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Vibrio abundance in modified extensive culture ponds of *Penaeus monodon*

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ABSTRACT

The population of *Vibrios* in water and hepatopancreas of *Penaeus monodon* collected from modified extensive culture ponds situated at Raigad, Maharashtra were studied. All animals collected were associated with more than one *Vibrio species.* Bacterial identification showed presence of four species of *Vibrios* namely, *Vibrio parahaemolyticus, V. alginolyticus, V. anguillarum* and *V. vulnificus.* The overall CFU values of *Vibrios* noted for pond water were in the range of 1.8×10^1 to 7.8×10^4. The number of *Vibrios* increased with length of rearing time and the highest level was found on 122th day of culture. Diseased shrimps showed higher *Vibrio* count (8.3×10^6 cfu/g of hepatopancreas) than healthy shrimp (2.6×10^6 cfu/g of hepatopancreas).

Introduction

One of the most important challenges faced in shrimp farming is the disease outbreak leading to mass mortality. *Vibrio* species are found to be frequent and apparently opportunistic pathogens of penaeid shrimp (Lightner, 1988). As the prevalence of pathogenic *Vibrios* appears to be influenced by the physico-chemical features of the environment (Cheng and Chen, 1998), area specific research is very important to assess the presence of pathogens and the environmental quality of the area where aquaculture is practised. In India, despite the rapid growth of aquaculture industry and the frequent outbreak of microbial diseases in the stock, only in recent years some attention was focused to undertake investigations on microbiological aspects of the culture systems (Anand *et al.,* 1996; Janakiran *et al.,* 2000). In the present study, changes in the population of the *Vibrio* community, in relation to physio - chemical parameters of pond are documented.

Materials and methods

The modified extensive monoculture shrimp ponds located at Raigad, Maharashtra were selected. On April 16, 1999, virus free (PCR tested) *Penaeus monodon* postlarvae (PL 17) were stocked in the culture pond. The three culture ponds selected for this study were of the same size (1 hactre), and were identically managed, in terms of stocking density, feed type, feeding schedule and aeration. The climatic conditions were almost same due to the colse physical proximity of culture ponds.

Water samples from three different ponds (1,2,3) were collected from subsurface of the ponds using a sterile 250-ml plastic bottle. The water samples

collected from different points in each pond were mixed and transported to the laboratory in ice.

Samples of water and shrimp were collected every 20 days for the first 100 days and thereafter, on day 115 and finally on day 122 (day of harvesting). Infected shrimp observed near the pond edges were collected manually on day 80 and transported live in oxygenated water to the laboratory for enumeration and identification of *Vibrios*. The results of *Vibrios* count (CFU) are given as per gram of hepatopancreas.

The *Vibrios* were enumerated using Thiosulfate-citrate-bile-sucrose (TCBS Himedia) agar by spread plate technique. For enumeration of *Vibrios* in the hepatopancreas, shrimp were surface disinfected by wiping with sterile cotton swab soaked in 75% alcohol (Sung and Hong, 1997). Hepatopancreas from five shrimps were removed, pooled together to form one sample, weighed and homogenized in sterile saline. A series of 10-fold dilutions were made using sterile saline solution as dilution blanks, and 0.1 ml from each dilution was plated on agar plates by the spread plate method (Brown and Poxton, 1996).

Water samples were serially diluted upto 10^{-5} using sterile saline water as dilution blanks, and 0.1 ml from each dilution was plated on Thiosulfate-citrate-bile-sucrose (TCBS Himedia) agar plates by pour plate method (Brown and Poxton, 1996). All plates were incubated at 30^0C for 24hr and the numbers of the bacterial colonies were counted and expressed as colony forming units (CFU) (Janakiram *et.al.*, 2000).

To determine the composition of the *Vibrio* population of each sample, 10-20 bacterial isolates were selected from TCBS plates containing 30-300 colonies. Selected colonies were subcultured and purified onto nutrient agar plates. Purified cultures were maintained on nutrient agar slants at 4^0C for further studies (Alvarez *et. al.*, 1998).

Species level identification of *Vibrios* was made by biochemical tests (Holt *et. al.*, 1984). Isolates showing typical morphological and biochemical characteristics of genus *Vibrio,* were referred to as *Vibrio* species. The extent of relationship between the bacterial load in water and physio-chemical parameters of water were assessed statistically by employing Pearson's correlation coefficient (r).

Results and discussion

In the present investigation an increasing trend was observed throughout the culture period in the *Vibrio* population in pond water and shrimp hepatopancreas (Table1). Bacterial population in shrimp tisses, pond water and bottom mud increase because of increasing metabolite and balance feed (Karunasager *et al.*, 1992). However, some investigations have reported that changes in bacterial population in pond water are erratic and they have attributed it to water exchange and frequent liming (Janakiram *et al.*, 2000; Anad *et al.*, 1996). The overall CFU values noted here for pond water falling within the range of 1.8×10^1 to 7.8×10^4 are comparable to that reported earlier in cultured ponds (Sung *et al.*, 2001; Janakiram *et al.*, 2000).

Vibrio count in pond water of all the three ponds are comparable during the first 40 days of culture (3.6×10^1 to 6.0×10^1 cfu/ml), but form day 60, pond 1 recorded high *Vibrio* count. Pond 1 had algal bloom which collapsed during this time and it might have resulted in increased *Vibrios*. The bacterial

TABLE 1. Vibrio counts *of water and shrimp hepatopancreas (HP)*

Days of culture	Pond number 1		Pond number 2		Pond number 3	
	Water CFU/ml	HP CFU/gm	Water CFU/ml	HP CFU/gm	Water CFU/ml	HP CFU/gm
0	3.6×10^1 ±1.14	N.R	5.6×10^1 ±0.54	N.R	1.8×10^1 ±1.30	N.R
20	2.0×10^1 ±1.22	N.R	3.6×10^1 ±0.89	N.R	3.2×10^1 ±0.83	N.R
40	6.0×10^1 ±1.22	4.8×10^2 ±1.30	6.8×10^1 ±1.78	3.0×10^2 ±1.22	2.0×10^1 ±1.22	2.8×10^2 ±0.83
60	3.2×10^3 ±0.83	9.6×10^2 ±1.14	2.6×10^2 ±1.14	8.8×10^2 ±0.83	7.0×10^1 ±1.58	5.0×10^3 ±0.70
80	6.0×10^3 ±0.70	3.8×10^4 ±0.44	7.6×10^2 ±1.67	2.2×10^2 ±0.83	3.0×10^2 ±1.22	7.2×10^2 ±0.83
100	2.2×10^4 ±1.64	6.0×10^3 ±0.70	4.8×10^3 ±0.83	3.2×10^3 ±1.30	2.6×10^3 ±0.89	3.4×10^3 ±1.14
115	5.2×10^4 ±0.83	2.8×10^4 ±0.44	6.8×10^2 ±1.30	5.4×10^3 ±1.81	8.0×10^3 ±1.22	5.4×10^3 ±1.14
122	7.8×10^4 ±0.83	7.4×10^4 ±1.14	2.0×10^3 ±1.22	5.8×10^3 ±1.30	2.2×10^3 ±0.83	7.8×10^3 ±1.09

N.R - Not Recorded

proliferations in pond water have an inverse relationship with phytoplankton bloom (Anand *et al.*, 1996;, Simidu *et al.*, 1977).

Vibrio count in shrimp hepatopancreas from all the three ponds showed similar pattern of developent until day 60. On day 80 shrimps from pond 1 had higher *Vibrios* than the other two ponds. The inrease inhepatopancraetic *Vibrios* was observed after increase in *Vibrio* count in water.

The health of the shrimp was monitored through periodic sampling and inspection. Infected shrimps were observed regularly in pond 1 from day 75 of culture and the pond was treated with oxytetracycline at a rate of 5g/kg of feed on days 83, 84 and 85 (Nash *et al.*, 1992). As the antibiotic was given through feed, it showed little or no effect on *Vibrio* population in water. Infected shrimps showed following signs: pink to red colouration, whitish muscle, lack of food in midgut and folded tail. The exoskeleton in some cases showed infection by protozoans, necrosis of appendages and broken antennae. They showed lethargic movements, reduced feeding and swimming near the pond edges. Among diseasd shrimps in pond 1, *V.vulnificus* and *V. anguillarum* were the dominant species. *Vibrio* species present in diseased shrimps were found in healthy shrimp also. Infected shrimps collected on day 80 from pond 1 showed higher *Vibrio* count of 8.3×10^6 cfu/g of hepatopancres than healthy shrimp collected from the same pond (2.6×10^3 cfu/g of hepatopancreas) (Table 2).

Of the *Vibrios* encountered *V.parahaemolyticus* and *V. alginolyticus* constituted major percent along with other unidentified *Vibrio* species in all

TABLE 2. *Percentage of* Vibrio *species isolated from healthy and diseased shrimp hepatopancreas on day 80 from culture ponds.*

	% of *Vibrio* species	
	(healthy)	(infected)
V. parahaemolyticus	25	5
V. vulnificus	--	45
V. alginolyticus	20	5
V. anguillarum	15	30
Other *Vibrio* Species	40	15

three ponds on the day of stocking. *V. vulnificus* was not detected prior to day 20 in pond number one and two (Table 3). From day 60 onwards *V. vulnificus* and *V. anguillarum* constituted major population in pond number one. Decrease in species diversity is suggestive of environmental stress (Sung *et al.*, 2001). Decrease in the diversity of the *Vibrio* communities was clearly seen in pond 1. In pond number two *Vibrio* species other than the four identified were seen to be dominating. Pond number three showed somewhat equal distribution of *V. parahaemolyticus, V. alginolyticus* and *V. anguillarum* on day 60.

Data on the physio-chemical parameters of water for the three ponds is given in Table 4. Gradual decrease in salinity due to rain was observed in all the ponds. A gradual increase in organic matter was observed in all three ponds,

TABLE 3. *Changes in the composition of the* Vibrio *species of pond water*

	% of *Vibrio* species							
Days of Culture	0	20	40	60	80	100	115	122
V. parahaemolyticus								
Pond 1	20	30	20	10	15	5	5	10
Pond 2	10	30	25	15	5	0	15	20
Pond 3	10	20	10	20	25	20	15	15
V. alginolyticus								
Pond 1	30	20	20	15	0	5	5	20
Pond 2	40	40	20	20	15	25	15	5
Pond 3	30	30	35	25	5	15	20	25
V. anguillarum								
Pond 1	10	10	35	30	30	25	25	30
Pond 2	0	0	5	15	20	15	20	25
Pond 3	0	10	15	25	5	15	20	25
V. vulnificus								
Pond 1	0	0	10	35	35	25	50	30
Pond 2	0	0	5	20	15	20	15	5
Pond 3	0	0	0	0	10	10	25	20
Other V. species								
Pond 1	40	40	15	10	20	40	15	10
Pond 2	50	30	45	30	45	40	35	45
Pond 3	60	40	40	25	45	50	35	25

TABLE 4. *Physico-chemical parameters of water and sediment organic matter*

Days of Culture	Water						Sediment								
	Temperature (^{0}C)			pH			Salinity (ppt)			Transparency (cm)			Organic matter (%)		
	Pond no.1	Pond no.2	Pond no.3	Pond no.1	Pond no.2	Pond no.3	Pond no.1	Pond no.2	Pond no.3	Pond no.1	Pond no.2	Pond no.3	Pond no.1	Pond no.2	Pond no.3
0	28.0	30.2	29.0	8.0	8.5	8.3	36	36	35	35	32	45	0.72	9.57	0.71
20	29.0	29.0	29.2	8.4	8.8	8.4	36	34	33	30	38	42	0.80	0.68	0.75
40	26.5	26.3	27.5	8.2	8.5	8.5	33	33	33	28	35	37	0.95	0.72	0.81
60	26.0	26.1	27.8	7.8	8.2	8.4	24	30	29	55	42	38	1.25	0.84	1.00
80	27.2	27.0	26.2	8.2	8.5	8.3	22	24	25	80	40	30	1.31	0.94	1.12
100	26.5	26.2	26.0	7.9	8.6	8.7	16	20	23	60	38	35	1.44	1.14	1.34
115	26.3	26.7	27.6	8.1	8.3	8.5	15	18	20	57	43	37	1.78	1.25	1.53
122	27.4	27.2	26.0	7.9	8.3	8.6	16	17	19	50	45	39	2.06	1.26	1.69

of which pond 1 showed the highest load (2.06%) on day 122. No relationship between *Vibrio* load in pond water and temperature, pH and transparency was found, as evidenced by the values of the correlation coefficient 'r'. The observations made during the present study are in accordance with Anand *et al.*, (1996) and Janakiram *et al.*, (2000), with no clear-cut correction, between *Vibrio* count and physiochemical parameters like temperature and pH. High *Vibrio* count was associated with high organic load in pond 1 and 3. Similarly, increase in *Vibrio* load coincided with decrease in salinity in all 3 ponds but organic load plays important role in *Vibrio* proliferation (Anand *et. al.*, 1996). It appears that the decomposition of excess feed and algal die-off resulted in proliferation of *Vibrios* masking the apparent relationships of the bacterial loads with physiochemical parameters.

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