E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2017; 5(2): 270-273

www.fisheriesjournal.com
Received: 07-01-2017
Accepted: 08-02-2017

Md Shariful Islam
Scientific Officer, Bangladesh Fisheries Research Institute, Shrimp Research Station, Bagerhat, Bangladesh

Subrina Khatun
Scientific Officer, Bangladesh Fisheries Research Institute, Shrimp Research Station, Bagerhat, Bangladesh

Syed Lutfor Rahman
Chief Scientific Officer, Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, Khulna, Bangladesh

Correspondence
Md Shariful Islam
Bangladesh Fisheries Research Institute, Shrimp Research Station, Bagerhat, Bangladesh

Comparison on bacterial load of black tiger shrimp (*Penaeus monodon*) farm in the southwest region of Bangladesh

Md Shariful Islam, Subrina Khatun and Syed Lutfor Rahman

Abstract
Shrimp farming is a promising sector in Bangladesh since return from export earning of this commodity is considerably high. With the rapid development of grow-out farms, good husbandry and environmental problems developed as the shrimps were stressed and weakened under adverse conditions. The total bacterial load during the sampling period as varied from $7.4*10^5$to $8.1*10^5$ CFU/g of Hepatopancreas, $7.10*10^5$ to $9.11*10^5$CFU/g of digestive tract, $7.1*10^4$ to $6.6*10^5$CFU/ml of water and $8.68*10^4$ to $4.88*10^5$ CFU/g of soil in diseased pond. At this time, the total bacterial load during the sampling period as varied from $9.4*10^3$to $7*10^4$CFU/g of Hepatopancreas, $7.2*10^3$to $4.8*10^4$CFU/g of digestive tract, $6.1*10^2$to $2.8*10^3$CFU/ml of water and $8.1*10^3$ to CFU/g of soil in non-diseased pond. Total number of bacteria in Hepatopancreas, digestive tract of shrimp, and water & soil of the diseased pond are differ distinctly from non-diseased pond. The pathogenic bacteria load during the sampling period as varied from $1.16*10^3$ to $1.9*10^3$CFU/g of Hepatopancreas, $1.29*10^3$ to $1.75*10^3$CFU/g of digestive tract, $1.1*10^3$ to $3.1*10^3$ CFU/ml of water and $7.5*10^2$ to $2.3*10^3$CFU/g of soil in diseased pond. At this time, the pathogenic bacteria load during the sampling period as varied from $1.39*10^2$to $4.3*10^2$CFU/g of Hepatopancreas, $4.2*10^2$ to $7.1*10^2$CFU/g of digestive tract, $7.5*10^1$to $5.1*10^2$CFU/ml of water and $3.2*10^2$to $5.1*10^2$CFU/g of soil in non-diseased pond. Total number of pathogenic bacteria in Hepatopancreas, digestive tract of shrimp, and water & soil of the diseased pond are differ distinctly from non-diseased pond. Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Disease (AHPND) is occurring for presence of V*ibrio parahaemolyticus*. That is a pathogenic bacteria. So, this study shows a positive indication for Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Disease (AHPND). Environmental factors such as water temperature, pH, dissolved oxygen, nutrients, organic substances etc. might be the causal factors of these bacterial load variations.

Keywords: *Penaeus monodon,* Shrimp farming, *Vibrio parahaemolyticus*

1. Introduction
There are about 1.5 million hectares brackish water *ghers* (large hydrological units protected by embankment with provisions of controlled drainage and irrigation infrastructures connecting with coastal rivers) in the southwest region of Bangladesh [1]. At present about 2, 17,000 ha areas are being used for brackish water aquaculture. Brackish water aquaculture in Bangladesh is mostly directed to traditional farming of brackish water shrimp, *Penaeus monodon*. The black tiger shrimp (*Penaeus monodon)* is a prominent marine shrimp with high economic value in Southeast Asia. Commercial production of the black tiger shrimp has been in a major decline due to higher incidents of viral and bacterial disease outbreaks [2]. Many studies in crustaceans, including the black tiger shrimp, focused on the development of probiotic applications to enhance disease resistance and growth [3]. However, a probiotic approach for the black tiger shrimp is challenging due to the lack of understanding of natural microflora and factors contributing to the diversity of the bacterial population. Being aquatic species, shrimps are constantly exposed to a variety of bacteria and viruses, which some can be pathogenic. Shrimp pathogens can orally enter, invade digestive tract system and cause infection [4]. Although shrimp has innate immune system to battle against the pathogen's invasion, it has been speculated that bacterial community in shrimp intestines may play protective roles as natural barriers.

Shrimp farming is a promising sector in Bangladesh since return from export earning of this commodity *is* considerably high. With the rapid development of grow-out farms, good husbandry and environmental problems developed as shrimp were stressed and weakened under adverse conditions. Moreover, shrimp is a highly putrescent fishery commodity as microbial activity is the most effective in producing undesirable odours and appearance in shrimp. Considering the recent outbreak of shrimp disease in the country, a preliminary investigation was designed to investigate the bacteriological status in farmed shrimp, *P. monodon* to know the distribution pattern of bacteria in different organs of shrimp.

2. Materials and methods

2.1 Soil and shrimp samples collection

Two Grow-out ponds (Diseased & Non-diseased) under Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, Khulna were selected for sampling of Black Tiger Shrimp, *P. monodon.* Soil & shrimp samples were collected carried to the Fish Disease Laboratory of Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, Khulna in a plastic container containing the same pond water of the respective samples.

2.2 Water quality parameters

Water quality parameters *viz.,* pH, Dissolved oxygen, Alkalinity, Salinity, NO^{2-}, NO^{3-}, NH_3, Sulfide were determined at twice in a month following standard methods (APHA 1992) of selected diseased and non-diseased pond.

2.3 Shrimp organs for bacterial load

Samples of Hepatopancreas & digestive tract were collected with a sterile loop in a pre-weighted test tube. Suspension of Hepatopancreas & digestive tract containing bacteria was made in physiological saline solution with the help of a vortex mixer. Necessary dilution was made by ten-fold dilution method. Inoculum (1 ml) was poured on TCBS plates & nutrient agar plates, incubated at 35 °C and colonies were counted after one days of incubation.

2.4 Bacterial count

Total Heterotrophic Bacteria (THB) and pathogenic bacteria mainly *Vibrio sp.* of both water and soil of the selected ponds were tested using pour plate bacterial culture method. Nutrient agar media and TCBS (Thiosulfate Citrate Bile Salt) agar media was used for culturing THB and *Vibrio sp.* respectively. Viable colony was counted using a colony counter.

Bacterial count (CFU/ml) = CFU × Dilution Factor

3. Results and Discussion

3.1 Bacterial load

The total bacterial load during the 1st sampling day was $7.4*10^5$, $7.10*10^5$, $7.1*10^4$ and $8.68*10^4$ in Hepatopancreas & Digestive tract of shrimp and water & soil of diseased pond, respectably. On the otherhand, The total bacterial load during the 1st sampling day was $9.4*10^3$, $7.2*10^3$, $2.5*10^3$ and $8.1*10^3$ in Hepatopancreas & Digestive tract of shrimp and water & soil of non-diseased pond, respectably. The total bacterial load during the 2nd sampling day was $7.6*10^5$, $9.15*10^5$, $6.9*10^5$ and $8.68*10^5$ in Hepatopancreas & Digestive tract of shrimp and water & soil of diseased pond, respectably. On the otherhand, The total bacterial load during the 2nd sampling day was $4.1*10^4$, $2.4*10^4$, $6.1*10^2$ and $3.2*10^4$ in Hepatopancreas & Digestive tract of shrimp and water & soil of non-diseased pond, respectably. The total bacterial load during the 3rd sampling day was $8.1*10^5$, $9.11*10^5$, $6.6*10^5$ and $4.58*10^5$ in Hepatopancreas & Digestive tract of shrimp and water & soil of diseased pond, respectably. On the otherhand, The total bacterial load during the 3rd sampling day was $7*10^4$, $4.8*10^4$, $2.8*10^3$ and $3.5*10^4$ in Hepatopancreas & Digestive tract of shrimp and water & soil of non-diseased pond, respectably (Table No-1).

Table 1: Variation of the total bacterial load in the hepatopancreas, digestive tract of *P. monodon* and pond water & soil of diseased and non-diseased pond.

Date	Diseased Pond				Non-diseased pond			
	Hepatopancreas	Digestive tract	Water	Soil	Hepatopancreas	Digestive tract	Water	Soil
22/06/16	$7.4*10^5$	$7.10*10^5$	$7.1*10^4$	$8.68*10^4$	$9.4*10^3$	$7.2*10^3$	$2.5*10^3$	$8.1*10^3$
24/06/16	$7.6*10^5$	$9.15*10^5$	$6.9*10^5$	$4.88*10^5$	$4.1*10^4$	$2.4*10^4$	$6.1*10^2$	$3.2*10^4$
26/06/16	$8.1*10^5$	$9.11*10^5$	$6.6*10^5$	$4.58*10^5$	$7*10^4$	$4.8*10^4$	$2.8*10^3$	$3.5*10^4$

The pathogenic bacterial load during the 1st sampling day was $1.16*10^3$, $1.29*10^3$, $1.4*10^2$and $2.3*10^3$ in Hepatopancreas & Digestive tract of shrimp and water & soil of diseased pond, respectably. On the otherhand, The pathogenic bacterial load during the 1st sampling day was $1.39*10^2$, $5.4*10^2$, $7.5*10^1$and $3.2*10^2$ in Hepatopancreas & Digestive tract of shrimp and water & soil of non-diseased pond, respectably. The pathogenic bacterial load during the 2nd sampling day was $1.9*10^3$, $1.59*10^3$, $1.1*10^3$and $7.5*10^2$in Hepatopancreas & Digestive tract of shrimp and water & soil of diseased pond, respectably. On the otherhand, The pathogenic bacterial load during the 2nd sampling day was $4.3*10^2$, $4.2*10^2$, $5.1*10^2$and $4.3*10^2$ in Hepatopancreas & Digestive tract of shrimp and water & soil of non-diseased pond, respectably. The pathogenic bacterial load during the 3rd sampling day was $1.8*10^3$, $1.75*10^3$, $3.1*10^3$and $1.9*10^3$in Hepatopancreas & Digestive tract of shrimp and water & soil of diseased pond, respectably. On the otherhand, The pathogenic bacterial load during the 3rd sampling day was $2.22*10^2$, $7.1*10^2$, $3.2*10^2$and $5.1*10^2$ in Hepatopancreas & Digestive tract of shrimp and water & soil of non-diseased pond, respectably (Table no-2).

Table 2: Variation of the pathogenic bacteria (*Vibrio sp.*) load in the hepatopancreas, digestive tract of *P. monodon* and pond water & soil of diseased and non-diseased pond.

Date	Diseased Pond				Non-diseased Pond			
	Hepatopancreas	Digestive tract	Water	Soil	Hepatopancreas	Digestive tract	Water	Soil
22/06/16	$1.16*10^3$	$1.29*10^3$	$1.4*10^2$	$2.3*10^3$	$1.39*10^2$	$5.4*10^2$	$7.5*10^1$	$3.2*10^2$
24/06/16	$1.9*10^3$	$1.59*10^3$	$1.1*10^3$	$7.5*10^2$	$4.3*10^2$	$4.2*10^2$	$5.1*10^2$	$4.3*10^2$
26/06/16	$1.8*10^3$	$1.75*10^3$	$3.1*10^3$	$1.9*10^3$	$2.22*10^2$	$7.1*10^2$	$3.2*10^2$	$5.1*10^2$

The total bacterial load during the sampling period as varied from $7.4*10^5$ to $8.1*10^5$ CFU/g of Hepatopancreas, $7.10*10^5$ to $9.11*10^5$CFU/g of digestive tract, $7.1*10^4$to $6.6*10^5$CFU/ml of water and $8.68*10^4$ to $4.88*10^5$ CFU/g of soil in diseased pond. (Table 1). At this time, the total bacterial load during the sampling period as varied from $9.4*10^3$to $7*10^4$CFU/g of Hepatopancreas, $7.2*10^3$to $4.8*10^4$CFU/g of digestive tract, $6.1*10^2$to $2.8*10^3$CFU/ml of water and $8.1*10^3$toCFU/g of soil in non-diseased pond (Table 1). Total number of bacteria in Hepatopancreas, digestive tract of shrimp, and water & soil of the diseased pond are differ distinctly from non-diseased pond. The pathogenic bacteria load during the sampling period as varied from $1.16*10^3$ to $1.9*10^3$CFU/g of Hepatopancreas, $1.29*10^3$ to $1.75*10^3$CFU/g of digestive tract, $1.1*10^3$ to $3.1*10^3$ CFU/ml of water and $7.5*10^2$ to $2.3*10^3$CFU/g of soil in diseased pond. (Table 2). At this time, the pathogenic bacteria load during the sampling period as varied from $1.39*10^2$to $4.3*10^2$CFU/g of Hepatopancreas, $4.2*10^2$to $7.1*10^2$CFU/g of digestive tract, $7.5*10^1$to $5.1*10^2$CFU/ml of water and $3.2*10^2$to $5.1*10^2$CFU/g of soil in non-diseased pond (Table 2). Total number of pathogenic bacteria in Hepatopancreas, digestive tract of shrimp, and water & soil of the diseased pond are differing distinctly from non-diseased pond.

Quantitative compositions of bacteria in different organs of the prawn investigated were found to vary with organs, pond water and soil also. In the study period, the bacterial loads in the hepatopancreas of prawn were higher than those in digestive tract or pond water or soil. Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Disease (AHPND) is occurring for presence of V*ibrio parahaemolyticus*. This study shows a positive indication for EMS. Environmental factors such as water temperature, pH, dissolved oxygen, nutrients, organic substances etc. might be the causal factors of these variations. Besides these supplementary feed to shrimp were the sources of organic substances. Bacterial growth in water was influenced by availability of carbon and energy source [5]. Udea *et al.* [6] mentioned that the microflora associated with the growing prawn significantly influenced by the environmental conditions.

3.2 Water quality parameters

Water quality parameters in shrimp ponds and their optimal ranges for shrimp culture are given in Table 3. Here shows that, pH is 7.8±0.5 which is more or less similar with optimum ranges. On the otherhand, Dissolved oxygen, alkalinity and salinity was 7.2±2.2, 121.6±37.9 and 10.7±9.3, all are in optimum ranges. But, $N0^{2-}$, $N0^{3-}$, NH_3 and sulfide level is in 0.32±0.02, 3.3±0.8, 2.5±0.7 and 0.4±0.5 respectively which are in higher than optimum ranges.

Table 3: Mean ± SD values of the water quality parameters of shrimp ponds. Optimum ranges are also given here.

Parameters	Mean±SD	Optimum ranges for shrimps
pH	7.8±0.5	7.5-8.5
Dissolved oxygen (mg/l)	7.2±2.2	5.1-5.9
Alkalinity (mg/l)	121.6±37.9	40-160
Salinity (ppt)	10.7±9.3	15-25
NO^{2-} (mg/l)	0.32±0.02	<1
NO^{3-} (mg/l)	3.3±0.8	<1
NH_3 (mg/l)	2.5±0.7	0.5
Sulfide (µg/l)	0.4±0.5	<0.03

Poor water quality and high organic loading are associated with shell lesion including bacterial growth in grow-out prawn. A variety of bacteria, producing extracellular lipases or protease such as *Aeromonas, Pseudomonas, Vibrio, Benekea* spp. have been implicated in shell disease [7]. Organic matter supplied as feed to pond and manure increased the bacterial load in pond water [8]. In most of the diseased ponds, total pathogenic bacteria(*Vibrio)* were high in the hepatopancreas of shrimp investigated which is similar with the findings of Lombardi and Labao [9, 10], mentioned *Pseudomonas, Vibrio* etc. as the possible causative agents of shrimp & prawn diseases. Although pathogenicity and antibiotic test were not performed in the present study, the isolated bacteria *Pseudomonas, Vibrio* and *Aeromonas* could be pathogens to the cultured shrimp which needs further study.

4. Acknowledgements

This work was supported by Chief Scientific Officer of Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, Khulna.

5. References

1. Anon. Compendium of Fish Fair 2005 (in Bengali). Department of Fisheries, Ministry of Fisheries and Livestock. 2005, 152.
2. Flegel TW. Current status of viral diseases in Asian shrimp aquaculture. Israeli J of Aqua. Bami. 2009; 61:229-239.
3. Rengpipat S, Rukpratanporn S, Piyatiratitivorakul S, Menasveta P. Probiotics in aquaculture: a case study of probiotics for larvae of the black tiger shrimp *Penaeus monodon*. In: Flegel, T.W. Ed., Advances in Shrimp Biotechnology. Proceedings to the Special Session on Shrimp Biotechnology. 5th Asian Fisheries Forum, Chiengmai, Thailand. 1998, 177-181.
4. Soonthornchai W, Rungrassamee W, Karoonuthaisiri N, Jarayabhand P, Klinbunga S *et al*. Expression of immune-related genes in the digestive organ of shrimp, *Penaeus monodon*, after an oral infection by Vibrio harveyi. Dev Comp Immunol. 2010; 34:19-28.
5. Marshal KC. Bacterial adhesion in oligotrophic habitats. Microbial. Sci. 1985; 2:321-322.
6. Udea R, Sagita H, Deguchi Y. Microflora associated with the developing gaint prawn, *Macrobrachium rosenbergii. In*: Proc. of the Third Asian Fisheries Forum, Singapore. 1992, 209.
7. Cook DW, Lofton SR. Chitinoclastic bacteria associated with shell disease- in *Penaeus* shrimp and the blue crab *Callinecte sapidus.}*. Wild Dis., 1973; 9:154-159.
8. Moriarty DJW. Bacterial productivity in ponds used for culture of Penaeid prawns. Microb. Ecol., 1986;

12(3):259-264.
9. Lombardi JV, Labao VL. Diseases and conditioning factors' of mortality in larval culture of prawns of the genus *Macrobrachium.* In: Proceedings of the 3rd Brazilian symposium on shrimp culture. Joao Pessoa, Paraiba, Brazil. 1991a, 401-408.
10. Lombardi JV, Labao VL, Diseases and other factors leading to mortality in juveniles and adults belonging to the genus *Macrobrachium.* In: Proceedings of the 3rd Brazilian symposium on shrimp culture. Joao Pessoa, Paraiba, Brazil. 1991b; 409-4:19