Introduction
1. Introduction

Aquaculture is one of the world’s fastest growing industry but disease outbreaks in cultured shrimp have caused serious economic losses. In Asia, mortalities of cultured shrimp because of white spot syndrome virus (WSSV) and yellow head virus (YHV) have resulted in economic losses of about $1 billion per year since 1994. Indian farmed shrimp production increased from about 30,000 tons in 1990 to around 1.15,000 tons during 2002–2003 (FAO, 2007). Due to the rapid expansion of shrimp aquaculture during early 1990s, the demand for post larvae (PL) could not be met by the hatcheries that existed in the country at that time. The import of PL from other Asian countries and poor management of the brood stock, the hatcheries and also the farms led to the outbreak of White Spot Syndrome Virus (WSSV) in 1994. The virus spread very rapidly, and the economic losses caused by mortalities were estimated at over US$ 200 million during 1999–2000 (FAO, 2007).

White Spot Syndrome Virus (WSSV) is the most serious pathogen of shrimp aquaculture in Asia. It first appeared in 1990s in Taiwan and China (Chou et al., 1995). The virus can cause 100% mortality within 3-5 days in infected shrimp (Karunasagar et al., 1997; Lotz & Soto, 2002). WSSV infects a wide range of wild crustaceans including crabs, lobsters and shrimp. The presence of WSSV has also been recorded from wild as well as hatchery reared post larvae. The simplest method to detect WSSV infection in shrimp is to observe for local lesions and white spots on the carapace. Sometimes a pink to reddish-brown coloration is seen on the shrimp due to the expansion of sub-cuticular chromatophores. Since the shrimps die after the appearance of symptoms, this method
cannot be used for diagnosis. Histological lesions include distinct hypertrophied nuclei containing margined chromatin and amphophilic central inclusions in the cuticular epithelial cells, connective tissue cells and hemocytes. Molecular methods like polymerase chain reaction (PCR) and Real time PCR are sensitive for detection of WSSV infection even in carrier animals that do not show any gross symptoms of the disease.

Vembanad Lake is an estuarine system located on the south-west coast of India. Many crustaceans spent part of their lifecycle in the estuarine system before migrating to the sea. The borders of the Vembanad Lake support extensive as well as semi-intensive aquaculture. WSSV infection of penaeid shrimp has a devastating influence on the shrimp aquaculture in the Vembanad estuarine system. Interventions in the form of reclamation and discharge of pollutants to the Vembanad Lake also have an adverse impact on the potential of the aquatic ecosystem that used to support high levels of bioproductivity and biodiversity. There has been very limited study on the occurrence of WSSV in decapods in the Vembanad estuarine system.

WSSV is an enveloped ovoid virus with rod shaped nucleocapsid with flat ends. The virus belongs to the genus Whispovirus under the family Nimiviridae. The complete genome sequence of WSSV has been determined for three different isolates with Gene bank Accession numbers AF369029, AF440570, AF332093 for viruses isolated from Thailand, Taiwan, and China respectively. The genome of WSSV is approximately 300 kb in length with 180 putative Open Reading Frames. Most of the Open Reading Frames of WSSV encode certain structural genes. Structural proteins of viruses are classified as envelope and non-envelope proteins. Envelope proteins play a vital role in virus entry assembly and release. Neutralization experiments of antibodies against six WSSV
Envelope proteins showed that the virus infection could be significantly delayed or neutralized by antibodies against three WSSV envelope proteins (VP68, VP281 and VP466) (Wu et al., 2005). Very little genetic variation has been recorded in the structural genes of WSSV from different geographical locations. There had been very limited work on the nucleotide sequence of the structural genes of WSSV isolated from India.

Monodon Baculovirus (MBV) can cause mortalities in hatchery reared larvae. Infection with MBV can cause severe mortalities in post larvae of hatcheries. Although MBV is relatively well tolerated by *Penaeus monodon*, it has been implicated in mass mortalities in shrimp cultured at high densities. Although good culture practices may enhance the survival of MBV-infected stocks, growth, crop value and performance may significantly be reduced and MBV may render the infected shrimp susceptible to other pathogens with higher mortality rates (Bower, 1996).

Hepatopancreas Parvovirus (HPV) is distributed worldwide and infects several penaeid shrimp including *P. monodon* (Lightner, 1996). HPV infected *P. monodon* is found to grow slowly compared to uninfected ones. Early juvenile stages are reported to have high levels of HPV infection. Two strains of HPV have been characterized at the molecular level. They are the HPVchin from *P. chinensis* from Korea and HPVmon from *P. monodon* from Thailand. The DNA sequence of HPVmon differs from HPVchin by almost 30% by examining the partial sequences for HPVchin (AY008257) and HPVmon (AF456476) available at GenBank.

Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) was first reported in juvenile *Litopenaeus stylirostris* in Hawaii (Lightner et al., 1983a, b). The virus has been detected in many penaeid shrimp around the world. Even though IHHNV
infection does not cause mortality in *Litopenaeus vannamei* and *P. monodon*: it results in a disease called Runt Deformity Syndrome in both species and hence causes substantial economic losses (Kalagayan *et al.*, 1991; Browdy *et al.*, 1993; Primavera & Quinitio, 2000). There had been limited work on the simultaneous occurrence of viral pathogens in the post larvae of paenaid shrimp in hatcheries.

PCR is the method of choice for detection of viral pathogens affecting shrimp aquaculture. PCR has been widely used for routine detection of WSSV in broodstock and post larvae. Although PCR is the method of choice for detection of viral pathogens, it cannot detect very low numbers of the virus. The routine screening PCR test used for detection of WSSV are able to detect about 10-50 copies in a PCR reaction. Moreover the conventional PCR method cannot quantify the amount of virus, but only the presence or absence of virus. Real-time quantitative PCR using SYBR Green as the fluorescence dye is a rapid and highly sensitive method for detection as well as quantification of WSSV. SYBR Green is a minor-groove DNA dye with a high affinity for dsDNA. In Real-Time PCR, the fluorescence of the SYBR Green dye is monitored at the end of each PCR cycle. The increase of fluorescence is dependant on the initial template concentration. Real-Time PCR can be utilized to screen for virus low grade infection in postlarvae, for the development of a specific pathogen free shrimp, screening of shrimp broodstock and to implement effectively the sanitary and phyto-sanitary regulations. Real-time PCR has been successfully employed for quantification of WSSV in different shrimp species (Dhar *et al.*, 2001; Durand and Lightner, 2002; Durand *et al.*, 2003; Powell *et al.*, 2006; Sritunyalucksana *et al.*, 2006c; Jang *et al.*, 2009)
The objectives of this study were

- To determine the prevalence of WSSV among the crustaceans in the Vembanad estuary, the shrimp aquaculture farms surrounding the estuary, and the sea off Cochin coast, India.
- To compare the sequence of six major structural proteins of WSSV: vp28, vp26, vp19, vp68, vp281, vp466 from different geographical locations with that of an isolate from India.
- To monitor the simultaneous occurrence of HPV, IHHNV, MBV and WSSV in postlarvae of *P. monodon* from hatcheries in India by Polymerase Chain Reaction.
- Development of a quantitative assay for WSSV infection.
- To determine the viral load of postlarvae from hatcheries in Kerala meant for aquaculture

**About this thesis**

In this thesis, the investigation has been dealt in the following manner:

A study was done to determine the prevalence of WSSV among the crustaceans in the Vembanad estuary, the shrimp aquaculture farms surrounding the estuary, and the sea off Cochin coast, India using two sets of nested PCR primers.

An investigation was also done to compare the sequence of six major structural proteins of WSSV: vp28, vp26, vp19, vp68, vp281, vp466 from different geographical locations with that of an isolate from India.
A study was also done to monitor the simultaneous occurrence of HPV, IHNV, MBV and WSSV in postlarvae of *P. monodon* from hatcheries in India by Polymerase Chain Reaction.

A real time PCR procedure was developed for the quantitative analysis of WSSV infection. The viral load of postlarvae from hatcheries in Kerala meant for aquaculture was also determined using the quantitative PCR.

The thesis is divided into five major chapters and each chapter is further divided into subheads. The first chapter highlights the identification of problem and with suitable objectives. The second chapter is the review of literature. The review includes a short description of the current status of shrimp aquaculture and the diseases affecting shrimp. A detailed review of spread of WSSV infection, genetic variability of WSSV strains, the structural proteins of WSSV, clinical signs and pathogenesis of WSSV infection, the host range, incidence of WSSV infection and diagnostic methods for WSSV. A review of the genome, the host range and detection methods for IHNV, MBV and HPV infection is also included. In chapter 3, materials and methods are discussed. Details of sample analysed and all the methods employed in the investigation are presented. In chapter 4, results and discussion are presented. The findings are discussed in detail. The results are discussed in tables and relevant photographs are also included.

A summary of the entire work is presented in chapter 5. A detailed bibliography of all the citations made in the thesis is given at the end of the thesis.

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