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Preliminary investigations on the geographical distribution of White Spot Syndrome Virus (WSSV) in black tiger shrimp *(Penaeus monodon)* brood stocks and post larvae in Sri Lanka and the relationship of WSSV with the occurrence of bacteria in grow-out ponds

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DECLARATION OF AUTHOR

I do here by declare that the thesis titled "Preliminary investigations on the geographical distribution of White Spot Syndrome Virus (WSSV) in black tiger shrimp *(Penaeus monodon)* brood stocks and post larvae in Sri Lanka and the relationship of WSSV with the occurrence of bacteria in grow-out ponds" is based on the work carried out by me under the supervision of Dr P.P.G.S.N Siriwardane Director General, National Aquatic Resources Research and Development Agency (NARA), Crow Island, Mattakkuliya, Colombo 15 and Dr. Ajantha de Alwis, Senior lecturer, Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda. It describes the results of my own investigation and independent research except where due references have been made in the text. No part of the thesis has been submitted earlier or concurrently for the same or any other degree to any other university.

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Signature of the candidate

We certify that the above statement made by the candidate is true and that this thesis is suitable for the submission to the university for the purpose of evaluation.

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Abstract

Sustainability of shrimp industry in Sri Lanka is threatened by White Spot Syndrome Virus (WSSV). However, the information pertaining to this virus in Sri Lanka is scarce. In addition to WSSV, the disease "Luminous Vibriosis" caused by species of the bacteria *Vibrio* also has been recently recognized as a serious threat affecting shrimp production in the Northwestern coast of Sri Lanka but this aspect also has been poorly dealt with.

In view of these two important diseases, present study was carried out to investigate some important aspects, namely,

a) the geographical distribution of WSSV in Black Tiger shrimp (*Penaeus monodon*) brood stocks and post larvae (PL), which is the most important commercial shrimp species of the country;

b) the occurrence of *Vibrio sp.* in grow-out ponds and the environmental parameters associated with them and

c) possible relationship between the occurrence of *Vibrio sp.* and WSSV disease in grow-out ponds.

The prevalence and geographic distribution of WSSV infection in *Penaeus monodon* shrimp brooders collected from Potuvil, Chilaw, Negombo, Hendala and Beruwala from June 2003 to January 2004 period were studied using two step Polymerase Chain Reaction (PCR) method. A total of 100 samples of brooders shrimp were screened for WSSV. As for the post larvae, 90 samples (each containing 100 post larvae of age 10-15 days) that were obtained from the brooders taken from the same collecting centers were screened. According to the study, brooders taken from Hendala were the significantly highest WSSV infected compared to the brooders taken from other locations ($P<0.01$).

Similarly, the post larvae obtained from brooders bought from Hendala were also the significantly highest WSSV infected PL compared with the PL obtained from others ($P<0.01$; Chi-square test). Brooders taken from Potuvil or their post larvae were not infected with WSSV. These results suggest that the brood stocks have acted as a route of infection of WSSV.

The occurrence of *Vibrio* bacteria species which was detected through TCBS selected media method was evident in all intake water sources and relevant grow-out ponds which also showed a statistical significance ($P< 0.05$). This suggests that the infection of *Vibrio* sp. spreads through the intake water into the grow-out ponds. There was a significant relationship recorded between the WSSV infection and the occurrence of green consisting of *Vibrio mimicus, Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio harveyi* and yellow consisting of *Vibrio cholerae, Vibrio alginolyticus, Vibrio fluvialis, Vibrio metehnikovii* type *Vibrio* sp ($P<0.05$). According to the results in the present study, determination of the maximum level of *Vibrio*-green and yellow colonies of the intake water at 50% threshold value was possible which was 55 colonies/ ml and 29 colonies/ ml respectively.

These results also confirm some important management aspects that have been suggested in shrimp farming industry. They are as follows;

- Checking the brooders by using two step PCR method before taken into the hatchery and screening post larvae before stocking in grow-out ponds are two important aspects in health management of shrimps.
- *Vibrio* counts of intake waters and grow-out pond should be kept at a maximum level of 55 colonies/ ml for green colonies and 29 colonies/ ml for yellow colonies.

- Establishing high standards in water quality and health management practices for hatcheries and grow-out ponds to prevent cross contamination and spread of the WSSV infection in the environment should be a priority to revive the shrimp industry in Sri Lanka.

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LIST OF ABBREVATIONS

APW	Alkaline Peptone Water
BMNV	Baculo Midgut Necrosis Virus
bp	Base Pairs
BWSS	Bacterial White Spot Syndrome
CBV	China Baculo Virus
CDNA	Complementory DNA
CFU	Colony Form Unit
DNA	De-oxy Ribo Nucleic Acid
DNTP	dinucleotide Tri Phosphate
DO	Dissolved Oxygen
EDTA	Ethidium Dichromate Tri Acetate
HPV	Hepatopancreas Parvolike Virus
IHHNV	Infectious Hypodermal Haemocytic Necrosis Virus
LOVV	Lymphoid Organ Vaculization V irus
LPM	Long-term Preservation Medium
MBV	Monodon Baculo Virus
NaCl	Sodium Chloride
PCR	Polymerase Chain Reaction
RNA	Ribo Nucleic Acid
RT-PCR	Reverse Trancriptase Polymerase Chain Reaction
TCBS	Thiosulphate Citrate Bile salt Sucrose
TBE	Tri Borate EDTA
TSI	Triple Sugar Iron
UV	Ulta Violet

CHAPTER 01

1.0 Introduction

The giant black tiger shrimp *Peneaus monodon* is one of the most important culturable species, among nearly 136 species of edible peneaied shrimps. Its large size, fast growth rate, high survival rate, resistance to handling, successful breeding in captivity, high price and high market potential, makes it one of the predominant culture species in the Indo-Pacific region (Hambrey, 1996). Table 1.1 shows the major culturable Shrimp Species around the World.

Asia is the largest shrimp farming region and has produced about 83.3% of the world's total shrimp production in 1994 (Anon, 1997), but the level decreased to 72% in the year 2000 (Anon, 2000). The western world has contributed about 16.7% in 1994 (Anon, 1997) but it increased up to 28% in 2000 (Anon, 2000). Thailand has been the leading country in marine shrimp farming. In the year 2000, it has exported about 250,000 metric tons of shrimps (Anon, 2001). The second largest producer is Equador.

The rapid expansion and intensification of shrimp farming worldwide has been accompanied by the occurrence of diseases, which has threatened the development of the industry (Bonzie, 1988; Lightner & Redman, 1998; Hossain *et al.*, 2001) and it is the main constraint to the sustainable shrimp aquaculture (Subasinghe and Barg, 1998). The viral & bacterial infections have been found to cause most of the production losses in

different parts of the world (Boonyratpalin *et al.*, 1992; Wongteerasupaya *et al.*, 1995; Bachere, 1998).

Table 1.1 Major culturable Shrimp Species around the World

Species	Common name	Major countries that culture the species
Penaeus monodon	Tiger shrimp	Asia, Africa, Australia with all other countries of cultured shrimps
P. merguiensis	Banana shrimp	Indonesia, Malaysia, Vietnam, Thailand
P. indicus	Indian white shrimp	India, Malaysia, Saudi Arab, Thailand
P. chinensis	Chinese white shrimp	China, Taiwan
P. japonicus	Kuruma shrimp	Japan, Taiwan, Australia
P. penicillatus	Red tailed shrimp	Taiwan
P. vannamei	Western white shrimp	Central America and North America, Brazil
P. stylirostris	Blue leg shrimp	USA, Central America, Brazil
P. esculentus		Australia
P. brasiliensis	Spotted pink shrimp	Brazil
P. setiferus		USA
Metapenaeus ensis	Sand shrimp	Vietnam, Malaysia

Source: World Shrimp Farming (1999).

1.1 History of shrimp farming

1.1.1 History of shrimp farming in Sri Lanka

Until early 1960's the shrimp fishery in Sri Lanka was an entirely lagoon and estuary by-catch fishery with an approximate production of 1000 metric tons per year which increased to 4000 metric tons per year at the beginning of the 1980's (Siriwardane, *and* De Alwis, 1988). In the last decade, the rearing techniques for shrimps have made a large progress and have reached an industrial dimension in Asia where it has always been a subsistence activity. Demand for shrimps also has been rising rapidly in the international market during this period. Shrimp farming on a commercial scale in Sri Lanka was first started by Messrs Lever Brothers in a farm covering 0.7ha, in 1977. However, real expansion of farmi,ng in Sri Lanka occurred in mid 1980's (Siriwardane,1988; Jayasinghe ,1997). Since then, it has become a main non-traditional foreign exchange earner (Siriwardane, 1988; Jayasinghe 1997a,1997b).

Among the 136 edible coastal shrimp species of Sri Lanka, Black Tiger shrimp (*Peneaus monodon),* is being used in commercial aquaculture (Siriwardane, 2000). In order to face the demand in the three major markets, namely, Japan, USA, Europe and largely in response to high export prices, a number of small scale entrepreneurs and a few large multinational companies embarked in shrimp farming. Realizing the importance of increasing shrimp production and the availability of unpolluted brackish water sources and suitable land that can be utilized for pond construction, the Government of Sri Lanka