E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2017; 5(2): 200-204

www.fisheriesjournal.com
Received: 19-01-2017
Accepted: 20-02-2017

Tausif Ahmad
MFSc. Student, Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, West Bengal, India

Dr. Koel Bhattacharya Sanyal
Senior Research Fellow, Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, West Bengal, India

Debapriyo Mukherjee
Senior Research Fellow, Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, West Bengal, India

Dr. TJ Abraham
Professor, Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, West Bengal, India

Dr. Gadadhar Dash
Professor, Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, West Bengal, India

Correspondence
Dr. Gadadhar Dash
Professor, Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, West Bengal, India

Detection of white spot virus (WSV) in *Litopenaeus vannamei* from shrimp aquaculture farms in East Midnapore district, West Bengal (India)

Tausif Ahmad, Koel Bhattacharya Sanyal, Debapriyo Mukherjee, TJ Abraham and Gadadhar Dash

Abstract
White spot syndrome virus (WSV) infection is one of the most virulent pathogenic and devastating viruses of the cultured penaeid shrimps. It leads to severe disruption and loss of economy every year. The present study was carried out to detect the presence of WSV in *Litopenaeus vannamei* from cultured farms in East Midnapore district, West Bengal for the first time. Out of 8 shrimp samples from different places two samples of *Litopenaeus vannamei* were found to be WSV positive from Rasulpur and Basanti areas of the district. The confirmation of WSV was detected through two steps PCR reaction. Conventional diagnosis fails to detect early stages of infection, and are also time consuming and less sensitive. Whereas Nested PCR or 2nd step PCR is a powerful and sensitive diagnostic tool for identification of WSV even at a very early stage (asymptomatic/ carrier stage) of infection. Furthermore histopathological studies of gills revealed the presence of developing and fully developed intranuclear inclusion bodies, muscles showed infiltration of haemocytes, necrosis, inflammation with degeneration and inflammation & extensive necrosis of hepatopancreas and degeneration of tubular lumen; thereby confirming the WSV infection among the *Litopenaeus vannamei.*

Keywords: Histopathology, inclusion bodies, *Litopenaeus vannamei*, PCR, WSV

1. Introduction
Shrimp farming is one of the most profitable and fastest-growing sectors of the aquaculture industry. *Litopenaeus vannamei* is currently emerging cultured shrimp species in West Bengal. Due to its faster growth rate with comparatively low feed conversion rate (FCR) and higher survival percentage during the culture attracting the farmers. In India, shrimp culture contributing a significant portion towards national income through export earnings. India stands as the fifth highest producer of farmed shrimps [1]. The farming systems currently employed in India can be broadly divided into six basic categories traditional, improved traditional, extensive, improved extensive, semi-intensive and intensive methods [2, 3, 4]. There are two distinct seasonal shrimp crops. The first crop starts in February or March and ends in May or June. The second crop starts in August or September and ends in November or December. Typically the first crop produces a much higher yield than the second crop [5].
West Bengal is having huge potential of brackish water area in India, spreading over three districts namely East Midnapore, North 24 Parganas and South 24 Parganas. Scientific culture of shrimp started in West Bengal during the mid-1980s and by 2010 more than 54,000 hectare area are under shrimp culture. The current aquaculture production of shrimp in West Bengal increased from 26,800 tons in 2001-2002 to 57,369.77 tons in 2014-2015 [6].
Due to the modernization of culture from traditional system pave the way for disease outbreak among the shrimps. High stocking density and use of compounded pelleted feed in order to achieve higher production rates impose stress on the shrimps, making them susceptible to diseases [7]. The diseases may be caused by various etiologic agents such as viruses, bacteria, fungi, parasites, algal toxins, nutritional deficiency or the adverse environment. Diseases caused due to virus such as WSV is the major challenging problem of shrimp culture across India.

White spot virus (WSV) is one of the most serious pathogen affecting shrimp aquaculture industry, and it has caused severe disruption to the industry since the early 1990s [8, 9]. The WSV name is derived from the clinical sign that has been reported in some susceptible penaeid shrimp hosts, for example the presence of white spots associated with calcium deposition on the inner surface of cuticle [10] and shrimp mortality can reach upto 100% within 3 to 7 days of infection, which causes great economic losses to the industry [11].

In India, WSV was first reported in 2011 on white leg shrimp (*Litopenaeus vannamei*) from farm located in Bhimavaram, West Godavari district, Andhra Pradesh [12].It has a broad host range, infecting all cultured shrimp as well as other invertebrate aquatic organisms such as crab and crayfish [13]. Common characteristics of the disease include presence of white spots on the carapace and the body colour of diseased shrimp becomes pale or reddish in colour with sever mortality.

The present study is an attempt to study WSV prevalence in *Litopenaeus vannamei* using a combination of molecular and histopathological techniques for the first time in East Midnapore district of West Bengal.

2. Materials and methods

2.1 Sample collection

Healthy and diseased shrimp samples (*L. vannamei*) were collected from different shrimp farms of East Midnapore district [Lat. 21º48'57.57"N; Long. 87º43'7.09"E], West Bengal, India. Individual shrimp samples fresh or moribund were collected within U-V sterilized polypropylene bags were brought to the laboratory of Aquatic Animal Health at Faculty of Fishery sciences, West Bengal University of Animal and Fishery Sciences, West Bengal within 4h of collection in insulated ice box. Shrimp samples were examined at site for behavioral changes, abnormalities, gross and clinical signs. For WSV detection gills and pleopods of shrimps were dissected and preserved in 70% ethanol for processed in next day. Gills and hepatopancreas from samples are dissected and kept in Davidson's fixative for histopathological examination.

2.2. Genomic DNA preparation

Genomic DNA was prepared from gill or pleopod tissue of each shrimp samples using commercially available kit (Genei™, Merck, Bangalore, India). Briefly, 20-30 mg of gill or pleopod tissue was taken in 1.5 ml Eppendorf tubes and grinded well with 200 µl of DNA extraction buffer (supplied in the kit) followed by incubation at 95 °C for 10 min. The tubes were then centrifuged at 10,000 rpm for 10 min at room temperature. A quantity of 50-100 µl of supernatant containing genomic DNA was collected and kept in separate tubes for further PCR reaction.

2.3. WSV screening by first step PCR

The genomic DNA was tested for the presence of WSV in a single-first PCR using a commercially available kit (GeNei™, Merck, Bangalore, India). The first PCR premix supplied with the kit contained external primer, dNTPs, buffer and $MgCl_2$. The PCR was carried out in a thermocycler (PTC100; MJ research Inc., St. Bruno, Quebec, Canada) with 25µl reaction mixture containing 1µl of genomic DNA, 23 µl of first PCR premix and 1µlTaq DNA polymerase (GeNei™, Merck). The mixture was thoroughly mixed and followed through thermal cycle as follows: the initial activation step at 95 °C for 3 min followed by 25-28 cycles of 30 sat 95 °C, 30 s at 58 °C and 30 s at 72 °C and finally last extension step of 5 min at 72 °C. PCR products were electrophoresed in 2% agarose gel and visualized the DNA bands by staining with ethidium bromide (10mg/100 ml). The gel photograph was documented in a gel documentation system (Gel doc 1000; BioRad, Hercules, CA, USA).

2.4. WSV screening by Nested PCR or 2nd step PCR

Conventional diagnosis fails to detect early stages of infection, and are also time consuming and less sensitive. Whereas Nested PCR or 2nd step PCR is a powerful and sensitive diagnostic tool for identification of WSV even at a very early stage (asymptomatic/ carrier stage) of infection. The first PCR product was first centrifuged for 30 seconds at 10,000 rpm. The first PCR premix supplied with the kit contained internal primer, dNTPs, buffer and $MgCl_2$. The PCR was carried out in a thermocycler (PTC100; MJ research Inc., St. Bruno, Quebec, Canada) with 25 µl reaction mixture containing 1µl of genomic DNA, 23 µl of Nested PCR premix and 1 µl Taq DNA polymerase (GeNei™, Merck). The mixture was thoroughly mixed and followed through thermal cycle as follows: the initial activation step at 95 °C for 3 min followed by 25-28 cycles of 30 s at 95 °C, 30 s at 58 °C and 30 s at 72 °C and finally last extension step of 5 min at 72 °C. PCR products were electrophoresed in 2% agarose gel and visualized the DNA bands by staining with ethidium bromide (10 mg/100 ml). The gel photograph was documented in a gel documentation system (Gel doc 1000; Bio Rad, Hercules, CA, USA).

2.5. Analysis of PCR products by gel electrophoresis

5 µl of PCR products from the above processes i.e. First & Nested PCR were loaded into 2% agarose gel with loading buffer and allowed to run at 100-120 volts and stop after 1 hour. The gel photograph was documented (Gel doc 1000; Bio Rad, Hercules, CA, USA) for further analysis.

2.6. Histropathological examination

Fixed *L. vannamei* samples in Davidson's fixative were processed and sectioned and stained for histopathological studies as per reference [14]. The sections were further examined and photomicrographs were taken using a trinocular research microscope (Olympus, Model BX51, Japan).

3. Results

3.1. PCR

The positive control for WSV showed a band both in first PCR (650 bp) and nested PCR (300 bp). Out of 8 samples of *L. vannamei* from different places of East Midnapore district, West Bengal, 2 samples were found positive for WSV with low infection from two different places i.e. Rasulpur and Basanti and others were found to be negative for WSV infection. Nested PCR assays showing bands at 300bp conforming mild infection of WSV among the two shrimp samples (Fig.1).

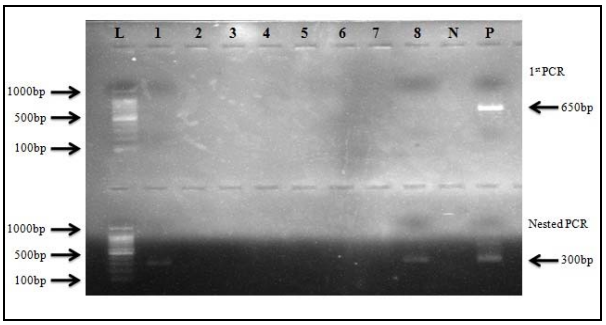

Fig 1: Agarose gel showing PCR detection of WSV (L: Ladder, N: Negative control, P: Positive control, 1-8: Samples)

3.2. Histopathology

The gills of WSV infected *Litopenaeus vannamei* showing; the presence of developing and fully developed intranuclear inclusion bodies and inflammation of gill lamellae. Also necrosis and degeneration of gill lamella were evident. The section of the gill from *Litopenaeus vannamei* infected with WSV revealed hypertrophy of the cells with the presence of inter-nuclear eosino-basophilic bodies (Fig. 2 a, b). The muscle showing WSV intranuclear inclusion bodies, infiltration of haemocytes, necrosis and inflammation with degeneration of muscle. The degenerated muscle fibers clearly separated from the striated muscle fiber forming a vacuolar space. The epithelial muscle layers were completely damaged compared to rest of the layers (Fig. 2c). The transverse section of hepatopancreas showing inflammation and extensive necrosis of hepatopancreas cell and degeneration of tubular lumen. Hepatopancreas of diseased *Litopenaeus vannamei* infected with WSV showed histopathological alteration like degeneration of tubular lumen, sloughing of cells with necrosis, extensive fibrosis, cell elongation, detachment of hepatopancreatic cells and necrosis of connective tissues and hepatopancreas (Fig2. d, e, f).

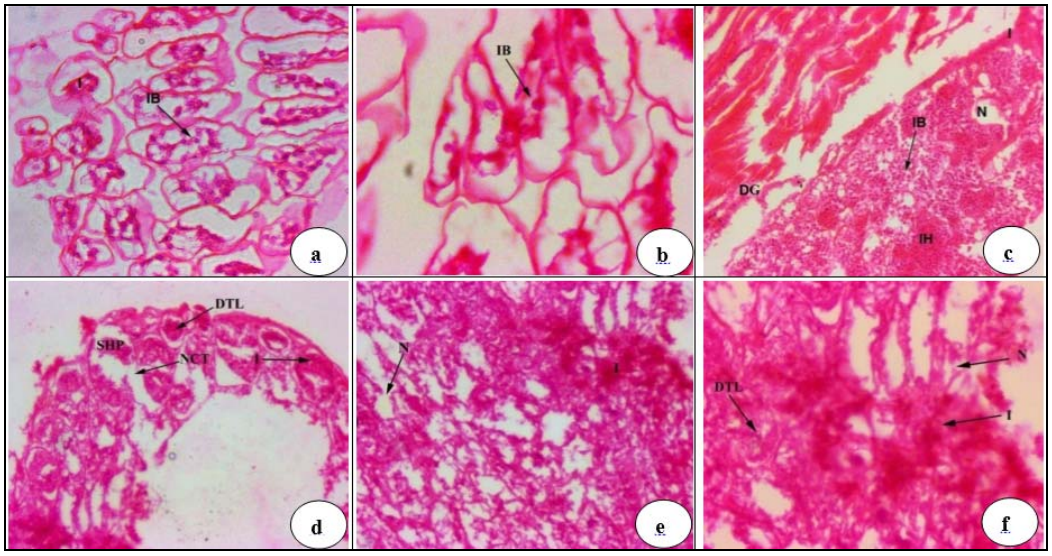

Fig. 2. Histopathological observation of WSV infected *Litopenaeus vannamei*: (a) The gills of WSV infected *Litopenaeus vannamei* showing; the presence of developing and fully developed intranuclear inclusion bodies (IB) and inflammation (I) of gill lamellae. [H&E stain 200X]. (b) The gills of WSV infected *Litopenaeus vannamei* showing; infected cells showing intranuclear inclusion bodies (IB) (H&E stain 400X]. (c) The muscle of diseased *Litopenaeus vannamei* showing; WSV intranuclear inclusion bodies (IB), infiltration of haemocytes (IH), necrosis (N), inflammation (I) with degeneration (DG) of muscle [H&E stain 100X]. (d) Histopathological changes in the transverse section (TS) of hepatopancreas of diseased *Litopenaeus vannamei* showing; inflammation (I) with sloughing of hepatopancreas cell (SHP), degeneration of tubular lumen (DTL), and necrosis of connective tissues (NCT) [H&E stain 100X].(e) The transverse section (TS) of hepatopancreas of diseased *Litopenaeus vannamei* showing; inflammation (I) and extensive necrosis of hepatopancreas cell. [H&E stain 100X]. (f) The transverse section (TS) of hepatopancreas of diseased *Litopenaeus vannamei* showing; inflammation (I) and extensive necrosis of hepatopancreas cell and degeneration of tubular lumen (DTL). [H&E stain 200X].

4. Discussion

During the past three decades shrimp culture has been emerging as one of the major industries in the tropical and subtropical areas of the world, and it also serves as a major source of earning for the poor coastal population [15]. In India, commercial shrimp farming started gaining momentum during the mid 1980's. Shrimp production over the past 10 years is rapidly increasing and according to the Food and Agriculture Organization (FAO) by 2011[16], India has become third largest aquaculture shrimp producer. Furthermore, the impact of disease due to virus, bacteria, parasites and environmental stress plays a significant role in shrimp production. Among all diseases WSV is highly pathogenic and seriously affecting the shrimp production with massive losses throughout the year. Several studies have addressed various aspects of WSV during the past one decade [9]. However, WSV epizootic has remained the most serious challenge to the shrimp farming industry, yet to be resolved. Since there are no therapeutic treatments currently available for WSV, the best management strategy is to prevent WSV from entering a shrimp farming facility [17] or use of optimal shrimp culture conditions.

In the present study, WSV prevalence was carried out among *L. vannamei* from cultured shrimp farms at different locations along East Midnapore district of West Bengal. Out of 8 *L. vannamei* samples from different locations only 2 samples were found Positive for WSV with low infection from the

location i.e. Rasulpur and Basanti with asymptomatic clinical signs and other were found to be Negative for WSV infection. The result was confirmed by PCR detection (band at 300bp) confirming mild infection of WSV among the two shrimp samples (Fig 1). The present results corroborate with the previous results [12], they first reported presence of WSV on white leg shrimp (*Litopenaeus vannamei*) from farm located in Bhimavaram, West Godavari district, Andhra Pradesh. Similar results were also reported WSV from *L. vannamei* from shrimp farms from South Cotabato [18]. Asymptomatic positive WSV might be due to the carrier state of infection, which is similar to the earlier report from Taiwan [19, 20]. Previous studies also reported acute infection of WSV associated with mass mortality (100%) and without clinical symptoms [21, 22]. Likewise, juvenile and sub-adult shrimp (*L. vannamei*) accompanied with severe mortality due to the high virulence of WSV, but were not observed with white spots on the carapace [23].

Moreover, histopathological studies of the gills of WSV infected *Litopenaeus vannamei* showing; the presence of developing and fully developed intranuclear inclusion bodies and inflammation of gill lamellae. The muscle showing WSV intranuclear inclusion bodies, infiltration of haemocytes, necrosis and inflammation with degeneration of muscle. The transverse section of hepatopancreas showing inflammation and extensive necrosis of hepatopancreas cell and degeneration of tubular lumen (Fig 2). The results of the present study is similar to the others [24], who reported histopathological analysis of gill tissues (tissue disorganization, cellular degeneration) from WSV infected shrimps from Nellore, AP, India, showing characteristic intranuclear eosino-basophilic bodies. Moreover other studies [25] also reported destruction of hepatopancreas tissue and demolition of cells with nucleus hyper-trophy, cellular degeneration, intra-nuclear inclusion bodies, nucleus pyknosis, karyorrhexis from WSV infected shrimp tissue from Iran.

The poor management practices in the culture system could induce stress in shrimp population and if the virus is latent in the population or present in the environment, there may be outbreak of the disease. Furthermore, it is alarming that WSV infection was detected in *L. vannamei*, which are supposed to be shrimp pathogen free (SPF) as these were imported from other countries through different quarantine test. So, this study is focused on explicating the risk related to diseases in cultured shrimps of West Bengal and this deserves more focus towards research on specific diseases and technologies for rapid diagnosis.

5. Acknowledgments

The authors gratefully acknowledge the assistance extended by the Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, West Bengal, India for providing necessary facilities for undertaking the work. Special thanks to National Bureau of Fish Genetic Resources (NBFGR), Lucknow, Uttar Pradesh, India and National Fisheries Development Board (NFDB), Hyderabad, Telangana, India for financial support during the study.

6. References

1. Felix S, Jawahar G, Venktramani VK. Application of Coastal Aquaculture Technologies, Success stories of Thoothukudi District, Tamilnadu. In: Fishing chimes. 2007; 26(10):46-49.
2. Abraham TJ, Sasmal D. Incidence of different disease conditions in shrimp culture systems of West Bengal with special reference to white spot syndrome virus infection. Journal of the Inland Fisheries Society of India. 2008; 40(2):1-6.
3. Raja RA, Kumar S, Sundaray JK, De D, Biswas G, Ghosal TK. Haematological parameters in relation to sex, morphometric characters and incidence of white spot syndrome virus in tiger shrimp *Penaeus monodon* (Fabricius, 1798) from Sunderban, West Bengal. Indian Journal of Fisheries. 2012a; 59(4):169-174.
4. Raja RA, Panigrahi A, Kumar S. Epidemiological investigation of brackish water culture systems in West Bengal, India. Journal of Applied Aquaculture. 2012b; 24(1):49-59.
5. Suresh AV. Development of the aqua feed industry in India. In: FAO Fisheries Technical Paper. 2007; 497:221-243.
6. MPEDA. Annual Report 2014-2015, Marine Products Export Development Authority, Cochin. 2015.
7. Alavandi SV, Vijayan KK, Rajendran KV. Shrimp diseases, their prevention and control. CIBA Bulletin. 1995; 3:1-17.
8. Lightner DV. Biosecurity in shrimp farming: pathogen exclusion through use of SPF stock and routine surveillance. Journal of the World Aquaculture Society. 2005; 36(3):229-248.
9. Flegel TW. Detection of major penaeid shrimp viruses in Asia, a historical perspective with emphasis on Thailand. Aquaculture. 2006; 258:1-33.
10. Escobedo-Bonilla CM, Alday-Sanz V, Wille M, Sorgeloos P, Pensaert MB, Nauwynck HJ. A review on the morphology, molecular characterization, morphogenesis and pathogenesis of white spot syndrome virus. Journal of Fish Diseases. 2008; 31(1):1-18.
11. Lightner DV. Virus diseases of farmed shrimp in the Western Hemisphere (the Americas): a review. Journal of Invertebrate Pathology. 2011; 106(1):110-130.
12. Balakrishnan G, Peyail S, Kumaran R, Theivasigamani A, Srinivasan N. First report on White Spot Syndrome Virus (WSSV) infection in white leg shrimp *Litopenaeus vannamei* (Crustacea, Penaeidae) under semi intensive culture condition in India. Aquaculture, Aquarium, Conservation & Legislation-International Journal of the Bioflux Society (AACL Bioflux). 2011, 4(3).
13. Wang YC, Lo CF, Chang PS, Kou GH. Experimental infection of white spot baculovirus in some cultured and wild decapods in Taiwan. Aquaculture. 1998; 164(1):221-231.
14. Bell TA, Lightner DV. A handbook of normal penaeid shrimp histology. World Aquaculture Society, Baton Rouge, LA. 1988.
15. Adger W. Sustainability and social resilience in coastal resource use. In: GEC-1997-23, CSERGE working paper. 1998.
16. Fish Stat J. Fisheries and Aquaculture Department. Food and Agriculture Organization of the United Nations (FAO). 2011. Available at: http://www.fao.org/fishery/statistics/software/fishstatj/en, 1 December, 2011.
17. Lotz JM. Viruses, biosecurity and specific pathogen-free stocks in shrimp aquaculture. World J Microbiol Biotechnol. 1997; 13:405-413.
18. Maralit BA, Caipang C, Santos MD, Maningas MBB.

PCR detection of white spot syndrome virus (WSSV) from farmed Pacific white shrimp (*Litopenaeus vannamei*) in selected sites of the Philippines. Aquaculture, Aquarium, Conservation & Legislation-International Journal of the Bioflux Society (AACL Bioflux). 2011, 4(4).

19. Hsu HC, Lo CF, Lin SC, Liu KF, Peng SE, Chang YS *et al*. Studies on effective PCR screening strategies for white spot syndrome virus (WSSV) detection in *Penaeus monodon* brooders. Diseases of aquatic organisms. 1999; 39(1):13-19.
20. Peng SE, Lo CF, Lin SC, Chen LL, Chang YS, Liu KF *et al*. Performance of WSSV-infected and WSSV-negative *Penaeus monodon* post larvae in culture ponds. Diseases of Aquatic Organisms. 2001; 46(3):165-172.
21. Lightner DV. A handbook of shrimp pathology and diagnostic procedures for diseases of cultured penaeid shrimp. World Aquaculture Society, Baton Rouge, Louisiana, USA. 1996, 304.
22. Lightner DV. The penaeid shrimp viruses TSV, IHHNV, WSSV, and YHV: current status in the Americas, available diagnostic methods, and management strategies. Journal of Applied Aquaculture. 1999; 9(2):27-52.
23. Pazir MK, Afsharnasab M, Jalali Jafari B, Sharifpour I, Motalebi AA, Dashtiannasab A. Detection and identification of white spot syndrome virus (wssv) and infectious hypodermal and hematopoietic necrosis virus (ihhnv) of *Litopenaeus vannamei* from bushehr and sistan and baloochestan provinces (Iran), during 2009-2010. Iranian Journal of Fisheries Sciences. 2011; 10(4):708-726.
24. Mishra SS, Shekhar MS. White spot syndrome virus isolates of tiger shrimp *Penaeus monodon* (Fabricious) in India are similar to exotic isolates as revealed by polymerase chain reaction and electron microscopy. Indian Journal of Experimental Biology. 2005; 43(7):654.
25. Pazir MK, Afsharnasab M, Niamaymandi N, Khadem H, Akbarpour E, Zendebudi AA. Histopathological Observation of White Spot Syndrome Virus and Infectious Hypodermal and Hematopoietic Necrosis Virus in Shrimp Farms, *Litopenaeus vannamei*, in Bushehr Province, Iran. Asian Journal of Animal Science. 2012; 6(5):209-219.