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Studies on luminous, *Vibrio harveyi* associated with shrimp culture system rearing *Penaeus monodon*

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Abstract: *Microbiological studies in a modified extensive shrimp culture system at Nambuthalai, southeast coast of India were carried out for a period of 120 days. Population dynamics and distribution profile of luminous bacteria and total heterotrophic bacteria in the water, sediment and animal samples were monitored. Luminous bacteria associated with exoskeleton, gills and gut were isolated and quantified. The total heterotrophic bacterial counts ranged from 1.3 x 10^4 to 25.3 x 10^4 CFU ml^{-1} in water and 1.5 x 10^6 to 26.2 x 10^6 CFU g^{-1} in sediment. The V. harveyi population density varied between 0.6 x 10^4 and 8.8 x 10^4 LCFU ml^{-1} in water and from 1.2 x 10^6 to10.4 x 10^6 LCFU g^{-1} sediment respectively. The gut of the animal was found to harbor high density of V. harveyi than gills and exoskeleton. The total heterotrophic bacteria and V. harveyi population density showed increasing trend during the culture period. The high V. harveyi density observed in this study at the end of the culture period correlated with the outbreak of white spot disease.*

Key words: *Luminescent bacteria, Vibrio harveyi, Shrimp culture, White spot disease*
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Introduction

Aquaculture is one of the fastest growing food production sectors in the world (Subasinghe *et al*., 1998). Currently, aquaculture industry in India and other parts of the world has been facing serious problems due to microbial diseases. In aquatic environments, diseases in fishes and shrimps are caused by opportunistic pathogens (Lundin, 1996). Thus, health management is of major importance in aquaculture.

Species from *Vibrio* are the most common bacterial pathogens causing some of the most serious diseases, growth retardation and sporadic mortalities in *Penaeid* shrimp (Lavilla-Pitago *et al*., 1998; Vandenberghe *et al*., 1998). Of these, the luminous *V. harveyi* is the causative agent of luminous disease. *V. harveyi* are found in coastal and marine waters, in association with surface and gut of marine and estuarine organisms and also in shrimp pond water and sediment (Otta *et al*., 1999). *V. harveyi* was also reported as the causative agent of vibriosis in tiger prawn (*Penaeus monodon*), kuruma prawn (*Penaeus japanicus*), pearl oyster *(Pinctada maxima*), (Pass *et al*., 1987; Lavilla- Pitago *et al.,* 1990; De la Pena *et al.,* 1993). Mass mortalities in shrimp hatcheries and culture ponds in Asian countries were reported by Sunaryanto and Mariyam (1986), Baticados *et al*. (1991). In India, several studies have reported V. *harvey*i-associated mass mortalities of hatchery reared *P. monodon* post larvae (Sivasankar and Jayabalan 1991; Pillai and Jayabalan, 1993; Karunasagar *et al*., 1994; Abraham *et al*., 1997). The bacterial disease control in aquaculture hatcheries has reached a stage where bacteriophages were proved to be an effective alternative for antibiotics to control infectious bacterium such as *V. harveyi* (Karunasagar *et al*., 2007). Prior to all, an effective management of the environment yields better control of diseases. This is possible through understanding the parameters causing the proliferation of pathogenic bacteria. The present research was focused to evaluate the prevalence of *V. harveyi* in a semi intensive culture system rearing *Penaeus monodon*.

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Materials and Methods

Water and sediment samples were collected every ten days from three different ponds A, B and C of a modified extensive farm located at Nambuthalai, which is a coastal village of Tamil Nadu state in the southeast coast of India. The stocking density was 10 individuals m^{-2} of *Penaeus monodon*. The study was initiated in November 2005 and continued till for 120 days. For bacteriological analysis, the subsurface water sample was collected in 125 ml sterile screw-capped PVC bottles taking necessary precautions to avoid cross contaminations. Water samples for the estimation of physico-chemical parameters were also taken from the same area. Physico-chemical parameters such as temperature, salinity, light extinction coefficient and pH of the water samples were measured with the help of thermometer, refractometer, secchi disc and pH meter respectively. Dissolved oxygen was estimated as per the procedure given by Strickland and Parsons (1976).

Sediment samples were collected using an alcohol-rinsed, air-dried, vertical corer. The central portion of the collected sediment

samples were aseptically transferred into sterile polythene bags. Total heterotrophic bacterial population density was estimated by pour plate technique with Zobell's marine agar. Plates in duplicate were incubated at 28 ± 2°C for three days and the colonies that developed on the plates were counted and expressed as CFU ml^{-1} or g. The enumeration and isolation of *V. harveyi* in water and sediment samples were made using *V. harveyi* agar (VH Agar) by spread plate technique as previously described (Harris *et al*., 1996).

From 50th day onwards the animal samples were collected. They were kept in sterile polythene bags and brought to the laboratory as quickly as possible. For the isolation of *V. harveyi*, swabs (1 cm^2) obtained from the gut, exoskeleton, and gills were inoculated on the VH Agar and incubated at 27°C. The presence of the luminous bacteria *V. harveyi* was observed in the plates after 72 hr in the darkness and their population density was expressed as luminous colony forming unit (LCFU). The isolated bacterial strains were confirmed as *V. harveyi* following the procedure given by Baumann and Schubert (1984).

Results and Discussion

Pond as a confined environment, all environmental parameters (temperature 25 - 29°C; salinity 22 - 37 ppt; pH 7.8-8.4, light extinction co-efficient 38 to 69 cm and dissolved oxygen 3.1 - 7.8ml l^{-1}) were being maintained through water exchange and lime application and they were monitored (Fig. 1-6). The total heterotrophic bacteria (THB) and *V. harveyi* load was increasing with increasing cultivation period in water, sediment and in animal samples collected from culture ponds (Fig. 7-10). In the present study the THB count varied from 1.3 x 10^4 to 25.3 x 10^4 CFU ml^{-1} in water and 1.5 x 10^6 to 26.2 x 10^6 CFU g^{-1} in sediment (Fig. 7, 8). The luminous bacteria *V. harveyi* population density varied between 0.6 x 10^4 and 8.8 x 10^4 LCFU ml^{-1} in water and in sediment it varied from 1.2 to10.4 x 10^6 LCFU g^{-1} (Fig. 9). The *V. harveyi* population density in the animal samples fluctuated between 0.5 x 10^4 and 7.3 x 10^4 LCFU (Fig. 10). The total heterotrophic bacterial count and *V. harveyi* population density was higher in sediment than water (Fig. 7, 9). The bacterial count in the sediment increases with increasing detritus organic load at the pond bottom. A short decrease in THB load

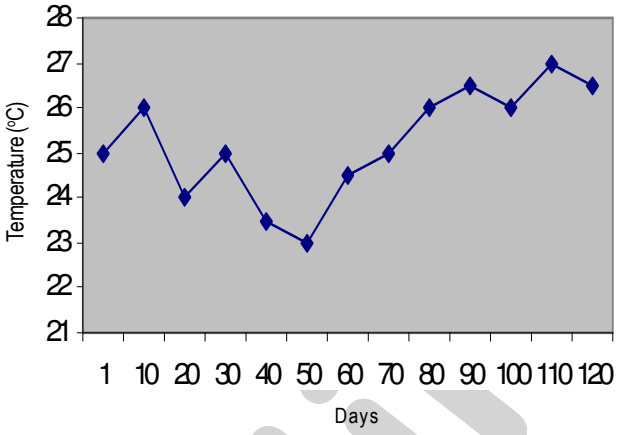

Fig. 1: Variations in atmospheric temperature

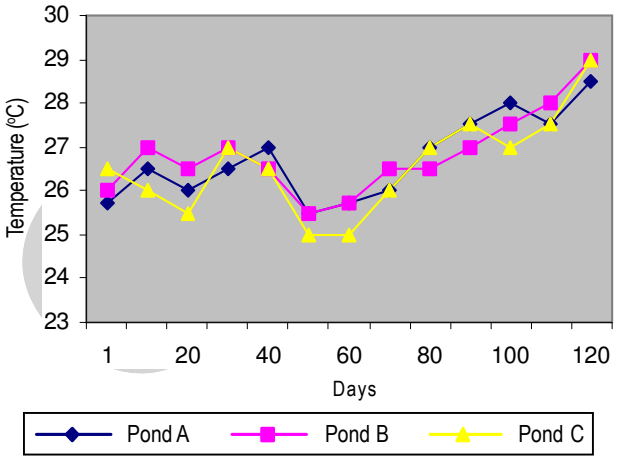

Fig. 2: Variations in surface water temperature

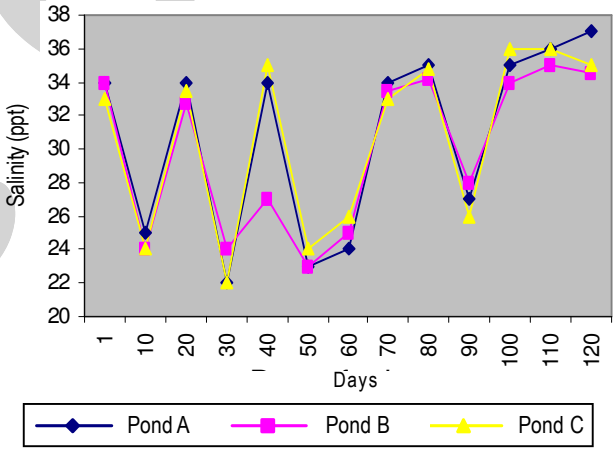

Fig. 3: Variations in salinity of water

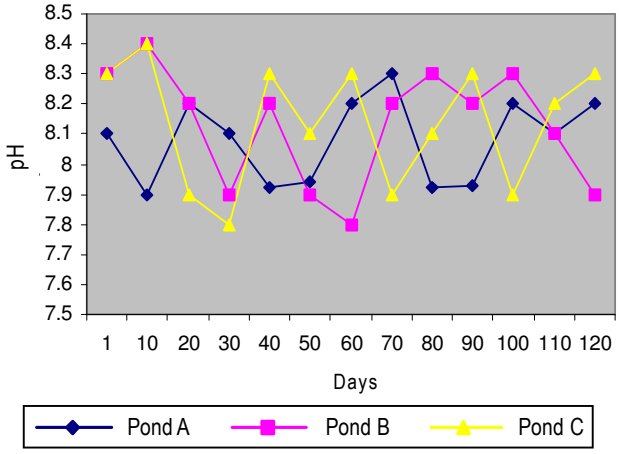

Fig. 4: Variations in pH value of water

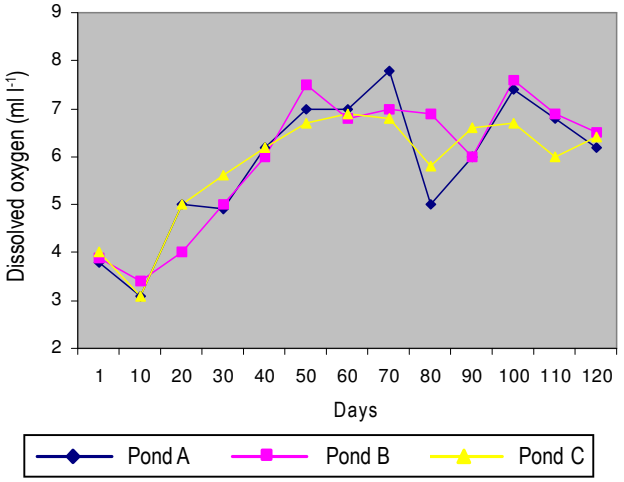

Fig. 5: Variations in dissolved oxygen of water

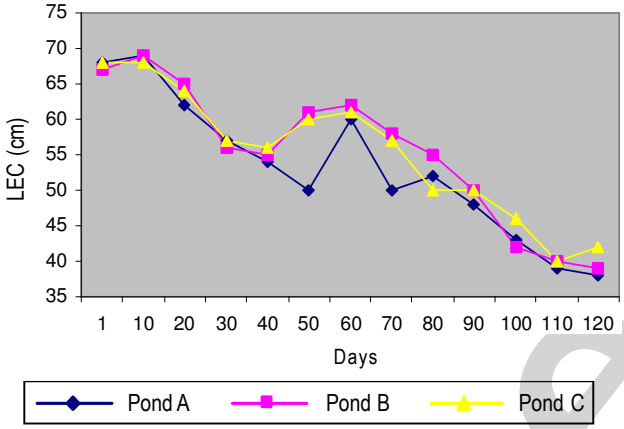

Fig. 6: Variations in light extention coefficient (LEC) of water

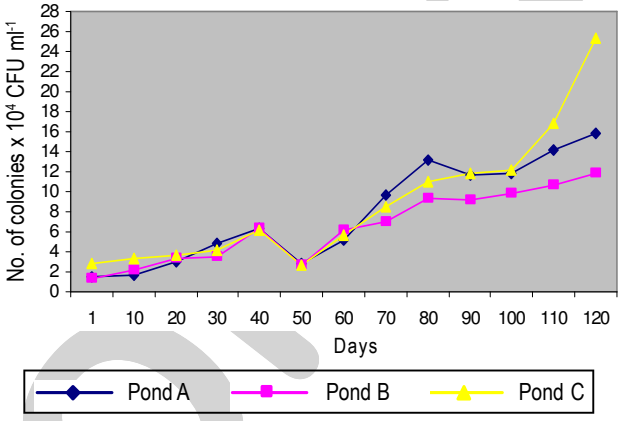

Fig. 7: Total hetrotrophic bacteria (THB) population density in water

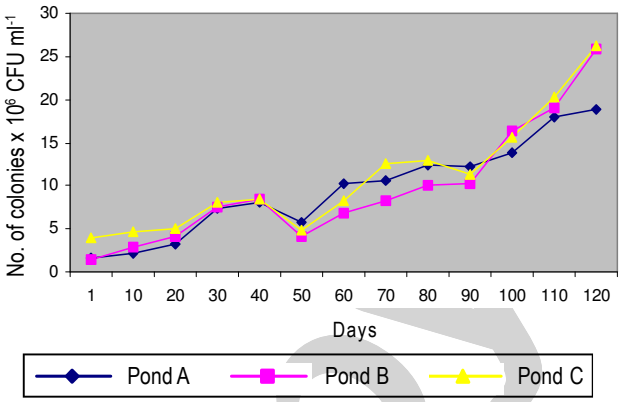

Fig. 8: Total hetrotrophic bacteria (THB) population density in sediment

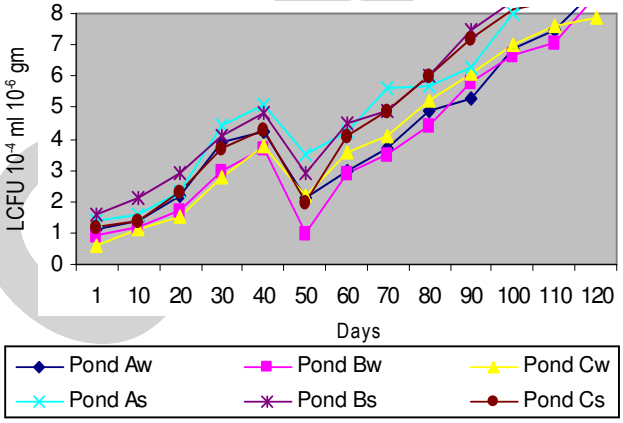

Fig. 9: *Vibrio harveyi* population density (LCFU) in water(W) and sediment (S)

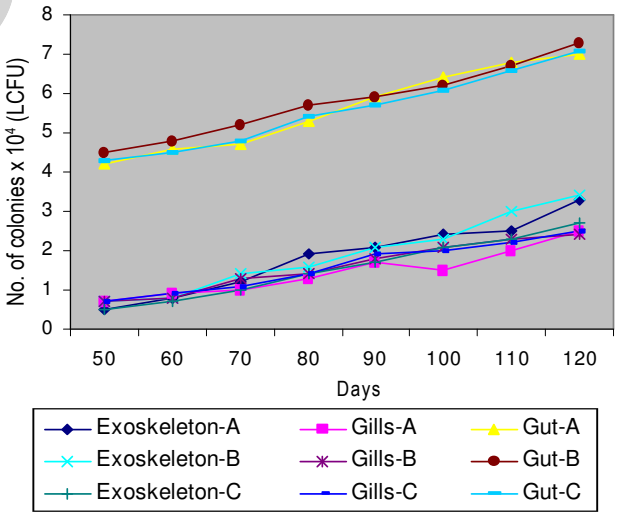

Fig. 10: Variations in *Vibrio harveyi* population density (LCFU) in animal samples collected from ponds A,B and C

in water (2.7 x 10^4 CFU ml^{-1}) and in sediments (4.2 x 10^6 CFU g^{-1}) was observed on 50th day. On the same day, the *V. harveyi* density also showed marginal decrease in water (1.0 x 10^4 LCFU ml^{-1}, Fig. 9) and in sediments (2.0 x 10^6 LCFU g^{-1}, Fig. 9). The shrimps cultured in pond had more *V. harveyi* load towards the later stage of culture and the carapace recorded the highest number of *V. harveyi*. The mean density of *V. harveyi* in gut (5.7x 10^4 LCFU), gills (1.54 x 10^4 LCFU), and exoskeleton (1.8 x 10^4 LCFU) was recorded at the end of the culture period (Fig. 10).

Proportional increase in the THB and *V. harveyi* density could be attributed to the increasing organic load because of the addition of artificial feed, fertilizers and vitamins. This observation reconciles with the earlier findings that the artificial feed and other organic additions in the aquaculture farms enhanced the heterotrophic bacterial population (MacLean *et al*., 1994; Sharmila *et al*., 1996; Dalmin *et al*., 2002). Environmental control on the bacterial density is ruled out as the pond is a confined one and the parameters were monitored and kept under control through exchange of water and lime application. Generally, the minimum population density of THB and *V. harveyi* was recorded in the month of November on the first day and the maximum population density was observed in the month of March on 120th day of cultivation. The minimum density may be due to the application of lime during the pond bottom preparation and low level of organic matter in the pond. As culture period progresses, accumulation of organic wastes in the shrimp pond takes placed due to the application of organic manure and fertilizers, feed waste, fecal matter and algal bloom (Moriarty *et al*., 1984; Laoberra *et al*., 1991; Chen, 1992).

In general, the results of our study indicate that bacterial load of shrimp pond water and sediment increases with increasing organic load, which is in agreement with previous reports by Horsley (1997), Dalmin *et al*. (2002). Higher abundance of the THB than the *V. harveyi* could be due to the accumulation of organic detritus at the pond bottom. Application probiotics on 50th day of culture may be one of the reason why the THB and *V. harveyi* density were low at that time. Vaseeharan and Ramasamy (2003) also reported that the growth of pathogenic *V. harveyi* isolated from *Penaeus monodon* with black gill disease was controlled by non-pathogenic *B. subtilis* BT23 as probiotic bacteria under *in vivo* and *in vitro* conditions.

V. harveyi remained a dominant bacterial form in the shrimp culture environment throughout the culture period is in agreement with the earlier observations (Ping and Meimei, 1994; Sharmila *et al*., 1996). *V. harveyi* play a vital role in shrimp culture ecosystem as opportunistic pathogens causing diseases and mortalities of varying intensities (Lightner, 1983, 1998; Sunaryanto and Mariyam, 1986; Lavilla-Pitago *et al*., 1990,1992; Baticados *et al*., 1991).

Occurrence of white spot disease was recorded from few shrimps at the end of culture operation. The field observations indicated that the shrimps were under stress and when the DOC was 120, the culture operation faced serious white spot disease outbreak when more populations of total heterotrophic bacteria and luminous *V. harveyi* were observed. Hence the culture organisms were harvested immediately in order to prevent the further infection to other organisms and avoid economic loss. Luminous bacterial load especially *V. harveyi* was found to be high during later stages of culture at the time of disease outbreak. Observations on the higher number of *V. harveyi* at the end of the cultivation period agrees with the previous work of Nash *et al*. (1992), Karunasagar *et al*. (1994). Further, they suggested that *Vibrios* can cause secondary infection in *P. monodon*. The present study reveal that the *V. harveyi* might have facilitated white spot disease causing virus to enter the chitinous body of shrimp due to its chitinolytic activity. The study demonstrates the relationships between the physico-chemical conditions of shrimp culture environment, the abundance of *V. harveyi* and consequent outbreak of viral diseases in farmed animals. Thus, monitoring physico-chemical parameters and the bacterial population especially the *V. harveyii* is crucial for a healthy aquaculture system. This study emphasise the indiscriminate usage of feed and higher stocking density of shrimp will trigger the high BOD and the related stress reactions make the animals more susceptible for the attack of opportunistic pathogens.

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