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Comparative Microbial Ecology of Fresh Water and Brackish Water Prawn Farms*

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Microbiological characteristics of fresh and brackish water prawn farms along with their physico-chemical parameters have been investigated. Even though water quality was within the accepted limits in the two farms, there were appreciable variations between the two in respect of pH and dissolved oxygen. Total bacterial counts of the water, mud and the cultured prawn from fresh water farm registered lower values, compared with those from the brackish water farm. However indicator bacteria like total coliforms and faecal coliforms were high in number in the fresh water farm. Human pathogenic bacteria like *Salmonella*, Enteropathogenic *E. coli* O157:H7, *Listeria monocytogenes*, *Vibrio cholerae*, *Aeromonas*, Enterotoxigenic *Bacillus cereus*, *Plessiomonas shigelloides* and other pathogenic *Vibrios* were also monitored in respect of two types of farm environments. Antibiotic resistance pattern of the bacterial cultures from the two farms towards seven commonly used antibiotics showed comparable values.

Key words: Microbial ecology, prawn farms, Salmonella, Vibrio, Bacillus, antibiotic resistance

Aquaculture has come up recently as an alternate solution to the decline in the marine resources. Shrimp is the most widely cultured variety in India. Tiger prawn (*Penaeus monodon*) and naran (*Penaeus indicus*) are cultured in brackish water systems, while scampi (*Macrobrachium rosenbergii*) is grown mainly in fresh water farms. A wide variety of culture methods are adopted in each system.

The ecology of any existing farming system is rendered very complex by the diversity of the biota that co-exist and interact in the aquatic environment characterized by numerous physico-chemical factors, weather variables and soil conditions. Apart from these intrinsic factors, other extrinsic factors such as human interventions contribute further to the complex nature of farm ecology.

It is generally accepted that the environment reflects on the microflora associated with fish (Horsley, 1971; Shewan, 1962). Even for the same fish species, significant variations in the microflora under different culture methods have been observed (Nedoluha & Westhoff, 1997).

The microbiology of farmed shrimp has been a subject of interest in India and abroad as evidenced by numerous publications (Nayyarahamed, *et al.*, 1995, Budisusilowati & Haryani 1995, Leangphibul, *et al.*, 1986, Reilly & Twiddy, 1992, Twiddy & Reilly, 1995). In this investigation, the microbiological features and physico-chemical parameters of the fresh water and brackish water prawn farms were studied and the differences between the two systems were evaluated. The information will be useful for designing HACCP protocol and also for ensuring the microbial safety of aquaculture products.

Materials and Methods

The fresh water farm selected for the study was at Ollur in Trichur district, situated in an area surrounded by brick making yards on either side and an

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uncultivated paddy field on the third side. The water for irrigating the farm was received from the canal on the other side. The brackish water farm selected was at Chellanam in Ernakulam district. It was surrounded on all the three sides by backwaters (brackish), except an uncultivated field on one side. In both the farms there was no human habitation in the immediate vicinity. In both farms, semi-intensive aquaculture was practised. Each farm had an area of about 5 ha. and a water column depth of 4 to 6ft. While the water supply for the brackish water farm was controlled by tidal currents, in the fresh water farm, water was controlled by pumping daily.

Two series of sampling were carried out for each system, one in June 1995 and the second in January 1996. Shrimp samples were collected by cast nets, packed in ice and brought to the laboratory as soon as possible. The water samples collected from four corners and the centre of the farm were pooled before analysis. Sediment samples were scooped out and put in sterile polythene bags for analysis.

The microbial analysis was carried out as per the methods outlined in U.S FDA (1984). Total plate count (TPC) was estimated by pour plating serial decimal dilutions in normal saline (0.85% NaCl in distilled water) with tryptone glucose extract agar (Oxoid) and incubating at 37°C for 48h. Total coliforms, feacal coliforms and *E. coli* were determined by MPN method using MacConkey broth, followed by inoculation to BGLB 2% and other tests as per U.S FDA (1984). Coagulase positive staphylococci and faecal streptococci were enumerated using Baird Parker agar and KF agar, respectively. *Salmonella* was determined by the U.S FDA (1984) method. *Listeria* was detected by enrichment in selective media followed by plating on Listeria selective agar (Oxoid, U.K.) and also by biochemical reactions (Lovett, 1988; McLaughlin, 1987). *Salmonella* and *Listeria* in the samples were tested by ELISA method also (Organon Teknika, USA). Enteropathogenic *E. coli* O157: H7 was determined by enrichment in modified E.C broth supplemented with novobiocin, followed by plating on Sorbitol MacConkey agar (SMA), (Okrend, *et.al.*, 1990). Typical colonies on SMA were confirmed by latex agglutination, using *E. coli.* O157 Test kit, (Oxoid, U.K.) specific for *Enteropathogenic E.coli* O157: H7. *Bacillus cereus* was estimated by the method of Szabo *et al.* (1984), using polymyxin-pyruvate-egg yolk-mannitol-bromocresol purple agar (PEMPA). The *B. cereus* isolates were tested for the production of diarrhoeal enterotoxin by Reverse Passive Latex Agglutination (RPLA) technique with specific antibody (Oxoid, U.K.). *Aeromonas, Vibrios* and *Plessiomonas shigelloides* were determined as per the procedure outlined in U.S FDA (1984).

The bacterial colonies isolated from TPC plates were purified by repeated streaking on TGA plates and identified as per the scheme of Surendran & Gopakumar (1981). The isolates were screened for resistance towards seven commonly used antibiotics by standard disc assay technique. The antibiotic discs (Oxoid, U.K.) were dispensed on the surface of seeded Muellor-Hinton agar plates using Oxoid antibiotic disc dispenser (Oxoid, U.K.). The behaviour of bacterial isolates towards individual antibiotic discs was interpreted using standard methods (Bauer & Kirby, 1966). The water samples collected from the farms were analysed for pH using a pH meter (Systronics, Bombay) and dissolved oxygen and salinity by the method of Stainton *et al.* (1974).

Results and Discussion

The water quality parameters are given in Table 1. Water in fresh water farm had slightly higher pH when compared with brackish water. However both farms registered pH within acceptable limits. The dissolved oxygen level of fresh water farm was higher than that of the brackish water farm. These results indicated that even though water quality was within limits in the

two farms, there were appreciable variations between the two.

Table 1. pH, Salinity and Dissolved Oxygen levels in the fresh water and brackish water farms studied.

Test	Fresh water farm	Brackish water pond
PH	8.1	6.7
Salinity (ppt.)	0.03	8.65
D.O.(mg/l)	8.9	4.3

The total plate count, counts of coliforms, faecal coliforms, *E. coli*, streptococci and staphylococci are presented in Table-2.

It was observed that the TPC of water and mud of fresh water farm was lower than that of the water and mud from brackish water farm. Corresponding decrease in the TPC of prawn muscle was also noted for *M. rosenbergii*, the freshwater prawn. However, intestinal bacterial count of *M. rosenbergii* was higher than that of *P. monodon* from the brackishwater farm. Since the bacterial counts of water, mud and muscle of the prawn from the two farms were in comparable ranges, the higher bacterial count of the intestine of *M. rosenbergii* probably reflected the nature of feed intake. In a similar study carried out in two shrimp farms in the West coast of India, 35 Km from Mangalore, Nayyarahamed *et al.* (1995) noted TPCs of the order of 10^2 to 10^3 cfu/ml for water, 10^3 to 10^4 cfu/g for sediment and 10^4 to 10^5 cfu/g for whole shrimp. Lloberra *et al.* (1990) and Budisusilowati & Haryani (1995) also reported TPCs of shrimp in similar range.

Levels of total coliforms, faecal coliforms and streptococci were high in the fresh water farm compared to the brackish water farm. The freshwater farm was surrounded by uninhabited yards on all sides and there was no possibility of faecal pollution except through canal water or during farm management. The brackish water farm, though encircled by brackish water bodies, was found to be relatively free from these organisms. The high salt level prevailing in the brackish water environment or the water currents due to tidal effects could be the reasons for the lower values of faecal pollution in the brackish water pond. The coliform levels reported by Nayyarahamed *et al.* (1995) was low, ranging from 0.078/g to >240/g for total coliforms and 0 to 2.7/g for faecal coliforms in sediment, water and shrimp gut. An *E. coli* count of < 3/g for shrimp and sediment samples from brackish water farms in Indonesia has been reported (Budisusilowati & Haryani 1995).

Variations in the incidence of human pathogens in the fresh and brackish water pond systems are given in Table 3.. In fresh water farm, *Salmonella* was detected in farm water only whereas in brackish water pond,

Table 2. Total plate count, total coliforms and faecal coliform levels in water and prawn in the fresh water and brackish water farm studied.

Sample	Fresh water pond				Brackish water pond			
	TPC	TC (MPN)	FC (MPN)	EC (MPN)	TPC	TC (MPN)	FC (MPN)	EC (MPN)
Water (count/ml.)	$.4x10^4$	1100	460	93	$8.5x10^5$	40	93	93
Mud (count/g)	$2.8x10^4$	14	11	0.75	$7.1x10^6$	2.1	1.2	1.2
Prawn muscle Peeled&deveined) (count/g)	$3.2x10^5$	1.4	0.93	0.09	$1.1x10^6$	1.2	0.75	0
Instestine with contents(count/g)	$4.2x10^8$	>1400	120	21	$3.8x10^7$	140	110	46

TPC = Total Plate Count; TC = Total Coliforms; FC = Faecal Coliforms: EC = Escherichia coli.

Table.3. Levels of pathogenic bacteria in the water and prawn from the farms

Bacteria	Fresh water pond				Brackish water pond			
	Water (/ml)	Mud (/g)	Prawn (/g)	Intestine (/g)	Water (/g)	Mud (/g)	Prawn (/g)	Intestine (/g)
Salmonella	A	P	A	A	A	A	P	P
Listeria	A	A	A	A	A	A	A	A
Sulphite-reducing Clostridia	P	P	P	P	P	P	P	P
Plesiomonas shigelloides	A	A	A	A	P	A	A	A
Enteropathogenic *E. coli* O157:H7	A	A	A	A	A	A	A	A
Aeromonas	$5.4x10^2$	$2.1x10^4$	$4.7x10^4$	$1.2x10^5$	$1.0x10^3$	$1.7x10^4$	$2.6x10^4$	$1.1x10^6$
Bacillus cereus	10	$1.0x10^4$	$1.0x10^2$	A	A	$1.0x10^4$	A	A
Vibrio	A	A	A	A	$2.2x10^3$	$5.6x10^4$	$5.9x10^4$	$2.0x10^5$

P = Present; A = Absent.

this microorganism was present in mud, water, prawn meat and intestine. Leangphibul *et al.* (1986) and Rattagool *et al.* (1990) reported that 5% of water samples and 1% of sediment samples of the brackish water shrimp farms of Thailand were contaminated by *Salmonella.* A comprehensive study in South East Asia reported incidence of *Salmonella* in 16% of prawn samples and 22% of water and sediment samples (Reilly & Twiddy, 1992). According to Wyatt *et al.* (1979) temperature of water, content of organic matter, stocking level and even size of the fish play a role in the density of *Salmonella* in fish pond. One important point to be noted in our study was that the *Salmonella* in prawn meat, intestine and mud were detected by ELISA method only; no typical colonies for *Salmonella* were observed by conventional culture method. Chances of *Salmonella* contamination from human sources in these two farms were remote, because of the lack of human habitation in the immediate vicinity. The materials used during pond preparation such as fertilizers of animal origin could be the source of contamination. It is also noteworthy that the contamination by *Salmonella* was more common in brackish water system than in fresh water, while the coliform levels showed a reverse trend.

Vibrios are autochthonous to the saline water; hence the recovery of *Vibrio* species from brackish water pond and their total absence in the fresh water system is quite natural. Random isolation and identification of these vibrios showed that *Vibrio alginolyticus* and *V. parahaemolyticus* were the dominant *Vibrio* species of the brackish water farm. *V. vulnificus* and *V. campbelli* were also isolated. Similar results have been reported by Nayyarahamed *et. al.* (1995) for brackish water shrimp and suggested that these vibrios could be normal inhabitants of the shrimp gut.

Aeromonas and *Plessiomonas* are also bacteria of aquatic origin and the former has been consistently isolated both from the fresh water and brackish water systems, with counts almost parallel, in these two environments. *Plessiomonas shigelloides* was found to be absent in these systems except in the case of water sample from brackish water pond. Twiddy & Reilly (1995) reported *Aeromonas* count of 10^3 to 10^5 cfu/ml for pond water and sediment, and 10^3 to 10^4 cfu/g for fish flesh. *P. shigelloides* was detected in the fish flesh in two out of nine samples analysed in Indonesian farms (Twiddy & Reilly, 1995).

Table 4. Antibiotic resistance pattern of the bacteria isolated from water, mud and prawn from the farms.

Antibiotics	Fresh water pond				Brackish water pond			
	Water (N=20)	Mud (N=16)	Prawn (N=24)	Intestine (N=20)	Water (N=24)	Mud (N=18)	Prawn (N=30)	Intestine (N=24)
Chlortetracycline	15	18	16	10	12.5	17	13	8
Kanamycin	30	24	40	35	25	22	17	12.5
Cloxacillin	75	72	84	95	50	55.5	33	67
Gentamycin	15	18	8	5	12.5	28	33	25
Ampicillin	80	72	68	70	67	83	67	75
Erythromycin	90	94	96	100	100	89	100	92
Chloramphenicol	15	12	25	0	12.5	6	7	4

N= Number of cultures tested; * Values given are percentage of cultures showing antibiotic resistance.

Listeria and enteropathogenic *E. coli* O157: H7 were not detected in the water, mud or prawn from both the environments. However water, mud and prawn meat from the fresh water farm carried *Bacillus cereus.* But in the brackish water system, mud alone carried this organism. Sulphite-reducing *Clostridia* were abundant in all the samples from both the systems, peak values being observed in the mud samples.

Qualitatively, bacterial flora of the water, mud prawn muscle and intestine of the fresh water system was mostly Gram positive, comprising *Micrococcaceae, Bacillacae* and *Arthrobacter* species. Gram negatives, comprising less than 30% of the total population, were mainly composed of members of Enterobacteriaceae, *Pseudomonas* and *Aeromonas* species.

Data on the incidence of antibiotic resistance of bacteria in the bacterial flora of these culture farms are presented in Table 4. Maximum number of bacterial strains showed resistance to erythromycin, followed by ampicillin and cloxacillin. Least resistance (less than 20%) was noticed towards gentamycin, chloramphenicol and chlortetracycline. The antibiotic resistance pattern of bacteria from the two farming systems was very much comparable.

The development of antibiotic resistance among bacterial populations from farm environment has been reported by researchers from Denmark, Finland and South East Asian countries. (Bjorklund *et. al*, 1990; Spanggaard *et al.* 1993, Twiddy & Reilly, 1995) Comparison becomes difficult as the antibiotics selected for the study differed in each case. The antibiotics are presumed to enter the farming system either through drugs used for prophylactic measures or as incorporated into feed (Twiddy & Reilly, 1995). It is a matter of relief that bacterial strains from the aquaculture farms have not so far developed resistance to life saving drugs like gentamycin and chloramphenicol.

It is evident from this study that the brackish and fresh water farms differed significantly in physical and microbiological features; but there was very little difference in the antibiotic resistance pattern. The study also points to the advantage of using advanced technique like ELISA for detection of pathogens. A more detailed study encompassing farms of wider area for longer duration may be required to identify the source of pollutants or pathogens and also to establish the trend in the antibiotic resistance pattern of bacteria.

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