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Prevalence of coliform and *Salmonella* in cultured prawn (*Macrobrachium rosenbergii*) ghers of different management conditions at Dumuria, Khulna, Bangladesh

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Abstract

An investigation was carried out to enumerate the coliform bacteria (Total coliform and faecal coliform) and to detect the pathogenic bacteria, *Salmonella* in cultured prawn at Dumuria, Khulna region from August to October, 2011. Fifty four prawn samples were collected from eighteen prawn farms under three management conditions *viz* traditional, improved traditional and semi-intensive management system. The samples were analyzed in Biochemistry and Molecular Genetics laboratory of Fisheris and Marine Resource Technology Discipline at Khulna University. The average number of total coliform was observed 15.33 MPN/g, 87.00MPN/g and 355.84 MPN/g and the average faecal coliform was observed 4.83 MPN/g, 10.00 MPN/g and 22 MPN/g in semi-intensive, improved traditional and traditional management farms respectively. The TC and FC of semi-intensive and improved traditional management system were within the good quality range (<100 MPN/g and 11 MPN/g) but in traditional management system it exceeded the limit. *Salmonella* spp. was absent both in semi- intensive and improved traditional management system but found only in traditional management system where raw cow dung, poultry faeces, raw meats were used. Bacterial loads of prawn samples were different because the management conditions were not similar and the water sources of the farms were different.

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Introduction

Aquaculture is one of the fastest-growing food-producing sectors throughout the world. Now aquaculture plays a vital role to fulfill our domestic demand of protein and also we earn a lot of foreign exchange by exporting fishes and fisheries product. Prawn sector plays a major role in public health sector in Bangladesh. It contributes a lot in the national economy generating employment and foreign exchange earnings. About two million people are associated with prawn sector for employment and foreign exchange earnings (Azim, *et al.*, 2002). In 2008-2009 Bangladesh earned TK.2744.12 crore by exporting 50368 tons prawn (DoF, 2008). The culture of giant freshwater prawn is being practiced on commercial scale in several parts of the world especially in south East Asia. Bangladesh with its favorable resources and agro-climatic conditions is widely recognized as one of the most suitable countries in the world for giant freshwater prawn *Macrobrachium rosenbergii*, (De Man, 1879) farming. Despite of this, very little attention has been paid to the bacterial flora associated with cultured *M. rosenbergii* (Miyamoto *et al.*, 1983) and its influence on the initial quality of this prawn species.

For the favorable soil and climatic condition the area of the freshwater prawn culture is expanding every year. The pace of adoption increased dramatically, and this technology spread to other southwest districts such as Khulna and Satkhira. Since 2000, the increased demand for prawn in the international market has attracted many fish farmers to prawn cultivation in freshwater and low-saline waters in different parts of Bangladesh, mainly Noakhali, Patuakhali, Mymensingh, Khulna and Bagerhat districts (Asaduzzaman *et al.*, 2007). In Khulna Region Dumuria is the potential place for prawn culture due to the availability of freshwater. In Bangladesh, most of the farmers use organic manures like cow dung and poultry faeces in addition to chemical fertilizers. It creates the chance of integration of *Salmonella, Cholera* and other pathogenic bacteria in the ponds. In the foreign markets, fresh water prawns of Bangladesh have been rejected mainly due to the contamination of *Salmonella,* and faecal coliforms and rarely for *Vibrio cholera,* but in the major cases for *Salmonella*. In the fish inspection and quality control laboratory in Khulna under the department of fisheries, in the year 2008, samples from 2479 consignments (freshwater prawns and fin fishes) ready for shipment were tested bacteriologically and 31 consignments were rejected due to the presence of *Salmonella,* 147 due to the excess content of total bacterial load (SPC and faecal coliforms). And in the year 2009, out of 2770 consignments, 46 consignments were rejected due to the presence of *Salmonella,* 123 due to the excess content of total bacterial load (SPC, and faecal coliforms). Among these rejected consignments majority were the consignments of fresh water prawn (*(M. rosenbergii*). The rejection of prawn consignment both internally and in the foreign market is involved with huge amount of financial loss to the nation which ultimately severely affects the prawn farmers and all other stakeholders.

Types and levels of bacterial populations associated with farmed giant fresh water prawn (*M. rosenbergii)* are the important indicators for the assessment of quality and safety of prawns. In addition, most diseases in *M. rosenbergii* are caused by opportunistic pathogens which are prevalent in the rearing environment (Janaki Ram & Madhavi, 1999). Therefore, it is essential to investigate the quantitative and qualitative aspects of bacterial flora associated with cultured fresh water prawn and particularly those of public health concern in order to develop a “Good Aquaculture Practice” (GAP) for the production of prawn safe for human consumption and for prevention of prawn diseases. Prevention or control of fish disease is essential to the success of the large-scale, production of fish. Generally, the prawn farmers do not know about the bacterial status of farmed prawn and also do not know the sources of bacterial contamination in their culture ponds. Therefore, to enrich the knowledge of the fresh water prawn farmers and to develop a good management practice for the production of prawn

safe for human consumption and for the prevention of diseases, it is important to study the bacterial contents of prawn and water of the pond during culture. Considering the importance, the study has been undertaken to enumerate the total coliform and faecal coliform bacteria and to detect *Salmonella* in cultured prawn of different management condition.

Materials and methods

Study area

Dumuria upazilla under Khulna district was selected for the study purpose in where huge numbers of fresh water prawn ghers are available. In this area fresh water prawns were cultured under different management conditions such as, semi-intensive, improved traditional and traditional.

Sample collection

For the purpose of the study 54 samples of harvestable sized giant fresh water prawns from 18 farms were collected from three different types of managed farms. Eighteen samples of fresh water prawns were collected from traditional farm, eighteen samples were collected from improved traditional farm and eighteen samples were collected from semi-intensive farm. The selected farms were not situated in the same area or not nearly adjacent to each other. Samples of harvestable size prawn (10 to 20 prawns / kg) were collected from three management condition and the weight of the one sample unit was 50 -100g. The prawns were harvested by using cast net and samples were handled with hand gloves and were collected in sterilized polythene bags by sealing the bags. Collected samples were kept in insulated sample collection box instantaneously and the temperature was maintained below 5°c using ice in the sampling box. Samples were transported to the Biochemistry and Molecular Genetics laboratory of Khulna University within one hour after sampling.

Condition of Prawn Farms

In semi intensive system, fertilizer was used and nutritional feed was supplied. Predators and competitors were controlled, water quality and prawn health were monitored regularly. Production cost was high, it included the elimination of predators and competitors and controlled over all aspects of water quality. In improved traditional system, fertilizer was also used and a balanced feed was supplied. Predators and competitors were also controlled, sometimes water quality and prawn health were monitored. Production cost was not so high. In traditional or extensive culture system cow dung, poultry faeces were used as manure. There was no control of water quality, the growth or mortality was not monitored; supplemental feed and fertilization was rarely applied.

Analytical Method

The experiments of bacteriological parameters of collected samples were performed in the Biochemistry and Molecular Genetics laboratory of Khulna University. Bacteriological parameters of prawn samples were determined by following the ISO methods. All the tests were done in triplicate.

Enumeration of Total Coliforms and Faecal Coliforms by MPN Method

This method is based on MPN procedure using Lauryl Sulphate Triptose Broth (LSTB) in a presumptive test, followed by confirmation of gas positive tubes using Brilliant-Green Lactose Bile Broth (BGLBB), each being incubated at 37°C ± 1°C and 44 °C_ ± 0.5°C for 48 hrs respectively for LSTB and BGLBB and formation of indole in triptone water. The procedure was followed according to the ISO 4831 (third edition) of bacteriological method.

Detection of Salmonella in Freshwater Prawn

The method used for the detection of Salmonella is a qualitative method and the result is reported as Salmonella detected or not detected in the certain quantity of sample taken. The procedure was followed according to the ISO method, 6579 (fourth edition).

Results and discussion

In this study quantitative and qualitative analyses of bacterial content of public health concern of cultured giant fresh water prawn *(M. rosenbergii*) was carried out. In semi- intensive management condition of fresh water prawn samples, total coliform range were (9 -23) MPN/g and faecal coliform range were (3- 7) MPN/g, in improved traditional management condition total coliform range were (53 -160) MPN/g and faecal coliform range were (7- 15) MPN/g and in traditional management condition total coliform range were (120 -616) MPN/g and faecal coliform range were (14-28) MPN/g. The highest number of total coliform (616 MPN/g) and faecal coliform (28 MPN/g) were enumerated in traditional or extensive management system of farm. On the other hand, the lowest number of total coliform (9 MPN/gm) and faecal coliform (3 MPN/gm) found in the semi-intensive management system and *Salmonella* species found in the sample of traditional culture system which has been shown in the Table 1.

Table 1. Bacterial flora found in cultured fresh water prawn

Pond type	Pond No.	Total Coliform/gm	Faecal Coliform/gm	*Salmonella* /25 gm
Semi-intensive	1	15	5	Absent
	2	12	3	Absent
	3	19	5	Absent
	4	14	3	Absent
	5	09	6	Absent
	6	23	7	Absent
Improved traditional	1	76	9	Absent
	2	109	12	Absent
	3	66	9	Absent
	4	160	7	Absent
	5	53	15	Absent
	6	58	8	Absent
Traditional	1	243	19	**Present**
	2	616	25	**Present**
	3	220	21	**Present**
	4	730	28	Absent
	5	120	14	Absent
	6	206	25	**Present**

Total coliforms

The average count of TC in semi- intensive management system, improved traditional management system, and traditional management system were 15.33 MPN/g, 87.00MPN/g and 355.84 MPN/g respectively (Fig. 1). The highest number of Total Coliform was observed in traditional farm (355.84 MPN/g) and the lowest number of Total Coliform was observed in semi-intensive farm (15.33MPN/g). The TC of semi- intensive management system and improved traditional management system were within good quality limit (<100 MPN/g) but in traditional management system TC exceeded the limit.

Jayaweera and Subasinghe (1990) stated that Coliform counts of raw materials ranged from <3 MPN/g to 100 MPN/g with 50% of the counts in the 3-10 MPN/g range. From the present investigated result it is confirmed that the number of TC was lower in those samples which were collected from semi- intensive and improved traditional systems where management systems were better and TC was higher in those samples which were collected from traditional systems where management system was poor.

Faecal coliforms

The average count of FC in semi- intensive management system, improved traditional management system, and traditional management system were 4.83 MPN/g, 10.00 MPN/g and 22.00 MPN/g respectively (Fig.2). The highest number of FC was observed in traditional management system (22.00 MPN/g) and the lowest number of FC was observed in semi- intensive management system (4.83MPN/g). The numbers of FC of semi- intensive and improved traditional management system were within good quality limit (11 MPN/g) but in traditional management system FC exceeded the limit.

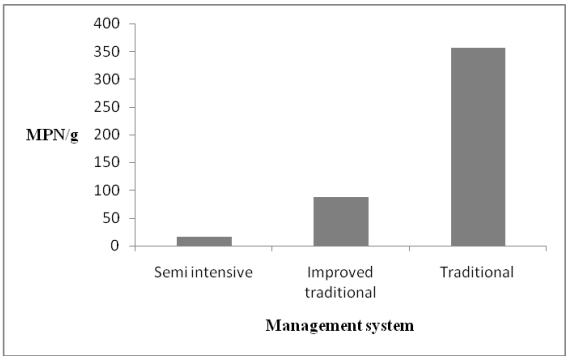

Fig. 1. Total coliform in three different management systems.

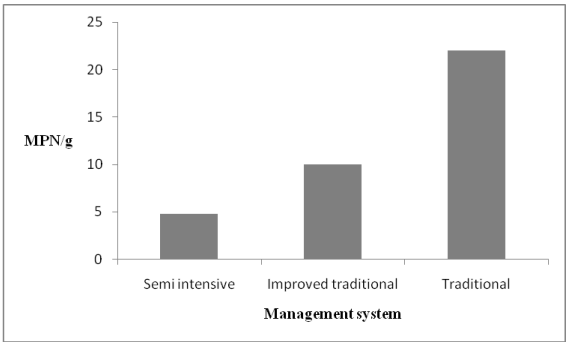

Fig. 2. Faecal coliform in three different management systems.

The international accepted limit of FC recommended by ICMSF (1986) and EU (2005) for fresh and frozen fish is 11 MPN/g. From the present investigated result it is confirmed that the number of FC was lower in those samples which were collected from semi- intensive and improved traditional systems where management systems were better and FC was higher in those samples which were collected from traditional systems where management system was not better.

Salmonella spp

The recommended limits for *Salmonella* sp. by ICMSF (1986) & EU (2005) is 0 (zero). It is clear from the table-1 that the amounts of *Salmonella* sp. of the samples that were collected from semi-intensive and improved traditional management system were in acceptable limit. But in traditional management system some were not in acceptable limit.

Fig- 1 and fig-2 showed that the bacterial loads (total coliform and faecal coliform) were higher and table 1 showed that salmonella was also present in traditional culture system where cow dung and poultry feces were used. Therefore, cow dung and poultry faeces might be the possible source of *Salmonella* and might have the role to increase the bacterial loads. This finding relates to the findings of (González *et al*, 1999), they mentioned that the composition and level of the microorganisms associated with the intestinal tract is related to the environment as well as to the food consumed by fish. Few studies have been conducted on the enumeration and detection of bacterial flora associated with giant freshwater cultured prawn (*M. rosenbergii).* The enumerated total coliform, faecal coliform and detected *Salmonella* in the present study was almost similar to those reported for farmed giant freshwater prawn (*M. rosenbergii*) cultured in earthen ponds in Saudi Arabia (Naim and Harbi, 2005), "Bacterial micro flora associated with farmed freshwater prawn (*M. rosenbergii)* and the aquaculture environment" (Kuttanappilly *et al.,* 2004), "Enteric bacteria and Water Quality of Freshwater Prawn (*M. rosenbergii)* in Culture environment from Kerala", India (Yathavamoorthi *et al.,* 2010). Total bacterial count and coliforms observed in the present study were also comparable to that reported for farmed freshwater prawn in India (Nayyarahamed & Karunasagar, 1995); (Surendran *et al.,* 1995). In the present study, faecal coliforms levels in *M. rosenbergii* were high as

previously reported for tiger prawn farms in India and in Philippines (Surendran *et al.,* 1995). This microbial group is important in foods as indicator of hygienic quality of foods and also as spoilage flora, (Gennari *et al.,* 1999). If the influent water does not contain high numbers of these organisms, incidence of such high numbers of these organisms in prawn may be attributed to the feed or animal manure commonly used to fertilize ponds. The composition of the bacterial micro flora found in *M. rosenbergii* farms is typical of freshwater environments and as generally recognized, is dominated by Gram-negative bacteria (Austin and Allen-Austin, 1983). The results found in this study are comparable and similar to the results of the studies that have been conducted in different countries by different researchers that has already been discussed. In the year 2010, a total of 2770 consignments processed shrimps and fish products of different fish processing plants were tested bacteriologically in the Fish Inspection and Quality Control (FIQC) laboratory, Khulna, under the department of fisheries and *Salmonella* were detected only in 46 consignments and no *V. cholerae* was detected (DoF). Therefore, the detection of *Salmonella in* this study correlates with the result findings of most of researchers and also with the results of FIQC laboratory of Khulna, Bangladesh. From the study it was found that the lowest number of total coliform and faecal coliform were found in the semi- intensive management system. On the other hand, the highest number of total coliform and faecal coliform were enumerated in traditional or extensive management system. *Salmonella* spp. was not identified in semi- intensive and improved traditional management system but observed only in traditional management system where raw cow dung, poultry faeces, raw meats were used. Therefore, use of raw cow dung, poultry faeces and raw meats, might be the possible source of pathogenic bacteria in freshwater prawn farms.

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