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Short Communication

Distribution profile of *Vibrio harveyi* in *Panulirus homarus*

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Abstract

Microbiological studies in lobster culture rearing system at Kovalam laboratory, Chennai was carried out for a period of 150 days. The infected tissues, gills, gut and the exoskeleton of the diseased animal were subjected to various microbial analysis to study the distribution profile of luminous bacterial. The total heterotrophic bacteria in the water, sediment and animal samples were also determined. The total heterotrophic bacterial counts ranged from 1.6 x 10^3 to 26.3 x 10^3 cfu/ml in water, 2.4 x 10^5-24.2 x 10^5 cfu/ml in sediment and 3.4 x 10^6 to 4.2 x 10^6 cfu/ml in infected animals. The Vibrio harveyi population density varied between 0.8 x 10^3 to 9.6 x 10^3 LCFU/ml in water, 1.8 x 10^5 to 12.5 x 10^5 LCFU/ml in sediment and 1.3 x 10^6 to 2.2 x 10^6 LCFU/ml in animal. The high amount of luminescent bacteria accounted to the outbreak of tail fan necrosis in Panulirus homarus when grown in artificial rearing system.

Key words: Distribution profile, *luminous bacterial*, *heterotrophic bacteria*, *Panulirus homarus.*

Introduction

Prevention of diseases and early diagnosis are very important in the cost effective management of aquaculture systems. Most of the disease situation, both non-infectious and infectious disease of unknown aetiology can be kept in limits of by proper management and husbandry techniques.

Infectious diseases and adverse environmental conditions might produce similar clinical symptoms in lobsters making the exact diagnosis difficult. However the primary cause of infections (by virus, bacteria or fungus) or infestations by algae, parasites might be the deteriorating environmental conditions. Hence precise information regarding the history of disease is very important in ascertaining the aetiology of diseases in lobster stock. Failure to adjust and adapt to environmental stresses like crowding, poor nutrition, low levels of dissolved oxygen, sudden changes in salinity and temperature weakens the innate immunological resistance and the opportunistic pathogens make progression to cause diseases in invitro conditions.

Hence trials were run in laboratory, at a shore-based experimental live-holding facility (LHF) to determine the influence of sea-post-fattening system, stocking density, temperature and other advent conditions necessary for the disease manifestation and growth of the lobsters.

Microbial biomass in aquatic habitat plays an important role in accordance to the host and environment relationship when such a balance is disturbed it results in disease outbreak, which affects adversely the host. In aquatic environment, the host intestine access begins when pathogens colonize in the gills and hence dissemination of different routes occur. On the other hand researchers also suggest a number of shell disease, a condition which occurs as a result of stressed condition during captivity. One such shell disease is the tail fan necrosis which has become a concern recently due to massive epidemics and economic loss during the culture and fattening process in lobsters.

Tail fan disease is a catch all term for a group of symptoms that affects *Panulirus homarus*. It is characterized by erosion and necrosis of the exoskeleton. The lesions are typically recognized in early stages as dark areas on the exoskeleton which are sometimes and other times may be pitted. The darkened spots are caused by melanization of the affected area. This is an immune response intended to contain the infection. In most cases, this is effective and the infection is lost when the animal molts. However, in severe cases, the infection can continue to the membranous layer of the exoskeleton or deeper into the tissue of the organism. This will sometimes lead to fatality, most likely due to secondary infections, or the infected shell may adhere to the membranous layer of the animal inhibiting molting. When the infection is able to penetrate the outer layers of the exoskeleton, it appears that it may spread laterally and undermine the inner layers of the shell. This undermining leads to a soft, somewhat spongy feeling when such an animal is handled, and an exoskeleton in this condition is easily cut or torn. Hence this type of shell disease has also been shown to render an organism more vulnerable to predation. A greater effort to characterize the microbial flora associated with the lesion are subjected with microbiological testing methodologies.

Chitinolytic bacteria presumably causes tail fan necrosis. These bacteria secrete chitinases that break down the exoskeleton and lead to the lesions. There are several potential mechanisms of entry of these bacteria. The pore canal and sensory hairs of the exoskeleton may become infected. The epicuticle may be breached chemically via lipolytic and/or by proteolytic may be breached due to physical damage. Physical damage exposes the underlying chitinous makes to degradation. Species of Vibrio are the most common bacterial pathogen causing some of the most serious diseases of these, the luminous *V. harveyi* is the causative agent of luminous disease. 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*[1]. The present research was focused to evaluate the presence of *V. harveyi* in a artificial fattening system of *P. homarus*.

Material and Methods

Live lobsters of the size ranging carapace length (CL) from 45mm to 50mm were obtained from Kasimedu Landing centre at Chennai, South India and brought to the Kovalam laboratory. They were acclimatized to 28+1 °C temperature and a salinity of 35 ppt for 5 days.

The lobsters were then stocked in FRP tanks according to their sizes and were fed with clams for fattening. The study was conducted for a period of 150 days.

The total number of lobsters (*Panulirus homarus*) taken in the study were 100 pieces. The stocking density in each FRP tank (1 tonne capacity) was 50 individuals that are 12 lobsters /sq.ft. Equal population of males and females were stocked in the tanks. The period of study was from Sep. 2010 – Jan.2011. During this period the water in the tank and the animals were acclimatized by luminescent vibriosis. This pathogenic vibriosis infected the tissues, gills, gut and exoskeleton, 43 numbers of lobsters showed clinical manifestation of tail fan necrosis. Of the total lobsters reared 12 lobsters died during the study period of 150 days.

Water exchange was done every day and the water used for rearing the lobster was treated through Biological filter and then rapid sand filter. Physico-chemical parameters such as temperature, salinity and pH of the water samples were measured with the help of thermometer, refractomeer and pH meter respectively.

The water temperature ranged between 23°C - 27°C, pH between 7.2 – 7.8 and the salinity ranged between 30 – 32 ppt.

The bacteriological analysis, the subsurface water sample was collected in 100 ml sterile screw – capped Tarson sampling bottles taking necessary precautions to avoid cross contaminations. Sediment samples were collected using an alcohol-rinsed, air dried vertical corer. The central portion of the collected samples was aseptically transferred into sterile polythene bags. Total heterotrophic bacterial population density was estimated by pour plate technique with Zobell's marine agar. Plates in duplicates were incubated 30°C for 1 day and the colonies were counted and expressed as CFU/ml^{-1}. The enumeration and isolation of *V. harveyi* was done in sea salt water agar using spread plate and streak plate method as described by Harris[2].

Luminescent bacteria were seen predominately in the animal by the second month of rearing. From the 50th day onward the samples were collected from the animals. Physical examination revealed the of erosive lesions on the tail, blistering of ventral surface to ragged edges of the tail, uropod ulceration, sometimes complete loss of peripod.

Exoskeleton bacterial isolates were obtained by abrasion, after sterilizing the surface of a small area of the lesion with alcohol. The scrapings were then transferred to 1 ml of sterile saline solution, homogenized and then spread onto Zobell's marine agar and incubated at 24°C for 1-2 days. Bacterial colonies were counted and separated according to colony morphology and presumptive identification test. Isolation of samples was also performed using gut and gill samples. The presence of luminous bacteria *V. harveyi* was observed in the plates after 72 hours in darkness and their population density was expressed as luminous colony forming unit (LCFU) when inoculated in sea salt water agar. The isolated bacterial strains were confirmed as *V. harveyi* following the procedure given by Baumann and Schubert[3].

Results and Discussion

Macroscopically, tail fan necrosis in *Panulirus homarus* was characterized by erosions of the dorsal and ventral side of the uropods and telson. Most of the specimens showed lesions confined to the outer layers of the exoskeleton and characterized by erosion and blister appearance of the tail, evident at the tips. Complete loss of periopod and ulceration of uropod tissue was also observed. The irregular blackened appearance of the eroded cuticle on the surface of the lesions, indicaed the activation of a phenolic oxidation process (melanization). This pattern of shell disease lesion, characterized by progressive loss of the tail fan with associated melanization is defined as tail fan necrosis[4] and is considered one of the most common shell diseases among lobsters. Studies indicate that the uropods and telson are not well perfused with hemolymph and hence they are subjected to less limiting immunological response. Thus the bacteria found in shell lesions are ubiquitous in the marine environment and are found on the exoskeleton of healthy as well as diseased lobsters[5] and become pathogenic when stressing factors are present.

Lobster health is influenced by a range of factors. One of the most important of which is stress. Stress responses are normal physiological reactions to changes in environmental conditions.

These conditions include a wide range of factors such as water quality parameters like oxygen levels, pH salinity, temperature, presence of toxins and physical factors like handling, injury, air exposure, nutrient availability. The environmental conditions can induce the survival of different kinds of pathogen which may induce disease in lobsters.

Exposure to stressors leads to short and long term changes in respiratory function, energy metabolism, fluid and ionic balance, acid-base balance and immunity[6-9]. If the stressor is mild and of short duration, the physiological disturbances are temporary. However, if the stressor is extreme, or if there is prolonged exposure even to a mild stressor, detrimental long-term effects can occur. These include reduced resistence to disease, reduced growth, impaired reproduction and reduced survival[10,11]. Hence the present study care was taken to avoid the stress given by environmental parameters and the rearing tanks were stabilized to a temperature of 23°C - 27°C, salinity ranging from 30 – 32 ppt and pH within 7.2 – 7.8.

The total number of lobsters taken in the study was 100. During the course of the study 43 number of lobsters showed clinical manifestation of tail fan necrosis. Of the total lobsters reared, 12 died during the study period.

Bacteriological analysis for the water sediment, animal showed the following results. The total heterotrophic bacterial counts ranged from 1.6×10^3 to 26.3×10^3 cfu/ml in water, 2.4×10^5-24.2×10^5 cfu/ml in sediment and 3.4×10^6 to 4.2×10^6 cfu/ml in infected animals. The *Vibrio harveyi* population density varied between 0.8 x 103 to 9.6×10^3 LCFU/ml in water, 1.8×10^5 to 12.5×10^5 LCFU/ml in sediment and 1.3×10^6 to 2.2×10^6 LCFU/ml in animal. The high amount of luminescent bacteria accounted to the outbreak of tail fan necrosis *Panulirus homarus* when grown in artificial rearing system. Hence a successful rearing system is able to balance the trade-off between operational cost and stock health in such a way to maximize financial returns from the operation. In poorly designed or maintained systems, on the other hand, stock health is comprised resulting in reduced production, poor product quality and reduced profits.

Figure-1
Lobster showing clinical manifestation of tail fan necrosis

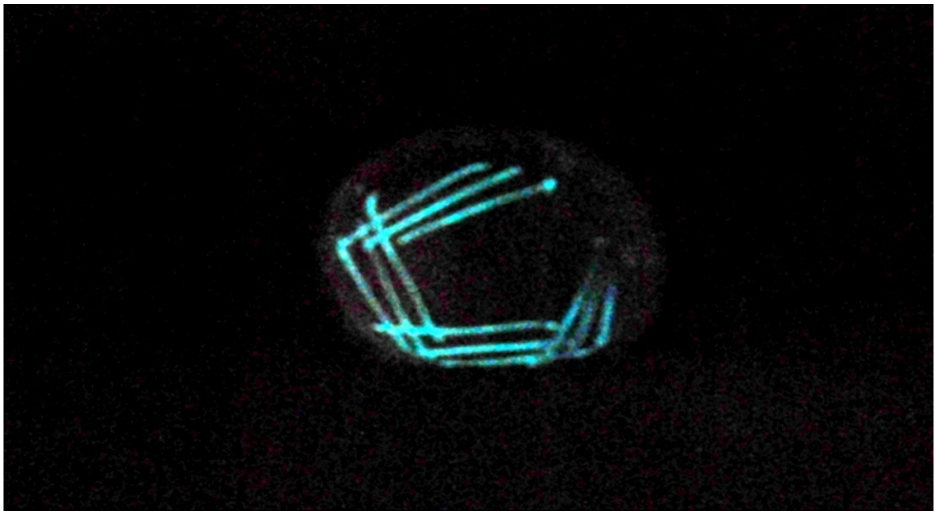

Figure-2
Luminescent *V. harveyi* in sea salt water agar

Conclusion

Lobster rearing systems are prone to various types of diseases caused by abiotic or biotic factors. Prevention of disease and yearly detection are the prerequisites to initiate management strategies to limit the damages that may occur due to disease situation. Luminous ***Vibrio harveyi*** included an important pathogen contributing the tail fan necrosis disease in ***Panulirus homarus*** should be deducted as early as possible to prevent it venture to economic losses. Maintenance of water quality and good husbandry are also required for successful rearing.

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