Loose Shell Syndrome (LSS) in Litopenaeus vannamei grow-out Ponds and its Effect on Growth and Production

Kuzhanthaivel Raja*, A Gopalakrishnan, Rajkumar Singh and R Vijayakumar
Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai 608 502, Tamil Nadu, India

Abstract
Loose shell syndrome is a chronic disease in shrimp industries, in the present study, two different L. vannamei grow-out ponds (normal and LSS infected ponds) were selected to study their water quality parameters. Average Body Weight (ABW) and Daily Growth Rate (DGR) in Prakasam district of Andhra Pradesh. The results of the present study showed, there was no much variation in water quality parameters between both the ponds except pH. The daily growth rate and average body weight was higher in the normal pond than LSS infected one. The maximum LSS prevalence was reached up to 20%. The infected shrimps became sluggish and negative allometric growth. The results of present study revealed that the LSS infected shrimps showed lower production in the grow-out pond.

Keywords: Loose shell syndrome; Litopenaeus vannamei; Average body weight; Daily growth rate; Length and weight

Introduction
Rapid development together with the intensification of farming practices since last two decades has created several problems in the shrimp industry. Disease outbreaks in cultured shrimps are the major concern which resulted in severe economic losses. In addition to existing disease problems, several diseases of unknown etiology remain to be studied to determine the causative agent and mechanisms of infection. In which, LSS is one of the important chronic disease. This is due to the poor bottom conditions of the ponds. The affected shrimp has a soft carapace with a gap between the muscle tissue and exoskeleton, shrunken hepatopancreas [1].

Loose Shell Syndrome (LSS) has been reported in the cultured Penaeus monodon since 1998 in India [2]. It is a chronic disease of farmed shrimps in Tamilnadu, southeast coast of India. Due to LSS, the feed conversion efficiency of shrimp substantially declines, leading to poor meat quality and affected ponds suffer poor survival and chronic mortalities. Society of Aquaculture Professionals (SAP) in 2002 recorded the incidence of LSS in more than 50% of the farms (>1100 ha surveyed) in coastal of Andhra Pradesh. The knowledge on LSS in L. vannamei in grow-out pond is meager as on date. Hence the present study attempts to study the LSS infection in L. vannamei and its effect on their production in grow-out ponds.

Materials and Methods
The ponds selected for study located along the banks of Musi creek, Ananthavaram (15°20’27.15” N 80°05’03.27”E) in Prakasam district of Andhra Pradesh. Two ponds were selected (normal pond and LSS infected pond) among the 12 ponds in the cluster for the comparison. The size of the culture ponds (Creek fed brackish water) were of 0.85 ha Water Spread Area (WSA) each. Post larvae (PL 7) purchased from commercial L. vannamei Specific Pathogen Free (SPF) status as per (CAA) Coastal Aquaculture Authority, India regulation and stocked with the density of 40/m². Feeding for the first month was based on the seed count. This also known as blind feeding as there was no scope for the verification of the feed consumption by the shrimp. After 30 days the pellet feed was introduced and feed adjustment and check tray feeding are based on feed consumption and the water quality of ponds.

Water quality parameters like temperature, pH and salinity were analyzed as per standard methods and Dissolved Oxygen (DO) of the shrimp ponds were analyzed by [3]. The growths of the shrimps were monitored once in a week since 35 days of culture (DOC). At the same time the animal’s health, length and weight were monitored. Average Body weight (ABW), Daily Growth Rate (DGR), the length-weight relationship was calculated using the conventional formula.

\[ W = a.L^b \]

Where was the derived weight of shrimps (g), L was the standard length (cm), coefficient a was the intercept in the axis and the regression coefficient b was an exponent indicating isometric growth when close to 3.

The normal and acute LSS infected shrimps were transported to the laboratory in live condition for histo pathological investigation. The samples were preserved in the Davidson’s fixative and the sections were done according to the methodology described by [4].

Results
The temperature was ranged between 27.4 and 29.6°C in normal pond and 27.3 and 29.7°C in LSS affected pond (Figure 1A). The salinity in the both the ponds ranged between 15 and 26ppt (Figure 1B). The pH in the normal pond was varied between 7.63 and 8, minimum was recorded on 133rd DOC and the maximum was on 112th DOC (Figure 1D). The DO level in the normal pond was ranged between 2.8 and 4.9 ppm, minimum was recorded on 133rd DOC and the maximum was on 63rd DOC (Figure 1C). The Body Weight (ABW) and Daily Growth Rate (DGR) in Prakasam district of Andhra Pradesh. The results of the present study revealed that the LSS infected shrimps showed lower production in the grow-out pond.

*Corresponding author: Kuzhanthaivel Raja, Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai 608502, TamilNadu, India, Tel: 04144-238043; E-mail: k.raja722@gmail.com

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The Daily Growth Rates (DGR) in normal pond was ranged between 0.12 and 0.31 g, the minimum was recorded on 35th DOC and maximum was on 105th DOC, in the LSS infected pond it was varied between 0.07 and 0.28 g, minimum was recorded on 98th DOC and maximum was on 199th DOC (Figure 1E). In the normal pond the L. vannamei reached the maximum growth 32.35 g on 133rd DOC, whereas in LSS infected pond it was 25.19 g on 133rd DOC (Figure 1F). The prevalence of LSS infection was 0% in normal pond and it was varied between 2 and 20%, minimum was observed on 84th DOC and maximum was noted on 115th DOC (Figure 2).

The LSS was first observed since 84th day of culture onwards, while the average body weight of the shrimps were about 15 g. Infected shrimps were sluggish, with soft muscle and exoskeleton, spongy, flaccid and feed poorly (Figure 3A), exoskeleton of the infected shrimps became damaged, the colloidal mat present on the surface, the shrimp does not moult for a long time. The hepatopancreas was melanized, shrunk and smaller in size when compare with healthy shrimps (Figure 3B). The intestine of the infected shrimps turns into opaque milky colour (Figure 3C). Gap found between the muscle and exoskeleton could be evidenced clearly (Figure 3D).

The cross section of normal hepatopancreas clearly shows hepatopancreas tubules (T), vacuolated B-cells (B) and haemal space (Figure 4A) but in case of LSS infected shrimps, the hepatopancreas tubules were degraded and haemal space was enlarged (Figure 4B). The cross of the normal muscle showing well arrangement of fiber cells (Figure 4C), but in the LSS infected shrimps shows; loss of muscle fibers structure and an expansion of the sarcomeric space (Figure 4D). The longitudinal section of normal gill showed well arrangement of gill filaments whereas the gill filaments where damaged in the LSS infected shrimps (Figure 4E,4F). The length and weight relationship showed that the b values of the normal pond was $b=2.886$ ($r^2=0.819$) (Figure 5A), whereas in the LSS infected pond $b=2.223$ ($r^2=0.617$) (Figure 5B).

**Discussion**

*L. vannamei* is a euryhaline species it can tolerate the wide range of salinity between 2 and 45 ppt [5-7] reported maximal growth between 5-15 ppt and least growth was reported at 49 ppt. In the present study the salinity fluctuation was not much. Because of the modern and intensive culture techniques, the animal vulnerable to sever several diseases including loose shell syndrome.
The loose shell syndrome characterized by a flaccid spongy abdomen due to muscular dystrophy, lethargic and flaccid. The exoskeleton forms a sort of loose covering over the abdominal musculature, with a gap between the muscle and shell in *P. monodon* by [1,2,8-10]. In the present study the same symptoms were found in *L. vannamei*, where the LSS infected shrimps became sluggish, soft exoskeleton, gap between muscle and shell, pigmented hepatopancreas and microbes fouling on the exoskeleton.

The result of the present study showed that the water quality parameters such as salinity, dissolved oxygen and temperature were not influence much for the LSS infection in *L. vannamei* but in case of pH play the major role, the pH raised at the time of infection began in LSS infected pond was due to settled down of the excess feed leads to higher organic load results in eutrophication leading to blooming. [11] observed the reason for the LSS infection due to the factors such as nutritional deficiency, pesticide pollution and poor water quality and certain management practices.

The daily growth rate was begin to decline on 77th DOC in the LSS infected shrimp pond than the normal one, it’s revealed that the LSS infection reduce the normal growth of the shrimp. Due to the decline of DGR evident in the lower average body weight than the normal normal pond at the time of harvest. Similarly [2] reported that the loose shell infected shrimp (*P. monodon*) shows minimum mean daily growth rate than the normal shrimps in the culture pond. The average body weight was higher in the normal pond than the LSS infected pond similarly.

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The LSS infected shrimps hepatopancreas, muscle and gills were totally damaged, gill fouling also occur which leads to reduction of respiration. The hepatopancreatic tubules of LSS infected shrimps (P. monodon) are mostly disarranged and disrupted; the compressed hepatopancreas tubules are also reported by [2].

In the present study the b value was lower in the LSS infected shrimps showed the negative allometric growth than the normal shrimps where isometric growth, its strongly revealed that the growth of the shrimps affected by the LSS infection in the culture pond. Positive correlation was found between the length and weight of the cultured shrimps from both the ponds but infected shrimps showed low positive correlation value than the normal one, it’s because of the changes in the weight according to the length due to the LSS infection. Similarly [2] reported the b value of LSS infected (P. monodon) is lower (b=2.2) than the normal one (b=2.7).

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