SURVEY AND DISEASE SURVEILLANCE OF NEWLY INTRODUCED *L.VANNAMEI* (BOONE, 1931) IN INDIA.

by

ROSHAN MARIA PETER

(2009 – 14 – 103)

Thesis submitted in partial fulfilment of the requirement for the degree

Master of Fisheries Science in Aquaculture

Faculty of Fisheries

Kerala Agricultural University, Thrissur

2011

DEPARMENT OF AQUACULTURE

COLLEGE OF FISHERIES

PANANGAD, KOCHI

DEDICATED TO

My Guide Dr. Devika Pillai

My loving Mom, Dad ,Brother

And

All Aqua Farmers

DECLARATION

I hereby declare that this thesis entitled **"SURVEY AND DISEASE SURVEILLANCE OF NEWLY INTRODUCED *L. VANNAMEI* (BOONE, 1931) IN INDIA"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship, or other similar title, of any other university or society.

Panangad

Date: 06.08.2011

ROSHAN MARIA PETER

(2009 – 14 – 103)

Dr. Devika Pillia
Associate Professor
Department of Aquaculture
College of Fisheries
Kerala Agricultural University
Panangad, Cochin

Date:

CERTIFICATE

Certified that this thesis entitled **SURVEY AND DISEASE SURVEILLANCE OF NEWLY INTRODUCED *L. VANNAMEI* (BOONE, 1931) IN INDIA"**is a record of research work done independently by **Ms. ROSHAN MARIA PETER (2009-14-103)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to her.

Dr. Devika Pillia
Chairman
Advisory Committee

Panangad

CERTIFICATE

We the undersigned members of the advisory committee of Ms. **Roshan Maria Peter (2009-14-103)** a candidate for the degree of Masters of Fisheries Science agree that this thesis entitled "**Survey and Disease Surveillance of newly introduced *L.vannamei* (Boone, 1931) in India**" may be submitted by Ms. **Roshan Maria Peter (2009-14-103)**, in partial fulfillment of the requirement for the degree.

Dr. Devika Pillai
Associate Professor
Department of Aquaculture,
College of Fisheries, Panangad, Kochi
(Chairperson)

Prof. Dr. C. M. Nair
Professor and Head
Department of Aquaculture
College of Fisheries Panangad, Kochi
(Member)

Dr. P.M. Sherief
Professor and Head
Department of Processing Technology,
College of Fisheries, Panangad, Kochi
(Member)

Mr. Mathew Sebastian
Associate Professor
Department of Management Studies
College Of Fisheries,
Panangad, Kochi ,
(Member)

ACKNOWLEDGEMENT

Many people from inside and outside College of fisheries, Panangad have assisted me during my study and in the preparation of the thesis. So, I would like to express my sincere thanks and appreciation for the following people.

I am fortunate to have benifitted from the constructive guidance and constant unconditional support and encouragement of my major advisor Dr. Devika Pillai, Assiociate Professor of Aquaculture, College of Fisheries, Panangad; during the course of my research and post graduation. I indebted to her for every possible help extended, valuable suggestions and meticulous scrutiny, which helped me immensely in completing my research and preparing and submitting the thesis.

I owe a great deal to Dr. C. Mohanakumaran Nair , Pro. Vice Chancellor, Kerala University of Fisheries and Ocean Studies & former Dean, College of fisheries valuable help and critical suggestions during my work. His timely guidance has helped me in successfully completing my research work.

I wish to express my gratitude to Mr. Mathew Sebastian, Associate Professor, Dept. of Management Studies, for his carefull attention and corrections which has enable me to arrive at meaningful conclusions. My

sincere thanks to him for his inestimable suggestions, which helped me a lot in preparing the thesis.

I feel great pleasure in expressing my regards and profound indebtness to my Advisory committee member Dr. P. M Sherief , Professor, Head Dept. of Processing Technology for his valuable suggestions and helps during my study.

I am grateful to Dr. B. Madhusoodana Kurup, Vice Chancellor and Dr.Thressa Registrar Kerala University of Fisheries and Ocean Studies .

I acknowledge deeply my debts to Dr. Shyama Associate Professor, Dr. K. Dinesh, Asst Professor, ,Dept of Aquaculture, Dr. K.V. Jayachandran, Professor and Dr. T.M Jose, Retd Professor, Dept of Fishery Biology, DR. N.N Raman Asst Professor, Dept of Fishery Hydrography and Mrs. Tessy K. Thomas, Dept. of Management Studies, College of Fisheries, Panangad for their generous help and support during my thesis preparation.

The assistance rendered by library staffs of College of Fisheries, Panangad and specially the advices given by Sri V.S. Kunjumuhamed are gratefully acknowledged.

I am thankful to Dr. Juliet Joseph, Divya Haridas, Dr. Manoj .C.K and Navay Teaching Assistants, College of Fisheries, for their valuable suggestions, help and guidance during my course work. I never forget to give thanks to them for their help, cooperation, encouragement and support in my work and study.

I express my sincere appreciation and heart felt thanks to my seniors, Katya Sir, Avinash, Vijaya kumar Reddyfor their assistance and encouragement through out my work.

I take this opptunity to express my infringed gratitude to my classmates and juniors specially Vimala, , Trinayan, Kranthi, Pratap, Aditya, Shelma, Jyasree, Devraj, and Ansar, for the generous help and wholehearted support who spared their valuable time when needed.

I wish to thank N.Verabhadra Rao, SurayaRao, and all the Aqua farmers of Nellore Dist. Andhra adesh for their help and pateince during sampling.

I express my deep sense of gratitude to my Mom, DaD and Brother for their love, constant support, encouragement, inspiration, faith and prayers without which, I would not have been able to complete my study.

Finally I bow my head before my God, whose blessing have made reach this far. Thanks God.

Panangad *Roshan Maria Peter*

Date:

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

TAAPs/TAADs	Trans-boundary Aquatic Animal Pathogens/Diseases
TADs	Trans-boundary Animal Diseases
DIAS	Database of Introduced Aquatic Species
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
WSSV	White Spot Syndrome Virus
TSV	Taura Syndrome Virus
IHHNV	Infectious Hypodermal haematopoietic Virus
LOVV	Lymphoid Organ Vacuolization Virus
YHV	Yellow Head Virus
MBV	Monodon Baculo Virus
HPV	Hepato Pancreactic Virus
PCR	Polymerase Chain reaction
RT-PCR	Reverse Transcription Polymerase Chain reaction
CAA	Coastal Aquaculture Authority of India
ETS	Effluent Treatment System

INTRODUCTION

1. INTRODUCTION

Aquaculture is a dynamic field that is advancing at a rapid pace and shrimp is the most important commodity in international seafood trade. However, volatility of international prices of shrimps and fluctuating foreign exchange rates, uncertainty of climatic conditions, susceptibility of shrimps to diseases due to trans-boundary movement continues to be major areas of threat for the industry. Disease is now recognized as a primary constraint to sustainable aquaculture and is responsible for severely impeding both economic and socio-economic development of many countries of the world. The diseases of cultured penaeid shrimp are due to infectious and non-infectious aetiologies. Among the infectious diseases viral and bacterial pathogens are of economic importance to culture as 60% of losses were attributed to viruses and about 20% to bacteria (Lightner and Redman, 1998; Flegel *et al.*,2008).

Aquaculture is faced with what is known as trans-boundary aquatic animal pathogens/diseases(TAAPs/TAADs), similar to the trans boundary animal diseases (TADs) in the livestock sector. (FAO/NACA, 2000; Bondad-Reantaso *et al.*,2001). The FAO database of introduced aquatic species (DIAS) reports that aquaculture development has been the primary reason cited for most introductions, which has increased exponentially since 1940. Most of these introductions are of fish, with only 6% or 191 records being of crustaceans. Such movements have been facilitated by recent advances in transport, rapid global development of aquaculture and the demand for new species to culture (Fegan *et al.*, 2001). Recent examples of major losses suffered by aquatic animals from pest or disease spread include the carp mortalities in Java, the infectious salmon anaemia outbreaks (and subsequent disease control programs) in Norway, the United Kingdom and North America, the white spot syndrome virus and Taura syndrome virus epidemics in shrimp aquaculture, the spread of epizootic

ulcerative syndrome (EUS) in Asia and Akoya disease in Japanese pearl oysters (Beers *et al.*, 2005). It is widely believed that Yellow head Virus (YHV 1992),White Spot Syndrome Virus (WSSV 1994) and Taura Syndrome Virus (TSV 1993) are the three most economically significant viral pathogens which have been introduced to the Asian and Latin American countries. These countries are suffering direct losses through the careless introduction of live shrimp stocks. (Briggs *et al.*, 2004).

The shrimp aquaculture sector, in particular, suffered losses estimated at US$ 3019 million, on a global scale, affecting major shrimp producing countries such as Bangladesh, China, India, Indonesia, Ecuador, Mexico, Philippines, Taiwan Province of China, Thailand and Vietnam (Israngkura and Sae-Hae, 2002, Briggs *et al.,* 2005). The lack of cohesive policies and regulatory frameworks in most Asian countries, as well as inadequate technical information to develop guidelines for safe trans-boundary movement of live aquatic animals, are major factors (Bondad-Reantaso and Subasinghe, 2005).

Black tiger shrimp *Penaeus monodon* was the most widely cultured species in India since the past two to three decades. However, in recent years, its culture has been beset with disease problems especially due to the white spot syndrome virus (WSSV) which wiped out entire culture stocks causing widespread economic loss to the shrimp farmers. India's shrimp production has stagnated at around 150,000 metric tonnes annually (Shrimp News International, 2008). Recently, shrimp exports from the country dropped from 35, 699 tonnes in 2005 down to 20,776 tonnes in 2007 (Business standard, 2010). The production costs of *P. monodon* is higher but the yields are lower than that of *Litopenaeus vannamei* and therefore, the introduction of *L. vannamei* is expected to help shrimp farmers reduce costs and increase production. The perception of the private sector is that the potential advantages outweigh the disadvantages, thus justifying the importations (Briggs *et al.*, 2005). This led to the introduction of the American

white shrimp *L.vannamei* from Hawaii via Taiwan Province of China. Initially, the Government of India granted permission only to two firms to import PCR tested SPF broodstock of *L.vannamei*. The initial successes of *L.vannamei* culture led to more widespread culture of the species using locally reared broodstock. The permission for import of *L.vannamei* broodstock is granted by the Coastal Aquaculture Authority of India (CAA) in conjugation with National Fisheries Development Board, Marine Products Export Development Authority (MPEDA) and Central Institute of Brackish water Aquaculture (CIBA).

Introduction of an alien species into the country has potential risks as *L.vannamei* are known to be carriers of WSSV (white spot syndrome virus), IHHNV (Infectious Hypodermal haematopoietic Virus), LOVV (Lymphoid Organ Vacuolization Virus)and TSV (Taura Syndrome Virus); the latter has not been reported in India so far. Although it is argued that Government allows only SPF stocks to be imported, illegal importations of non-SPF stocks are likely to happen. Moreover, SPF stocks may be susceptible to other pathogens during the culture period. TSV resistant strains combined with biosecurity measures to reduce infection due to other viruses could greatly assist the development of *L. vannamei* industry in Asia (Briggs *et al.*, 2005). Although the Government of India has formulated guidelines and frameworks to restrict the movement of shrimp species, the initial success of *L.vannamei* culture in the country has led to widespread import of the species into the country, using legal or illegal methods. There is a potential threat of TSV or other disease outbreak in the country. Thus the study was undertaken to understand the extent of *L.vannamei* introduction in the country and the potential risks faced.

The objectives of the present investigation were:

1. To survey the extent of introduction of *L.vannamei* in
   Nellore district of Andhra Pradesh, India and the level of acceptance by farmers.

2. Screening for the presence of potential disease causing agents (viruses/bacteria) in *L. vannamei.*

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 OVERVIEW OF SHRIMP INDUSTRY

2.1.1 World

The long and colourful history of shrimp farming can be divided into three distinct eras. During the 'Start-up Era' nearly all stocking material was wild post larvae (PL) gathered from the sea. In each hemisphere, shrimp farming was based on use of native species i.e., in Asia, *P. monodon* dominated while in the West, the industry used *Litopenaeus vannamei*. During this era, annual production increased rapidly (~100%/year) driven by demand for farmed shrimp product and a relative absence of disease which allowed simple pond culture methods to succeed (Wyban, 2009).

In the second era i.e., 'Hatchery Era' (1988-96), post larvae were produced in land-based hatcheries and shrimp farming in each hemisphere continued to use native species. Asian shrimp production was at least five times greater than Western production throughout this era, so global production statistics were dominated by *P. monodon* (Wyban, 2009).

During the 'Hatchery Era', total world production only increased from 604 to 693 thousand MT resulting in an average annual gain of just 2%/yr. The main obstacle to growth in this era was widespread shrimp disease due to improper pond practices (Lightner 1996). Wyban (2007) proposed that shrimp production from farming during this era reached a "carrying capacity" for use of wild, non-domesticated, non-SPF animals. While farmers tried increasing stocking densities to increase yields and profits, their use of wild animals precluded these attempts and prevented industry growth (Wyban, 2009).

In the third era, named 'Breeding Era' (1996 to 2007), industry production grew from about 700,000 MT to 3.2 MMT with sustained annual growth of more than 20% per year. In 2007 the increments of industry crop value over 10 years directly resulted from domestication, breeding and widespread adoption of *L. vannamei* in Southeast Asia (China, Thailand and Indonesia), which accounted for more than 75% of total world production(FAO 2008). Use of *L.vannamei* spread from the West into Asia in this era, so it could also be called the *'L. vannamei* Era'. (Wyban, 2009). Over the last decade, shrimp farming annual crop value has tripled as a result of the widespread use of domesticated *L. vannamei*. In 1997, global farmed-shrimp annual production of 700,000 MT had a total crop value of about US$3.5 billion based on an average price of $5/kg (FAO 2008)." In 2006, the production of this species reached 2.1 million MT, up from 1.6 million MT in 2005.

2.1.2 Shrimp Farming Scenario In India

Shrimp aquaculture has become an important economic activity in maritime states of India, particularly in the State of Andhra Pradesh, since the early 1990's. The giant tiger shrimp, *Penaeus monodon* (Fabricius) popularly called the black tiger, has been, and continues to be, the leading cultured species in India. Superior growth rate and a high market demand have made it a good candidate among the available marine shrimp species in India for commercial aquaculture. Production loss due to various diseases especially white spot disease (WSD) caused by white spot syndrome virus (WSSV) is of major concern to this Indian shrimp farming sector, since its first occurrence in 1994 (Manohar *et al.*,1996; Karunasagar *et al.*, 1997; Mohan *et al.*, 1998; Shankar and Mohan, 1998; Madhavi *et al.*, 2002). According to the estimates by Marine Products Export Development Authority (MPEDA), on an average, 10,000 to 15,000 metric tonnes of shrimp production, worth about US$ 60-70 million is lost annually due to disease problems. It is in this context that the industry actively promoted the introduction of a non-native species, the Pacific white shrimp, *Litopenaeus vannamei*, as an alternative to *P.*

monodon for commercial aquaculture operations in the country (Rajeev and Prasad, 2003).

2.2 *LITOPENAEUS VANNAMEI*

Litopenaeus vannamei (Boone, 1931) is native to the Pacific coast of Mexico and Central and South America as far south as Peru, in areas where water temperatures are normally over 20^{O}C through out the year (Wyban and Sweeny, 1991; Rosenberry, 2002).

2.2.1 Biological Features

Similar to all penaeid shrimp, the rostrum is moderately long with 7-10 dorsal and 2-4 ventral teeth. In mature males (20 g) petasma is symmetrical and semi-open and females (28 g) have open thelycum. Life cycle consists of six nauplii, three protozoeae and three mysis stages as seen in all penaeid shrimps. Colouration is normally transluscent white, but can change depending on substratum, feed and water turbidity. Maximum size is 23 cm, with maximum carapace length (CL) of 9cm. Females grow faster and larger than males (FAO, 2000).

2.2.2 Historical Background

In 1973 spawning of *L.vannamei* was achieved for the first time in Florida followed by commercial culture of this species in South and Central America. The discovery of unilateral ablation to promote maturation in Panama in 1976 led to widespread pond culture of the species. By early 1980s, the intensive breeding and rearing techniques led to its culture in Hawaii, mainland United States of America and much of Central and South America. The rapidly increasing trend was punctuated by declines co-incident with disease outbreaks. Asia has seen a phenomenal increase in production. Though no production was reported to FAO in 1999, it rose to nearly 1,11,6000 tonnes by 2004 and had overtaken the

production of *P.monodon* in China, Taiwan province of China and Thailand. The reason for the dramatic increase in production of *L.vannamei* (whiteleg shrimp) was that it was more profitable to tiger shrimp. The culture period is only 90-110 days with more stocking density and a feed with only 20-35% protein. (FAO, 2000).

2.3 *LITOPENAEUS VANNAMEI* IN INDIA

It has been estimated that in 1994 alone, the losses due to disease outbreaks in the country totalled over US$17.6million (Subasinghe *et al.* 1995), which made the industry to actively promote the introduction of a non-native species, the Pacific white shrimp, *Litopenaeus vannamei*, as an alternative to *P. monodon* for commercial aquaculture operations in the country (Rajeev and Prasad 2003).The giant tiger shrimp, *Penaeus monodon* (Fabricius) has been the leading cultured species in India. However, the species has been observed to be vulnerable to several viral and bacterial pathogens that have resulted in the recurrence of diseases, such as White Spot Syndrome Virus (WSSV) and Vibriosis in many regions of India, from Andhra Pradesh on the east coast to Kerala on the southwest coast, thereby devastating the shrimp culture industry in India and inflicting losses amounting to approximately US$21 million (Raghavan *et al.*, 2006). CAA gave licenses for 1,208 aqua farmers in 2009 to take up *L.vannamei* culture in 2,500 hectares belonging to them (The HINDU report, 2009).

During 2001 & 2002, both scampi and tiger shrimp culture had come down due to severe disease out breaks in Andhra Pradesh. Two companies in Andhra Pradesh namely M/s Sharath Industries and M/s BMR were the only ones granted permission to introduce *L.vannamei* in India by the Ministry of Agriculture (CAA report). They also got permission to culture *L.vannamei* as a pilot project in the year 2003. During the first crop, harvest size was 20 g, survival rate was 90 % & their FCR was 1.5. In 2004, M/s Sharath Industries supplied seed to two farms in

Gangapatnam, who then started culture with 40 pc/m^2. Harvest size was 20 g with 130 days of culture (DOC). M/s BMR supplied seed to other farms in Iskapalli village for stocking at a density of 20 to 30 pc/m^2 & obtained a good crop. In 2008, the Supreme Court issued the orders to close the farms in this particular village, as the farmers had to face an unidentified disease outbreak. Ministry of Agriculture issued notice through CAA to both Sharat Sea Foods and BMR to stop all their activities before the end of June 2009. Fisheries authorities served notices to about 70 aqua farmers, who had started *L.vannamei* culture without permission (Chinarong and Yamuna Krishna, 2011). The government has stipulated various conditions for the farmers shifting to *L.vannamei* culture. Not withstanding the conditions laid down by the Coastal Aquaculture Authority (CAA) to take up *L.vannamei* shrimp (American white shrimp) culture, several aqua farmers of Nellore district have already ventured into the culture without obtaining mandatory license (HINDU report, 2009).

The Union government allowed cultivation of white prawn (*L.vannamei*) in India from February 2010 (CAA report). India's *Business Standard(2010)* states that shrimp culture in the country, predominantly black tiger shrimp, has declined from 106,165 tonnes in 2007-08 to 75,996 tonnes in 2008-09, a fall of 28.4 %. Keeping in view the demand for quality shrimp seed, the NFDB has decided to set up a specific pathogen-free (SPF) shrimp seed plant for the black tiger prawn project at Mulapolam village in Srikakulam district of Andhra Pradesh together with Moana Technologies, a Hong Kong-based company with a proven record in SPF research for the species. NFDB's role was to create hatchery infrastructure and then lease out the facilities to Moana Technologies, which will have a production capacity of three billion SPF seed a year. However, this project has not made much progress (*Business Standard, 2010*).

The Centre created a quarantine facility in Chennai and approved 24 hatcheries to supply *L.vannamei* seeds (HINDU report, 2009; Business standard,

2010). However, without proper biosecurity measures, producing consistent post larvae will be difficult and will affect the aqua farm economics.

2.4 *P.MONODON* v/s *L.VANNAMEI*

There are many reasons for the introduction of *L.vannamei* into areas where they are not indigenous, the main reasons being the problems faced with the culture of indigenous species and the perceived (rightly or wrongly) production benefits of the alien species.

2.4.1. Growth rate

L.vannamei has the potential to grow as fast as *P. monodon* (@ to 3 g/wk) up to 20 g , the maximum size of *L.vannamei* usually cultured under intensive culture conditions (up to 150/m2) (Wyban and Sweeny, 1991). In contrast, the growth (and survival) rate of *P. monodon* has been declining in recent years from 1.2 to 1 g/wk (and 55 % to 45 % survival) over the last five years in Thailand (Chamberlain, 2003) due, perhaps to disease load and/or genetic inbreeding. *L.vannamei* capture foods faster than all of the other shrimp species (*Metapenaeus brevicornis, M. ensis, Penaeus merguiensis,* and *P. monodon*), outcompeting them by 80-100%. (Chavanich *et al.*,2009).

2.4.2 Stocking density

L.vannamei are amenable to culture at very high stocking densities of upto 150/m^2 in pond culture, and even as high as 400/m^2 in controlled recirculated tank culture, resulting in better productivity per unit area than that currently achievable with *P. monodon* in Asia. (Briggs, *et al.* 2005).

2.4.3. Salinity tolerance:

L.vannamei tolerates a wide range of salinities, from 0.5-45 ppt, is comfortable at 7-34 ppt, but grows particularly well at low salinities of around 10-15 ppt (Wyban and Sweeny, 1991).

2.4.4. Temperature tolerance

Although *L.vannamei* will tolerate a wide range of temperatures, it grows best between 23-30 °C. They will also tolerate temperatures down to 15°C and up to 33°C without problems, but at reduced growth rates (Wyban and Sweeny, 1991). *L.vannamei* can thus be profitably cultured during the cool season in Asia (October-February).

2.4.5 Dietary protein requirement

Compared with other species, *L.vannamei* requires a lower protein (and hence cheaper) diet (20-35 %) during culture than *P. monodon, P. chinensis* or *P. stylirostris* (36-42%), and are more able to utilize the natural productivity of shrimp ponds, even under intensive culture conditions (Wyban and Sweeny, 1991). In Mainland China, Indonesia and elsewhere, it has been shown that even lower protein levels of 20 % or less can be used successfully with *L.vannamei.* The natural bacterial productivity of the ponds is correctly stimulated (McIntosh *et al.*, 1999).Recent commercial results from Indonesia have shown that *L.vannamei* growth, survival and production rates, all slightly increased using 30-32 % compared to 38-40 % protein diets in intensive ($60/m^2$) culture (Taw *et al.*, 2002).

2.4.6 Ease of breeding and domestication

L.vannamei have an open thelycum , which enables induced mating and spawning easily in captivity (unlike the closed thelycum in *P. monodon*). This feature permits much more control and enhancement of the cultured stock and allows the development of SPF and SPR stocks, which in turn reduces the

expense, disease implications, environmental concerns, unpredictability and unreliability and expense of relying on wild broodstock.

2.4.7 Larval rearing

Larval survival rates during hatchery rearing are generally higher (50-60 %) with *L.vannamei* than with *P. monodon* (20-30 %) (Rosenberry, 2002).

2.4.8. Disease resistance

L.vannamei is generally considered to be more disease resistant than other white shrimp (Wyban and Sweeny, 1991), although it is highly susceptible to White Spot Syndrome Virus (WSSV), Taura Syndrome Virus (TSV), IHHNV which results in runt deformity syndrome (RDS) and Lymphoid Organ Vacuolization Virus (LOVV). Injection of WSSV into *L.vannamei* was shown to result in 100% mortality within 2-4 days, proving that its infectivity and pathogenicity was similar to that found with *P. monodon, M. japonicus* and *P. chinensis* (*P. orientalis*) (Tapay *et al.*, 1997). Additionally, the generally higher water temperatures experienced in tropical Asian countries may have helped to limit mortalities due to WSSV in *L.vannamei* (compared to Latin America) since WSSV has been shown repeatedly to lose its virulence in water over 30°C.

2.4.9. Specific Pathogen Free (SPF) shrimp

The main advantages of culturing the shrimp species *L.vannamei* is that these species are commercially available as high health animals from SPF stocks. *P.monodon* have very limited or no availability from SPF stocks, but this may well change in the near future as such stocks are currently under development (Briggs *et al.*, 2005).

2.4.10. Post-harvest characteristics

After harvest, if well treated with plenty of ice, *L.vannamei* are particularly resistant to melanosis and keep a good appearance three to four days after

defrosting. Another advantage is that *L.vannamei* have a higher meat yield at 66-68 % than *P. monodon* at 62 %.

2.4.11. Marketing advantages

White shrimp, such as *L.vannamei* and *P. stylirostris*, are the preferred species for consumption for the world's largest shrimp market – the USA (Briggs *et al.*, 2005), thus increasing international export and adding to nations economy.

2.5 CONSTRAINTS FOR SHRIMP TRANSBOUNDARY MOVEMENT

Various codes and guidelines have been laid down in Asia-Pacific region to improve safe transboundary movement of live aquatics and most of those efforts have been largely ineffective at preventing the spread of alien shrimp and their viral pathogens.(Briggs *et al.*, 2005).

2.5.1 Lack Of Understanding Pathogen Transfer Pathways

The pathways of viral pathogen transfers in shrimp are still far from clear, making it difficult to design protocols for testing imported products (Flegel, 2003). Imported shrimp are subjected to thorough analysis for known viral pathogens, but there may exist many "hidden" or "cryptic" viruses within, which may not infect or cause disease and mortality in one species, may have other effects in other native species. For example, SPF shrimp shipped
from Hawaii resulted in the contamination of shrimp in Brazil and Colombia with TSV (Brock *et al.*, 1997), because at that time, TSV was not known to have a viral cause and therefore went unchecked in SPF protocols. Unfortunately, current understanding of the effects of many viruses on different shrimp hosts is limited, making such disease testing even more difficult (Briggs *et al.*,2005).

2.5.2 Producer Driven Importations

Governments have formulated guidelines and/or regulatory frameworks to restrict the movement of shrimp species, but the private sector in most countries

where *L.vannamei* is imported has gone ahead by using illegal or illicit import procedures which is almost impossible to stop (Briggs *et al.*, 2005). This is exacerbated by the multi-functional structure of the private sector, where different stakeholders may have drastically different motivations and attitudes to risk aquatic disease emergencies (Fegan, 2005). The situation is very similar in India.

2.5.3 Limitations On Law Enforcement

Legislation for transboundary movements has been enacted, but the extensive borders, lack of resources, a lack of desire or reluctance (or pressure) not to interfere with the competitiveness of the commercial sector, lack of clear understanding and knowledge and weak regulatory structures of many countries make enforcement very complex, expensive and sometimes impossible (FAO, 2005). In India, legislation for transboundary movement is not strictly implemented. With its large number of airport and shipping ports, strict implementations of quarantine measures will involve prohibition costs. However, since the potential losses far outweigh the costs involved in establishment, enforcement and dissemination of laws and guidelines, its imperative that health certification, enforcement of health code practise of OIE and quarantine measures have to be placed without further delay (Bernoth *et al.*, 2008).

2.5.4 Inadequate Testing Facilities And Protocols For Viral Pathogens

According to Briggs *et al.* (2005), ahough SPF (Specific Pathogen Freee) broodstock and PL of *L. vannamei* and *P. stylirostris* (unlike *P. monodon*), are commercially available but shrimps are characterized by persistent viral infections that often produce gross signs of disease or mortality especially when shrimps are stressed. Thus to detect these pathogens sophisticated procedures such as Polymerase Chain Reaction (PCR) methodologies or other molecular diognostics tools and quarantine facilities are required. Though such facilities exist,

competent and accurate analysis of the disease status of the imported shrimp is still not always possible due to reason already mentioned.

2.5.5. Perceptions Of SPF And SPR Shrimp

A common perception amongst farms is that SPF and SPR shrimp are 'disease free'. SPF refers only to the present pathogen status for specific pathogens and not to pathogen resistance or future pathogen status (Lotz, 1997). Although the original stock of SPF or SPR shrimp may be certified as clear of specific pathogens or resistant certain specific strains (TSV, YHV, IMNV, IHHNV, WSSV , BP and NHPV), the animals produced from this stock may not be so reliable, particularly if the biosecurity of the producing facility is poor (Briggs *et al.*, 2005).

2.6 TRANSBOUNDARY MOVEMENTS OF AQUATIC ANIMALS

Baldock (2002) defined trans-boundary animal diseases (TADs) as epidemic diseases that are highly contagious or transmissible, with the potential for rapid spread irrespective of national borders and that cause serious socio-economic and possibly public health consequences.. Aquaculture is faced with trans-boundary aquatic animal pathogens/diseases (TAAPs/TAADs), similar to the TADs in the livestock sector (FAO/NACA, 2000; Bondad-Reantaso *et al.* 2001; FAO/NACA, 2001). Aquaculture production and invasive species are intricately linked. Live plants and animals introduced for economic production escape their confines and establish in the wild (Lee and Gordon 2006). The traded animals may be of a species that is new to the importing country and hence immunologically naïve to resident aquatic animal pathogens and vice-versa. In many cases it will not be known whether aquatic animal species in the importing country are susceptible to the pathogens that imported animals may carry, or whether the imported animals

are susceptible to resident pathogens, before such unintended "field trials" are conducted (Bernoth *et al.* 2008).

According to DIAS (2008), data on the reasons given for introducing marine and brackishwater species, introductions of new species for aquaculture development (701 of 1738 cases, 40.3%) is most frequently cited, followed by capture fisheries development (346 cases, 19.9%). Other reasons cited include accidental releases (6.3%), diffusion (5.2%), biocontrol (5.1%), ornamentals (3.0%), research (2.9%), other reasons (2.7%) and unknown (14.4%).

The disease risk inherent in the translocation of live aquatic animals has been well known and documented especially because of past experience and devastating socio-economic consequences (Bondad-Reantaso *et al.*, 2005). The Office International des Épizooties (OIE, the World Organisation for Animal Health) lists 30 pathogens/ diseases of finfish, molluscs and crustaceans that fit the criteria of being of socio-economic and/or public health importance and significant in the international trade of aquatic animals and aquatic animal products (OIE 2002). At global level the number of countries providing estimates of losses due to disease is increasing and the combined estimated losses in production value due to shrimp diseases from 11 countries for the period 1987 to 1994 (i.e., Chinese Taipei-1987, Philippines-1989, Indonesia-1991, China- 1992, Ecuador-1992, USA-1993, Bangladesh-1994, India-1994, Mexico-1994, Thailand-1994 and Vietnam- 1994) were in the order of US$ 3,019 million (Israngkura and Sae-Hae 2002). Some of these movements are regarded as having caused not just localised outbreaks but even pandemics, for example, furunculosis, crayfish plague and epizootic ulcerative syndrome (Roberts, 2003). Examples of such spread in the Asian region in recent years are koi herpesvirus disease (KHVD), WSS and Taura syndrome. KHVD was first reported in Israel and United States in 1998. In the Asia-Pacific region, infection with koi herpesvirus (KHV) has been reported from China, Indonesia, Japan, Republic of Korea, Philippines, Singapore, Taipei China and Thailand

(NACA/FAO, 2005a,b; OIE, 2005a,b). Taura syndrome was first reported in shrimp farms in Ecuador in 1991-1992 and spread throughout the America, Indonesia, Malaysia (suspected), Myanmar, Taipei China, Thailand and Vietnam (OIE, 2005 a, b; NACA/FAO, 2005a,b) through shipments of infected post-larvae and broodstock, causing mass mortality of cultured shrimp. The spread of WSSV infection throughout most of Asia during the mid-1990s and subsequently to the Americas from 2001 was explosive and was almost certainly the consequence of a prolific international trade in live shrimp and other crustacean seed and broodstock (Lightner *et al*, 1997).

2.7 INTRODUCTION & IMPACT OF *L.VANNAMEI* IN OTHER COUNTRIES

Introduction of non-native species into ecosystems that may have both negative and positive impact(s) to the environment. However, introduced species both intentionally and unintentionally can also result in the loss of native species, changes in community structure and function, and alterations of the physical structure of the systems(Lambert *et al*, 1992; Ceccherelli *et al*, 2000; Grosholz *et al*, 2000).

2.7.1 Taiwan

The first commercial shipment of SPF *L. vannamei* broodstock from the Americas to Asia was from Hawaii to Taiwan Province of China in 1996, but in early 1999 TSV began to cause significant (80 % in 3 days) mortality of juvenile shrimp in ponds in Taiwan Province of China (Tu *et al.*, 1999; Yu and Song, 2000; Wyban, 2002). Thus, importation of TSV-contaminate *L. vannamei* post larvae or spawners was probably the main origin of the TSV outbreak in Taiwan (Tu *et al.*, 1999).

2.7.2 Korea

The nucleotide sequences of the partial capsid protein VP1 of 2 Korean isolates were 99% identical to each other and 96 to 99% identical to those of TSVs isolated from the Americas, Taiwan, and Thailand which suggests the possibility that TSV has been introduced via the imported stock of *L. vannamei*.(Do *et al.*, 2006)

2.7.3 Indonesia

The Pacific white shrimp (*Litopenaeus vannamei*) was unofficially introduced to Indonesia in 1999 into Java island, and received government approval in 2001. Taura syndrome (TS) disease was detected in Indonesia in 2002 and the disease currently affects at least ten provinces. Infectious myonecrosis (IMN) is an emerging *L. vannamei* disease, first detected in Indonesia in May-June 2006 (Taukhid and Nuraini 2001). Similar genome sequences of IMNV from Indonesia and Brazil may be yet another example of the unfortunate transfer of shrimp pathogens over large geographical distances by careless movement of contaminated stocks for aquaculture (Flegel, 2006; Senapin *et al.*, 2007).

2.7.4 Brazil

Brazil began importing non-indigenous shrimp in 1980 and *L. vannamei* and *P.stylirostris* in 1983 from all over Latin America. This resulted in the introduction of various viral diseases including IHHNV, TSV and NHP. Recent studies have revealed geographic variations in IHHNV isolates, which suggested that the Philippines were the source of the original infection of IHHNV in Hawaii, and subsequently in most shrimp farming areas of Latin America (Tang *et al.*, 2002).

2.7.5 Thailand

American White Shrimp was first introduced into Thailand in 1999 from Hawaii, Mexico and China (Briggs *et al.*,2004) .The Shrimp fry was illegally brought for culture to Surajthani province, Southern Thailand and the culture was reported successful. Since 2001, however the fry are illegally brought into the country for culture from time to time until 2002. Following the outbreak in 2003, the Taura syndrome virus (TSV) and IHHNV was detected in *L. vannamei*, and *Penaeus monodon* (Nielson *et al*, 2005; Phalitakul *et al.*, 2006).

2.8 RISK ANALYSIS

Risk analysis is an old concept newly applied to aquatic animal health to assess disease introduction through international trade in animals and animal products, i.e. import risk analysis (IRA) (MacDiarmid 1997; Arthur et.al 2004; Bondad-Reantaso, 2004; Peeler *et al.*, 2007). The purpose of risk analysis is to provide a structured, internationally agreed-upon means to assess disease risks objectively and transparently so that (i) the risks that serious pathogens and diseases will be transferred between trading partners are minimized, (ii) applied sanitary measures (e.g., restrictions on species and/or sources of origin, health certification requirements, quarantine, treatment, etc.) can be justified; and (iii) restrictions to trade are minimized (WTO, 1994).

To reduce and hopefully eliminate the accidental transfer of pathogens resulting from aquaculture activities, various national and international organisations *viz.* FAO, the OIE, WTO, NACA, ASEAN, ICES SEAFDEC and the GAA have described codes of conduct and management guidelines (BMPs) supported by disease surveillance protocols and tools for transboundary importation of alien shrimp and their subsequent culture (Briggs *et al.*, 2005).

The key advantage to using risk analysis is that it provides a comprehensive and disciplined framework for decision-making that produces transparent and

defensible results. Guidelines for IRA provided by the OIE (O.I.E., 2009), for example, stipulate that data used and assumptions made in an analysis are comprehensively documented to ensure rigour and transparency. There are two components to a hazard which contribute to its risk: its likelihood and the consequences resulting from the hazard occurring (Mark *et al.,* 2011).

2.9 DISEASES OF PENAEIDS

Shrimp diseases have increased steadily over the past decade and is now perhaps the largest obstacle to sustainability of the industry (Rosenberry, 1993)

2.9.1 VIRAL DISEASES

In 1989, six viruses were known to affect penaeid shrimp, but by 1997 more than 20 viruses were identified as having affected wild stocks and commercial production (Fulks *et al,* 1992; Bower *et al,* 1994; Hernandez-Rodriguez *et al.*, 2001). The OIE now lists seven viral diseases of shrimp in the Aquatic Animal Health Code (OIE, 2003), which are considered to be transmissible and of significant importance to socio-economic and/or public. These viral diseases are: white spot disease (WSSD), Yellowhead disease (YHD), Taura syndrome virus (TSD), spawner-isolated mortality virus disease (SMV), tetrahedral baculovirosis (*Baculovirus penaei* - BP), spherical baculovirosis (*Penaeus monodon*-type baculovirus) and Infectious hypodermal and haematopoietic necrosis (IHHNV) (OIE, 2003). *L.vannamei* are known to be carriers of the following viral diseases: WSSV, BPV, IHHNV, Reo virus, LOVV and TSV (Overstreet *et al.*, 1997; Flegel, per. com.). *Penaeus monodon* are known carriers of: WSSV, YHV, MBV, IHHNV, BMNV, GAV, LPV, LOVV, MOV and REO (Lightner, 1993; Flegel, 2003).

2.9.1.1 Baculoviral Midgut Gland Necrosis Virus (BMNV)

BMNV is a type C, non-occluded gut infectious baculovirus (72×310 nm) causing Baculoviral Midgut Gland Necrosis (BMN) as serious epizootic in

hatchery reared penaeids. The cumulative mortality reaches upto 100%. Severely affected PL are easily distinguished by inactive floating on surface exhibiting white turbid hepatopancreas (mid gut). The virus have been reported from wild and cultured Penaeus sp. in Japan, Korea, Australia, Indonesia, and Philippines (Shankar and Mohan, 2002).

2.9.1.2 Monodon Baculovirus (MBV)

MBV is a type A Occluded baculovirus, (42×246 nm) having ds DNA. The virus is wide spread all over the world. Moderate to heavy infection affecting hepatopancreas and anterior midgut of all stages except nauplius and protozoea stage are reported (Shankar and Mohan, 2002).

2.9.1.3 *Taura Syndrome Virus (TSV)*

Taura Syndrome Virus was first identified from farms around the Taura river in Ecuador in 1992(Jimenez, 1992; Brook *et al.,* 1995) and hence given the name. Taura syndrome (TS) is a virus disease of penaeid shrimp caused by ss RNA picorna virus (30-32 nm) (Lightner, 1996; Bonami *et al.,* 1997; Fauquet *et al.,* 2001). TSV is widely distributed in the shrimp farming regions of America and South East Asia (Brock, 1997; Hasson *et al.,* 1999; Bondad- Reantaso *et al.,* 2001; Chang *et al.,* 2004). The biggest concern to Asian countries already or currently wanting to import *L. vannamei* is the possibility of introducing TSV.

TS has three distinct phases, acute, transition and chronic (Lightener, 1996; Hasson *et al.,* 1999). Acute phase displayed by moribund *L.vannamei* include expansion of red chromotophores giving an overall pale reddish coloration and making the tail fan and pleopods distinctly red with 80- 95% mortality; hence referred as 'red tail' diseases (Lightner *et al.,* 1995). In transition and chronic phase there are no pathognomic lesions and molecular and antibody-based methods of diagnosis is necessary (Lightner, 1996). The prevalence of TSV has been found to range from 0 to 100% (Brook, 1997; Laramore, 1997; Jimenez *et al.,* 2000).

2.9.1.4 *Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV)*

Infectious Hypodermal and Haematopoietic Necrosis (IHHN) is caused by Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV), a small (22nm), ss DNA parvovirus(Lightner, 1983; Bonami *et al.*, 1990; Bonami and Lightener, 1991; Lightner, 1993). IHHNV is now commonly found in cultured and wild Penaeid (Brook and Lightner, 1990; Owens *et al.*, 1992; Lightener, 1996). It has been reported from Pacific coast of Latin America from Mexico to Peru and Indo-Pacific region, but not yet from the Atlantic coast of the Americas (Brock and Main, 1994; Lightner, 1996; Bondad-Reantaso *et al.*, 2001). IHHNV was first discovered in *L. vannamei* and *P. stylirostris* in the Americas in 1981, starting in Hawaii and had significant negative consequences for cultured *L. vannamei* in the Americas during the 1990s (Lightner, 1996a; Lightner, 2002). In *L.vannamei* IHHNV causes the chronic disease called the runt-deformity syndrome (RDS) in which irregular growth and cuticular deformities, rather than mortalities are the principle effects (Kalagayan *et al.*,1991; Browdy *et al.*,1993; Castille *et al.*, 1993; Bray *et al.*, 1994).

IHHNV has probably existed for some time in Asia without detection due to its insignificant effects on *P. monodon*. Recent studies have revealed that Philippines were the source of the original infection in Hawaii, and subsequently in most shrimp farming areas of Latin America (Tang *et al.*, 2002). IHHNV is being increasingly reported from India too, both from *P.monodon* hatcheries and farms.

2.9.1.5 *White Spot Syndrome Virus (WSSV)*

White Spot Syndrome is a viral disease of penaeid shrimp caused by infection due to White Spot Syndrome Virus (WSSV). WSSV is a large double-stranded DNA non- occluded baculovirus (Lightner, 1996). In Asia (since 1992) and Latin America (since 1999), this virus is now and has been the most serious threat facing the shrimp farming industry. It is an extremely virulent pathogen with a large number of host species (Flegel *et al.*, 1997; Lightner and Redman, 1998b).This disease is probably the major cause of direct losses of up to US$1

thousand million per year since 1994 in Asia (Briggs *et al.,* 2005). Similar problems have occurred throughout Central and South America, with the exception of Brazil and Venezuela, which remain WSSV-free due to the prompt and effective closure of their borders to all crustacean imports in 1999. Prevalence is highly variable from <1% in infected wild population to upto 100% in captive population (Lo and Kou, 1998).

WSSV infects many types of ectodermal and mesodermal tissues especially the cuticular epithelium and subcuticular connective tissues (Momoyama *et al.*, 1994; Wongteerwsupaya *et al., 1995).*

Regardless of the origin, isolates of WSSV have shown little genetic or biological variation,

suggesting that the virus emerged and was spread from a single source (Lightner, 2002). WSSV was first reported in farmed *M. japonicus* from Japan in 1992/93, but was thought to have been imported with live infected PL from Mainland China. (Briggs *et al.*, 2004). White spots (3mm dia) embedded within exoskeleton is the most commonly observed clinical sign and mortality rates reaches upto 100% in 3-10 days. High degree of colour variation with predominance of reddish or pinkish discoloration are seen in diseased population which show lethargic behaviour and cumulative mortality typically reaches 100% within two to seven days of infection (OIE, 2009).

2.9.1.6 *Yellow Head Virus (YHV)*

YHV infect cultured shrimp from late PL stage onwards. Cessation of feeding, congregation at pond edges, generally bleached appearance and discoloration of hepatopancreas are features of YHV outbreaks. YHV is a rod shaped virus measuring 44×173 nm.(Limsuwan, 1991; Chantanachookin *et al.,* 1993) .YHV out breaks have been reported only in *P.monodon* and *L.vannamei* (Boonyaratpalin *et al.,* 1993; Chantanachookin *et al.,* 1993; Sittidilokkratna *et al.,* 2009).

YHV can cause 100% mortality in infected ponds within 3-5 days of first appearance of clinical signs (Chantanachookin *et al.,* 1993). It has been reported in China, India, Indonesia, Malaysia, Philippines, Sri lanka, Thailand and Vietnam (Wang *et al.,* 1996; Lightner, 1996; Albaladejo *et al.,* 1998; Mohan *et al.,* 1998; Wang and Chang, 2000). Yellow Head Virus was the first major viral disease problem to affect Asian shrimp farms when it was diagnosed as causing extensive losses in Thailand starting in 1990/91. YHV and its close relatives Gill associated virus (GAV) and Lymphoid Organ Vacuolization Virus (LOVV) are single strand RNA viruses, similar to TSV. (GSMFC website; Flegel *et al.*, 1997; Lightner and Redman, 1998b).

2.9.1.7 *Lymphoid Organ Vacuolization Virus (LOVV)*

Lymphoid Organ Vacuolization Virus was first noted in *L. vannamei* farms in the Americas in the early 1990s (Brock and Main, 1994). It was later discovered in Australia, along with the other TSV-like virus GAV (Lightner and Redman, 1998b). An RNA viral pathogen very similar to LOVV in *L. vannamei* has recently been discovered in Thailand in the lymphoid organ of *P. monodon* (Lightner, 2002).

2.9.2 BACTERIAL DISEASES:

Many different forms of bacteria can potentially infect shrimp, frequently as opportunistic follow-on to viral infection or environmental stress. Most of the disease outbreaks to date have been concentrated in the following categories: Vibrio bacteria including luminous bacteria, *Leucothrix sp* and *Thiothrix sp*. Many of these bacteria can infect a broad range of shrimp species and exist naturally all over the tropics (Fulks *et al*, 1992, Bower *et al,* 1994).

2.9.2.1 Vibriosis

Vibrio spp. are among the chitinolastic bacteria associated with shell disease (Cook & Lofton 1973) and responsible for mortality of cultured shrimp

worldwide (Lightner & Lewis, 1975; Adams, 1991; Lightner *et al.*, 1992; Lavilla-Pitogo *et al.*, 1996; Chen *et al.*, 2000). Outbreaks may occur when environmental factors trigger the rapid multiplication of bacteria already tolerated at low levels within shrimp blood (Sizemore & Davis, 1985), or through gills as they are covered by thin exoskeleton (Taylor & Taylor, 1992), or by bacterial penetration through wounds in the exoskeleton or pores (Jiravanichpaisal & Miyazaki, 1994; Alday-Sanz *et al*,. 2002).

Vibrio species are part of the natural microflora of wild and cultured shrimps (Sinderman, 1990). They become opportunistic pathogens when natural defence mechanisms are suppressed (Brock and Lightner, 1990). Major epizootics of vibriosis mostly caused by a number of Vibrio species of bacteria, including: *V. harveyi, V. vulnificus, V. parahaemolyticus, V. alginolyticus, V. penaeicida* (Brock and Lightner, 1990; Ishimaru *et al.*, 1995) have been reported for *P. monodon* from the Indo-Pacific region, *M. japonicus* from Japan, and *L. vannamei* from Ecuador, Peru, Colombia and Central America. There have been occasional reports of vibriosis caused by *V. damsela, V. fluviatalis* and other undefined vibrio species (Lightner, 1996).

According to Jayasree *et al* (2006), occurrence of five types of diseases: tail necrosis, shell disease, red disease, loose shell syndrome (LSS) and white gut disease (WGD) by Vibrio spp. in *Penaeus monodon* is noticed from culture ponds of coastal Andhra Pradesh. Among these, LSS, WGD, and red disease caused mass mortalities in shrimp culture ponds. Six species of Vibrio- *V. harveyi, V. parahaemolyticus, V. alginolyticus, V. anguillarum, V. vulnificus and V.splendidus* are associated with the diseased shrimp.

Adult shrimps suffering vibriosis may appear hypoxic, show reddening of the body with red to brown gills, reduce feeding and may be observed swimming lethargically at the edges and surface of ponds (Anderson *et al.*, 1988; Nash *et al.*, 1992). Vibrio spp. also cause red-leg disease, characterised by red colouration of

the pleopods, pereopods and gills, in juvenile to adult shrimps and may cause mortality of up to 95% during the warm season (Chen, 1992). Eyeball necrosis disease is caused by *V. cholerae.* The eyeballs of infected shrimps turn brown and fall away and mortality occurs within a few days (Chen, 1992).

2.9.2.2 Black gill disease

Black gills in shrimp can be caused by accumulation of debris in gills, bacteria or fungus Fusarium sp. This disease is epizootic and can cause mass mortalities in the initial stages of this disease, the gills turn orange-yellow or light brown. Eventually, the gills turn darker until they are black (Felix *et al.,* 2001).

2.9.2.3 Black or Brown Spot disease

It is also called as shell disease, rust disease, burned spot disease and necrosis. Infestation is caused by bacteria belonging to *Vibrio, Aeromonas and Pseudomonas.* All stages of life are affected by this disease. Gross signs are appearance of brownish to black erosion of carapace, abdominal segments rostrum, tail, gills and appendages (Lightner *et al.,* 1992).

2.9.2.4 Filamentous bacterial infection

The bacteria in this infection is *Leucothrix* species, which affect larvae, PL, juveniles, and adults. Signs are the presence of fine colourless, thread-like growth on the body surface and gills. Interference with locomotory and moulting process results in heavy mortality in post- larvae (Felix *et al.,* 2001).

2.10 MOLECULAR DIAGNOSIS FOR SHRIMP DISEASES

Serology cannot be used to detect existing or prior infections with shrimp viruses because of the absence of adaptive immunity in invertebrates (Fearon and

Locksley, 1996). To control and prevent diseases, molecular methods are finding increasing application for differential diagnosis, epidemiological investigations and screening of infections in hatcheries and on farms. Methods such as the polymerase chain reaction (PCR), dot-blot hybridisation (DBH) and *in situ* hybridisation (ISH) have now been developed for a wide range of shrimp viruses and bacterial pathogens (Lightner and Redman, 1998). Virus propagation *in vitro* has had very limited application because of the absence of suitable cultured cell lines. Thus PCR would be a very useful tool for rapid and sensitive detection of pathogens in shrimp. DNA-based detection methods for the most important viral pathogens (IHHNV, HPV, SMV, TSV, YHV, GAV/LOV, WSSV, MBV, and BP) have been reported in the literature and some DNA based diagnostic methods are commercially available (Lightner, 1999). PCR and RT-PCR methods are important in helping to control the spread of major shrimp disease agents, but they have the disadvantage of requiring sophisticated equipment and highly trained personnel (Flegel *et al.*, 2008).

Introduction of *L.vannamei* in India provides significant economic and other benefits to those directly involved in the trade, as well as to governments and to the public. It is therefore realistic to accept that the trade will continue due to advantages over *P.monodon*.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Survey of *L.vannamei farms*

Survey of 32 *L.vannamei* farms (Plate 3) in Gangapatnam, Mudivarthi, Nedumusali and Utukur (Nellore district, Andhra Pradesh) was done with the help of a questionnaire (Appendix 1), to find out the level of acceptance by shrimp farmers. A comparison of *L.vannamei* culture practice with that of *P.monodon* was made.

3.1.2 Sample Collection

Shrimp samples (*Plate 4)* for the present study were collected from four places of Nellore, Andhra Pradesh *viz.,* Gangapatnam, Mudivarthi, Nedumusali and Utukur. Plates 1 & 2 show the location and area of sampling. Collection was carried out twice in a year (ie. during July 2010 and June 2011). A total of 127 samples were collected that comprised 39 samples from Gangapatnam, 37 from Mudivarthi, 36 samples from Nedumusali, and 15 from Utukur.

The shrimp samples were collected in self sealing polythene bags and were transported to the lab in insulated box filled with ice gel packets and ice. Direct contact between ice and the shrimps was avoided and special care was taken to bring the samples to lab in ice. The samples, on arrival in the lab were packed into small quantities, labelled properly and then stored in a deep freezer (-20°C). Care was taken to minimise repeatedthawing and freezing of the isolates.

Andhra Pradesh

Nellore

Plate 1 *India Map showing location site*

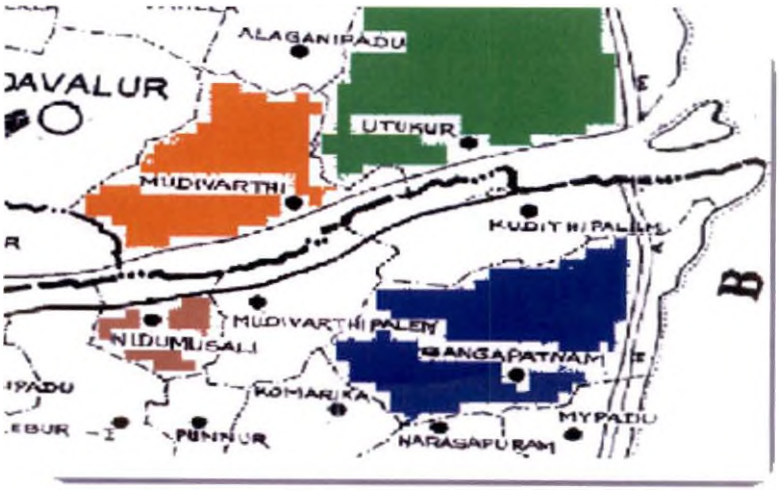


Plate 2 *Map showing area of sampling*

Plate 3 *L.vannamei pond*

Plate 4 *L.vannamei (healthy specimen)*

Redman, 1998; Lo *et al.*, 2007) in disease screening studies. Frozen samples were thawed and tissue of 40mg was minced properly using a tissue grinder and placed in lysis buffer (1ml). The lysis buffer was prepared by mixing 2M Tris, 0.5M EDTA and sterilized by autoclaving. Then 10% SDS was added after dissolving it by heating to 60^0 C. Proteinase K @ 10µg/ml (5µl) was added and the tissue was incubated at 65^0 C for 2 h.

The DNA was then isolated by adding equal volume (500µl) of phenol: chloroform: isoamyl alcohol(25: 24:1) to the tubes, as reported by Taggart *et al.*(1992) and Sambrook and Russell (2001). After inverting the tubes a few times, they were centrifuged for 5 min at 9,000g. The top aqueous layer was recovered into a new tube and 500µl of chloroform: isoamyl alcohol (24:1) was added. After mixing properly it was centrifuged at 9,000g for 5 minutes. The supernatant recovered was transferred into a fresh tube. Then, 0.1 volume of 6M NaCl and 3 volumes of absolute alcohol were added to precipitate the DNA, inverting the tubes several times. The DNA precipitate was recovered by centrifugation at 12,000 g for 10 minutes. After discarding the supernatant, the DNA pellet was washed briefly in 70% ethanol, air dried and resuspended in 50µl nuclease free distilled water. After completely dissolving, the DNA was stored at - 20^0C. A spectrophotometer (Biophotometer plus, Eppendorf) was used to evaluate the total amount of obtained DNA and to analyse the purity of DNA. Samples are considered to be of adequate purity if A_{260}:A_{280} >1.5 was obtained.

3.2.2 RNA EXTRACTION FROM SHRIMP TISSUE

Pleopods were used as target tissue in RNA extraction for diagnosis of TSV (OIE, 2009). 40-50 mg of pleopods was homogenized quickly in 1ml of trizol reagent (Sigma-Aldrich Co., USA) using RNAse free tissue grinder. After incubation at room temperature for 5 min, 200µl chloroform was added to the sample and mixed well by inverting the tubes up and down for 15 seconds. Kept for 2-3 minutes and then centrifuged at 13,000 rpm for 15 min at 4^0C. The

supernatant was carefully removed to a fresh tube and the RNA precipitated with 500µl isopropyl alcohol. The tube was mixed gently and kept at -20^{0}C overnight for RNA precipitation. It was then centrifuged at 13,000 rpm for 15 min at 4^{0}C and the supernatant discarded. The pellet was washed with 1ml of ice-cold 75% ethanol and then centrifuged at 9300 rpm for 5 minutes. The supernatant was decanted and the pellet air dried. The isolated RNA was then dissolved in 50µl sterile distilled water and stored at -80^{0}C (Pillai *et al.*, 2006).

3.2.3. PCR ANALYSIS FOR WSSV

The PCR protocol described by Lo *et al.* (1997) was used in the present study for WSSV detection. Initially, a master mix was prepared in a PCR tube containing 1X buffer, Primers (0.5 µM), *Taq* enzyme (1.5U), dNTPs (200 µM each) and water. The primer sequences used were as follows: Forward primer 5' GAC-AGA-GAT-ATG-CAC-GCC-AG 3' and Reverse primer 5'ACC-AGT-GTT-TCG-TCA-TGG-AG 3'. 24 µl of master mix was aliquoted to each of the PCR tubes and 1 µl of DNA template (100 pg/l) was added. A known positive sample, negative sample and a 'no template' controls were included.

Reactions were performed in a thermal cycler (Biorad) using the following conditions: Initial denaturation for 4 min at 95^{0}C followed by 35 cycles of denaturation for 1 min at 95 ^{0}C; annealing for 1 min at 54 ^{0}C; extension for 2 min at 72 ^{0}C. The reaction ended with a final extension at 72 ^{0}C for 5 min. After completion of PCR run, agarose gel electrophoresis was carried out at 70 volts using 12 µl of sample (10µl of amplified sample DNA+ 2 µl of loading dye) in 1% agarose gel containing 0.05µg/ml of ethidium bromide. Gel Doc (Biorad) was used to visualise and analyse the DNA bands.

3.2.4 DIAGNOSTIC PCR FOR IHHNV

The method outlined by Nunan *et al.* (2000) was followed for analysis of IHHNV. The DNA was extracted using Phenol:Chloroform:Isoamyl method as described earlier. Intially, a 'master mix' of 24µl was prepared. The master mix consisted of sterile water, 1X PCR buffer, dNTPs (200 µM each), 0.5 µM of the two primers (Forward – 5' TTG-GGG-ATG- CAG- CAA- TAT-CT-3'; Reverse -- 5' GTC- CAT- CCA- CTG- ATC- GGA- CT- 3'), and Taq enzyme (1.5 U). For 25 µl of PCR reaction mixture, add 24 µl of reaction mixture and 1 µl of sample DNA template. A known positive sample, negative sample and a 'no template' controls were included. Reactions were performed in a thermocycler using the following conditions: 35 cycles of initial denaturation for 5 min at 95^0C; denaturation for 30 sec at 95 ^{0}C; annealing for 30 min at 55 ^{0}C; extension for 1 min at 72 ^{0}C with additional final extension for 7 min at 72 ^{0}C. After completion of cycle, agarose gel electrophoresis was done with 1% agarose gel. 12 µl of samples (10 µl amplified sample DNA + 2 µl loading dye) were loaded in the wells containing 0.05µg/ml of ethidium bromide to stain the DNA. DNA bands were visualised using a Gel-doc system (Bio-Rad).

3.2.5 RT-PCR ANALYSIS FOR TSV

The RT-PCR method used for TSV detection in the study followed the method used in Nunan *et al.*(1998). Initially, the RNA sample was subjected to reverse transcription. For this, 5 µl of reaction mixture consisting of 3 µl of RNA, 1 µl each of 0.5 µM of forward and reverse primer were added to PCR tubes and kept in thermocycler at 25^0 C for 10 min and 60^0 C for 15 min. Later, into the 5 µl of reaction mixture tube, 10X RT buffer (1 µl), dNTPs (200 µM each) (1.5 µl), 10 U/ µl RT enzyme (0.5 µl) and sterile water (2.0µl) were added. This reaction mixture was incubated in thermocycler at 42^0 C for 2 min and at 94^0 C for 2 min followed by sudden chilling at -20^0 C. The optimized master mix concentrations (final concentration in 25 µl total volume) for PCR used for detection of TSV in

shrimp samples (cDNA) were: primers (0.5 µM each), dNTPs (150 µM each), Taq polymerase enzyme (0.5 units), buffer (1X). For 25 µl of PCR reaction mixture, add 24 µl of reaction mixture and 1 µl of sample DNA template. The primers used were Forward -- 5' AAG TAG ACA GCC GCG CTT3' and Reverse – 5' TCA ATG AGA GCT TGG TCC 3'. A known positive sample, negative sample and a 'no template' controls were included. The PCR conditions used were: Initial denaturation at 95^0 C for 5 minutes; denaturation at 94^0 C for 45 sec; annealing at 60^0 C for 45 seconds; extension at 72^0 C for 45 seconds for 40 cycles ending with an additional elongation step at 72^0 C for 10 min. After completion of reaction, 12 µl (10 µl amplified sample DNA+ 2 µl loading dye) amplified product was analyzed by electrophoresis at 70 volts on a 1% agarose gel containing 0.05µg/ml of ethidium bromide to stain the DNA. The DNA bands were visualised using a gel documentation system (Gel Doc –Bio rad make).

3.2.6 PCR ANALYSIS FOR HPV

The PCR protocol and primer sets for HPV were described by Phromjai *et al.* (2002). The PCR reactions were carried out in a 25 µl reaction mixture that consisted of 1X PCR reaction buffer, 10 p mol each primer, 200 µM dNTPs, 0.9 units of Taq DNA polymerase, 2 µl of template DNA and sterile distilled water to adjust the volume to 25 µl. The cycling conditions for the reaction consisted of 30 cycles of initial denaturation at 95 ^{0}C for 1 min, annealing 60 ^{0}C for 1 min and extension at 72^0C for 1 min, followed by a final extension at 72^0C for 7 min. After completion of reaction, 12 µl each of the samples (10 µl amplified sample DNA+ 2 µl loading dye) were analyzed by electrophoresis on a 1% agarose gel wells containing 0.05µg/ml of ethidium bromide to stain the DNA. The samples were run for at constant voltage of 70 volts. DNA was visualised in the gel documentation system (Gel doc system, Biorad).

3.2.5. DIAGNOSTIC PCR FOR MBV

The kit supplied by M/s Bangalore Genei Pvt. Ltd., Bangalore was used to screen samples for the presence of MBV. The reaction conditions for PCR were: Initial denaturation for 5 min at 95^{0}C followed by denaturation for 30 sec at 95 ^{0}C; annealing for 45 sec at 55 ^{0}C and extension at 72 ^{0} C for 45 seconds (40 cycles) ending with an additional final extension at 72^0 C for 5 min. 12 µl samples (10 µl amplified sample DNA+ 2 µl loading dye) were analyzed by electrophoresis at constant voltage (70 volts) on a 1% agarose gel containing 0.05µg/ml of ethidium bromide and visualised in the gel documentation system.

3.2.6 METHODS FOR BACTERIAL ANALYSIS

3.2.6.1 Bacterial Isolation

For isolation of the bacteria present in diseased sample (*Plate 5),* Total Plate Count (TPC) method was followed as it gives an estimate of viable (live) cells in the sample. 12 g of the sample (muscle, gills, exoskeleton) was macerated well with 0.85% of saline (autoclaved). Serial decimal dilutions upto 10^{-6} was prepared for TPC sampling. 0.5 ml of each dilution was inoculated onto respective plates in duplicate using a sterile bent glass rod. After about 30 min the plates were incubated at 37°C overnight (18-24 h). After the incubation, characteristic colonies were inoculated into sterile nutrient broth and incubated at 37°C for 18-24 h. From broth the culture was inoculated onto nutrient agar plates and TCBS agar (Surendran *et al.,* 2006) and incubated at 37°C for 18-24 h

3.2.6.2 Bacterial identification

A series of biochemical reactions as described by Farmer and Hickman-Brenner (1992) was followed with some modifications. Only preliminary method was performed for identification of bacteria until genus level.

3.2.6.2.1 Gram Staining

Gram staining was performed using a staining kit (Nice Chemicals, Cochin). The slide was observed under oil immersion objective to record the gram reactions.

3.2.6.2.2 Motility test

Tryptone Soy Broth NaCl was used to find the motility of the bacteria. 16 to 18 hours grownculture of the isolates were tested for motility by hanging drop technique, using cavity slide.

3.2.6.2.3 Oxidase test

Oxidase reagent

N,N,N,'N'- tetramethyl-p-phenylenediamine 2HCL - 0.1g

Distilled water - 1ml

Whatman 6mm disc were sterilized in hot air oven at 140°C for 1 h. The disc was dipped in the oxidase reagent and allowed to absorb, dried and stored in dark bottle at 4°C.

Procedure

Cytochrome oxidase test was performed using pre-moistened filter paper strips soaked with 1% oxidase reagent. Young colonies from TCBS were spotted on to the disc with a glass rod. Development of dark purple colour within 10s indicates positive reaction.

3.2.6.2.4 Oxidation/ Fermentation test

Hugh- Leifson O/F media

Peptone 2g

Yeast extract	5 g
Nacl	20 g
Glucose	10 g
Bromocresol purple	0.015 g
Agar	3 g
Distilled water	100 ml

The ingredients were dissolved by boiling and the pH was adjusted to 7.4 ± 0.2 and dispensed in 5ml volumes, autoclaved at 110°C for 10 min.

Procedure

This test was conducted to test whether the isolates were fermentative, oxidative or inert. Two O/F tubes were inoculated by stabbing. One was overlaid with paraffin. Glucose is utilized by fermentation in the tube with the over layer by oxidation in the other tube. Fermentative or oxidative activity was recorded by change in colour of the medium from purple to yellow due to acid production from glucose. Inert organisms fail to produce acid and hence media colour remains unchanged.

3.2.6.2.5 Amino acid decarboxylase test

Basal medium for decarboxylase test

Peptone	5 g
Yeast extract	3 g
NaCl	15 g
Glucose	1 g
Bromocresol purple	0.016 g

The ingredients were dissolved and the pH was adjusted to 7.4 ± 0.2. The basal medium was divided into 4 parts and amino acid lysine, ornithine and arginine were added to each part at the rate of 1%. The fourth part served as control. The medium was dispensed in 3 ml volumes and autoclaved for 10 min at 110°C.

Procedure

Ability of the micro-organism to decarboxylase the amino acids such as lysine, ornithine and arginine was tested by inoculating test cultures into set of four test tubes, three containing each of the amino acids in separate tubes and a fourth one with only basal medium which served as control. After overlaying with sterile paraffin, all the tubes were incubated at 37 °C. When the colour of the medium changed to yellow and returned to purple, the reaction was recorded as positive, whereas the control tube without amino acid remained yellow.

3.2.7 Water Analysis

Water testing kit of Tanschem Agri.tech Ltd (Gujarat) and Biosol (A.A. Biotech, Chennai) was used to analyse water samples collected from the culture ponds. Instructions in the manual were followed to analyse pH, hardness, alkalinity and presence of ammonia in the collected water samples. Salinometer was used to measure salinity in the water sample.

3.2.8 Economic analysis

The economic indicators like gross profit, net profit, total cost /kg, revenue /kg, break even production, break even price, input- output ratio, return on investment cost and return on operating cost were calculated (Shang, 1990). Interest was calculated at 7% on the cost of investment items (sluice, generator, aerator, shed and pump). In owner operated farms, opportunity cost was taken and in leased farms lease amount was taken. Variable cost includes feed, seed, labour, chemicals, fuel, electricity charges, repair charges etc.

Fixed cost = Lease / opportunity cost + interest + depreciation

Depreciation = Cost of investment item / Life span

Total cost = Fixed cost + Variable cost

Gross profit = Revenue – Total variable cost

Net profit = Revenue – Total operating cost

Total cost per kg = Total cost / Total production

Revenue per kg = Total revenue / Total production

Break even production = Total operating cost / Unit price

Break even price= Total operating cost / Quantity produced

Input-output ratio = Total revenue / Total operating cost

Return on investment = Net profit / Total investment

Return on operating cost = Net profit / Total operating cost

RESULTS

RESULTS

4.1 Survey of *L.vannamei* farms

Thirty four *L.vannamei* farms were surveyed using the questionnaire prepared for the study (Appendix 1) and the following data were collected.

In Nellore district semi-intensive monoculture was followed in *L.vannamei* farms. Culture duration varied between 90 to 110 days, thus 2 cycles / year were being carried out from March-July and August-December. Pond preparation was similar to *P.monodon* culture. Bird fencing was observed only during initial phase of culture (when PL was stocked). 29 of aqua farmers procured SPF seed (from certified hatcheries) and stocked them at a density of 20/m^2. Commercial starter and grower type of pellet feed with lower protein levels were used. The percentage of protein ranged from 32-35%. Check tray (1-2/ha), line feeding and broadcasting were the feeding methods followed by farmers. By this, they were able to obtain growth of 2-2.5 g/ week. In most of the farms, the source of water was bore well, although some used creek water. There was no water exchange in most of the farms during the culture period. These farms used probiotics once in 10 days. However, in some farms water exchange was done after 2 months of stocking and further water exchange was done only if required. Chemicals like lime, superphosphate, agricultural lime and zeolite were used to maintain water quality management. Some farmers used cow dung as fertilizer to enhance plankton production. As stocking density is very high, aerators are a necessity. A commonly encountered problem in *L.vannamei* culture was low dissolved oxygen levels in farms where sufficient number of aerators were not incorporated.

Among the farms culturing *L.vannamei,* only 33% had obtained permission from the Coastal Aquaculture Authority (CAA) for culture. The mandatory requirement of having biosecurity measures (fencing of the farm, reservoirs for water intake, bird scares/bird netting, separate implements for each pond) were followed only by 41% (Plate 3). A mere, 10% of the farms surveyed had the

facility for Effluent Treatment System, 53% practised chlorination and de-chlorination of water during water exchange and 38% farms maintained trained personnel. 22% had culture of indigenous species *P. monodon* in adjacent ponds. It was observed during the survey that only 84% procured tested SPF seed from certified hatcheries. While 90-92% depended on pelleted feed, only 80-85% of the farms surveyed carried out regular monitoring every 1-2 weeks (by experienced staff provided by the feed company itself).

Plate 6 L.vannamei pond without proper inlet and crab fencing

Plate 7 L.vannamei pond without crab fencing

4.2 Analysis of water quality parameters

Water samples collected from 34 farms were analysed for water quality and the results were as follows:

Table 1. Results obtained in water analysis.

PARAMETERS	RANGE
P^H	7.1 - 8.2
Alkalinity	75 - 225 mg/l as $CaCO_3$
Ammonia	0.1-0.2 ppm
Salinity	4- 35 ppt

4.3 Screening of the samples for viruses

Shrimp samples (*L. vannamei)* (127) collected from four different areas of Nellore, Andhra Pradesh *viz.,*.Gangapatnam, Mudivarthi, Nedumusali and Utukur were used in the present study. As mentioned earlier, samples were collected twice. During the first collection, (May - June, 2010), the shrimp samples looked healthy without any signs of disease (Plate 4). However, an year later during rainy season (June-July, 2011), mortality and signs of disease were being reported. Incidence of WSSV was reported in *L.vannamei* ponds that were adjacent to WSSV affected *P.monodon* culture ponds (Mohandas, per.com). Hence, a second phase of sample collection was done during July 2011. The shrimps in many of the farms were looking unhealthy. The shrimps were seen swimming along the sides of the pond during day, a clear sign of onset of disease problem. In the problematic ponds, shrimp died during moulting. The dead shrimp had empty gut, with light pink coloration over the abdomen and tail region (Plate 5). Mortality occurred in large numbers, almost doubling every day. Once these initial signs were observed i.e., shrimps swimming near aerators and dead pinkish colour shrimp near bunds farmers harvested their stock. A large amount of organic matter, blackish in colour, could be observed at the bottom of the pond during harvest.

Sixty samples were tested for the presence of WSSV, IHHNV, HPV, MBV and TSV viruses by molecular method. Being DNA viruses, PCR was carried out for WSSV, IHHNV, HPV and MBV, while RT-PCR was used for TSV as it is an RNA virus.

4.3.1 Screening for WSSV

The samples initially collected were screened for WSSV by single step PCR and those collected in the second phase, having an unhealthy appearance, were screened by 2-step PCR to detect the presence of the virus even if present in very low numbers. Fig. 1 shows the PCR analysis for WSSV. Lane 5 shows the positive control with the amplified DNA fragment at the expected molecular

weight of 650 bp. Absence of any band in the negative control in lane 4 indicates that there was no contamination during analysis. All the samples were negative for WSSV as no band was visible in any of the samples.

4.3.2 Screening for IHHNV

The samples initially collected were screened for IHHNV by single step PCR and those collected in the second phase, having an unhealthy appearance, were screened by 2-step PCR to increase the sensitivity of the reaction. Fig. 2 shows the PCR analysis for IHHNV. Lane 3 shows the positive control with the amplified DNA fragment at the expected molecular weight of 813 bp. Absence of any band in the negative control in lane 2 shows that there was no contamination during analysis. All the samples were negative for IHHNV as no band was visible in any of the samples.

4.3.3 Screening for HPV

The samples collected were screened for HPV by PCR to detect the presence of the virus. Fig. 3 shows the PCR analysis for HPV. Lanel shows the positive control with the amplified DNA fragment at the expected molecular weight of 414 bp. Absence of any band in the negative control in lane 3 confirms that there was no contamination during analysis. All the samples were negative for HPV as no band was visible in any of the samples.

4.3.4 Screening for MBV

The samples collected were screened for MBV by PCR to detect the presence of the virus. Fig. 4 shows the PCR analysis for MBV. Lane 5 shows the positive control with the amplified DNA fragment at the expected molecular weight of 480 bp. Absence of any band in the negative control in lane 6 indicates that there was no contamination during analysis. All the samples were negative for MBV as no band was visible in any of the samples.

4.3.5 Screening for TSV

The samples initially collected were screened for TSV by single step PCR and those collected in the second phase by 2-step PCR, to detect the presence of the virus even if present in very low numbers. Fig. 5 shows the PCR analysis for TSV. Lane 7 shows the positive control with the amplified RNA fragment at the expected molecular weight of 230 bp. Absence of any band in the negative control in lane 6 confirms that there was no contamination during analysis. All the samples were negative for TSV as no band was visible in any of the samples.

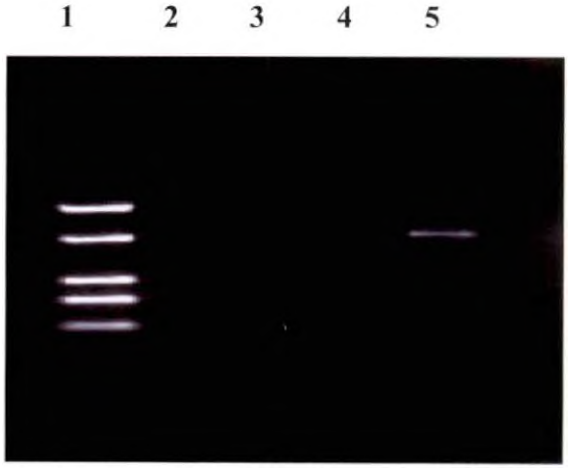


Plate No.8 **Agarose gel electrophoresis of samples screened for WSSV by PCR. 1% agarose gel. Lane 1- Mol. Wt. marker 1000bp ladder; Lane 2 and 3- representative samples; Lane 4- negative control; Lane 5- positive control (650 bp)**

1 2 3 4 5 6 7 8 9 10 11 12 13

Plate No.9 Agarose gel electrophoresis of samples screened for IHHNV in 1% agarose gel. Lane 1 to 10 representative of samples; 11- positive control (813bp); Lane 12-negative control; Lane 13 - Mol. Wt. marker (1000bp ladder).

1 2 3 4 5 6 7 8

Plate No.10 PCR analysis for HPV in shrimp samples. 1% agarose gel. Lane 1- Mol. Wt Marker (1000bp ladder) ; Lane 2- positive control (414bp) ; Lane 3- negative control; Lane 4 to 8- samples

Plate No.11 Agarose gel electrophoresis pattern (1% agarose) for MBV screening . Lane 1- Mol. Wt. Marker (1000bp ladder); Lane 2 to 4- samples; Lane 5- positive control (480 bp); Lane 6- negative control

Plate No.12 **Screening for TSV-Analysis of PCR products using 1% agarose gel. Lane 1- Mol. Wt. Marker (1000bp ladder); Lane 2 to 5- representative samples; Lane 6- negative control; Lane 7- positive control (230 bp)**

4.4 Incidence of bacteria

Since the shrimps collected during the second phase had obvious signs of disease, they were then screened for bacteria. White, smooth and slightly flattened colonies were observed on Total plate count agar. Single isolated colonies were cultured in nutrient broth and further streaked on nutrient agar and TCBS agar. Routine microbiological analysis was carried out and the results are interpreted in Table 1.

4.4.1 Gram reaction and motility test

The colonies observed in objective were gram negative short curved rods and actively motile.

4.4.2 TCBS agar

In the TCBS agar typical colonies which were large (2-3 mm dia), smooth, yellow, slightly flattened were observed.

4.3.3 Oxidase test

Dark purple colour developed on the oxidase disc indicated a positive reaction.

4.4.4 Oxidation/ Fermentation test

The change in the colour of the media from purple to yellow in both the tubes was recorded as positive reaction.

4.4.5 Amino acid decarboxylase test

In this test the media which changed to yellow and then returned to purple was interpreted as positive reaction. In lysine decarboxylase and ornithine

decarboxylase media, the yellow colour returned to purple, indicating a positive reaction while in arginine dihydrolase medium, yellow colour was retained indicating negative reaction.

Table 2. Summary of the results obtained in the biochemical tests conducted on the isolated bacteria

TESTS	TYPICAL REACTION
Gram stain	Gram (-) rods
Motility	Motile
Oxidase test	+
Oxidation/ fermentation test	+
Arginine dihydrolase	–
Lysine decarboxylase	+
Ornithine decarboxylase	+

4.5 Economic analysis of *L.vannamei farms*

Economic analysis of semi-intensive farming of *L.vannamei* was done based on the data collected from 34 farms on an annual basis.

Table 3. Economic analysis of *L.vannamei* farms (Annual basis)

INDICATORS	*L.VANNAMEI*
Yield (Kg / ha / yr)	15,941
Total cost (Rs / ha/ yr.)	21,92,114
Total revenue (Rs/ ha/ yr)	35,24,529
Profit (Rs / ha/ yr)	13,32,415
Total cost (Rs / kg)	151
Revenue (Rs / kg)	235
Profit (Rs / kg)	84
Break even production (Kg/ha)	9,842
Break even price (Rs/ kg/ ha)	151
Input output ratio	2.3
Return on investment	7.7
Return on operating cost	1.3

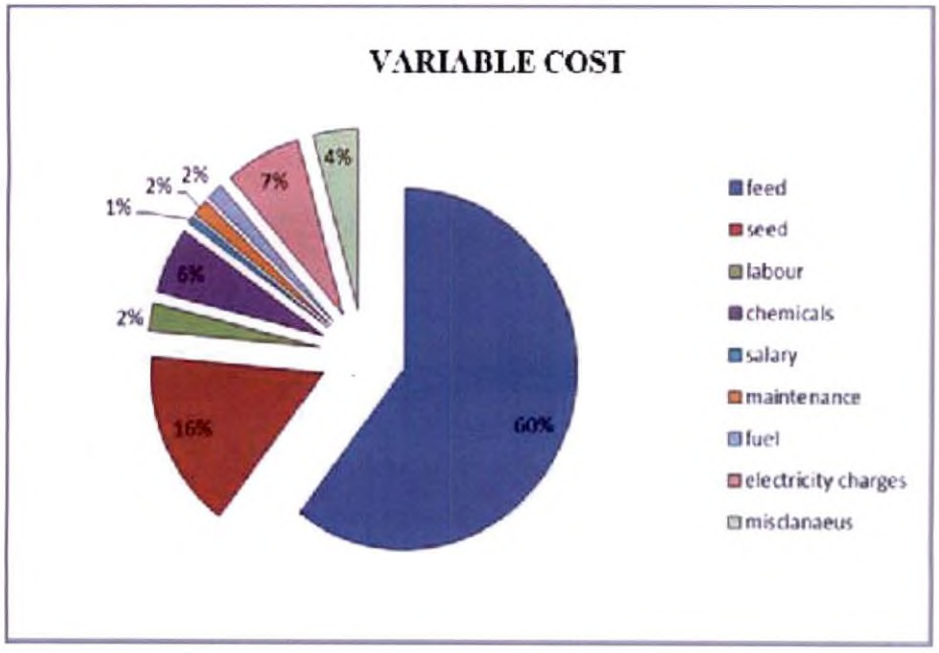


Fig. 1 Components of operating cost in *L. vannamei* farms

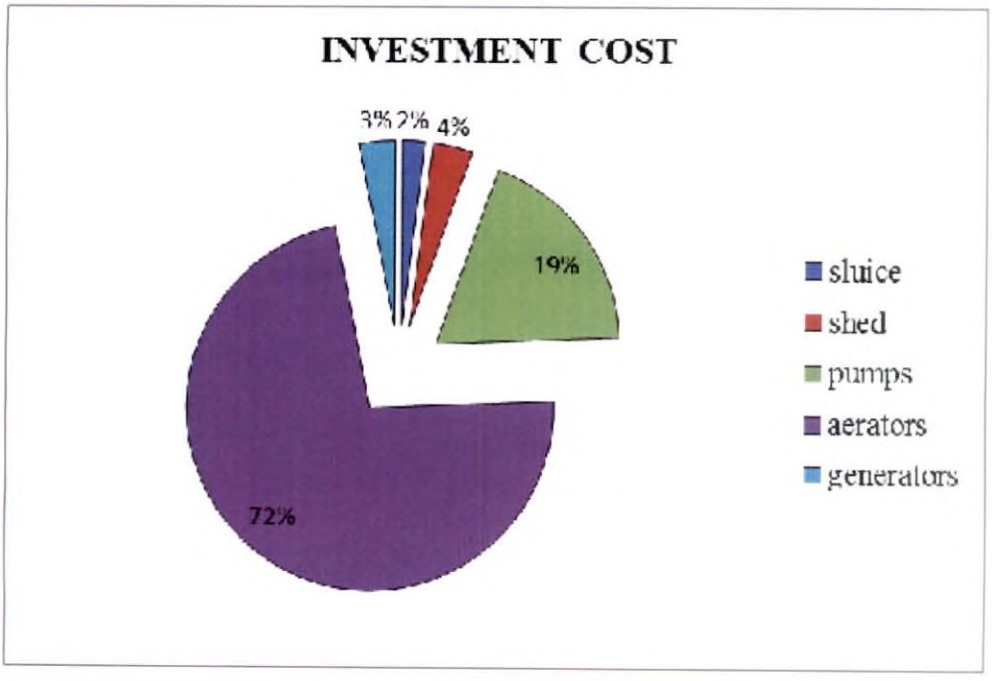


Fig. 2 Components of fixed cost in *L. vannamei farms*

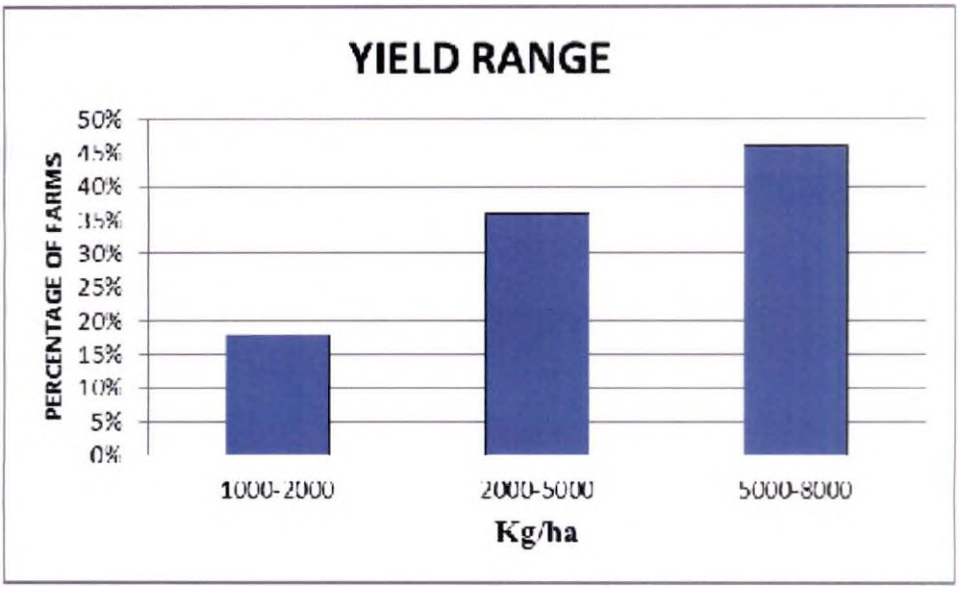


Fig. 3 Yield range in *L. vannamei farms*

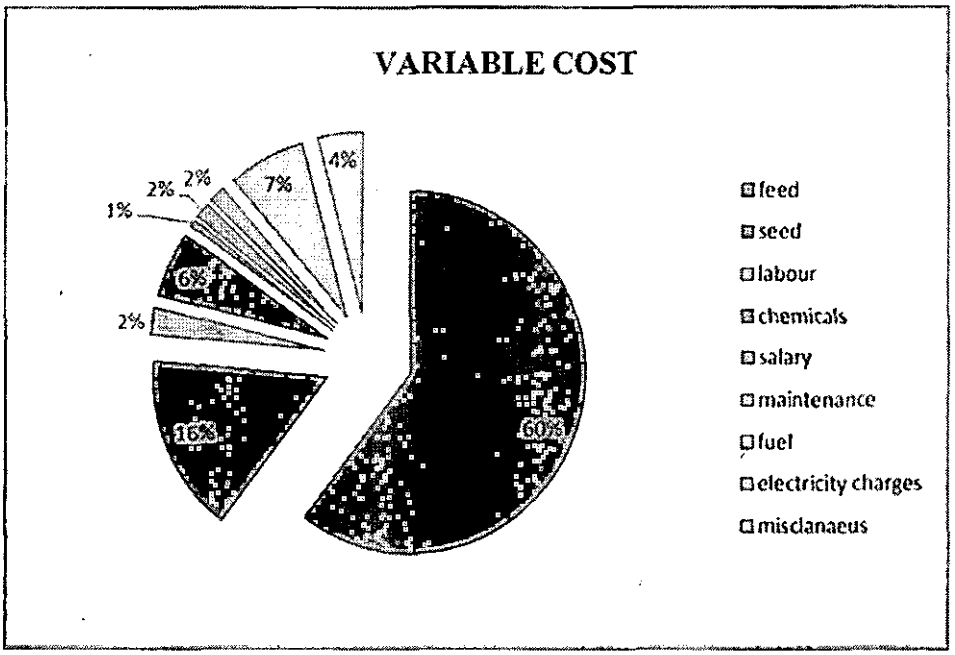
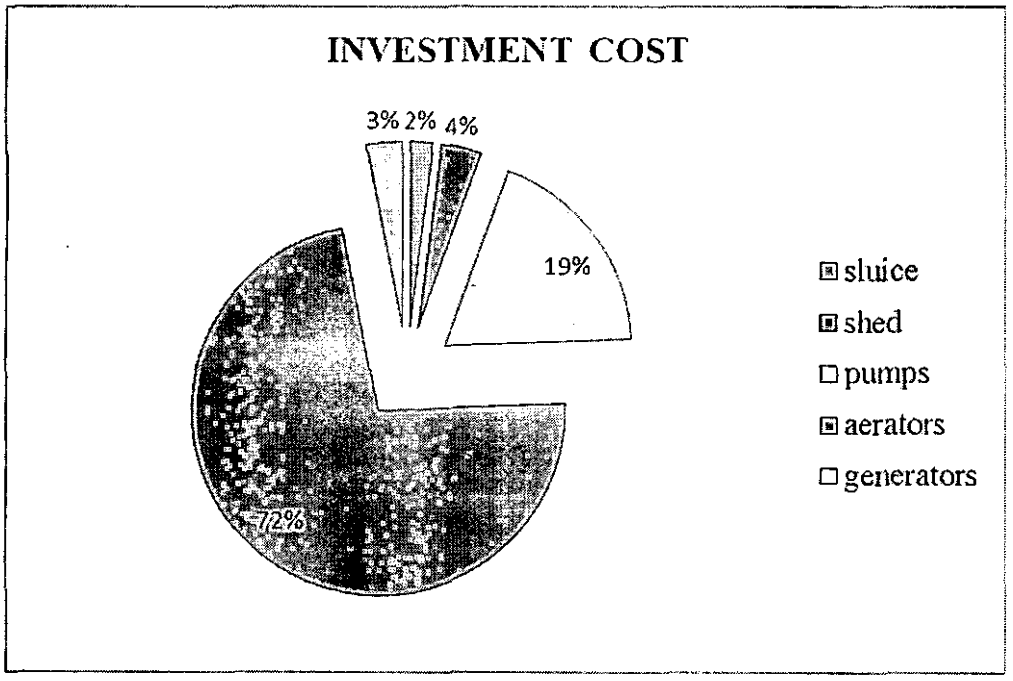


Fig. 1 Components of operating cost in *L.vannamei farms*



DISCUSSION

5. DISCUSSION

5.1 Survey of *L.vannamei* farms

P. monodon (tiger shrimp), a native species and backbone of shrimp industry in India is being increasingly replaced by *L. vannamei,* an exotic species, due to its positive traits over tiger shrimp, namely availability of SPF stock, less number of days of culture (DOC) and demand of American whiteleg in the international market. FCR of *P.monodon* is 1.5-1.8 while that of *L.vannamei* is 1.5-1.6.

Table 4. Comparison of *L.vannamei* and *P.monodon*

PRACTICES	*L.VANNAMEI*	*P.MONODON*
Stocking density	20/m^2	8/m^2
Aerators	8/ha	4-6/ha
Prophylactic measures	Reservoirs & treating ponds	Much less
Water exchange	No	Yes
Diseases observed	DO problem, WSSV	WSSV
Days of culture	90-110	120-130
Farm gate price	Rs 210 (30)/Kg	Rs 270 (30)/Kg

Of the 34 farms surveyed in the present study (which is only a small percentage of the total farms in Nellore district), it was seen that only 41% of these farms were following biosecurity measures. This clearly points to the risk faced by the shrimp farming industry. New diseases may emerge from mutations of previously non pathogenic organisms. Although 84% of the farms procured tested SPF seed from certified hatcheries, about 16% of farmers are culturing non-SPF stock indicating that there are chances of introduction of alien pathogens and it is realistic to accept that once an aquatic animal pathogen gets established in a new location, it is very difficult to eradicate. For instance, in Thailand, TSV in wild populations of *P. monodon* and other species of shrimps confirmed that TSV has already spread into the Bangpakong river and the Gulf of Thailand through illegal introduction of alien species (Senanan *et al.*, 2009). Similarly, during early 1999, TSV was imported with wild brood stock from Latin America, Taiwan Province of China (Tu *et al.*, 1999; Yu and Song, 2000). An important characteristic of any transboundary animal disease is the speed with which it can spread to other farms, villages, districts, the entire country, and even beyond that country's borders and it is a permanent, non-reversible form of pollution. In the aquatic world, the situation is worse: aquatic pathogens spread quickly through waterways that know no political boundaries (Bernoth *et al.,* 2008). Bijukumar (2009) states that transboundary diseases due to introduction of exotic species is the second greatest biodiversity menace after environment pollution. When the patterns of spread of diseases and pathogens of shrimp are examined, especially those for viral pathogens, there is convincing evidence that most major disease outbreaks are associated with the movement of live shrimp (FAO, 2005)

Only 10% of the farms had the facility for Effluent Treatment System (ETS), which indicates that a very high percent (90%) of the farms dispose off waste/used water into open waters without proper treatment. This may eventually lead to introduction of alien pathogens and may affect the wild indigenous species. 53% are practising chlorination and de-chlorination of water during water exchange. If the alien shrimp *L.vannamei* gets introduced into open water, there

are all possibilities of competition among other indigenous species. The introduction of *Oreochromis niloticus* in the 1950s together with *Lates niloticus* allowed commercial fisheries to develop, but at the same time, the native tilapia disappeared from the reported commercial fisheries. At the same time release, of fish/shellfish into the wild can also have genetic impacts at three levels: on the individuals released, on specific native individuals or on closely related indigenous species, and the effects can be either direct or indirect (Dabbadie and Lazard 1999). It should be emphasised that environmental support is seldom accounted for in aquaculture production and generally not perceived in aquaculture management, although it is basic to the survival of the industry. Hence, if the ecosystem–industry interdependence is not recognised, aquaculture will be limited by the impact of its actions on the environment, as in the case of intensive shrimp farming (Emerson and Rajalakshmi, 2002).

84% of farms procured tested SPF seed from certified hatcheries but it may be pointed out that SPF animals are those which are free from specific diseases for which they have been checked. New, hidden or "cryptic" viruses may be present, which will/may escape detection. To cite an example, SPF shrimp shipped from Hawaii resulted in the contamination of shrimp in Brazil and Colombia with TSV (Brock *et al.,* 1997), since TSV was not identified/reported as a pathogen. Another matter of concern about SPF stock is that once this 'high health' shrimp moves out of healthy controlled biosecure conditions, it is vulnerable to pathogens in culture ponds that do not follow proper biosecurity norms.

It was observed in the present study that only 33 % of farmers have CAA permission for *L.vannamei* culture. Therefore, authorities cannot trace or take necessary action during the hours of crisis. Only a small proportion of financially stable farmers, are in a state to practise or implement biosecurity measures including ETS. If a potentially dangerous situation similar to the widespread disease outbreak due to WSSV is to be avoided, it may be necessary on the part of the government to extend financial assistance to poor and marginal farmers for establishing biosecurity and ETS facilities. Registration of all shrimp farms

should be made mandatory. In the present study, it was seen that most of the aqua farmers are ignorant of the threat caused due to illegal importations of exotic species and the necessity of implementing biosecurity and ETS in their farms. So, government should organise awareness programmes, aqua clubs etc., for the benefit and awareness of the aqua farmers.

Biosecurity in aquaculture production is a program for protecting cultured aquatic organisms from harmful effects of introduced diseases (Scarfe, 2003). Through implementation of biosecurity measures, the United States managed to eradicate WSSV from its shrimp culture industry in 1997 after initial losses (Lightner, 2002). This clearly suggests that by following best management practises we can reduce the incidence of diseases and can sustain the shrimp industry from economic losses. Scarfe (2003) also suggested that to be maximally effective, frameworks for aquatic animal biosecurity need to inter alia adhere to the principle of vertical (local, state, national, international) and horizontal (local to local, state to state, etc.) integration, application, and agreement (standardisation and harmonisation). The old adage of "prevention is better than cure" certainly holds true.

5.2 Water quality parameters

Alkalinity, pH and ammonia were found to be in normal range in all the water samples collected. However, the salinity in some of the ponds was observed to be as low as 4 ppt. *L.vannamei* tolerates a wide range of salinities, from 0.5-45 ppt, but grows particularly well at 10-15 ppt (Wyban and Sweeny, 1991).

5.3 Surveillance for diseases in *L.vannamei*

In the present study, 60 samples of *L.vannamei* were screened for viral and bacterial pathogens. No viral diseases have been diagnosed although there have been unconfirmed reports of WSSV incidence in farms culturing *L. vannamei* adjacent to *P.monodon* farms (Mohandas, per.com).

Opportunistic bacteria such as Vibrio sp. are common in shrimp culture ponds and they tend to cause disease when conditions are favourable for them. The organic load in shrimp culture ponds is quite often heavy. As a result, the bacterial load also increases alarmingly within a short time. At the slightest incidence of stress, the bacteria can then cause disease in shrimps. Problems due to low dissolved oxygen levels and signs of disease were particularly noticed in farms that did not have sufficient number of aerators. Thus, vibriosis observed in the present study is not surprising. As already mentioned, SPF shrimp are also susceptible to various pathogens once they leave the confines of the biosecure environment in which they were produced. Although the survey and disease surveillance done in the present study was on a limited scale, observations made during the study indicate that if the present conditions under which *L. vannamei* is being cultured in Andhra Pradesh continue, there is a potential risk of disease outbreak in the region.

Hence, disease surveillance should be an integral and key component of the government aquatic animal health services. This is important for early warning of diseases, planning and monitoring of disease control programs, provision of sound aquatic animal health advice to farmers, certification of exports, international reporting and verification of freedom from diseases (Baldock, 2004). A more structured and science-based approach to introductions and transfers of aquatic species is necessary, including the use of science-based risk assessment procedures (import risk analysis, IRA) prior to the trans-boundary movement of live aquatic animals or their products (Arthur and Bondad-Reantaso, 2004). The progressive exploitation of the ecosystem to expand the economy has made it necessary to redevelop the ecosystem in order to maintain the economy (Regier and Baskerville, 1986). Past experience has shown that good health management is a key to success in aquaculture. Preventing and controlling disease, avoiding the international and domestic spread of pathogens, developing and adopting better farm management practices, capacity building and targeted research for producing stress tolerant organisms are all essential to good health management in

collaboration with regulatory frameworks and enforceable laws (Subasinghe, 2005). It is realistic to accept that trade will continue to occur, and despite all precautions, diseases will continue to be spread internationally. However, the risk of this happening can be reduced, and the effects can be mitigated, if all parts of the chain of trade, from producer to international organisations, accept their responsibility to cooperate in providing an unbroken chain of biosecurity (Bernoth *et al.* 2008).

5.2 A COMPARISON OF THE ECONOMICS OF *L.VANNAMEI* CULTURE WITH THAT OF *P.MONODON*

A comparison of the economics of *L.vannamei* culture calculated from the questionnaire used in the present study (Appendix 1) with the results obtained in semi-intensive farming of *Penaeus monodon* by Sathiadhas *et al.* (2009) is given in Table 3. The total cost of production of whiteleg shrimp is 2.3 times more than that of tiger shrimp. The investment requirements like aerators and generators are more in whiteleg shrimp. Due to the high stocking density practised in whiteleg shrimp, the seed cost is also on the higher side. The annual net profit obtained from the farming of whiteleg shrimp is 1.5 times more than that of tiger shrimp. The break even production is 62% of the actual production in whiteleg shrimp. The break-even price for the whiteleg shrimp is comparable with that of tiger shrimp.

The input-output ratio shows that *L.vannamei* shrimp culture is economically better than that of *P.monodon.* Many economic indicators like annual net profit, break even price and input-output ratio favour whiteleg shrimp culture over tiger shrimp culture. Though the cost of production is higher for *L.vannamei* culture, the risk factor (disease incidence) is much less.

Table 5: Comparison of the Economics of *P.monodon* (Sathiadhas *et al.* 2009) and *L.vannamei*

PARAMETERS	*L. vannamei*	*P.monodon*
1. Total costs (Rs. Lakh/ha)	21.92	9.64
2. Annual production (kg/ha)	15,942	6000
3. Annual net profit (Rs. Lakh/ha)	13.32	8.36
4. Break-even production(Kg./ha)	9,842	875
5. Break-even price (Rs./Kg/ha)	151	161
6. Input-output ratio	2.3	1.8

SUMMARY

7. SUMMARY

Peneaus monodon an indigenous species of our country was the major shrimp species cultured and the most important commodity in shrimp industry. This is being increasingly replaced by *Litopenaeus vannamei* (Boone 1931), a native of the Pacific coast of Mexico, Central and South America because of its superior traits over tiger shrimp, namely availability of SPF stock, reduced days of culture (DOC), lesser protein requirement (28-35%) and demand in the international market.

The present study was carried out to find out the extent of introduction of *L.vannamei* in Nellore district of Andhra Pradesh, India and the level of its acceptance by farmers. A questionnaire was used to survey *L. vannamei* farms. Water samples collected from these farms were analysed. Surveillance for the presence of any potential disease causing agents, especially, viruses (WSSV, IHHNV, HPV, MBV and TSV) or bacteria in *L. vannamei* was also carried out. PCR, RT-PCR and conventional microbiological analysis were used to screen the viruses and bacteria in the collected samples. Economic analysis was worked out and compared with tiger shrimp culture.

The results are summarized as follows:

1. Most of the farmers are not culturing this exotic species based on the laws laid out by CAA (33% are registered farms, a mere 10% have ETS facility and only 41% are practising biosecurity measures).
2. Availability of SPF stock, reduced days of culture (90-110 days), possibility to grow at high stocking densities and international demand are luring aqua farmers to venture into *L. vannamei* culture
3. Salinity of water maintained in many farms were not in the desired range, as recommended by CAA.

4. No potential viral diseases (WSSV, IHHNV, HPV, MBV and TSV) have been diagnosed by PCR and RT-PCR analyses.
5. *Vibrio sp.* has been detected from samples exhibiting signs of bacterial disease.
6. Economic analysis confirms that culture of *L. vannamei* is better than *P.monodon* culture.

REFERENCES

REFERENCE

Adams, A. 1991. Response of penaeid shrimp to exposure to Vibrio species. *J. Fish Shellfish Immunology* 1:59–70.

Alday- Sanz. V., Roque, A., Turnbull, JF. 2002. Clearing mechanisms of Vibrio vulnificus biotype I in the black tiger shrimp Penaeus monodon. *J Dis Aquat Org* 48:91–99.

*Anderson,I.G., Shamsudin, M.N. and Shariff, M. 1988. Bacterial septicemia in Juvenile tiger shrimp, Penaeus monodon, cultured in Malaysian brackishwater ponds. *J.Asian Fis.Sci.* 2: 93-108.

Albaladejo, J.D., Tapay L.M., Migo V.P., Alfafara C.G., Somga J.R., Mayo S.L., irandar.C., Natividad K., Magbanua F.O., Itami T., Matsumura M., Nadala E.C.B. Jr & Loh, P.C. 1998. Screening for shrimp viruses in the Philippines. In: Flegel T.W.(ed.), *Advances in Shrimp Biotechnolog.* National Center for Genetic Engineering and Biotechnology, Thailand, pp 251–253.

Arthur, J.R., Bondad-Reantaso, M.G., Baldock, F.C., Rodgers, C.J. and Edgerton, B.F. 2004. Manual on risk analysis for the safe movement of aquatic animals. Asia-Pacific Economic Cooperation Fisheries Working Group 01/2002. APEC/DoF/NACA/FAO, NACA, Bangkok, Thailand. 59 pp.

Baldock, C. 2002. Health management issues in the rural livestock sector: useful lessons for consideration when formulating programmes on health management in rural, small-scale aquaculture for livelihood.p. 7-19. In: Arthur, J.R., Phillips, M.J., Subasinghe, R.P., Reantaso, M.B and MacRae, I.H. (eds.), *Primary Aquatic Animal Health Care in Rural, Small-scale, Aquaculture Development.* FAO Fish. Tech. Pap. No. 406: 38 pp.

Anonymous. 2011, Jan. 15. White Shrimp Hits the market. Available:

www.businessstandard.com.

Basheer K.P.M., 2009, April.26. Vannamei shrimp may enter farms soon. *The Hindu.* Available : http:// www.epaper.thehindu.com

Beers, P., Findlay, V., and. Perrera, R. 2005. A New Word for an Old Concept. In. Walker, P., Lester, R., and Bondad-Reantaso, M.G. (eds.), *Diseases in Asian Aquaculture.* Fish Health Section, Asian Fisheries Society, Manila. pp. 3-13.

Bernoth, E M., Chavez, C., Chinabut, S., and Mohan, C.V. 2008. International trade in aquatic animals -- a risk to aquatic animal health status? pp. 53-70. In Bondad-Reantaso, M.G., Mohan, C.V., Crumlish, M. and Subasinghe, R.P. (eds.), *Diseases in Asian Aquaculture VI.* Fish Health Section, Asian Fisheries Society, Manila, Philippines. 505pp.

Briggs, M., Funge-Smith, S., Subasinghe, R.P. and Phillips, M. 2005. Introductions and movement of *Penaeus vannamei* and *Penaeus stylirostris* in Asia and the Pacific. Food and Agriculture Organization of the United Nations Regional Office for Asia and Pacific. RAP Publication 2004/10. Bangkok, 2004 pp. 79.

Boonyaratpalin S., Supamattaya K., Kasornchandra J., Direkbusaracom S., Ekpanithanpong, U. and Chantanachooklin, C. 1993. Non-occluded baculo-like virus, the causative agent of yellow head disease in the black tiger shrimp (Penaeus monodon). *Fish Pathology* **28**,103–109.

Bondad-Reantaso .M.G. 2004. Trans-boundary aquatic animal diseases/pathogens.

In: J.R. Arthur and M.G. Bondad-Reantaso. (eds.), *Capacity and Awareness Building on Import Risk Analysis for Aquatic Animals. Proceedings of the Workshops* Bangkok, Thailand, pp. 9-22.

Bondad-Reantaso, M.G., McGladdery, S.E., East, I. & Subasinghe,R.P.(eds). 2001. Asia Diagnostic Guide to Aquatic Animal Diseases. FAO Fish. Tech. Pap. No. 402, pp. 236

*Bondad-Reantaso, M.G., Subasinghe, R.P., Arthur, J.R., Ogawa, K., Chinabut, S., Adlard, R., Tan, Z. and Shariff. M. 2005. Disease and health management in Asian aquaculture. *Vet. Parasitol.* 132: 249-272.

Bonami J.R., Brehelin M., Mari J., Trumper B. & Lightner D.V. 1990. Purification and characterization of IHHN virus of penaeid shrimps. *J. Gen. Virol.*, **71**: 2657–2664.

Brock, J.A., Gose, R.B., Lightner, D.V. and Hasson, K. 1997. Recent developments and an overview of Taura Syndrome of farmed shrimp in the Americas. In: Flegel, T.W. and MacRae, I.H. (eds.), *Diseases in Asian Aquaculture III.* Fish Health Section, Asian Fisheries Society, Manila, the Philippines, pp. 275-284.

Brock, J.A, Lightner , D.V. 1990.Diseases of crustacea. In: Kinneo (ed.), *Diseases of marine animals, Vol III* Biologische Anstalt Helgoland, Hamburg, pp. 245-349.

Brock, J.A. & Main, K.L. 1994. A guide to the common problems and diseases of cultured *Penaeus vannamei.* World Aquaculture Society, Baton Rouge, Louisiana,USA, pp.242

Browdy, C.L., Holloway J.D., King C.O., Stokes A.D., Hopkins J.S. & Sandifer P.A. 1993. IHHN virus and intensive culture of *Penaeus vannamei*: effects of stocking density and water exchange rates. *J. Crustacean Biol.*, **13**: 87–94.

Castille, F.L., Samocha, T.M., Lawrence A.L., He H., Frelier, P. & Jaenike, F. 1993.Variability in growth and survival of early post larval shrimp (*Penaeus vannamei* Boone 1931).*Aquaculture,* **113**, 65–81.

*Ceccherelli, G., Paizzi, L. and Cinelli, F. 2000. Response of the non-indigenous Caulerpa racemosa (Forsskal) J. Agardh to the native seagrass Posidonia oceanica (L.) Delile: effect of density of shoots and orientation of edges of meadows. *J. Exp. Mar. Biol.* 243: pp. 227-240.

Chamberlain, G. 2003. World shrimp farming: progress and trends. World Aquaculture, Salvador, Brazil, May 20, 2003.

Chen, D. 1992. An overview of the disease situation, diagnostic techniques, treatments and preventatives used on shrimp farms in China. In:. Fuls, W and Main, K.L. (eds.), *Diseases of Cultured Penaeid Shrimp in Asia and the Unites States,* The Oceanic Institute, Hawaii. pp. 47-55.

Chantanachookin, C., Boonyaratpalin, S., Kasornchandra , J., Direkbusarakom, S., Aekpanithanpong, U., Supamattaya, K., Sriuraitana S. & Flegel, T.W. 1993. Histology and ultrastructure reveal a new granulosis-like virus in *Penaeus monodon* affected by yellow-head disease. *Dis. Aquat. Org.*, **17**, 145–157.

Chen, FR., Liu, PC., Lee, K.K. 2000. Lethal attribute of serine protease secreted by Vibrio alginolyticus strains in Kurama Prawn Penaeus japonicus. *Zool Naturforsch* 55:94–99.

Do, J.W., Cha, S.J., Lee, N.S., Kim, Y.C., Kim, J.W., Kim, J.D. and Park, J.W. 2006.Taura syndrome virus from Penaeus vannamei shrimp cultured in Korea. *Dis Aquat Organ.* 70(1-2): pp.171-4.

FAO/NACA. 2000. Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals and the Beijing Consensus and Implementation Strategy. FAO Fish. Tech. Pap. No. 402. 53p

FAO/NACA. 2001. Manual of procedures for the implementation of transboundary animals in Asia. Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals. FAO Fish. Tech. Pap. No. 402 (1): 106 pp.

FAO.2006. Cultured Aquatic Species Information Programme. *Penaeus vannamei.* Cultured Aquatic Species Information Programme.Text by Briggs, M. In: *FAO Fisheries and Aquaculture Department* Rome. http://www.fao.org/fishery/culturedspecies/Litopenaeus_vannamei/en

Fegan, D. 2000. International trade in live shrimp: *Global Aquaculture Advocate* Part I., pp. 22-23.

*Fegan, D.F. 2005. Preparedness and response to aquatic disease emergencies: the relevance and role of the private sector. Regional Workshop on Preparedness and Response to Aquatic Animal Health Emergencies in Asia FAO fisheries proceedings no. 4, pp. 113-121.

Fegan, D., Arthur, J.R., Subasinghe, R.P., Reantaso, M.B., Alday de Graindorge, V. & Phillips, M.J. 2001. Consultant report: A review of transboundary aquatic animal pathogen introductions and transfers. In: *Report of the Puerto Vallata Expert Consultation.* pp. 132-175.

Flegel, T.W. 1997. Major viral diseases of the black tiger prawn (*Penaeus monodon*) in Thailand. *World Journal of Microbiology and Biotechnology* 13, 433-442.

Flegel, T.W., Boonyaratpalin, S. & Withyachumnarnkul, B. (1997). Current status Of research on yellow-head virus and white-spot virus in Thailand. *In:* Flegel, T.W. & MacRae, I.H., (eds.), *Diseases in Asian Aquaculture III.* Fish Health Section, Asian Fisheries Society, Manila, the Philippines. pp.285–296.

Flegel, T.W. 2003. Problematic transfer of viruses amongst Penaeid shrimp. Presentation at the Aquamarkets Shrimp Session, Manila, Philippines in The special danger of viral pathogens in shrimp translocated for aquaculture. Science Asia 32: 215-221.

*Flegel T.W. & MacRae I.H. (eds.), Diseases in Asian Aquaculture III. Fish Health Section, pp. 243-254. Manila, Asian Fisheries Society.

Grosholz, E.D., G.M. Ruiz, C.A. Dean, K.A. Shirley, J.L. Maron, and P.G. Connors. 2000. The impact of a non-indigenous marine predator in a California bay. *Ecology*. 81:1206-1224.

Hasson K.W., Lightner D.V., Mohney L.L., Redman R.M., Poulos B.T. & White B.L. (1999). Taura syndrome virus (TSV) lesion development and the disease cycle in the Pacific white shrimp Penaeus vannamei. *Dis. Aquat. Org.*, **36**, 81–93.

Hernández-Rodríguez, A., Alceste-Oliviero, C., Sánchez, R., Jory, D., Vidal, L. &

Constain-Franco, L.F. 2001. *In:* R.P. Subasinghe, P. Bueno, M.J. Phillips, C. Hough, S.E. McGladdery & J.R. Arthur, (eds.), *Aquaculture development trends in Latin America and the Caribbean*. pp. 317-340.

Ishimaru, K., Akarawa-Matsushita, M., Muroga, K. 1995. Vibrio penaeicida sp., nov., a pathogen of kuruma shrimps (Penaeus japonicus). *Int. J. Syst. Bacteriol.*43: 8-19.

Israngkura, A., and S. Sae-Hae. 2002. A review of the economic impacts of aquatic.animal diseases. p.253-286. In Arthur, J.R., Phillips, M.J., Subasinghe, R.P., . Reantaso , M.B and MacRae, I H. (eds.), *Primary Aquatic Animal Health Care in Rural, Small-scale, Aquaculture Development.* FAO Fish.Tech. Pap. No. 406.

*Jayasree, L., Janakiram, P and Madhavi, R. 2006. Characterization of Vibrio spp. Associated with Diseased Shrimp from Culture Ponds of Andhra Pradesh (India).*J of the World Aquaculture Society*, 37 (4): 523.

Jimenez R., Barniol R., De Barniol L. & Machuca M. 2000. Periodic occurrence of epithelial viral necrosis outbreaks in *Penaeus vannamei* in Ecuador. *Dis. Aquat. Org.*, **42**, 91–99.

Jiravanichpaisal, P and Miyazaki, T. 1994. Histopathology, biochemistry and pathogenicity of Vibrio harveyi infecting black tiger shrimp Penaeus monodon. *J. Aquat. An. Health* 6: 27-35.

Kalagayan, H., D. Godin, R. Kanna, G. Hagino, J. Sweeney, J. Wyban, and J. Brock. 1991. IHHN virus as an etiological factor in runt-deformity syndrome (RDS) of juvenile Penaeus vannamei cultured in Hawaii. *Journal of the Word Aquaculture Society* 22:235-243.

Karunasagar, I., Otta, S.K. and Karunasagar, I. 1997. Histopathological and bacteriological study of white spot syndrome of *Penaeus monodon* along west coast of India. *Aquaculture* 153, 9-13.

Lambert, W. J., Levin, P.S and Berman, J., 1992. Changes in the structure of a New England (USA) kelp bed: the effect of an introduced species? *Mar. Ecol. Prog. Ser.* 88: 303-307.

Lavilla-Pitogo, CR., Leano, EM., Paner, MG. 1998. Mortalities of pond-cultured juvenile shrimp Penaeus monodon associated with dominance of luminescent vibrios in the rearing environment. Aquaculture 164:337–349.

Lee, D.J. and Gordon, R.M. 2006.Economics of aquaculture and invasive aquatic species--an overview. *Aquaculture Economics & Management* 10 (2), pp. 83-96.

Lightner, D.V., Lo, C.F. and Owens, L. 2008. Shrimp disease control: past, present and future, pp. 355-378. *In* Bondad-Reantaso, M.G., Mohan, C.V., Crumlish, M. and Subasinghe, R.P. (eds.), *Diseases in Asian Aquaculture VI. Fish Health Section.* Asian Fisheries Society, Manila, Philippines. pp. 355-361.

Lightner, D.V., Redman, R.M., Poulos, B.T., Nunan L.M., Mari, J.L. and Hasson, K.W. 1997. Risk of spread of penaeid shrimp viruses in the Americas by the international movement of live and frozen shrimp,Rev. *Sci. Tech. Off. Int. Epizoot.*16: 146–160.

Lightner, D.V. and Lewis, D.H. 1975. A septicemic bacterial disease syndrome of

penaeid shrimp. *Mar. Fish. Rev.* 37(5-6): 25-28.

Lightner, D.V. (ed.), 1996. A Handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Cultured Penaeid Shrimp. World Aquaculture Society,Baton Rouge, LA, USA.

Lightner, D.V., Bell, T.A., Redman, R.M., Mohney, L.L., Natividad, J.M., Rukyani, A. and Poernomo, A. 1992. A review of some major diseases of economic significance in penaeid shrimps/shrimps of the Americas and Indo-Pacific. In: Shariff, M., Subasinghe ,R. and. Arthur, J.R .(eds.) , *Proceedings 1st Symposium on Diseases in Asian Aquaculture.* Fish Health Section, Asian Fisheries Society, Manila, Philippines. pp. 57-80.

Lightner, D.V. and R.M. Redman, 1998. Shrimp diseases and current diagnostic methods. *Aquaculture,* **164:** 201-220.

Lightner, D.V. (1996). A Handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Cultured Penaeid Shrimp. World Aquaculture Society, Baton Rouge, LA, USA. 304 p.

Lightner, D.V. 1996. Epizootiology, distribution and the impact on international trade of two Penaeid shrimp viruses in the Americas. Revue Scientifique et Technique, *OIE* 15 (2):579-602.

*Lightner D.V. & Redman, R.M. 1998 . Emerging crustacean diseases. *In*: Third International Symposium on Aquatic Animal Health, Aug 30-Sept 3,Baltimore, Maryland, USA. 6 pp.

Lightner, D.V., Redman, R.M. and Bell, T.A. 1983. Infectious hypodermal and hematopoietic necrosis, a newly recognized virus disease of penaeid shrimp. *J. Invertebr. Pathol.* 42:62-70.

Lightner, D.V. 1993. Diseases of cultured Penaeid shrimp. *In*: James P. McVey, (ed.), *CRC Handbook of Mariculture, 2nd Edition, Volume I,* Crustacean Aquaculture. Boca Raton, Florida. Pp. 393-486.

Lightner, D.V. 1995. Taura syndrome: an economically important viral disease impacting the shrimp farming industries of the Americas including the United States. *In:* Proceedings of the ninety-ninth annual meeting USAHA, Reno, Nevada. pp. 36-52.

Lightner, D.V. 2002. Introduction of non-indigenous shrimp viruses and their potential impact on farmed and native wild shrimp populations. *ISAAH,* pp 203.

Lo, C.F., Ho, C.H., Chen, C.H., Liu, K.F., Chiu, Y.L., Yeh, P.Y., Peng, S.E., Hsu, H.C., Liu, H.C.,Chang, C.F., Su, M.S., Wang, C.H. and Kou, G.H. 1997. Detection and tissue tropism of white spot syndrome baculovirus (WSBV) in captured brooders of *Penaeus monodon* with a special emphasis on reproductive organs. *J.Diseases of Aquatic Organisms* 30: 53-72.

Lotz, J.M. 1997. Disease control and pathogen status assurance in an SPF-based shrimp aquaculture industry, with particular reference to the United States. In: *Proceedings of the ninety-ninth annual meeting USAHA,* Reno, Nevada. pp. 36-52.

Limsuwan C. (1991). Handbook for Cultivation of Black Tiger Prawns.

Tansetakit, Bangkok, Thailand (in Thai).

Lo, CF. and Kou, G.H .(1998) Virus-associated white spot syndrome of shrimp in Taiwan: a review. *Fish Pathol* 33:365-371

MacDiarmid, S.C. 1997. Risk analysis, international trade, and animal health. p. 377-387. *In* V. Molak, (ed.) Fundamentals of Risk Analysis and Risk Management. CRC Lewis Publishers, Boca Raton, LA.

Madhavi, R., Janakiram, P., Jayasree, L. and Murthy, P.S.N. 2002. Occurrence of concurrent infections with multiple viruses in *Penaeus monodon* from culture ponds of north coastal Andhra Pradesh. *Current Science* 82: 1397-1400.

*Mark, A., Alexander, T. G., Murray, E.B., Wallace, S. and Peeler, E.J.,2011.The Application Of Risk And Disease Modelling toEmerging Freshwater Diseases In Wild Aquatic Animals. *Freshwater Biology* 56: 658–675.

Mohan, C.V., Shankar, K.M., Kulkarni S. and Sudha, P.M. 1998. Histopathology of cultured shrimp showing gross signs of yellow head syndrome and white spot syndrome during 1994 Indian epizootics. *Diseases of Aquatic Organisms* 34: 9-12

Mohandas, 2009. CP Company manager,.Personal communication.

Momoyama K., Hiraoka M., Nakano H., Koube H., Inouye K. & Oseko N. 1994.

Mass mortalities of cultured kuruma shrimp, *Penaeus japonicus*, in Japan in 1993: histopathological study. *Fish Pathol.*, **29**, 141–148.

NACA/FAO. 2001. Aquaculture in the Third Millennium. *In*: Subasinghe, R.P., Bueno, P.B., Phillips, M.J., Hough, C., McGladdery, S.E. & Arthur, J.R. (eds.), *Technical Proceedings of the Conference on Aquaculture in the Third Millennium*, Bangkok, Thailand, 471 pp.

NACA/FAO. 2005a. Quarterly Aquatic Animal Disease Report (Asia and Pacific Region), 2005/1, January-March 2005. Network of Aquaculture Centres in Asia-Pacific, Bangkok. Thailand. 58 pp.

NACA/FAO. 2005b. Quarterly Aquatic Animal Disease Report (Asia and Pacific Region), 2005/2, April-June 2005. Network of Aquaculture Centres in Asia-Pacific, Bangkok. Thailand. 65 pp.

Nash, G. Nithimathachoke, C., Tungmandi, C., Arkarjamorn, A., Prathanpipat, P and Ruamthaveesub, P. 1992. Vibriosis and its control in pond-reared Penaeus monodon in Thailand. In: M. Shariff, R.P. Subasinghe and J.R. Authur (eds.) Diseases in Asian Aquaculture 1. Fish Health Section, Asian Fisheries Society,Manila, Philippines. pp. 143-155.

Nielson, L., Sang-oum, W., Cheevadhamarak, S. & Flegel. T. 2005. Taura syndrome virus (TSV) in Thailand and its relationships to TSV in China and the Americas. *Dis. Aquat. Org.* 63:101-106.

Nunan L.M., Poulos B.T. & Lightner, D.V. 1998. The detection of white spot syndrome virus (WSSV) and yellow head virus (YHV) in imported commodity shrimp. *Aquaculture,* **160**, 19–30.

Nunan L.M., Poulos B.T. Lightner D.V. 1998. Reverse transcription polymerase chain reaction (RT-PCR) used for the detection of Taura Syndrome Virus (TSV) in experimentally infected shrimp. *Dis. Aquat. Org.*, **34**, 87–91.

Nunan L.M., Poulos B.T. & Lightner D.V. (2000). Use of polymerase chain reaction (PCR) for the detection of infectious hypodermal and hematopoietic necrosis virus (IHHNV) in penaeid shrimp. *Mar. Biotechnol.*, **2**, 319–328.

OIE. 2002. International Aquatic Animal Health Code and Diagnostic Manual for Aquatic Animal Diseases, 5th Edn. Office International des É pizooties, Paris. http://www.oie.int/eng/normes/en_acode.htm

OIE 2003, Manual of Diagnostic Tests for Aquatic Animals, Office International des Epizooties, Paris, 4: pp.358.

OIE. 2005a. Quarterly Aquatic Animal Disease Report. January – March 2005 (Asian and Pacific Region). World Organisation for Animal Health Regional Representation for Asia and the Pacific, Tokyo, Japan. 43 pp.

OIE. 2005b. Koi herpesvirus in Singapore. Information received on 23 September 2005 from Dr Chua Sin Bin, Director, Veterinary Public Health and Food Supply Division, Agri-food and Veterinary Authority of Singapore. 66 pp.

OIE. 2005c. HandiStatus. Available: http://www.oie.int/hs2/report.asp?lang=en).

O.I.E. 2009. Aquatic Animal Health Code. World Organisation for Animal Health. pp.345-390

Overstreet, R.M., Lightner, D.V., Hasson, K., McIlwain, S. & Lotz, J.M. 1997. Susceptibility to Taura Syndrome Virus of some Penaeid shrimp species native to the Gulf of Mexico and the Southeastern United States. Journal of *Invertebrate Pathology* 69: 165-176.

Owens L., Anderson I.G., Kenway M., Trott L. & Benzie J.A.H. 1992. Infectious hypodermal and hematopoietic necrosis virus (IHHNV) in a hybrid penaeid prawn from tropical Australia. *Dis. Aquat. Org.*, **14**, 219–228.

Peeler E.J., Murray A.G., Thebault A., Brun E., Giovaninni A. & Thrush M.A. 2007. The application of risk analysis in aquatic animal health management. *Preventive Veterinary Medicine*, 81: 3–20.

Pillai, D., Bonami, J.R., Widada, J.S., 2006. Rapid detection of *Macrobrachium rosenbergii* nodavirus(*MrNV)* and Extra small virus (XSV), the pathogenic agent of white tail Disease of *Macrobrachium rosenbergii (De Man),* by loop mediatted isothermal amplification. *J. Fish. Dis.* 29, pp. 275-283.

Phalitakul, S., Wongtawatchai, J., Sarikaputi, M., Viseshakul, N. 2006. The molecular detection of Taura syndrome virus emerging with White spot syndrome virus in penaeid shrimps of Thailand. *Aquaculture* 260 :pp. 77-85.

*Raghavan, R.P., Prasad, G., Rajeev Raghavan, P. 2006. A needless diversification:

A perspective on the unregulated introduction and culture of the Pacific white shrimp, Litopenaeus vannamei, in India. *World Aquaculture [World Aquacult.]* 37 (1) pp. 8-20

Roberts, R.J. 2003. Biosecurity – pandemics and the aquatic environment.

In Lee, C. and P.J. O'Bryen (eds.), *Biosecurity in Aquaculture Production Systems Exclusion of Pathogens and Other Undesirables.* The World Aquaculture Society, Baton Rouge, Louisiana 70803, USA. pp 5-11

Riji John, K. 2001. Viral Diseases of Shellfish and their management. National Training course on *Fish Disease Diagnosis and Health Management,* pp. 42-49.

Rosalind George, M. 2001. Bacterial Diseases of Shellfish and their management. National Training course on *Fish Disease Diagnosis and Health Management,* pp. 26-33.

Rosenberry, B., (ed.), 1993. World Shrimp Farming. *Shrimp News International,* December. pp. 156-175.

*Rosenberry, B. 2002. World shrimp farming 2002. *Shrimp News International,* 276 pp.

Shang, Y.C. 1981. Aquaculture economics pp 47- 60.

*Sambrook, J. and Russell, D. W. 2001. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Press, New York.

Sathiadhas, R., Najmudeen, T. M. and Prathap, S. 2009. Break-Even Analysis And Profitability Of Aquaculture Practices In India. *Asian Fisheries Science* 22:667-680

Scarfe, A.D. 2003. State, regional, national, and international aquatic animal health

policies: Focus for future aquaculture biosecurity. *In* Lee, C. and O'Bryen P.J. (eds.), *Biosecurity in Aquaculture Production Systems. Exclusion of Pathogens and Other Undesirables*. The World Aquaculture Society, Baton Rouge, USA. pp .233-262.

Senapin, S., Phewsaiya, K., Briggs, M., Flegel, T.W. 2007. Outbreaks of infectious myonecrosis virus (IMNV) in Indonesia confirmed by genome sequencing and use of an alternative RT-PCR detection method. *Aquaculture.* 266, (1-4) pp. 32-38.

Shankar and Mohan, 2002. Fishand Shell fish Health Management. Manaual pp 21 -50.

Sindermann, C.J. 1990. Principal Diseases of Marine Fish and Shellfish, 2 (2 ed.) edition. Academic Press, New York.

*Sittidilokratna, N., Chotiwiwatthanakun, C., Wijegoonawardane, P. K. M., Unajak, S., Boonnad, A., Wangnai, W., Cowley, J. A. and Walker, P. J. 2009. A virulent isolate of yellow haed nidovirus contains a deformed envelope glycoprotein gp116. *Virology, 384:* pp. 192-200

Sizemore RK, Davis JW (1985) Source of Vibrio spp. found in the hemolymph of the blue crab Callinectes sapidus. J Invertebr Pathol 46:109–110.

Surendran, P. K., Thampuran, N., Nambiar, V. N., Lalitha, K. V. 2006. Laboratory Manual On Microbilogical examination of seafood, Central Institute of Technology, India

Taggart, J.B., Hynes, R, A., Prodohol, P.A. and Ferguson, A. 1992. Asimplified protocol for routine total DNA isolation from salmonid Fishes. *J. Fish Biol. 40:*963-965

Tang, K.F.J., Durand, S.V., White, B.L., Redman, R.M., Mohoney, L.L. & Lightner, D.V. 2002. Induced resistance to white spot syndrome virus infection in *Penaeus stylirostris* through pre-infection with infectious hypodermal and haematopoietic necrosis virus – a preliminary study. *Aquaculture* 216(1-4):19-29 pp.

Tapay, L.M., Lu, Y., Gose, R.B., Brock, J.A. & Loh, P.C. 1997. Infection of whitespot baulovirus-like virus (WSBV) in two species of Penaeid shrimp Penaeus stylirostris (Stimpson) and P. vannamei (Boone). In: Flegel ,T.W. & MacRae, I.H. (eds.), *Diseases in Asian Aquaculture III.* Fish Health Section, pp. 297-302. Manila Asian Fisheries Society.

Taukhid and Yani Lestari Nuraini, 2001, Infectious Myonecrosis Virus (IMNV) in Pacific White Shrimp (*Litopenaeus vannamei*) in Indonesia. *SEAFDEC International Workshop on Emerging Fish Diseases in Asia pp.* 255

Taw, N., Srisombat, S. & Chandaeng, S. 2002. L. vannamei trials in Indonesia. Global Aquaculture Advocate, December 2002, pp. 20-22.

Taylor, H.H., Taylor, E.W. 1992. Gills and lungs: the exchange of gases and ions. In: Harrison, FW. And Humes, AG. (eds) ,*Microscopic anatomy of invertebrates* 10. Wiley-Liss, New York, pp 203–293.

Tu, C., Huang, H.T., Chuang, S.H., Hsu, J.P., Kuo, S.T., Li, N.J., Hsu, T.L., Li, M.C. & Lin, S.Y. 1999. Taura syndrome in Pacific white shrimp *Penaeus vannamei* cultured in Taiwan. *Diseases of Aquatic Organisms* 38: 159-161.

Wang y, C. & Chang, P.S. 2000. Yellow head virus infection in the giant tiger prawn *Penaeus monodon* cultured in Taiwan. *Fish Pathol.*, **35:** 1–10.

Wang, C.S., Tang, K.F.J., Kou, G.H. and Chen, S.N. 1996. Yellow head disease-like virus infection in the kuruma shrimp *Penaeus japonicus* cultured in Taiwan. *Fish Pathology* 31:177-182.

*Wongteerasupaya, C., Vickers, J.E., Sriurairatana, S., Nash, G.L., Akarajamorn, A., Boonsaeng, V., Panyim, S., Tassanakajon, A., Withyachumnarnkul, B. and Flegel, T.W. 1995. A non-occluded, systemic baculovirus that occurs in cells of ectodermal and mesodermal origin and causes high mortality in the black tiger prawn *Penaeus-monodon*. *Diseases of Aquatic Organisms*21: 69-77.

WTO (World Trade Organization). 1994. Agreement on the Application of Sanitary and Phytosanitary, Measures. WTO, Geneva.

Wyban, J.A. & Sweeney, J.N. 1991. Intensive shrimp production technology. *High Health Aquaculture Inc.*, Hawaii. 158 pp.

Wyban, J. 2002. White shrimp boom continues. *Global Aquaculture Advocate*, December, 2002, pp. 18-19.

*Wyban, J.2009. World Shrimp Farming Revolution :IndustryImpact Of Domestication, Breeding and Wide spread Use of Specific pathogen free *Penaeus vannamei* .In:. Browdy. C.L. and Jory, E.S.(eds.), *The Rising Tide, Proceedings of the Special Session on Sustainable Shrimp Farming,* World Aquaculture. The World Aquaculture Society, Baton Rouge Louisiana USA. pp. 12 - 21

Yu, C.I. & Song, Y.L. 2000. Outbreaks of Taura syndrome in pacific white shrimp *Penaeus vannamei* cultured in Taiwan. *Fish Pathology* 35(1): 21-24.

* Not referred in original.

ABSTRACT

ABSTRACT

P. monodon (tiger shrimp) was the most preferred cultured species in India, but repeated occurrence of diseases especially white spot disease (WSD) caused by white spot syndrome virus (WSSV) had a severe setback to the shrimp culture industry. This prompted the industry to actively promote the introduction of a non-native species, the Pacific white shrimp, *Litopenaeus vannamei*, as an alternative to *P. monodon* for commercial aquaculture operations in the country. The authority to issue licenses to take up *L.vannamei* culture was vested with the Coastal Aquaculture Authority (CAA), who issued licenses for 1,208 aqua farmers in 2009. Availability of SPF stock, ability to grow well even at high stocking densities, reduced days of culture (DOC), lesser protein requirement (28-35%) and demand in the international market attracted aquafarmers to switch to culture of *L.vannamei.* A survey carried out in the Nellore district of Andhra Pradesh to understand the level of acceptance and the nature of culture practice among the farms culturing *L.vannamei* revealed that only 33% of the farms surveyed had obtained permission from the CAA, 41% followed biosecurity measures and merely 10% had the facility for Effluent Treatment System. 53% practised chlorination and de-chlorination of water during water exchange and only 38% farms maintained trained personnel. It was observed during the survey that only 84% procured tested SPF seed from certified hatcheries. While 90-92% of yhe farms depended on pelleted feed, only 80-85% of the farms surveyed carried out regular monitoring every 1-2 weeks. Disease surveillance in the farms surveyed did not detect the presence of any of the major shrimp viruses (WSSV, IHHNV, MBV, HPV and TSV) in the 60 samples screened using molecular methods. However, *Vibrio sp.* was detected in samples exhibiting obvious signs of bacterial disease. High stocking densities without adequate number of aerators can be stressful to the shrimps and might be the reason for bacterial infection.

Economic analysis indicates that *L.vannamei* culture is more profitable than *P.monodon* culture.

APPENDIX - 1

QUESTIONNAIRE

FARM

A) General:

1. Name & address of farmer:

2. Area under cultivation:

3. No. of ponds (each area):

4. Species cultivated:

5. Type of culture:

Monoculture / polyculture-

Intensive/ semi/ extensive-

6. Source of PL:

7. Stocking density:

8. Date and stage of stocking:

9. Harvesting time:

Culture cycle:

ılture duration:

efore which culture (reason for shift?):

Prophylactic measures taken:

) Pond Management:

1. New or old pond:

2. Pond drying:
   - Chemicals used:

- How many times:
- Duration:

3. Fertilizers: inorganic/organic
4. Sluice :
5. Fencing:
6. Water exchange:
7. Filters:
8. Water quality:
    a. PH
    b. Salinity-
    c. Temperature-
    d. Source-
9. Water: recycled/ discharged?

C) Feed:

. Feed given:

a. Commercial/farm made
b. Starter/ grower
c. Type

. Frequency:

. Feeding method:

. Additional feed:

. Probiotics / Antibiotics used:

') Health Management:

1. SPF Stocks:
   - yes/ no-
   - source

2. Sanitation measures:

3. Sampling interval:

4. Diseases observed:

5. Chemicals/Antibiotics:

E) OTHERS:

1. Staff no. :
   - Technical –
   - Labour –

2. MPEDA Permission: Y/N

3. Pumps (capacity, no.,)

4.Aerators:

5. Other observations: