives dose reported for Kanagawa negative cells is of the order of 10^{10} cells.g^{-1} (Sakazaki *et al.*, 1968) in volunteers, whereas the minimum infective dose of Kanagawa positive strains varied from 10^5 to 10^7 cells (Sanyal & Sen, 1974; Sanyal *et al.*, 1973). In the light of the above findings, even if one consumes 100 g fish per diet, the maximum number of *V. parahaemolyticus* ingested comes to 10^5 to 10^6, assuming the average count to be in the order of 10^4.g^{-1} of fish and that of Kanagawa positives to about 20% of this count. It is far less than the infectives dose. This conclusively showed that there was no risk from consumption of seafood that carry *V. parahaemolyticus*, provided there is no chance of growth in later stages.

The EU regulations stipulate that this organism should not be present in the processed seafood. A limit of 100.g^{-1} of *V. parahaemolyticus* is allowed for the raw seafood exported to Japan. The limit of *V. parahaemolyticus* in fresh and frozen fish and shellfish is 100.g^{-1} as per ICMSF recommendation (ICMSF, 1974). A detailed collaborative study on the incidence of *V. parahaemolyticus* in finfish, shellfish and shrimp from Japan (Sakazaki *et al.*, 1979) provided data for setting hygienic standards for this organism. According to their study, the acceptance limit for *V. parahaemolyticus* in raw fish was fixed as $<10^4$.g^{-1}, as determined by MPN. The Kanagawa reaction was not taken into account when this limit was set. In a recent study, DePaola *et al.*, (2000) suggested that concentrations of *V. parahaemolyticus* cells

$>10^4.g^{-1}$ or $>10.g^{-1}$ of *tdh* or *trh* positive *V. parahaemolyticus* in the environment should be considered as extraordinary and a 100 - 1000.g^{-1} of *V. parahaemolyticus* level was almost common.

The authors are grateful to the Director, Central Institute of Fisheries Technology, Cochin, for permission to publish this paper. The first author is indebted to the Indian Council of Agricultural Research, New Delhi, for providing financial assistance for this study.

References

Asakawa, Y., Akahane, S. and Noguchi, M. (1974) in *International Symposium on Vibrio Parahaemolyticus,* (Fujino, T., Sakaguchi, G. & Takeda, Y., Eds.), p. 97-103, Saikon Publishing Co. Ltd., Tokyo

Bandekar, J.R., Chander, R., Nerkar, D.P. and Lewis, N.T. (1982) *Indian J. Microbiol.* **22**, 247

Daniels, N.A., Ray, B., Easton, A., Marano, E., Kahn, A., MacShan, L., Rosario, L, Baldwin, T., Kingsley, M.A. Puhr, N.D., Wells, J.G. and Angelo, F.G. (2000) *J. American Med. Assoc.* **284**, 1541

DePaola, A., Kaysner, C.A., Bowers, J. and Cook, D.W. (2000) *Appl. Environ. Microbiol.* **66**, 4649

Elliot, E.L., Kaysner, A.C. and Tamplin, M.L. (1995) in *US-FDA Bacteriological Analytical Manual*, 8th edn., Chapter 9, Association of Official Analytical Chemists International, Gaithersburg, MD, USA

El-Sahn, M.A., El-Banna, A.A. and El Tabey Shehata, A.M. (1982) *Can J. Microbiol.* **28**, 1261

Huq, A., West, P.A., Small, E.B., Huq, M.I. and Colwell, R.R. (1984) *Appl. Environ. Microbiol.* **48**, 420

ICMSF (1974) *Microorganisms in Food,* Vol **2**, The International Commission on Microbiological Specification of Foods, University of Toronto Press, Toronto

Karunasagar, I. and Mohan Kumar, K.C. (1980) *Indian J. Med. Res.* **72**, 619

Karunasagar, I., M.N. Venugopal, K.C. and Karunasagar, I. (1984) *Can J. Microbiol.* **28**, 1261

Kasper, C.W. and Tamplin, M.L. (1993) *Appl. Environ. Microbiol.* **59**, 2425

Lee, W.C., Lee, M.J., Kim, J.S. and Park, S.Y. (2001) *J. Food Protect.* **64**, 899

Matsumoto, C., Okuda, J., Ishibashi, M., Iwanaga, M., Garg, P., Ramamurthy, T., Wong, H.C., DePaola, A., Kim, Y.B., Albert, M.J. and Nishibuchi, M. (2000) *J. Food Prot.* **63**, 807

Monticelli, L.S. and Crisafi, E. (1995) *Microbiologica Bologna.* **18**, 289

Nair, B.G. (1985) *Indian J. Med. Res.* **82**, 281

O'Neil K.R., Jones, S.H and Grimes, D.J. (1992) *Appl. Environ. Microbiol.* **58**, 3257

Oliver, J.D. (1989) in *Food-borne Bacterial Pathogens*, p. 570-597, (Doyle, M.P., Ed.) Marcel Dekker Inc., New York

Sakazaki, R., Iwanami, S. and Tamura, K. (1968) *Jap. J. Med. Sci. Biol.* **21**, 313

Sakazaki, R., Karashimada, T. Yuda, K., Sakai, S. Asakawa, Y., Yamasaki, M., Nakanishi, H., Kobayashi, K., Nishio, T., Okazaki, H., Doke, T., Shimada, T. and Timura, K. (1979) *Arch. Lebensmittelhyg.*, **30**, 103

Sanjeev, S. and Stephen, J. (1993) *Fish. Res.* **16**, 273

Sanjeev, S. and Stephen, J. (1995) *Fish. Technol.* **32**, 64

Sanyal, S. and Sen, P.C. (1974) in *International Symposium on Vibrio parahaemolyticus*, (Fujino, T., Sakaguchi, G. and Takeda, Y., Eds.), p. 227-235, Saikon Publishing Co. Ltd., Tokyo

Sanyal, S., Sil, J. and Sakazaki, R. (1973) *J. Med. Microbiol.* **6**, 121

Thompson, C.A Jr., Vanderzant, C. and Ray, S.M. (1976) *J. Food Sci.* **41**, 117

Twedt, R.M. (1989) in *Foodborne Bacterial Pathogens*, (Doyle, M.P., Ed.), p. 544-562, Marcel Dekker Inc., New York

Wagatsuma, S. (1974) in *International Symposium on Vibrio parahaemolyticus* (Fujino, T., Sakaguchi, G. and Takeda, Y., Eds.), p. 91-96, Saikon Publishing Co. Ltd., Tokyo

Williams, L.A. and Lecock, P.A. (1985) *Appl. Environ. Microbiol.* **50**, 1490

Wong, H.C., Shieh, W.R. and Lee, Y.S. (2000) *J. Food Prot.* **56**, 980

Wright, A.C., Hill, R.T., Johnson, J.A., Roghman, M.C., Colwell, R.R. and Morris Jr., J.G. (1996) *Appl. Environ. Microbiol.* **62**, 712