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Enteric Bacteria and Water Quality of Freshwater Prawn *Macrobrachium rosenbergii* (De Man) in Culture Environment from Kerala, India

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Abstract: Enteric bacterial population associated with farmed freshwater prawn and its environment, water quality of prawn farm and the existing association between these parameters were studied. Microbiological parameters were determined following the United States Food and Drug Administration (USFDA) methods and the physico-chemical parameters as per the standard methods of American Public Health Association (APHA). Prawn samples yielded a mean Total Plant Count (TPC) in the range of 4.57 to 6.66 log cfu g^{-1} and was the highest among all other samples. Prawns followed by water samples had the higher level of enteric indicator organisms. Sediment showed higher count of sulphite reducing *clostridia.* Emerging pathogen *E. coli* O157:H7 were absent in all the samples analyzed. *Enterobacter* (31.5%) followed by *Citrobacter* (13.2%) and non enteric bacteria *Aeromonas* (11%) were the dominant flora recovered. *Escherichia*, *Klebsiella*, *Hafnia*, *Serratia*, *Salmonella* and *Shigella* were the other opportunistic enteric bacterial pathogens detected from this system. The rearing practices such as use of cow dung as fertilizer and microbiologically contaminated feed could have influenced the enteric flora. Study on various physico-chemical parameters of pond water revealed that they were within the suitable range for the freshwater prawn culture. Correlation analysis revealed a significant positive correlation between pollution indicator parameters such as Total Organic Carbon (TOC), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) with that of Total Plate Count (TPC) and Total Enterobacteriaceae Count (TEC) of the pond water and prawn samples. Presence of bacteria of public health significance in the aquaculture pond envisages strict hygienic handling, processing of prawn from this system and cooking prior to consumption to ensure public health safety.

Key words: Enteric bacteria, scampi, water quality, pollution indicators

INTRODUCTION

World aquaculture industry witnessed a phenomenal growth over the last 10 year period by way of increment in production from 24.38 million tones in 1995 to almost double in the year 2006 (51.70 million tones) (FAO, 2009). India with its aquaculture production of

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3.12 million tones is placed second only after China in the year 2006 (FAO, 2009). Freshwater crustaceans including prawns though produced in meager quantities (around 953 198 tones) have gained significance as high value commodity by standing as fifth highest valued species group. Farming of giant freshwater prawn *Macrobrachuim rosenbergii* popularly known as scampi is spreading fast to all Indian states due to its large size attainment, tolerance to water quality changes, ability to cope with handling stress and ability to feed on unconventional feeds. The farming of scampi received a big boost lately owing to the demand and attractive price fetched by the Indian scampi in the international market and the slump in tiger shrimp production due to disease. The production of scampi in India increased from 7150 tones during the year 1999-2000 to 27, 262 tones in 2007-2008 (MPEDA, 2008).

While realizing the potential of aquaculture as an important food producing enterprises, hand in hand there exist, the health risk associated with the aquaculture produce due to contamination of products by chemical and biological agents (Huss *et al*., 2000). Reviews on the microbiological evaluation of aqua cultured raw fish/shellfish and their growing environments emphasized the need for the assurance of the quality and safety before sending them to retail and export market (Reilly and Kaferstein, 1999). Shellfishes, the most internationally traded food of aquatic origin, are in the forefront of food safety and quality improvement due to stringent microbial quality and safety regulations enforced by international regulatory authorities. Majority of the bacteria implicated in the disease out break of *M. rosenbergii* and seafood borne bacteria of human health importance belonged to the mesophilic Enterobacteriaceae group (Brock, 1993; Ogbondeminu, 1993). They included pathogenic *E. coli*, *Salmonella*, *Shigella*, *Yersinia*, *Enterobacter*, *Citrobacter* etc., recovered from different aquaculture systems in India and other parts of the world (Anderson *et al*., 1989; Ogbondeminu, 1993; Jayasree *et al*., 1999). Threat due to these organisms is more, when earthen pond fertilized with animal manure, was used as the production systems (Ogbondeminu and Okaeme, 1986, 1989). Reports of the occurrence of pathogenic strains of *E. coli* and outbreaks of illness due to consumption of a wide range of fish and seafood products are common (Samadpour *et al*., 1994; Kumar *et al*., 2001; Teophilo *et al*., 2002). Among the different groups, *E. coli* O157, an Enterohemorrhagic *E. coli*, is an important emerging pathogen with very low infective dose (10-100 cells).

It was well established that rearing site water quality and microbiology play a significant role in overall well being of fish, nutrient status of pond, fish yield potential and most importantly quality and safety of fish raised from them (Reilly and Kaferstein, 1999). Therefore, monitoring of productive areas for microbial quality is a fundamental prerequisite to obtain safe produce from the farms. Different researchers reported a direct or indirect correlation between the microbial parameters and water quality parameters (Ogbondeminu, 1993; Jun *et al*., 2000; Surendraraj *et al*., 2009). In this study, we report the microbial and limnological quality of scampi farm, the existing correlation if any between this parameter and the presence of bacteria of public health significance to pay way to develop safe farm management practices.

MATERIALS AND METHODS

Description of the Sampling Site

The samples of prawn, pond water, source water, sediment and feed were obtained from a freshwater prawn farm situated in Kumarakom, Kottayam district of Kerala, India. The earthen pond sampled has an area of 0.90 ha with a stocking density of 40,000 Juveniles ha^{-1}.

The pond was surrounded by two rearing ponds in two sides, third and fourth side was covered by feeder canal and a transition pond, respectively. Pond dykes had plantations like coconut, banana trees etc and pond bottom was silty. The pond was fertilized with cow dung initially and continued every 15 days according to the nutrient status. Feeding was by the commercial formulated feed obtained from CP Aquaculture India Pvt. Ltd., Chennai. There was periodic water exchange by opening the inlet cum outlet to a small transition pond which was connected to the feeder canal, passing parallel to the farm. Feeder canal has direct contact to the natural freshwater water body.

Sampling

Five sampling was carried out on 50th, 71st, 127th, 155th and 227th day of culture. Prawn samples were harvested by cast net and put in sterile polythene bag. Water samples were collected from 4 different location of the pond by inverting the sterile polystyrene bottle to about 30 cm below the surface and a well-mixed homogeneous sample was used for analysis. Feeder Canal Water (FCW) samples were also collected in similar manner. Pond sediment were scooped out from four different locations of the pond and collected in a sterile polythene bag. All the samples were kept in ice box carried to laboratory. Analysis was initiated with in 2 h of sample collection. Sampling was done between 08:00 to 09:00 h Indian standard time.

Water Quality Parameters

Water temperature, pH and salinity were determined at the farm location by using thermometer, digital pH meter (Cyberscan, USA) and refractometer (Atago Co. Ltd., Japan), respectively. Transparency was measured using secchi disc (Trivedy and Goel, 1984). Total Suspended Solids (TSS) and Dissolved Oxygen (DO) were analyzed according to the standard methods for examination of water and wastewater (APHA/AWWA/WEF, 1998). TOC, NO_3-N (nitrate nitrogen), BOD, COD and surfactant, were measured by properly calibrated pastel UV spectrophotometer (SECOMAM RS 232, France) with a spectral range of 200 to 320 nm. Turbidity was measured by employing the digital turbidity meter (Merck, USA). Conductivity of the water was measured by electronic conductivity meter (CIFT, India).

Bacteriological Analysis

Ten grams of the prawn (headless) or sediment samples were homogenized for 1 min with 90 mL of saline (0.85% NaCl) in a stomacher 400 lab blender (Seward, London, UK). Prawn, sediment and water samples were serially diluted and used for analysis. The total plate count was enumerated by pour plating the samples on Tryptone Glucose Agar (TGA), Total Enterobacteriaceae count on Violet Red Bile Glucose Agar (VRBGA, Oxoid, Basingstoke, UK) and faecal *Streptococci* in Kenner Faecal streptococcus agar (KF) (USFDA, 2001).

Total coliforms, faecal coliforms and *E. coli* counts were estimated for prawn/sediment and water samples by a three-tube and five tube Most-Probable Number (MPN) procedure, respectively, with the following modification. Aliquots of serially diluted samples were inoculated into MacConkey broth (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 to 48 h. Positive tubes were (1) subjected to an MPN procedure in Brilliant Green Lactose Bile Broth (BGLB) (Oxoid, UK) at 37°C for 24 to 48 h and (2) subjected to Elevated Coliform (EC) broth (Difco, Becton Dickinson, Sparks, Md., USA) and indole (Difco, Becton Dickinson, Sparks, Md., USA) broths at 44.5°C. Positives in BGLB, EC broth and indole were noted

down and referred to McCardy's MPN table to determine the total coliforms, faecal coliforms and *E. coli* counts, respectively. A loopful of bacterial culture from indole-positive tubes was streaked on Eosine Methylene Blue agar (EMB agar, BBL, Becton Dickinson, Sparks, Md., USA) and characteristic *E. coli* colonies were isolated and confirmed by Indole-Methylred-Voges-Proskauer-Citrate (IMViC) tests (APHA/AWWA/WEF, 1998). Sulphite reducing clostridia were analyzed by MPN method using differential reinforced clostridia medium (USFDA, 2001).

Detection of *E. coli* O157:H7

Twenty five gram of the prawn or sediment and 25 mL of water samples were enriched in 225 mL of modified elevated coliform broth (Difco, USA) containing novobiocin (20 mg mL^{-1}; Sigma Chemical Company, St. Louis, Mo., USA) at 42 C with shaking (150 rpm). After an overnight incubation, diluted enrichment samples were plated on MacConkey sorbitol agar (Oxoid, UK) supplemented with cefixime (SR 0191, Oxoid, UK) and potassium tellurite (March and Ratman, 1986). Plates were incubated overnight at 37°C and sorbitol-negative colonies were isolated at a rate of 2-3 colonies per sample. After isolate purification, they were streaked on EMB agar and confirmed isolates were checked for MUG (methyl umbelliferyl-β-glucuroride) reaction and IMViC test. MUG and sorbitol-positive *E. coli* (ATCC 25922) were used as controls for checking sorbitol and MUG reactions. Isolates that were sorbitol and MUG negative were tested for latex agglutination with *E. coli* serotype O157 specific antisera as per manufacturer's instruction (Oxoid, UK).

Characterization of Enteric Bacteria

For identification, 2 to 5 well-separated typical colonies from VRBGA plates were selected using Harrison's disc method (Harrigan and McCance, 1976). These cultures were purified and stored for further study in nutrient agar slants. Altogether, 146 pure culture isolates were obtained and identified up to the genus level with the help of an identification scheme from web http// www.vet.uga.edu/WEBFILES/ in consultation with Edwards and Ewing (1972) and MacFaddin (1980). About, 5% of the isolates were crosschecked for identification using analytical profile index 20 E (API 20 E, bioMerieux).

Statistical Analysis

All the results were produced as mean and mean log±SD values for water quality and microbiological parameters respectively. Statistical analysis between the means was accomplished using Tukey's test and a two-tailed Pearson correlation analysis was carried out. The statistical package used in the study is SPSS, 10.

RESULTS AND DISCUSSION

Physico-Chemical Characteristics of Water

In general, water quality parameters showed a distinct variation among different sampling phases and with few exceptions they were within the optimum range reported for scampi culture (Table 1, 2) (Brock, 1993; Saxena, 2003). The temperature and pH of the Pond Water (PW) and FCW ranged between 28.97±0.06 to 31.08±0.06°C and 6.41±0.01 to 7.20±0.03, respectively. Salinity was increasing throughout the farming phase and reached the highest level (2.53 ppt) towards the end of the culture period in PW, which corresponds to the summer season. The observed values were similar to the one reported by Lalitha and Surendran (2004) study on the scampi culture pond.

Table 1: Water quality parameters of pond water used in giant freshwater prawn (*M rosenbergii*) farm*

	Pond water				
Parameters	Phase I	Phase II	Phase III	Phase IV	Phase V
Temperature (°C)	30.17±0.06^{b}	30.07±0.06^{b}	31.03±0.06^{a}	28.97±0.06^{c}	31.08±0.06^{a}
pH	7.11±0.02^{b}	7.03±0.01^{c}	6.69±0.03^{d}	7.20±0.03^{a}	6.74±0.01^{d}
Salinity (ppt)	0.10±0.00^{c}	0.33±0.06^{c}	0.73±0.06^{b}	0.90±0.20^{b}	2.53±0.12^{a}
TSS (ppm)	11.17±0.25^{c}	18.97±0.85^{b}	23.13±1.42^{a}	20.83±0.87^{b}	24.30±0.30^{a}
TOC (ppm)	1.67±0.12^{e}	2.47±0.06^{d}	3.47±0.25^{c}	5.70±0.00^{b}	6.17±0.06^{a}
NO_3-N (ppm)	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2
Surfactant (ppm)	Less than 0.5	Less than 0.5	0.90±0.10^{b}	1.60±0.10^{a}	Less than 0.5
COD (ppm)	5.27±0.06^{e}	7.43±0.15^{d}	10.13±0.73^{c}	16.20±0.20^{b}	17.60±0.60^{a}
BOD (ppm)	2.30±0.10^{d}	3.47±0.06^{c}	4.80±0.36^{b}	8.17±0.06^{a}	7.97±0.31^{a}
Turbidity (NTU)	7.33±0.58^{c}	12.00±0.00^{b}	11.67±0.58^{b}	11.67±0.58^{b}	37.33±1.15^{a}
SDT (cm)	119.33±1.15^{a}	99.33±1.15^{b}	63.67±1.15^{d}	84.67±0.58^{c}	65.67±1.15^{d}
Conductivity (milli mhos)	0.67±0.01^{a}	0.53±0.01^{a}	0.85±0.01^{a}	28.47±0.25^{c}	4.43±0.38^{b}
DO (ppm)	5.18±0.07^{a}	4.75±0.08^{b}	3.18±0.07^{d}	3.24±0.03^{d}	3.87±0.13^{c}

*Results are presented as Mean±SD. Means in a column with the same superscript letters are not significantly different ($p>0.05$). TSS: Total suspended solids; TOC: Total organic carbon; COD: Chemical oxygen demand; BOD: Biological oxygen demand; SDT: Secchi disc transparency; DO: Dissolved oxygen; ppt: Parts per thousand; ppm: Parts per million; NTU: Neplometric turbidity unit; cm: Centimeter

Table 2: Feeder canal water quality for giant freshwater prawn (*M rosenbergii*) farm*

	Feeder canal water				
Parameters	Phase I	Phase II	Phase III	Phase IV	Phase V
Temperature (°C)	30.10±0.10^{c}	30.23±0.06^{bc}	31.00±0.02^{a}	29.40±0.52^{d}	30.87±0.06^{ab}
pH	7.17±0.03^{a}	7.11±0.02^{b}	7.00±0.02^{c}	6.41±0.01^{e}	6.72±0.02^{d}
Salinity (ppt)	0.13±0.07^{c}	0.43±0.06^{c}	1.00±0.10^{b}	1.23±0.15^{b}	2.97±0.21^{a}
TSS (ppm)	9.63±0.40^{d}	11.10±0.89^{c}	12.87±0.42^{b}	14.23±0.31^{b}	26.43±0.35^{a}
TOC (ppm)	3.23±0.06^{c}	3.10±0.01^{c}	2.33±0.12^{d}	4.00±0.10^{b}	6.57±0.06^{a}
NO_3-N (ppm)	Less than 0.2	Less than 0.2	Less than 0.2	1.70±0.02^{b}	2.43±0.06^{a}
Surfactant (ppm)	1.50±0.01^{a}	Less than 0.5	Less than 0.5	Less than 0.5	Less than 0.5
COD (ppm)	6.57±0.06^{b}	6.30±0.10^{b}	4.47±0.30^{c}	10.03±0.31^{a}	10.20±0.44^{a}
BOD (ppm)	3.23±0.12^{b}	3.03±0.06^{b}	1.97±0.15^{c}	4.57±0.15^{a}	4.67±0.21^{a}
Turbidity (NTU)	8.00±0.20^{b}	7.67±0.38^{b}	7.67±0.58^{b}	16.67±0.40^{a}	15.33±0.52^{a}
Conductivity (milli mhos)	0.32±0.04^{b}	0.32±0.12^{b}	0.45±0.02^{b}	12.73±0.15^{a}	1.37±0.15^{c}
DO (ppm)	3.31±0.06^{ab}	3.41±0.03^{b}	3.22±0.03^{c}	4.84±0.06^{a}	2.29±0.11^{d}

*Results are presented as Mean±SD. Means in a column with the same superscript letters are not significantly different ($p>0.05$). TSS: Total suspended solids; TOC: Total organic carbon; COD: Chemical oxygen demand; BOD: Biological oxygen demand; DO: Dissolved oxygen; ppt: Parts per thousand; ppm: Parts per million; NTU: Neplometric turbidity unit; cm: Centimetre

The pollution indicator parameters, TSS, TOC, BOD, COD and turbidity values showed an increasing trend for pond water as culture progressed. As reported earlier, in our study also bacterial population increased positively with above parameters and hence, control of pollution parameters to the optimum level is important (Martinez *et al*., 2002). Decomposition of organic matter from aquatic plants and settling of left over feed and excreta from prawns might have contributed to the higher BOD and COD. However, in the case of FCW, similar trend was missing except for TSS. NO_3-N and surfactants were below the detection level in most phases both in PW and FCW and were within the permissible level (Brock, 1993). Lalitha and Surendran (2004) observed a DO level of 5 to 6 ppm, where as the DO in the present study varied between 3.18±0.07 to 5.18±0.17 ppm for PW and still lower in FCW. Chien *et al.* (1999) reported that high conductivity has a direct bearing on the survival of microbes. Conductivity values showed an increasing trend and were highest in phase IV. Though, the microbial counts were comparatively low in the last two phases, similar effect as reported by earlier author is missing in this study.

Table 3: Microbiology of freshwater prawn farm during different phase of culture*

Samples	TPC	TEC	TCC	FCC	ECC	FSC	SRCC	*E. coli* O157
	------- (log cfu g^{-1}) -----		---------- (log MPN g^{-1}) ----------			---------- (log cfu g^{-1}) ----------		
Prawn								
Phase I	4.57±0.06^{d}	2.69±0.01^{e}	1.33±0.06^{d}	0.56±0.03^{d}	0.29±0.04^{d}	Less than 2	1.12±0.12^{d}	Less than 2
Phase II	5.67±0.02^{c}	3.74±0.01^{d}	2.45±0.05^{c}	2.43±0.05^{c}	2.43±0.05^{b}	3.46±0.02^{c}	2.45±0.05^{c}	Less than 2
Phase III	6.19±0.10^{b}	4.34±0.02^{a}	3.71±0.09^{b}	3.56±0.03^{b}	3.37±0.06^{a}	4.01±0.36^{b}	4.09±0.08^{a}	Less than 2
Phase IV	6.66±0.03^{a}	4.17±0.02^{c}	2.42±0.05^{c}	0.65±0.09^{d}	0.42±0.05^{c}	2.93±0.03^{d}	1.16±0.08^{d}	Less than 2
Phase V	6.09±0.04^{b}	4.27±0.01^{b}	4.15±0.08^{a}	4.10±0.07^{a}	3.42±0.05^{a}	4.25±0.01^{a}	3.19±0.02^{b}	Less than 2
Samples	TPC	TEC	TCC	FCC	ECC	FSC	SRCC	*E. coli* O157
	------- (log cfu g^{-1}) -----		---------- (log MPN g^{-1}) ----------			---------- (log cfu g^{-1}) ----------		
Sediment								
Phase I	4.40±0.05^{d}	1.38±0.05^{c}	1.28±0.07^{a}	0.28±0.05^{a}	0.25±0.03^{a}	Less than 2	4.62±0.03^{a}	Less than 2
Phase II	5.08±0.04^{b}	1.60±0.04^{b}	0.45±0.05^{b}	0.22±0.02^{ab}	0.14±0.05^{b}	Less than 2	3.36±0.10^{c}	Less than 2
Phase III	5.51±0.01^{a}	2.15±0.03^{a}	0.39±0.01^{b}	0.21±0.02^{b}	0.19±0.04^{ab}	Less than 2	4.19±0.02^{b}	Less than 2
Phase IV	4.91±0.02^{c}	1.09±0.08^{d}	ND	ND	ND	Less than 2	3.09±0.08^{d}	Less than 2
Phase V	4.48±0.03^{d}	Less than 2	ND	ND	ND	Less than 2	1.64±0.03^{e}	Less than 2
Samples	TPC (log cfu mL^{-1})	TEC (log cfu 100 mL^{-1})	TCC	FCC	ECC	FSC	SRCC	*E. coli* O157
			--------- (log MPN 100 mL^{-1}) ---------			---------- (log cfu mL^{-1}) ----------		
PW								
Phase I	3.43±0.04^{e}	2.01±0.05^{b}	1.30±0.04^{b}	1.24±0.09^{b}	1.10±0.04^{c}	Less than 2	2.56±0.03^{b}	Less than 2
Phase II	3.97±0.02^{d}	1.90±0.02^{c}	1.84±0.02^{a}	1.83±0.02^{a}	1.68±0.05^{a}	Less than 2	1.73±0.02^{c}	Less than 2
Phase III	4.13±0.03^{c}	2.72±0.01^{a}	1.38±0.05^{b}	1.31±0.09^{b}	1.31±0.09^{b}	Less than 2	1.50±0.04^{d}	Less than 2
Phase IV	5.42±0.02^{a}	1.88±0.03^{c}	0.97±0.06^{c}	0.77±0.07^{c}	0.73±0.05^{d}	Less than 2	4.04±0.14^{a}	Less than 2
Phase V	4.66±0.03^{b}	2.77±0.02^{a}	1.33±0.08^{b}	1.24±0.09^{b}	1.20±0.10^{bc}	Less than 2	2.67±0.03^{b}	Less than 2
Samples	TPC (log cfu mL^{-1})	TEC (log cfu 100 mL^{-1})	TCC	FCC	ECC	FSC	SRCC	*E. coli* O157
			--------- (log MPN 100 mL^{-1}) ---------			---------- (log cfu mL^{-1}) ----------		
FCW								
Phase I	3.37±0.03^{c}	1.93±0.05^{d}	1.67±0.05^{c}	1.66±0.05^{c}	1.52±0.03^{b}	Less than 2	2.26±0.04^{c}	Less than 2
Phase II	3.34±0.02^{c}	2.45±0.08^{b}	2.34±0.05^{b}	1.85±0.01^{b}	1.60±0.07^{b}	Less than 2	1.73±0.04^{e}	Less than 2
Phase III	4.09±0.04^{b}	1.95±0.01^{d}	1.51±0.04^{d}	1.33±0.05^{d}	1.22±0.06^{c}	Less than 2	2.60±0.05^{a}	Less than 2
Phase IV	4.03±0.03^{b}	2.12±0.06^{c}	1.59±0.03^{cd}	1.38±0.05^{d}	0.82±0.07^{d}	Less than 2	2.40±0.04^{b}	Less than 2
Phase V	4.38±0.01^{a}	4.20±0.02^{a}	3.08±0.05^{a}	2.95±0.01^{a}	2.10±0.05^{a}	Less than 2	1.94±0.08^{d}	Less than 2

*Results are presented as Mean log±SD. ^{a,b,c,d}Means in a column with the same superscript letters are not significantly different ($p>0.05$). TPC: Total plate count; TEC: Total Enterobacteriaceae count; TCC: Total coliforms count; FCC: Faecal coliforms count; ECC: *E. coli* count; FSC: Faecal *Streptococci* count; ND: Not detected; SRC: Sulphite reducing *Clostridia* count; PW: Pond water; FCW: Feeder canal water; cfu: Colony forming unit; MPN: Most probable number

Microbiology of Freshwater Shellfish Farm

The TPC of prawn, sediment and PW samples were significantly different among the different sampling phases ($p<0.05$) and ranged between 3.34 cfu mL^{-1} and 6.66 cfu g^{-1} (Table 3). Prawn samples always had higher TPC than all other samples and were between 4.5 and 6.7 log cfu g^{-1} among different faming phases. Sediment TPC was 0.5 to 1.5 log cfu g^{-1} lower than that of prawn and for pond water it was varying from 3.40 to 5.42 log cfu mL^{-1}. The count observed in this study for prawns is slightly higher than the one reported for different types of fresh and processed samples (Bandekar *et al*., 2004; Lalitha and Surendran, 2006). Higher count for shellfish than sediment and PW samples were also reported by earlier studies (Lalitha and Surendran, 2006; Surendran *et al*., 2000).

As in TPC, the bacterial indicator organisms such as TEC, TCC, FCC, ECC and FSC were also found to be high in prawn samples than all other samples. The counts were significantly different among different sampling phases ($p<0.05$) and increased with progress in culture except in phase IV. Sediment samples recorded the least count for the above parameters and in the last two phases they were absent. The PW and FCW water were found to have been contaminated with all the above indicators but there was not much variation among faming phases ($p>0.05$). The present findings were comparable with the earlier reports on prawn

farms from Kerala (Surendran *et al*., 2000; Lalitha and Surendran, 2006). The microbial analysis of feed and fertilizer used in the pond showed a higher level of TPC, TEC, TCC and FSC. The higher count of TPC and other indicator organisms in prawn samples might be due to their detritus feeding habits and the use of contaminated feed and fertilizers.

Surendran *et al*. (2000) reported the recovery of Sulphite Reducing *Clostridia* (SRC) from all the samples of freshwater prawn, sediment and water. In our study also, SRC was detected in all samples. It was higher in sediment/FCW and was low in the case of prawn samples. Contamination by different pathogens such as *Salmonella*, *Vibrio cholerae* and pathogenic *E. coli* were increasingly reported from sediment samples of prawn culture pond (Surendran *et al*., 2000; Jeyasekaran and Ayyappan, 2002). However, Screening for the emerging pathogen *E. coli* O157 in this study revealed that they could not be detected in this culture system.

Composition of Enteric Bacteria in Scampi Farms

Characterisation 146 enteric bacterial isolates from scampi farm revealed that *Enterobacter* (31.5%) was dominant flora. This was followed by *Citrobacter* (13.7%) and non enteric bacteria *Aeromonas* (11%). Other genera recovered include *Serratia*, *Escherichia*, *Salmonella, Klebsiella, Shigella, Morganella*, *Plesiomonas* and *Providencia* at varying proportions. Earlier studies also reported the recovery of genera belonging to the family Enterobacteriaceae and Aeromonadaceae as dominant flora in the larval rearing and grow out culture environments of *M. rosenbergii* (Anderson *et al*., 1989; Lalitha and Surendran, 2004, 2006). Al-Harbi and Uddin (2004) detected *Salmonella*, *Shigella* and other pathogens from *Macrobrachium* culture ponds from Saudi Arabia region. Higher incidence of *Aeromonas* is of health and safety concern as there were reports of recovery of enterotoxigenic strains of *Aeromonas* sp. from *M. rosenbergii, M. malcomsonii* and from aquatic environment (Rahim and Aziz, 1994).

Feeder canal water harbor diverse group of bacteria and as many as 9 genera were detected. Prawn and water samples carried seven enteric bacterial genera each, whereas in sediment only four genera were detected. *Enterobacter* was the dominant genera detected in prawn, sediment and feeder canal water. In pond water, *Citrobacter* was the dominant genera recovered (Table 4). Studies on enteric bacterial composition for prawn are scanty. Reports on composition of enteric bacteria on water samples and meat products revealed a similar dominance as observed in our study and the genera recovered were also similar (Ramteke *et al*., 1992; Jimenez *et al*., 2003). In the present study, *Salmonella* (4.5%) was recovered from prawn samples and pond water had *Salmonella* along with *Shigella.* Previous studies could recover *Salmonella* from sediment samples in addition to prawn (Surendran *et al*., 2000; Jeyasekaran and Ayyappan, 2002). Table 5 shows the status of enteric bacterial pollution during different phases of culture period. Higher density of enteric bacterial genera was observed in phase III followed by phase II and phase IV. *Enterobacter* dominated from phase II onwards until the last phase with a percentage contribution ranging from 29 to 50.6. *Citrobacter* was the predominant genera in phase I of culture.

Correlation between Water Quality and Microbiology of Fish and Shellfish Farms

The relationship between physico-chemical parameters and bacterial count attracted much of attention (Ogbondeminu and Adeniji, 1984; Ferguson *et al*., 1996). In this study, correlation between freshwater prawn pond microbiological quality and physico-chemical parameters was established using the two tailed Pearson's correlation coefficient

Table 4: Composition of enteric bacteria among different samples

	Samples									
	Fish		Mud		PW		SW		Total	
Group	No.	%	No.	%	No.	%	No.	%	No.	%
Aeromonas	2	4.5	2	11.1	8	19.0	4	9.5	16	11.0
Citrobacter	4	9.0	-	-	16	38.0	-	-	20	13.7
Enterobacter	22	50.0	6	33.3	6	14.3	12	28.6	46	31.5
Escherichia	-	-	-	-	-	-	10	23.8	10	6.8
Hafnia	2	4.5	6	33.3	4	9.5	4	9.5	16	11.0
Klebsiella	-	-	4	22.2	-	-	2	4.5	6	4.1
Morganella	-	-	-	-	-	-	2	4.5	2	1.4
Plesiomonas	-	-	-	-	-	-	2	4.5	2	1.4
Providencia	2	4.5	-	-	-	-	-	-	2	1.4
Salmonella	2	4.5	-	-	2	4.8	4	9.5	8	5.5
Serratia	10	22.7	-	-	2	4.8	-	-	12	8.2
Shigella	-	-	-	-	4	9.5	2	4.8	6	4.1
Total	44	100.0	18	100.0	42	100.0	42	100.0	146	100.0

Table 5: Changes in the composition of enteric bacteria during different farming phase

	Farming phase				
	I	II	III	IV	V
Group	(Nos)				
Aeromonas	2	-	4	-	10
Citrobacter	12	4	4	-	-
Enterobacter	-	8	14	8	16
Escherichia	6	-	-	2	2
Hafnia	4	2	6	-	4
Klebsiella	-	-	6	-	-
Morganella	-	-	2	-	-
Plesiomonas	-	-	-	2	-
Providencia	-	2	-	-	-
Salmonella	-	8	-	-	-
Serratia	-	4	2	6	-
Shigella	-	-	2	4	-
Total	24	28	40	22	32

($p<0.05$ and 0.01). Earlier study observed a positive or negative correlation between temperature, salinity and fish pond microbial quality (Sugita *et al*., 1985; Markosova and Jezek, 1994; Ferguson *et al*., 1996). However in this study, no consistent relationship could be observed between pond water temperature, pH and salinity with that of microbial quality of pond water and prawns.

TOC, BOD and COD indicate directly or indirectly the organic pollution status and a change in these parameters in water from pond and feeder canal water of scampi culture systems positively affected TPC and TEC (significant at $p<0.05$ and 0.01) of prawn and pond water. Occasionally in this system similar findings were observed for TSS and turbidity also. It was reported that organic matter helps with greater survival of aquatic bacteria (Gerba and McLeod, 1976) and increase in the level of above parameters lead to the significant increment in density of microbial load (Sugita *et al*., 1985; Ferguson *et al*., 1996; Surendraraj *et al*., 2009). No consistent relationship could be seen between DO and microbial parameters. Several authors reported a significantly positive correlation between TPC of pond water and fish (Ogbondeminu, 1993; Apun *et al*., 1999). In the present study, pond water microbial parameters TPC, TEC showed a significant positive correlation with fish TPC, TEC, FSC ($p<0.01$), TCC and SRC ($p<0.05$).

CONCLUSION

The TPC and enteric bacterial counts were detected in higher numbers especially in prawn samples and some of the members recovered in this study like Salmonella, Shigella, Hafnia and Klebsiella were established prawn/human pathogens. Further studies on the pathogenic potential/toxin producing ability need to be studied for *Aeromonas* and *E. coli* for establishing actual threat posed by these organisms. However, detection of diverse group of enteric bacteria including potential pathogens in scampi culture pond suggests that strict hygiene procedures and proper cooking prior to consumption is essential to safe guard the consumers. Good correlation between the bacterial population and the water quality variables like TOC, BOD, COD opens up an avenue for research in this line to establish a water quality and microbial quality model useful in assessing the quality and safety status of farm prawn.

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REFERENCES

Al-Harbi, A.H. and M.N. Uddin, 2004. Quantitative and qualitative study of the bacterial flora of farmed freshwater prawn (*Macrobrachium rosenbergii*) larvae. J. Applied Ichthyol., 20: 461-465.

Anderson, I.G., M.N. Shamsudin and G. Nash, 1989. A preliminary study on the aerobic heterotrophic bacterial flora in giant freshwater prawn, *Macrobrachium rosenbergii*, hatcheries in Malaysia. Aquaculture, 81: 213-223.

APHA/AWWA/WEF, 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edn., American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC., USA., ISBN: 0-87553-235-7.

Apun, K., A.M, Yusof., J. Kumbang, 1999. Distribution of bacteria in tropical freshwater fish and ponds. Int. J. Environ. Health Res., 9: 285-292.

Bandekar, J.R., A.S. Kamat, M. Karani, V. Dhokane and R. Shashidhar *et al*., 2004. Bacteriological quality of farmed freshwater fish and shellfish meant for Export. Fish Technol., 41: 57-62.

Brock, J.A., 1993. A Synopsis of Pathology, Diseases and Problems of Cultured Macrobrachium, with an Emphasis on Experiences in Hawaiian Prawn Farming. In: CRC Handbook of Mariculture, Vol: I Crustacean Aquaculture, James, P. and Vey Mc (Eds.). CRC Press, Boca Raton, FL, USA., ISBN-10: 0849302552, pp: 361-391.

Chien, Y., H.T. Laia, H. Thi and S.M. Liub, 1999. Modeling the effects of sodium chloride on degradation of chloramphenicol in aquaculture pond sediment. Sci. Total Environ., 239: 81-87.

Edwards, P.R. and W.H. Ewing, 1972. Identification of Enterobacteriaceae. 3rd Edn., Burgess Publishing Co., Minneapolis.

FAO, 2009. The State of World Fisheries and Aquaculture 2008. Food and Agricultural Organization, Rome.

Ferguson, C.M., B.G. Coote, N.J. Ashbolt and I.M. Stevenson, 1996. Relationships between indicators, pathogens and water quality in an estuarine system. Water Res., 30: 2045-2054.

Gerba, C.P. and J.S. McLeod, 1976. Effect of sediments on the survival of *Escherichia coli* in marine waters. Applied Environ. Microbiol., 32: 114-120.

Harrigan, W.F. and M.E. McCance, 1976. Laboratory Methods in Food and Dairy Microorganisms. Academic Press, London.

Huss, H.H., A. Reilly and P.K.B. Embarek, 2000. Prevention and control of hazards in seafood. Food Control, 11: 149-156.

Jayasree, L., P.R. Janaki and R. Madhavi, 1999. Shell disease in the freshwater Prawn Macrobrachium rosenbergii (de. Man): Etiology, Pathogenicity and Antibiotic sensitivity. J. Aquac. Trop., 14: 289-298.

Jeyasekaran, G. and S. Ayyappan, 2002. Post harvest microbiology of Farm-reared, tropical freshwater prawn (*Macrobrachium rosenbergii*). J. Food Sci., 67: 1859-1861.

Jimenez, S.M., M.C. Tiburzi, M.S. Salsi, M.E. Pirovani and M.A. Moguilevsky, 2003. The role of visible faecal material as a vehicle for generic *Escherichia coli*, coliform and other enterobacteria contaminating poultry carcasses during slaughtering. J. Applied Microbiol., 95: 451-456.

Jun, X., F. Xiuzheng and Y. Tongbing 2000. Physical and chemical factors and bacteria in fish ponds. Naga: World Fish Centre Q., 23: 16-20.

Kumar, H.S., S. Ottu, I. Karunasagar and I. Karunasagar, 2001. Detection of Shiga-toxigenic *Escherichia coli* (STEC) in fresh seafood and meat marketed in Mangalore, India by PCR. Lett. Applied Microbiol., 32: 334-338.

Lalitha, K.V. and P.K. Surendran, 2004. Bacterial microflora associated with farmed freshwater prawn *Macrobrachium rosenbergii* (de Man) and the aquaculture environment. Aquac. Res., 35: 629-635.

Lalitha, K.V. and P.K. Surendran, 2006. Microbiological quality of farmed tropical freshwater prawn, *Macrobrachium rosenbergii* de Man. J. Aquat. Food Prod. Tech., 15: 71-82.

MacFaddin, J.F., 1980. Biochemical Tests for Identification of Medical Bacteria. 2nd Edn., Williams and Wilkins, Baltimore.

March, S.B. and S. Ratman, 1986. Sorbitol MacConkey medium for detection of *Escherichia coli* O157: H7 associated with hemorrhagic colitis. J. Clin. Microbiol., 23: 869-872.

Markosova, R. and J. Jezek, 1994. Indicator bacteria and limnological parameters in fishponds. Water Res., 28: 2477-2485.

Martinez, V., F. Abascal, M.V. Esteller, L. Bibiano and S. Bulbulian, 2002. Water quality in a reservoir used for carp production. Geofisca Int., 41: 421-427.

MPEDA, 2008. Role of MPEDA in Indian aquaculture in. http://www.mpeda.com/inner_home.asp?pg=# accessed on 01.01.2010.

Ogbondeminu, F.S. and H.A. Adeniji, 1984. Comparative study of bacterial flora in relation to water quality of fertilized and nonfertilized fish ponds in Nigeria. Kainji Lake Research Institute Annual Report, pp: 32-39.

Ogbondeminu, F.S. and A.N. Okoeme, 1986. Bacterial flora associated with an organic manure aquaculture system in kainji lake Basin, Nigeria. Int. J. Zoon., 13: 54-58.

Ogbondeminu, F.S. and A.N. Okaeme, 1989. Comparative analysis of bacterial flora associated with water and fish in manured pond. Biosci. Res. Comm., 1: 103-108.

Ogbondeminu, F.S., 1993. The occurrence and distribution of enteric bacteria in fish and water of tropical ponds in Nigeria. J. Aquac. Trop., 8: 61-66.

Rahim, Z. and K.M. Aziz, 1994. Enterotoxigenicity, hemolytic activity and antibiotic resistance of *Aeromonas* sp. isolated from freshwater prawn marketed in Dhaka, Bangladesh. Microbiol. Immunol., 38: 773-778.

Ramteke, P.W., J.W. Bhattacharjee, S.P. Pathak and N. Kalra, 1992. Evaluation of coliforms as indicators of water quality in India. J. Applied Bacteriol., 72: 352-356.

Reilly, A. and F. Kaferstein, 1999. Food safety and products from aquaculture. J. Applied Microbiol., 85: 249S-257S.

Samadpour, M., J.E. Ongerth, J. Liston, N. Tran and D. Nguyen *et al*., 1994. Occurrence of Shiga-like toxin-producing *Escherichia coli* in retail fresh seafood, beef, lamb, pork and poultry from grocery stores in Seattle, Washington. Applied Environ. Microbiol., 60: 1038-1040.

Saxena, V., 2003. Scientific Guidelines for farmers engaged in freshwater Prawn farming in India. Aquacult. Asia Mag., 8: 17-18.

Sugita, H., T. Fushino, K. Oshima and Y. Deguchi, 1985. Microflora in the water and sediment of freshwater culture ponds. Bull. Jap. Soc. Sci. Fish. NISSUISHI., 51: 91-97.

Surendran, P.K., N. Thampuran and N. Nambiar, 2000. Comparative microbial ecology of freshwater and brackishwater prawn farms. Fish. Technol., 37: 25-30.

Surendraraj, A., K.H. Sabeena Farvin, R. Yathavamoorthi and N. Thampuran, 2009. Enteric bacteria associated with farmed freshwater fish and its culture environment in kerala, India. Res. J. Microbiol., 4: 334-344.

Teophilo, G.N., R.H. Dos-Fernandes-Vieira, D. Dos-Prazeres-Rodrigues and F.G. Menezes, 2002. *Escherichia coli* isolated from seafood: Toxicity and plasmid profiles. Int. Microbiol., 5: 11-14.

Trivedy, R.K. and P.K. Goel, 1984.. Chemical and Biological Method for Water Pollution. Studies Environmental Pub., India.

USFDA, 2001. Bacteriological Analytical Manual. Revised. 8th Edn., AOAC, Washington DC, USA.