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Impact of mineral deposition on shrimp, *Penaeus monodon* in a high alkaline water

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Abstract

This study compares water quality parameters, shrimp growth and mortality rates, and biomass at harvest in two ponds of equal size, seeded with the same density (7 m^{-2}) of White Spot Syndrome Virus (WSSV) and Monodon Baculo Virus (MBV) negative post-larvae (PL)-20 of shrimp, *Penaeus monodon* in the Vellar estuary of South India. The primary difference between the ponds was the water source; one was filled from the estuary and the second with water from bore wells with high alkalinity. Temperature in both ponds was similar and reached 32°C after 185 days of culture. Dissolved oxygen (DO) levels were within the acceptable range although levels in the alkaline pond were near the lower limit for the last 90 days before harvest. Salinity levels were similar in both ponds, above optimal levels, and increased over the 185 days. Alkalinity in the estuarine water was typically < 50 ppm and again 200-320 ppm in the alkaline pond. In the alkaline pond, beginning on the 75th day mineral deposits was observed covering all parts of the shrimp including the eye and the inner gill chambers, and by harvest, 42% of the shrimp showed this coating. Elemental analysis identified the major constituents as calcium, phosphorus and manganese. Survival rates in the estuarine-water-fed pond was 92% with a total pond biomass at harvest of 1.65 tons ha^{-1} compared to survival of 79% in the alkaline pond and a biomass at harvest of 1.020 tons ha^{-1}. When well water must be used, its alkalinity should be monitored and diluted with water from other sources.

Key words

Shrimp culture, *Penaeus monodon*, Alkaline pond, Mineral deposit

Introduction

Since the mid-nineties of the previous century, shrimp aquaculture in Asia is subjected to many problems such as disease out-breaks, environmental degradation, poor pond soil and water conditions and is highly correlated with poor management practices in the pond (Lightner and Kumula, 1993; Lightner, 1996; Subasinghe, 1997; Gopalakrishnan and Parida, 2005; Fegan, 2007; Gopalakrishnan *et al*., 2008). Water quality plays an important role in shrimp culture and various aspects concerning the water quality have been reported recently (FAO, 2007). Since a decade, a few shrimp farmers of Tamil Nadu, India have been using saline bore-well water for shrimp culture, and they are able to avoid the outbreak of viral diseases (Gopalakrishnan *et al*., 2008). However when the bore-well water was used, mineral deposition was noticed on the farm implements like aerators, PVC pipes, electric wires, concrete structures (like sluices) and on the shrimps too (Gopalakrishnan *et al*., 2008). The mineral deposition made the shrimp's shells rough (rough shell disease), stunted the growth of the shrimp (Chanratchakool, 2003) and caused more mortality resulting in less survival rate.

The present investigation was undertaken to study the incidence of mineral deposition on the shrimps cultured in the bore-well-water-fed farms, to find out the growth-stage in which the mineral deposition become conspicuous, and compare the growth, survival, morphological changes, and the elemental composition of the shrimps in estuarine-water-fed and bore-well-water-fed farms.

Materials and Methods

Two shrimp culture ponds situated along the Vellar estuary (Lat. 11°27'38.93'N; Long. 79°43'10.05'E) were used. One was supplied with estuary water and the other (the "alkaline" pond) with bore-well-water of high alkalinity. Each pond was stocked with White Spot Syndrome Virus (WSSV) and Monodon Baculo Virus (MBV) negative post-larvae (PL)-20 of shrimp, *Penaeus monodon* on the same day and their size and age were the same. Water quality parameters (temperature, salinity, alkalinity, dissolved oxygen (DO) and pH were monitored every week throughout the culture period (185 days) at 06:00 hr. Salinity was measured using a hand refractometer (ATAGO, Japan) and pH using a pH pen, while total alkalinity (HCO_3 mg l^{-1}) and DO (Winkler's method) were determined according to Strickland and Parsons (1972).

Shrimp were fed with commercial pellet feed (CP Shrimp Feed, Thailand). To assess health, growth, and incidence of mineral deposition, about 200 shrimps were collected by cast netting from the four corners and the center of each pond. The collected shrimps were released back into the ponds after the observations. Samples were taken on the 25th day and then every 10 days until the end of the culture period. Special attention was paid to the condition of the exoskeleton and to any mineral deposition, which were noted qualitatively.

Shrimps showing obvious mineral deposits were examined using scanning electron microscopy (SEM) (JOEL JSM-5610-LV SEM, at the Central Instrumentation Facility, Annamalai University, India). The shrimps with appreciable mineral deposits were fixed immediately in 2.5% glutaraldehyde in 0.2 M phosphate buffer at pH 7.2. The samples were post-fixed with 1% osmium tetroxide in the same buffer, dehydrated through a graded series of ethanol, and critical-point dried. Samples were coated with gold and observed under SEM. To determine the elemental composition of mineral deposits, spectral analysis of anterior-lateral aspect of the shrimp carapace and the sixth abdominal segment were examined using an elemental energy-dispersive X-ray microanalyser (EDX) (JOEL JSM-5610-LV SEM). Total shrimp biomass, survival percentage, feed conversion ratio (FCR) = Food consumed (g) / Wet weight gain (g), daily growth rate (DGR) = Wet weight gain / experimental days and average body weights (ABW) were determined at the time of harvest.

Results and Discussion

Temperatures varied from 28.9-32.2 and 29.8-32.1°C, respectively in the estuarine-water-fed and bore-well-water fed ponds. Both ponds showed little difference in temperatures over the 185-day study period (Table 1). Salinity, however, was somewhat lower (31‰) in the estuarine-water-fed pond until day 90, after which it was similar, but higher on the day 185, the last of the study period, when a maximum salinity of 45.8‰ was recorded. Dissolved oxygen ranged between 3.4 - 5.9 and 3.2 - 5.7 ppm, respectively in the estuarine-water-fed and bore-well-water fed ponds. Dissolved oxygen levels in the ponds were similar to each other for the first 45 day, but then differed quite considerably from each other. The pH of the estuarine-water-fed pond gradually increased from 7.7 on the first day to pH 8.3 on the day of harvest. In contrast, while the "alkaline" pond showed pH ranging from 8.2 to 8.9, with a generally rising trend during the period. The alkalinity of the water in the alkaline pond was consistently much higher than the estuarine-water-fed pond. Minimum and maximum alkalinity values were respectively 35.1 - 87.11 ppm for the estuarine-water-fed pond and 197.0 - 321.33 ppm for the alkaline pond (Table 1).

The daily average body weight of shrimp of the two water system was steady and showed higher at harvest (32.54 g) in estuarine-water-fed pond in compared to 25.01 g in alkaline pond (Fig. 1 A). The daily growth rate showed a more complicated pattern (Fig. 1B), but was almost constantly higher in the shrimp in the "alkaline pond". Growth in the estuarine-fed pond declined

Table - 1: Comparison of water quality parameters in estuarine-water-fed and bore-well-water-fed ponds during the study period

Parameter	Estuarine-water-fed pond	Bore-well-water-fed pond
Temperature (°C)	28.9 - 32.2	29.8 - 32.1
Salinity (‰)	22.1 - 45.8	30 .0- 41.3
Dissolved oxygen (ppm)	3.4 - 5.9	3.2 - 5.7
pH	7.7 - 8.3	8.2 - 8.9
Alkalinity (ppm)	35.1 - 87.11	197.0 - 321.33

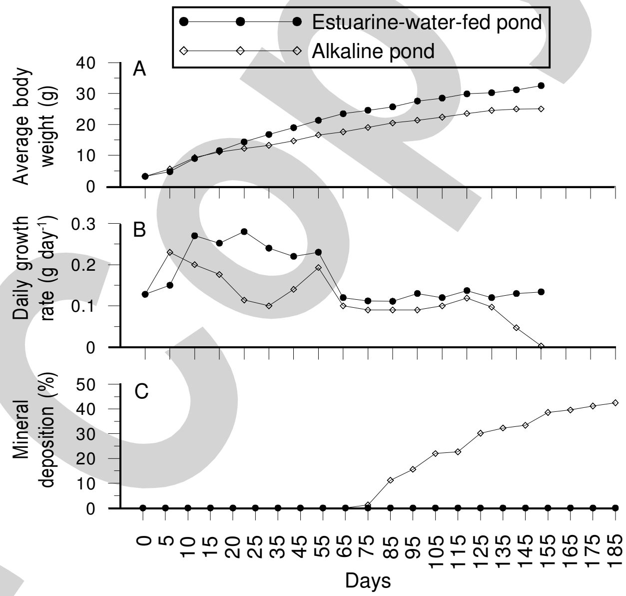

Fig. 1: Comparison of shrimp growth in the estuarine-water-fed and alkaline ponds during the study period. (A) Average daily body weight; (B) Daily growth rate; (C) Percentage of shrimp showing mineral deposits on their exoskeleton

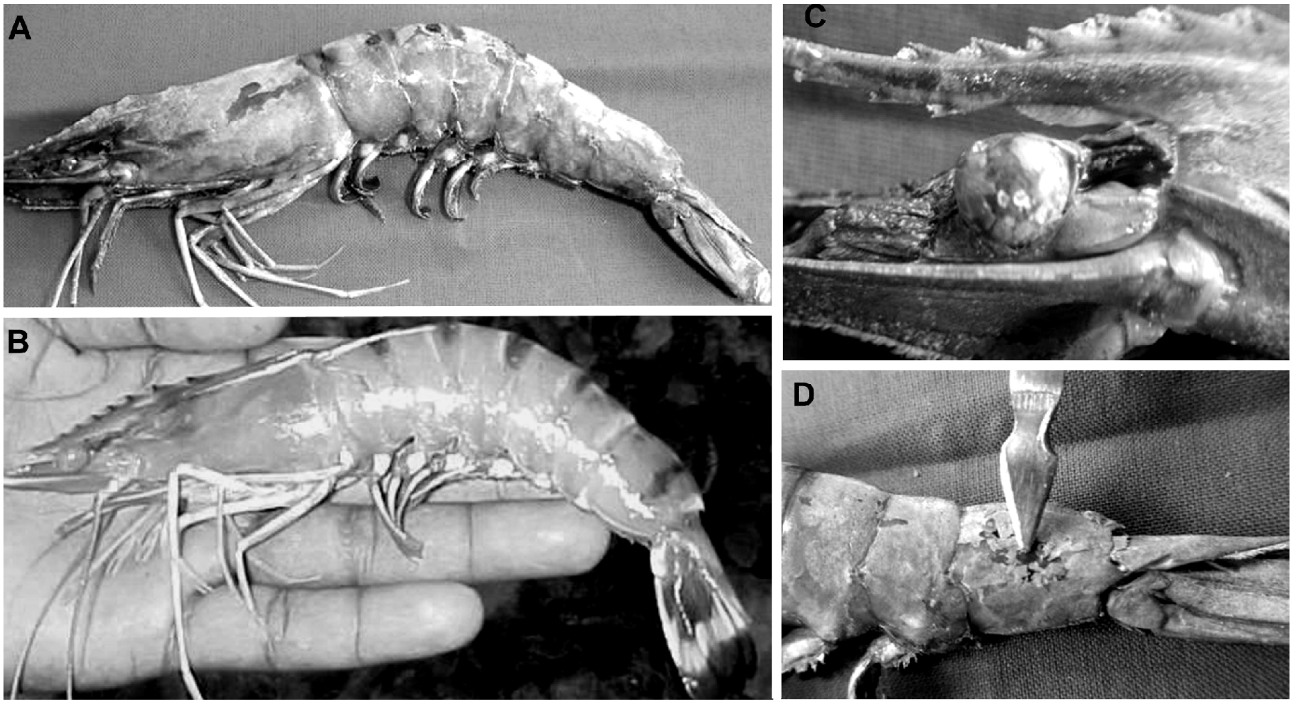

Fig. 2: Photographs showing mineral deposits on (A) shrimp from the alkaline pond, (B) shrimp from estuarine-water-fed pond lacking deposits, (C) deposits covering eye and (D) deposits on 6[th] abdominal segment

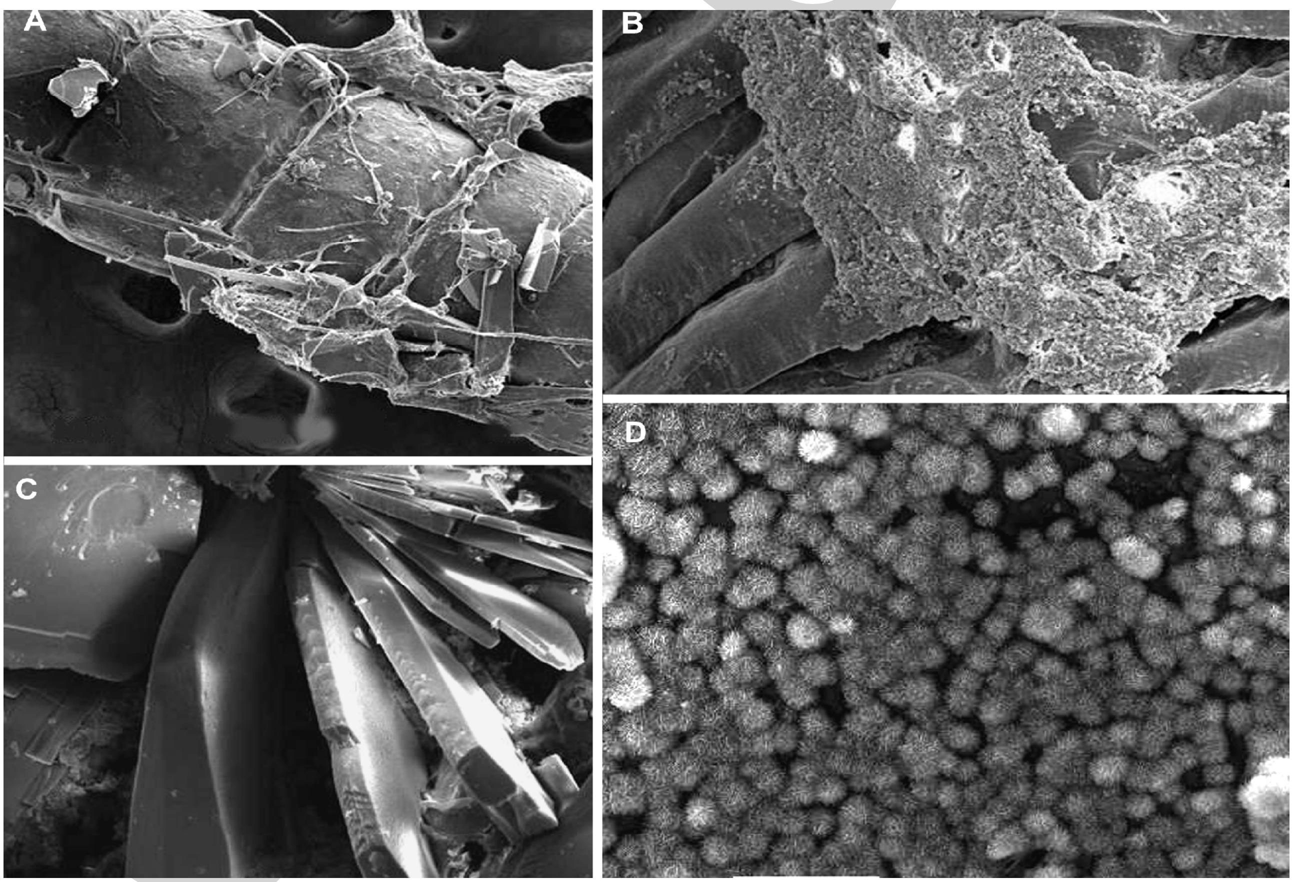

Fig. 3: Scanning electron micrographs showing (A) deposits on antenna, (B) deposits on gill, (C) fracture through deposit, (D) high magnification view of deposits. All scale bars = 100 μm

sharply after day 165 to 0 on last day of the study period, 185. The growth rate pattern is different to body weight due to the stress created by the mineral deposition on the shrimp, which ultimately reduce the consumption of feed. So, it's ultimately needs to poor growth.

The final biomass in the estuarine -fed pond was 1.635 tons ha^{-1} and only 1.020 tons ha^{-1} in the alkaline pond. The FCR of the estuarine-water-fed pond was 2.82 and 3.19 in the alkaline pond. The final survival in the estuarine-water-fed pond was 95.4 and 69.87% in the alkaline pond.

Mineral deposits were not observed on shrimp in either pond until day 75, when 1.2% of shrimps in the alkaline pond had deposits (Fig. 1C). By day 185, deposits affected 42.5% of shrimps in the alkaline pond (Fig. 2 A) but none of the shrimp in the estuarine -fed pond (Fig. 2B). Mineral deposition is automatically being calculated while analyzing the material by the EDX. The mineral deposits were clearly seen, even with the naked eye, on all body parts immediately after the shrimp were exposed to air (Fig. 2C). The heaviest deposition was observed on the sixth abdominal segment (Fig. 2D). SEM images of mineral deposition as a thin layer on the antenna and the inner side of the gill lamellae (Fig. 3 A and B). At higher magnification the deposits appear as multiple layers which fractured vertically during processing (Fig. 3C). Fig. 3D shows an undisturbed layer of mineral deposits from the carapace. Each ovoid crystal is approximately 8-12 µm in diameter and is composed of short rods. The elemental composition of mineral values of manganese, sodium, magnesium, aluminium, silica, phosphorus and calcium (%) contents (of carapace and abdomen 6th segment) were 12.87, 2.18, 0.51, 5.14, 6.55, 33.22, and 39.53 and 49.42, 0.52, 1.62, 2.16, 3.17, 32.35 and 10.76 respectively. EDX analysis of the deposits revealed they are composed primarily of manganese, phosphorus and calcium, with some variation between the carapace and abdomen. Calcium deposition was dominant on the carapace, while manganese was the most abundant element on the sixth abdominal segment. The other major elements recorded were phosphorus, magnesium and silica.

The biomass at harvest of *P. monodon* cultured in the two adjacent and similar ponds along the Vellar estuary in South India was significantly different. Both ponds were seeded with PL 20 at a density of 7 m^{-2} and differed primarily in the source of water. One pond supplied with estuarine-water-fed pond produced 1.635 tons ha^{-1} while the other “alkaline” pond, supplied with high-alkalinity bore-well water, produced only 1.020 tons ha^{-1}. Both harvests were lower than the 5-15 tons ha^{-1} reported using a higher stocking density of 30-70 m^{-2} (Liu and Manacebo, 1983). The FCR is an important tool in striving for cost reduction. Previous studies (Akiyama *et al*., 1991; Tacon *et al*., 1998) reported a global average FCR of 2.1 at a stocking density of 10-15 m^{-2}. In the present study, the FCRs in the estuarine-water-fed pond and alkaline ponds were 2.82 and 3.19, respectively. This suggests that the lower stocking density provided more food per shrimp. The growth rate in the alkaline pond was 0.23 g day^{-1} compared with 0.28 g day^{-1} in the estuarine-water-fed pond and both were low compared with a global average of 17 g day^{-1} (Chen *et al*., 1989).

Because water quality is essential for good shrimp production, several parameters were monitored daily and many were within optimal ranges for the shrimp in both ponds (Rutledge and Guest, 1977). Optimal temperature has been reported between 27-33°C (Maguire and Allen, 1992), and in our ponds the temperature was between 29 and 32.2°C. Optimal DO has been reported between 3-5 ppm and lower levels may result in mortality (Lee and Wickens, 1992; Maguire and Allen, 1992; Garcia and Bruna, 1999). Levels in both ponds ranged between 3.2 and 5.5 ppm, but during the last 90 days the alkaline pond consistently had DO levels near the bottom of this range. Optimal salinity has been reported between 15 and 25‰ and higher or lower levels may affect growth physiology (Garcia and Bruna, 1999). Higher salinity favors PL survival and resistance to WSSV (Liu and Manacebo, 1983; Alday-Sanz, 2006). Salinity in both ponds began above 25 and reached 40 and 45.8‰ by harvest in the estuarine-water-fed pond and alkaline ponds respectively. Both the pond exceeded the recommended level and comparatively higher salinity recorded in the alkaline pond. In the estuarine-water-fed pond, the water pH was always within optimal levels of 7.8-8.5 (Lee and Wickens, 1992) while alkaline pond water was at the upper end of this range at 8.2-8.9.

The water quality parameter that showed the greatest difference between the two ponds was alkalinity. General concerns about using ground and well water, and alkalinity were noted by Boyd (2005, 2007) and in our study the remarkably high alkalinity in the alkaline pond is most likely the major contributing cause for the lower productivity, the low growth rate, and the low survival of shrimp in this pond. Estuarine-water-fed pond was within the optimal levels while water in the alkaline pond was higher than previously reported from the zero-water exchange system in the Chilka lake area of Orissa, India where alkalinity ranges between 42 and 140 ppm (Balasubramanian *et al*., 2004).

A result of the high alkalinity in the water of the alkaline pond is the mineral deposition on the cuticle of the shrimp which was noticeable on every surface of the body including eyes and gills. Chanratchakool (2003) stated that the pond alkalinity above 150 ppm coupled with pH levels above 8.3 lead to the deposition of calcium on the exoskeleton. Studies on shrimp in similar habitats are limited and some parts of the carapace on the shrimp, *Rimicaris exoculata* living by hydrothermal vents are similar. These shrimp are exposed to mineral laden water, and bacteria associated with iron oxide deposits have been described on their branchial chambers (Corbari *et al*., 2008).

It is not clear how the thin mineral deposit over the abdomen and carapace causes physiological problems. We have no evidence that the deposits may be covering internal surfaces of the gut and at this time must assume the problem is related to depositions on the exoskeleton. Even a partial covering of the gills could diminish oxygen uptake especially during the last 90 days when DO levels were consistently low in the alkaline pond. Decreased blood oxygen levels decrease shrimp growth and immune function (Scholnick *et al.*, 2006).

This report documents for the first time deterimental effects of high alkaline water in shrimp culture. To manage this problem, when bore-well-water is used for culturing *P. monodon*, to the bore-well-water should be diluted with non-alkaline water and monitored carefully.

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