Fishery Technology
2002, Vol. 39(2) pp : 150 - 154

Prevalence of *Vibrio* species on Fish from Pelagic and Demersal Habitats

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The density and diversity in the *vibrio* species on the body parts of some commercially important fishes of India were studied with respect to the habitat viz pelagic and demersal. The distribution of vibrios on fish from these habitats showed wide variations both quantitatively and qualitatively. The results indicate that the density of vibrios on the skin and gills may be more in pelagic fish compared to demersal types. However the intestinal count of vibrios is similar in the two categories. The species diversity was more in the demersal fishes particularly in the intestinal samples.The vibrio species were in the order of dominance as *V. alginolyticus, V. orientalis,* and *V. campbelli.* Species like *V. alginolyticus, V. parahaemolyticus, V. vulnificus* and *V. mimicus* were among the pathogenic species isolated from the fish samples. The study reveals that *vibrios* constitute a major portion of the total bacterial flora in tropical fish.

Key words: *Vibrio* species, tropical fish, habitat

The genus *Vibrio* comprises species that are characterised by wide variations in the nutritional requirements, physiological traits and biochemical features. This suggests that different species may vary greatly in their potential to inhabit environments of differing nature. Besides their role as human pathogens, vibrios are reported to cause infections in aquatic creatures (Knappskog *et.al.* 1993, Austin *et.al.* 1993.) Some vibrios are also reported to be capable of causing spoilage of tropical fish and prawn (Surendran, 1980, Philip & Lakshmanaperumalsamy, 1992).

Tropical oceans and the creatures inhabiting such areas are reported to be a good reservoir of vibrios. Occurrence of vibrios in tropical marine environment and animals have been reported previously (Karunasagar *et.al* 1990, Prasad & Rao, 1994 Matte *et.al* 1994, Thampuran, *et al,* 1996, Thampuran & Surendran, 1998.). But such studies are limited to a particular species or a group only and information regarding the overall picture of the divergent members in the *Vibrio* population existing in the tropical climate is scanty. The aim of the present study was to investigate the occurrence and distribution of *Vibrio* species in the body parts of some commercially important fish in this area and to correlate their presence with habitat viz. pelagic/demersal and also with the feeding habit.

Materials and Methods

From pelagic habitat eight species viz., *Sardinella longiceps, Thryssa mystax, Rastrelliger kanagurta, Scomberomorus commerson, Mugil cephalus, Selar crumenophthalmus, Decapterus russelli* and *Strongilura strongilura* were selected and *Johnius dussumieri, Lates calcarifer, Nemipterus japonicus, Gerres filamentosus, Arius dussumieri* and *Lutjanus malabaricus* from demersal fishes. The study was carried out for a period of one year. Selected fish species included in the study were collected in very fresh condition from fish landing centres or from the vessels. Samples were collected in sterile polyethene bags and transported to the laboratory under ice for bacteriological examination. The total time-lapse between

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collection and analysis of the sample was 4-6 h.

Fishes were identified and categorized into pelagic and demersal according to the scheme of Fischer & Bianchi (1984). The scheme for analysis was generally based on the method outlined by USFDA (1995) with minor modifications. Skin surface with muscle (SM), gill (G) and intestine (I) of fishes were studied. Ten gram portions of each part were cut aseptically and blended with 90 ml sterile 3% NaCl diluent in a Stomacher 400 (Seward) for 1 minute. Care was taken while sampling the gills and intestine, to reduce surface contamination by swabbing the body surface with 70% ethanol before taking the specimen.

Each sample was analyzed for total halophilic bacterial count (THC) and total Vibrio count (TVC). Serial dilutions pour plated on trypticase soy agar (TSA) supplemented with 3% NaCl represented THC and TVC was estimated by direct plating on (TCBS, OXOID) thiosulphate citrate bile salt agar. The plates and tubes were incubated at a temperature of 37°C for 2days in the case of viable count and 24h for vibrio count. Depending on the number of colonies per plate upto 30 colonies were picked for identification from each plate. Pure cultures of presumptive colonies were identified to species level as per the scheme of Alsina & Blanch (1994). Randomly selected strains were also further checked for confirmation by use of API strips (bioMerieux, France). The count of individual members of the vibrios were determined as percentage from the total isolates of each sample.

Results and Discussion

During most part of the study the surface water temperature was found to be within 28 to 31°C. The pH of the water was around 8 and salinity between 29.4 to 33.6‰.

Data of the percentage distribution of total halophilic bacterial population arranged in different count ranges for both pelagic and demersal fishes are tabulated in Table 1. The mean log count per g for total halophiles on skin and muscle ranged from 4.7 to 7.8 in pelagic fish and 5.5 to 7.6 in demersal fish. In the intestine, the corresponding values were respectively 7.0 to 8.5/g and 7.1 to 8.4/g. Maximum number of SM, G and I samples of demersal fishes were found to belong to the count range of 7.0, but maximum samples of the pelagic fish belonged to count range of 6.0 to 7.0 for skin surface, 8.0 for gills and 7.0 for intestine.

Table 1. Total halophilic bacterial population in fish : Percentage distribution in different count ranges

Part of fish	Type of fish	Total halophilic bacterial % of samples with the log count/g of					
		3.0	4.0	5.0	6.0	7.0	8.0
Skin surface	Pelagic	Nil	12	12	38	38	Nil
Skin surface	Demersal	Nil	Nil	17	33	50	Nil
Gills	Pelagic	Nil	Nil	Nil	Nil	38	62
Gills	Demersal	Nil	Nil	Nil	17	66	17
Intestine	Pelagic	Nil	Nil	Nil	Nil	75	25
Intestine	Demersal	Nil	Nil	Nil	Nil	67	33

Regarding the total vibrio population, 50% of skin and muscle had a count in the range of 1 to 2 log values and the same percentage also came within the range of 3 to 4 log values. (Table 2) The majority of the demersal fishes (49%) were in the count range of 3.0-4.0 log values. This points to wide variation in the *vibrio* count of pelagic

Table 2. Total *vibrio* populations in fish : Percentage distribution in different count ranges

Part of fish	Type of fish	Total vibrio count (TVC) % of samples with the log count/g of						
		0	1.0	2.0	3.0	4.0	5.0	6.0
Skin surface	Pelagic	Nil	38	12	Nil	38	12	Nil
Skin surface	Demersal	Nil	17	34	49	Nil	Nil	Nil
Gills	Pelagic	Nil	12	Nil	38	12	26	12
Gills	Demersal	Nil	Nil	17	34	49	Nil	Nil
Intestine	Pelagic	Nil	12	Nil	25	63	Nil	Nil
Intestine	Demersal	Nil	Nil	17	33	17	33	Nil

fish varieties. For skin and muscle and gills, the peak values in count of *vibrios* was higher for pelagic than demersal; but for the intestine, the demersal fish were having a higher *vibrio* count. These results indicated that even though the total halophilic count is higher in demersal fish, the density of *vibrios* may be more in pelagic fish compared to the demersal types. However the intestinal density which is reflection of feed intake (Natarajan *et al* 1979), is similar in the two catagories and points to the selective growth of some species in the gut or common feed.

Densities of the halophilic bacteria and vibrios in the fishes of both habitats showed no consistent pattern of occurrence or distribution. Wide variations in both THC and TVC were observed among these samples especially in pelagic fishes. These variations in the count among different fishes have been attributed to factors like physiological differences among fishes, degree of development of digestive system (Sera & Ishida; 1972), adaptations in the gills of plantivores (Oliver *et.al* 1982) texture of the skin surface, seasonal variation (Thampuran & Surendran, 1998; De Paola *et.al*, 1994) feeding habits viz. diet (De Paola *et.al*, 1994, Natarajan *et.al*, 1979) and time after ingestion of food (Sera & Ishida, 1972). The present study indicates that the environment where the fish inhabits also is a deciding factor on the *vibrios*. Greater variations in the bacterial number and quality in pelagic fish compared to the demersal fish could be the result of the frequent disturbance in the pelagic water mass.

Quantitative distribution of individual *vibrio* species in pelagic (*Sardinella longiceps*) and demersal (*Arius dussumieri*) fishes are shown in Table 3. *V. alginolyticus V. mimicus* and *V. campbellii* were the most abundant species on the body surface and gills of pelagic fishes and *V. orientalis* constituted dominant member of the intestine of pelagic fish. *V. alginolyticus* and *V. campbellii* were major species in on S&M and gills of demersal fish while intestine carried *V. logei* and *V. orientalis* in large numbers. *V. parahaemolyticus* was more in S&M of pelagic fish while and *V. vulnificus* number was maximum in the intestine of the pelagic fish.

Table 3. Density* of Vibrio populations on body surface and intestine of fishes

Vibrio Species	Pelagic fish *Sardinella longiceps*		Demersal fish *Arius dussumieri*	
	Body Surface #	Intestine	Body Surface #	Intestine
V. orienalis	$8.95x10^3$	$1.61x10^6$	40	$3.0x10^3$
V. vulnificus	360	$3.0x10^3$	120	700
V. alginolyticus	$2.7x10^4$	126	$1.2x10^4$	2.0
V. campbelli	$2.4x10^4$	300	$1.7x10^4$	2.0
V. parahaemolytics	240	ND	150	ND
V. pelagius II	$2.4x10^3$	ND	3.2x102	ND
V. splendidus	$8.8x10^3$	ND	110	ND
V. mimicus	$3.0x10^4$	ND	ND	700
V. logei	ND	$2.1x10^5$	ND	$1.5x10^3$
V. marinus	3	ND	ND	300
V. natriegenes	ND	30	ND	ND
V. damsela	ND	ND	ND	300
V. harveyi	ND	ND	ND	900
V. carchariae	ND	ND	23	ND
V. mediterranei	3	ND	$2.3x10^3$	ND

* - Results from a typical study;
\# - Include skin with muscle and gills;
ND - Not detected

Table 4 indicates the prevalence of *vibrio* species on S&M, gills and intestine of the total samples of pelagic and demersal origin in the study.

There were striking variations regarding the distribution of these vibrio species on the body parts of both pelagic and demersal fishes. *Vibrio carchariae, V harveyi, V. proteolyticus* and *V. damsela* were isolated only from demersal fishes while *V. natriegens* could be isolated from the pelagic fish only (Table 4). Similarly the skin with muscle and the gills portions of pelagic fish carried a larger number of vibrio species than intestine while for demersal fish, the reverse was observed. Since bacterial flora of fish is a reflection of the environment from where it

Table 4. Species - wise distribution of *Vibrios* on various part of the fish body of pelagic/demersal fishes of the present study

Vibrio species	Skin & muscle		Gills		Intestine	
	P	D	P	D	P	D
V. campbelli	+	+	+	+	–	+
V. orientalis	+	+	+	+	+	–
V. vulnificus	+	+	–	–	+	–
V. pelagius II	+	+	+	–	–	+
V. natriegenes	+	–	–	–	+	+
V. alginolyticus	+	+	+	+	+	+
V. marinus	+	–	+	–	–	–
V. parahaemolyticus	+	–	+	–	–	+
V. mimicus	–	–	+	–	–	+
V. mediterranei	–	+	+	–	+	+
V. logei	–	–	–	+	+	+
V. damsela	–	–	–	–	–	+
V. harveyi	–	–	–	–	–	+
V. carchariae	–	–	–	–	–	+
V. proteolyticus	–	–	–	–	–	+
Total specis	8	6	8	4	6	12

P - Pelagic fish; D - Demersal fish; + present; – Absent

is caught, this evidently points that difference in the habitat of pelagic and demersal fish could be the reason for the variations in the distribution of vibrios. Even for the individual fish the microenvironment existing in the fish body leads to changes in relative occurrence of vibrio species. *V. parahaemolyticus* could be isolated from skin and muscle of pelagic fish, but not from that of demersal fish. Sakazaki and Shimada (1986) states that *V. parahaemolyticus* does not inhabit deep sea where high hydrostatic pressure and low temperature prevails. Hence the absence of this organism on the skin surface of demersal fish is justifiable. However this organism was recovered from the intestine of demersal fishes.

The intestine, which might be a reflection of the food, contained maximum Vibrio species. From demersal fishes, 12 species were isolated while only 6 vibrio species could be isolated from pelagic fishes. The fishes of the demersal group are carnivores and eat small prey fishes, juveniles of large varieties, crab and other invertebrates. An exception in this study is *Gerres filamentosus*, which is a detritus feeder. Even though there was no noticeable difference in the quantitative distribution of vibrios, qualitatively, intestine of carnivorous fishes were found to exhibit a greater species diversity among vibrios. De Paola *et.al* (1994) observed that in the Gulf Coast fishes, *V. vulnificus* was associated primarily with benthic species rather than the planktonic species and its number varied mostly in the case of fish species which feed on plant and organic detritus.

Among the pathogenic species, *V. parahaemolyticus, V. vulnificus, V. alginolyticus, V. mimicus, V. carchariae* and *V. damsela* were isolated. *V. harveyi*, a fish pathogen was also noted in this study. The presence of some of these species have been reported previously from the raw fish collected from the west coast of India (Natarajan *et.al.* 1979, Prasad & Rao 1984. Thampuran *et.al*, 1996). It is evident from this study that occurrence and distribution of vibrios associated with different fishes vary considerably depending on its habitat or feeding habit and they constitute a considerable part of the bacterial flora of their body. This necessitates that while taking precautionary measures or fixing limits of pathogenic species meant for export trade, the nature and origin of the raw material has to be taken into account.

The authors are thankful to Indian Council of Agricultural Research for the financial assistance under AP Cess fund Scheme for carrying out this work. They are also grateful to Director, Central Institute of Fisheries Technology, Cochin 682029, for permission to publish the paper.

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