BACTERIAL PROFILES IN SHRIMP CULTURE SYSTEMS AFFECTED BY WHITE SPOT EPIZOOTIC

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ABSTRACT

Severe economic losses were reported during 1996 as a result of whitel spot disease out-break in the shrimp culture industry of Sri Lanka. The estimated loss in foreign exchange during year 1996 was around Rs.1,000 million. The moribund shrimops affected by the disease were often found associated with visual clinical signs related to bacterial infections.

In the present study bacterial profiles of shrimp culture systems infected with White Spot Baculo Virus (WSBV) bordering the Dutch canal, Chilaw and Puttlam lagoons were investigated. Total Bacterial Counts (TBC), sucrose fermenting and sucrse non-fermenting Vibrio populations and luminescent baterial (Vibrio harveji) counts were studied in morbund shrimp with typical white spots, moribund shrimp without visual symptoms and in active shrimp together with sediment and water samples from infected ponds.

The infected ponds bordering the Dutch canal recorded the heighest TBC ($6.6x10^4$ ± 2.96/g, $7.3x10^6$ ± 2.18/g) sucrose fermenting Vibrio ($1.5x10^3$ ± 0.89/g, $6.8x10^3$ ± 3.76/g) and sucrose non-fermenting Vibrio ($5.9x10^3$ ± 1.01/g, $3.6x10^4$ ± 4.92/g) in water and sediments respectively when compared to the samples obtained from Chilaw and Puttalam Lagoons.

All bacterial populations were high int eh hepatopancreas of moribund shrimp with external WSBV signs and were followed by samples of carapace, muscles and gills. Luminescent bacteria were observed only in ponds bordering the Chilaw lagoon.

The TBC were relatively high in moribund shrimp with typical signs of white spots ($2.5x10^8$ ± 0.78/g) and this was followed by moribund shrimp without typical signs ($5.8x10^5$ ± 1.13/g) and healthy shrimps ($8.3x10^4$ ± 2.36/g).

All bacterial populations were found high in morbund shrimp with typical white spot systoms when compared to others. The bacterrial quality of farms bordering the Dutch canal appeqrs to be relatively poor when compated to other brackish water systems used for shrimp culture activities in teh North Western Province. **(SLJAP 2000. Vol. 1, No. 1: 61-68)**

Key word: *Shrimp culture, white spot, bacteria*

INTRODUCTION

Shrimp industry has become a main non-traditional foreign exchange earner in Sri Lanka. In 1996 it has contributed 2365 million rupees to the foreign exchange earnings of Sri Lanka from the export of 3155 metric tons of shrimp.

The frequent disease out-breaks and mass mortalities is one of the main factors that affects the sustainability of the industry. Viral outbreak which was commenced in June 1996 caused severe losses in cultured penaeus shrimp in Sri Lanka. It was characterized by obvious white spots on the carapace and appendages. White Spot syndrome associated Baculo Virus (WSBV) is the name given to the causative agent of the new viral disease which has caused high shrimp mortalities and severe loss to the local shrimp culture industry. The estimated loss to the Sri Lankan economy was 1000 million rupees in 1996 (Jayasinghe, 1997).

Microorganisms play major role in pond culture, particularly with respect to productivity, nutrient cycling, the nutrition of the cultured animals, water quality, disease incidences and environmental impact of the effluent (Moriarty, 1997). It is generally agreed that bacteria are opportunistic pathogens in shrimp only causing disease when the animals defenses are compromised (Guerra-Flores, A.L. and Gomez-Gil, B. (1996).

It has been found in a study (Nash, 1990) *Vibrio* as the dominant bacterial genus in all cases of moribund shrimps investigated. Bacterial infections with *Vibrio spp.* is one of the most serious disease problems in pond-reared tiger shrimp, and can result in considerable production losses with mortalities upto 100 percent (Lightner, 1988: Nash, unpublished reports, 1989-90.).

Information on bacterial profiles in shrimp culture systems is vital in building Hazard Analysis & Critical Control Point plans (HACCP) and determining final post harvest quality and losses. The present study is a part of an in-depth study to assess bacterial profiles in shrimp culture systems and concentrate on bacterial profiles during a major epizootic, White Spot Disease outbreak.

MATERIALS AND METHODS

The water samples, sediments, infected shrimp and shrimp without clinical signs in infected ponds (15-30 g weight group) were collected from rearing ponds bordering the Dutch canal, Puttalam lagoon and Chilaw lagoon during the period of WSBV outbreak (during June 1997 to December 1997). Visual symptoms associated with WSBV affected shrimp were also recorded. Gills, carapace, hepatopancreas and muscles of infected and healthy adults were aseptically obtained using sterile dissecting blades, forceps etc. and placed in a petridish. Sediment samples of these ponds and tissues obtained from shrimp were diluted with 2% Sodium Chloride included peptone water in ten folds and macerated. Ten fold serial dilutions were done to avoid overgrowth of bacteria. The diluted samples were inoculated on duplicate plates of TCBS agar by spread plate technique and on 2% nutrient agar by drop method technique. After inoculation TCBS plates were incubated at 37°C for 24 hrs and 2% NA plates at 30°C for 24 hrs.

Mean bacterial counts were subjected to log 10 transformation and data were analyzed using the minitab statistical analysis package.

RESULTS AND DISCUSSION

Enumeration data of Total Bacterial Counts (TBC) and total *Vibrio* counts for affected pond water and sediment are summarized in Fig. 1 and Fig. 2. TBC and total *Vibrio* counts were significantly higher ($p < 0.05$) in sediment than in water samples and also could be attributed to the presence of nutrients, in higher concentrations in sediments which enhances the bacterial growth.

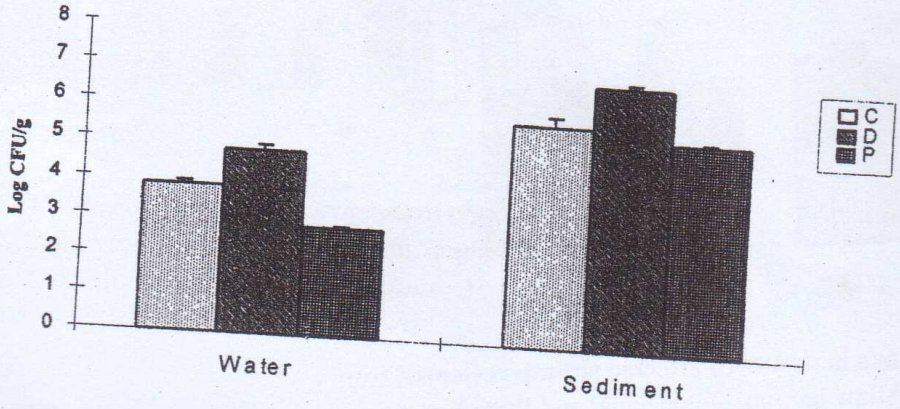

Fig.1 Total Bacterial Counts in water and sediments of shrimp culture ponds bordering Chilaw lagoon, Dutch canal and Puttalam lagoon. C- Chilaw lagoon, D- Dutch canal, P-Puttalam lagoon

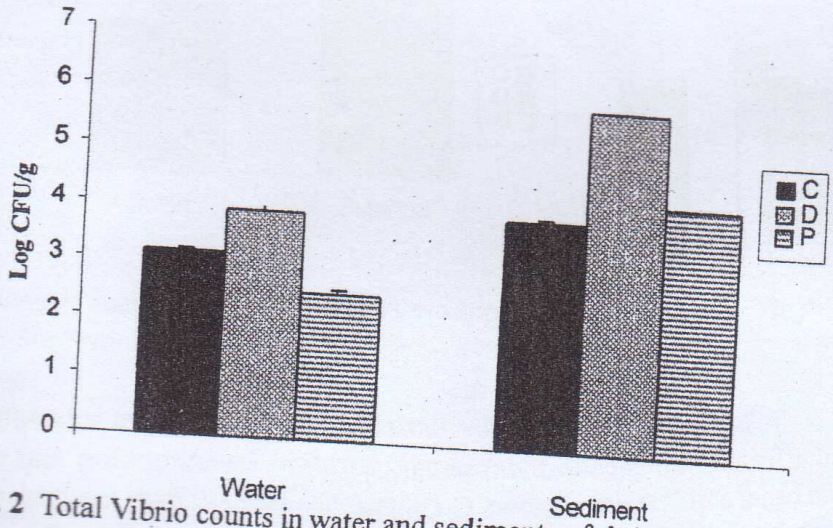

Fig. 2 Total Vibrio counts in water and sediments of shrimp culture ponds bordering Chilaw lagoon, Dutch canal and Puttalam lagoon. C-Chilaw lagoon, D-Dutch canal, P- Puttalam lagoon

Total bacterial counts as well as total *Vibrio* counts were significantly high ($p < 0.05$) in shrimp farms where the Dutch canal act as the main water source than in the farms bordering other water sources. It has been shown that the water quality in the Dutch canal has been polluted due to shrimp farms and the Dutch canal act as water source for nearly 70% of the total farm population (Corea *et al.*,1995).

TBC and total *Vibrio* counts in tissues (hepatopancreas, gills, carapace and muscles) of affected shrimp from farms bordering the Chilaw lagoon, Dutch canal and Puttalam lagoon are presented in Fig 3. and Fig. 4. TBC and total *Vibrio* counts were significantly high ($p < 0.05$) in affected hepatopancreas than in other tissues concerned except one occasion. TBC and total *Vibrio* counts of shrimp tissues from farms bordering the Puttalam lagoon were significantly high ($p < 0.05$) except few occasions.

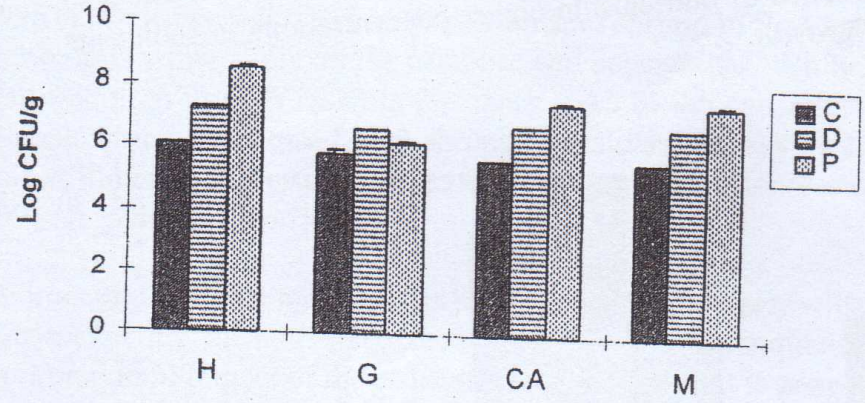

Fig. 3 Total Bacterial Counts in tissues of affected shrimp collected from farms bordering Chilaw lagoon, Dutch canal and Puttalam lagoon. C-Chilaw lagoon, D-Dutch canal, P-Puttalam lagoon H-Hepatopancreas, G-Gills, CA-Carapace, M-Muscle

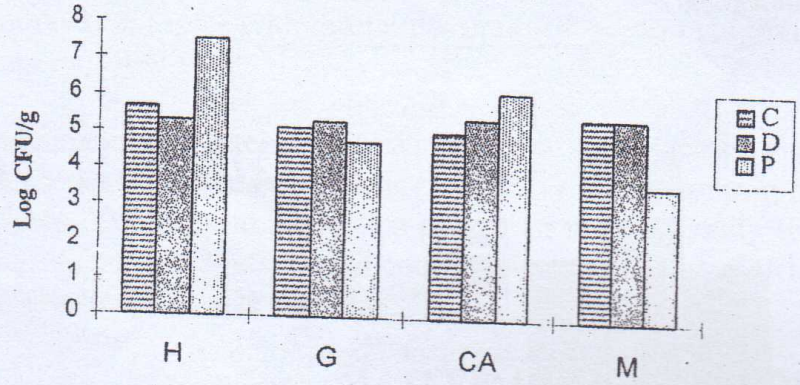

Fig.4 Total Vibrio Counts in tissues of affected shrimp collected from farms bordering Chilaw lagoon, Dutch canal and Puttalam lagoon. C-Chilaw lagoon,D-Dutch canal,P-Puttalam lagoon H-Hepatopancreas, G-Gills, CA-Carapace, M-Muscle

According to Nash (1990) hepatopancreas of the shrimp affected with viruses appears to be more susceptible to subsequent *Vibrio* infection which leads to a more severe chronic inflammatory response of nodule formation, which with septicemia are the eventual causes of death.

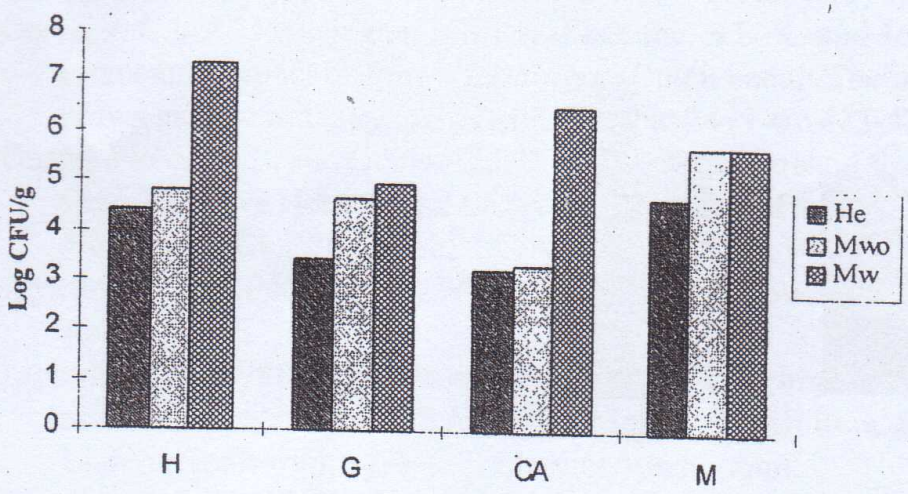

Fig.6 Comparision of total Vibrio counts in different tissues of shrimp. He-Healthy, Mwo- Moribund without visual symptoms, Mw- Moribund with symptoms, H- Hepatopancreas, G- Gills, CA- Carapace, M- Muscle

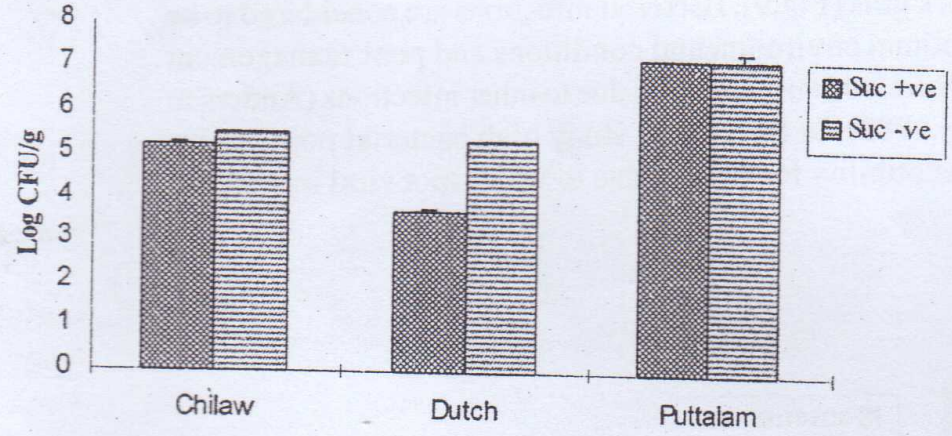

Fig. 7 Sucrose fermenting and sucrose non fermenting Vibrio spp. in WSBV affected hepatopancreas. Suc +ve- Sucrose fermenting, Suc -ve- sucrose non-fermenting

Total *Vibrio* counts of muscles from disease and apparently healthy shrimp were similar. In all the tissues *Vibrio spp.* predominated in shrimp tissues with white spots indicating higher bacterial burden on them than in other shrimp.

As shown in Fig. 7 and Fig. 8 sucrose non fermenting *Vibrio spp.* are high in WSBV affected shrimp than in shrimp without clinical signs. Nash (1990) has isolated *V. parahaemolyticus*, *V. damsela*, and *V. vunificus* as some of sucrose non fermenting *Vibrio spp.* in shrimp culture indicating that several important pathogens in shrimps are sucrose non fermenting category. Promotion of the growth of sucrose fermenting bacterial population is one of the management strategies followed by shrimp farmers to control bacterial disease in shrimp culture (Anonymus,1996).

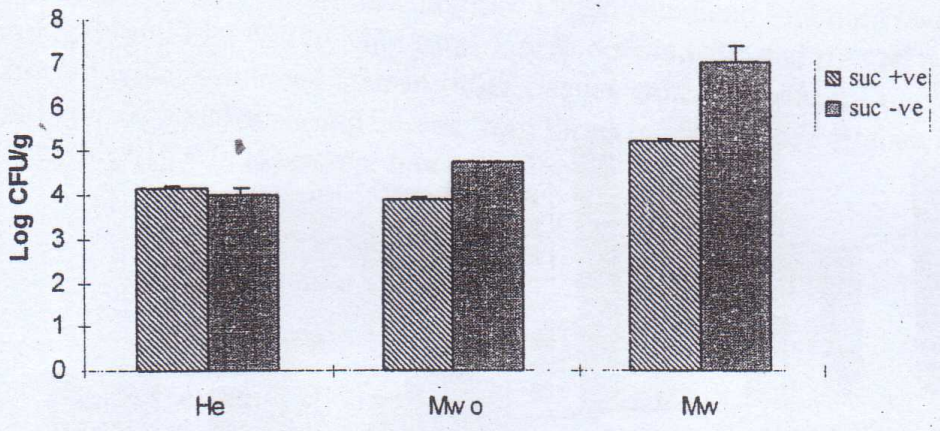

Fig. 8 Sucrose fermenting and sucrose non-fermenting Vibrio spp. in hepatopancreas of shrimp He-Healthy, Mwo-Moribund without visual symptoms, Mw- Moribund with symptoms Suc +ve- sucrose fermenting, Suc -ve-Sucrose non-fermenting

Antenna rot, black gills and tail rot were the most common signs related to bacterial infections observed in WSBV infected shrimps. Nearly 25% of the shrimp in the infected ponds showed antenna rot and black gills (Fig.9). Bacterial infections are considered to be related to stress caused by sub-optimal environmental conditions and poor management as well as prior cuticular injury or increased susceptibility due to other infections (Anderson *et al.*, 1988; Lightner, 1988, Chen.1989). In the present study high bacterial populations appear to be due to increased susceptibility to bacteria due to white spot viral infection.

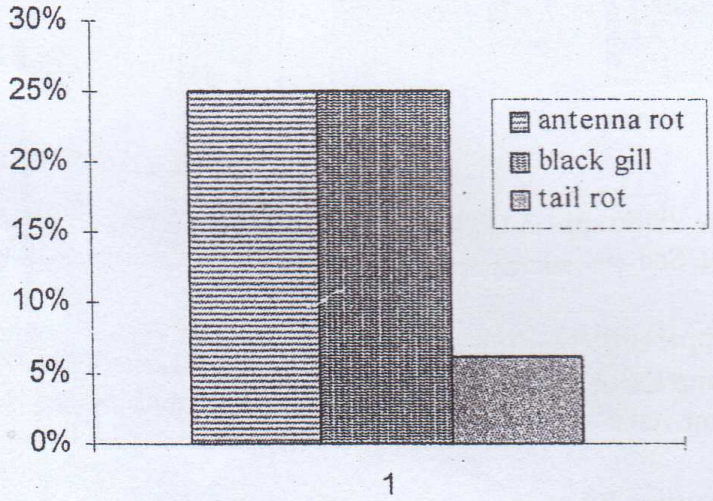

Fig. 9 Symptoms related to bacterial infections associated with WSBV

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