Short-Term and Long-Term Effect of Acidic and Alkaline p^H on the Mortality, p^H Tolerance, Growth and Development Pattern of Shrimp (Litopenaeus Vannamei)

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Abstract

In recent years, drastic alterations in hydrogen ion concentrations of the aquatic systems leading to environmental acidity and alkalinity, posing a severe problem to aquatic life causing decline and disappearance of many inhabitants in different parts of the globe, especially sustenance of shrimp culture. Hence, Short-term (24 hours) and long-term (90 days) effect of acidic and alkaline p^H on the mortality, p^H tolerance, growth and development pattern of Litopenaeus Vannamei has been studied. The sub-lethal limit was determined after exposing Litopenaeus Vannamei for 168 hours in all p^H media. Furthermore, from the experimental results, it has been observed that sub-lethal limit was from $6.5p^H$ to $8.5p^H$. The growth of shrimp was studied from 10 days up to 90 days period at different ranges, wherein the maximum reduction has been found in the first ten days only. However, at the end of the experiment there has been 12.41 percent decrease in the body weight at $8.5p^H$ and in total 16.58 percent productivity get decreased.

Keywords: Acidic, Alkaline, p^H, Short term, Long term, Sub-lethal.

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Introduction

A dynamic equilibrium exists in aquatic ecosystems between biotic and abiotic factors. The chemical and physical demands of life in water impose vigorous constrains on aquatic species. In aquatic habitats, variations occur usually in abiotic factors such as temperature, salinity, photoperiod, pH, turbidity and gaseous contents daily and seasonally. Each of these factors, single or together if altered can impose a considerable load of stress on the physiology of aquatic living beings. Stress is a best descriptive term for environmental pressures, which require physiological compensation in living material. The pesticides, organic pollutants, organic and inorganic acids from different sources frequently enter the aquatic systems and create a type of stress condition to the organisms.Shrimps perform all their bodily functions in water. This is because they are very dependent upon water to breathe, feed and grow, to excrete wastes, to maintain a salt balance, and to reproduce. Understanding the physical and chemical qualities of water is critical to successful aquaculture. Largely, water determines the success or failure of aquaculture operation. Very high (> 9.5) or very low (< 4.5) p^H values of media are unsuitable for most aquatic organisms. Young and immature stages of aquatic insects are extremely sensitive to p^H values. Moreover, high p^H levels (9.0 to 14.0) may cause harm to shrimps by denaturing cellular membranes. Changes in p^H can also affect aquatic life indirectly by altering other aspects of water chemistry. To the contrary, low p^H levels accelerate the release of metals from rocks or sediments in the stream. These metals can affect animal’s metabolism and their ability to take water in. At high p^H (>9) the ammonium in water is converted to toxic ammonia, which can kill organisms. Moreover, cyanobacterial toxins can also significantly influence the animal populations. Thus, p^H is important in aquaculture as a measure of the acidity of the water or soil. In addition, it is to be noted that aquatic organisms may not survive in waters below p^H 4.0 and above p^H 10.0 for long periods. The other major reason for the death of shrimp at altered p^H media was due to damage to the gill structure and due to broken gill lamellae. Another possible reason for mortality due to extreme acidic p^H may be due to loss of bicarbonate ions causing acidic and death of shrimp. Similar studies have reported causing the loss of sodium, calcium, chlorides and carbonate ions under altered p^H conditions (Beamish et al. 1975). In total, one can conclude that the mortality of shrimp was due to toxic effect hypoxia or ionic imbalance or both. The survival of shrimp varied at different p^H ranges. The stress for the animal is more at extreme p^H range. Moreover, it is also true for any toxin. In this experiment, the survival time of animal depends upon the intensity of lethality as found in the evidence through extreme p^H variations. It is because of its commercial and nutritive value several studies have been conducted on prawn (Itami, Takahashi & Nakamura, 1989; Lavilla-Pitogo et al. 1990; Itami et al. 1991; Vera et al. 1992; Lee et al. 1997) in various countries such as Australia (Pizzutto & Hirst, 1995), India (Karunasagar, Pai & Malathi, 1994), Indonesia, Thailand (Jiravanichpaisal, Miyazaki & Limsuwan, 1994), the Philippines (Baticados et al. 1990) and Taiwan. However, much work

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needs to be undertaken in order to assess the effects of altered pH medium on the mortality, growth and production of Litopenaeus Vannamei.

Literature Review

Altered pH medium also exerts equal stress condition on the aquatic living beings like temperature, salinity, oxygen and other physical conditions (McDonald et al. 1980). The important food organisms of aquatic animals are also affected by the acidification of lakes. Many studies have dealt with the relationships between mortality and the various environmental conditions in prawns, such as anatomical works on the general description of white shrimp (Young, 1959), the branchial organ of the tolerance and respiration of prawn on exposure to air (Egusa, 1961; Defur et al. 1988; Whiteley & Taylor, 1990; Whiteley, Al-Wassia & Taylor, 1990; Nakamura, 1994; Samet, Nakamura & Nagayama, 1996), relative humidity effect on kuruma prawn (Samet & Nakamura, 1997), histology with fine structure of the gills of various penaeid species (Talbot, Clark & Lawrence.1972; Couch, 1977).

The range of concentrations or intensities of environmental factors are variable within which an animal is able to survive for its approximate average survival time has been defined as the zone of tolerance (Fry, 1971).The resistance of various fresh water animals to strong alkalies in distilled and natural waters has been studied by many investigators (Eicher, 1946; Bhaskar et al. 1984). Other studies on the survival and mortality of fishes in response to pH stress include (Hill & Hampton, 1969; Beamish & Harvey, 1972; Dunson & Martin, 1973; Daye & Garside, 1975; Robinson et al. 1976; Speir, 1987; Hall Jr. et al. 1993). It is through the considerable data available on this aspect it can be generalized that the approximate lethal limits of pH ranges between 3.7 to 5.4 in acidic waters and between pH 9.5 to 11.1 in alkaline waters for different aquatic animals.

Other studies have determined the pH tolerance capacities of various developmental stages in different fish (Speir, 1987; Hall Jr. et al.1993) species such as larvae, young fry, embryos, and eggs (Mount, 1973; Daye & Garside, 1976; Daye & Garside, 1980) and tolerance to other stress conditions were reported in prawn (Defur et al. 1988; Whitely & Taylor, 1990; Samet, Nakamura & Nagayama, 1996; Samet & Nakamura, 1997). Although a number of studies exist on the mortality and survival of fishes due to the relative toxicities of various acids, much less work has been done to determine the physiological changes associated with pH stress of aquatic animals, especially prawns exposed to low environmental pH. At low pH, suffocation and excessive mucous secretions have also been reported by several workers (Daye & Garside, 1976). Decreased respiration was also reported in prawn under other stress conditions (Taylor & Whiteley, 1989; Whiteley & Taylor, 1990; Nakamura, 1994; Samet, Nakamura & Nagayama, 1996). The decreased ability of aquatic animals to extract oxygen from low pH waters is attributed to the decreased blood pH in acidic waters (Neville, 1979). The influence of pH on the exchange of

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sodium in fresh water animals is well-documented (Leivestad & Muniz, 1976).

Reduced growth of animals on exposure to acidic media was reported a number of workers (Menendez, 1976). Mount in 1973 demonstrated that both egg production and hatchability were reduced in Zebra fish. Acidic surface waters in combination with selected trace metals become toxic to various biological groups, particularly fish. Results from various studies have demonstrated that several anadromous and semi- anadromous (migratory) fishes to Chesapeake Bay may be adversely affected by surface water acidification (Buckler et al. 1987; Hall, 1987; Hendrey, 1987; Janicki & Greening, 1988).

Relevance

Litopenaeus Vannamei has been selected as the test animal because it is having high commercial and nutritive value along with tolerance capacity towards stress conditions. Hence, the study has selected to understand the short- term and long-term effect of altered pH on mortality, growth, and production of Litopenaeus Vannamei.

Objective

To assess the short-term and long-term effect of altered pH on mortality, growth and production of Litopenaeus Vannamei.

Materials & Methods

The shrimp Litopenaeus Vannamei has been selected for the present study in view of its high tolerance capacity towards different stress conditions, commercial and nutritive value. The shrimp has been exposed to different p^H levels in both acidic and alkaline media and determined the mortality and survival p^H ranges. In acidic medium p^H3.5 to 7.0 was observed, whereas in alkaline medium 7.0 to 10.5 p^H was recorded. The mean survival time of shrimp was drastically reduced at extreme p^H ranges and no mortality was observed at sub-lethal ranges which envisage the lethal p^H is more toxic. A dropping method has been designed for the maintenance of constant p^H in the medium, I N HCL has been used for the acidic medium and I N NaOH used for alkaline medium (Bhaskar et al. 1982) respectively. All animals were maintained at the rate of 50 animals for cubic meter water.

Results & Interpretations

In control shrimp, there was no mortality and mean survival time was found to be 166 hours (Table 1). In the acidic range at 6.5p^H also no mortality was observed. The mean mortality time was also same (166 hours). Interestingly, 50 percent mortality was noticed at 5.5p^H with mean survival time of 13 hours, but at 4.5p^H the

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mortality was 75 percent with a mean survival time of seven hours. At 3.5p^H all shrimps died within 1.69 hours. The sub-lethal limit was determined after exposing Litopenaeus Vannamei for 168 hours i.e., 7 days in all p^H media. It has been observed from aforementioned experimental results that sub-lethal concentrations of shrimp Litopenaeus Vannamei were found to be from 6.5p^H to 8.5p^H. In case of alkaline media, 100 percent mortality was noticed at 10.5p^Hbut at 9.5 p^H 50 percent mortality was found. No mortality was recorded at 8.5p^H even after 6.9 days in alkaline media. The mean survival time has been 1.75 hours at 10.5p^H, but at 9.5p^H the mean survival is only 76 hours.

LC50 values

The 100 percent survival has been recorded only between the 6.5 to 8.5 p^H after13hrs. In addition, LC 50 values are noticed in acidic media at 5.5p^H, but in alkaline media, at 9.5p^H. THE ANOVA results showed that there was a significant difference in the results between columns but not rows. Data on the effect of altered p^H media on the weight of Litopenaeus Vannamei during chronic exposure at different p^H values has been presented Table 1.2. It is during shrimp rearing, the growth of Litopenaeus Vannamei as indicated by the body weight in grams has been recorded. The data were collected at a frequency of 10 days up to 90 days period at different ranges. The results showed that at p^H levels 3.5, 4.5, 6.5, 9.5 and 10.5 all animals died, wherein the shrimp did not survive for even for 10 days. However, at 7.5 and 8.5p^H, all animals survived until the completion of the life cycle that is 90 days. In addition to this, under normal conditions (control) body weight was found to be 14.5 grams after completion of rearing for 90 days (Table-2). The data showed that body weight at 8.5p^H has been lesser than that of the body weight at 7.5p^H (control). It is after 10 days of rearing, there was 27.8 percent reduction in the body weight at 8.5p^H. The maximum reduction was found in the first ten days only. However, at the end of the experiment there has been 12.41 percent decrease in the body weight at 8.5p^H. The data regarding the economic characters such as productivity of shrimp at different p^H levels are indicated in Table 3. The shrimp count was 68.96 at 7.5p^H, but it increased to 78.74 at 8.5p^H. The survival of the shrimp was 86 percent at 7.5p^H. However, survival was only 80 percent at 8.5p^H. The total feed consumed for 1000 shrimps was 0.948 kg, but for the same number at 8.5p^H, consumed (1.23 kg.). This clearly shows that at higher p^H we have to give more food (1.285 kg). The productivity also decreased at 8.5 p^H (1.016 kg.), when compared to productivity of 1.218 kg at 7.5p^H. In total 16.58 percent, productivity was decreased. The total food conversation ratio was 1.28 at 7.5p^H. However, the same was found to be 0.823 at 8.5p^H. There are -35.95 percent reductions in the food conversation ratio at 8.5p^H. All the economic characters of the crop that were studied statistically decreased (significant at $p<0.005$).

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Table 1: Percentage Mortality and Mean Survival Period of Shrimp Litopenaeus Vannamei in both Acidic and Alkaline p^H Media

S. No.	p^H Media	p^H Value	Percent Mortality	Mean Survival Time (Hours)	Mean Survival Time (Days)
1	Control	7.5	0	166	6.910
2	Acidic	6.5	0	166	6.910
3		5.5	50	13	0.542
4		4.5	75	7	0.297
5		3.5	0	1.67	0.069
6	Alkaline	8.5	0	166	6.190
7		9.5	50	76	3.166
8		6.5	100	1.75	0.070

Table 1.1.: ANOVA

Source of Variation	SS	Df	MS	F Value	P Value	F Crit.
Rows	4803.889	7	686.2699	0.218146	0.974822	2.764199
Columns	28297.41	2	14148.7	4.497475	0.031008	3.738892
Error	44042.91	14	3145.922			
Total	77144.21	23				

Hypothesis

H_0: There is no significant difference between the data of rows and columns.
Result: Null hypothesis H_0 is rejected

Table 2: Effect of Altered p^H Media on Growth of Litopenaeus Vannamei during a Prolonged Period of Exposure

			Number of Days of Exposure								
S. No	P^H Media	P^H	10	20	30	40	50	60	70	80	90
			Weight In Grams								
1	Control	7.5	2.88	5.34	6.7	7.3	8.45	10.3	11.7	12.5	14.5
2	Acidic	6.5	AD	AD	AD	AD	AD	AD	AD	AD	AD
3	Acidic	5.5	AD	AD	AD	AD	AD	AD	AD	AD	AD
4	Acidic	4.5	AD	AD	AD	AD	AD	AD	AD	AD	AD
5	Acidic	3.5	AD	AD	AD	AD	AD	AD	AD	AD	AD
6	Alkaline	8.5	2.1	4.2	6.23	6.9	7.9	9.43	10.3	10.4	12.7
7	Alkaline	9.5	AD	AD	AD	AD	AD	AD	AD	AD	AD
8	Alkaline	10.5	AD	AD	AD	AD	AD	AD	AD	AD	AD
Percentage Change Over Control at $8.5p^H$			-27.0	-21.3	-7.01	-6.5	-6.51	-8.45	-1.97	-6.80	-12.4

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Table 3: Change in the Productivity of Shrimp Litopenaeus Vannamei during Chronic Exposure of Altered p^H Media

S. No.	Parameter	p^H 7.5 (Control)	p^H 8.5 (Experimental)	Percentage Change Over Control
1	Initial Stocking (Numbers)	1000± 43	1000± 23	NS
2	Density/m^2	50	50	NS
3	PL Stocking (Days)	PL15	PL15	NS
4	Harvest Size (gm)	14.5 ±0.7	12.7±1.2	-12.41 (P< 0.001)
5	Count (Numbers / Kg)	68.96 ±3.5	78.74 ±4.2	14.18 (P< 0.001)
6	Survival (%)	86 ±5.4	80±4.3	6.98 (P< 0.001)
7	Total Feed (Kg)	0.948 ±0.003	1.234 ±0.02	30.17 (P< 0.001)
8	Production (Kg)	1.218 ±0.021	1.016 ±0.012	-16.58 (P< 0.001)
9	Food Conversion Ratio	1.285 ±0.112	0.823 ±0.0065	-35.95 (P< 0.001)

± - Standard Deviation; P-Level of Significance; NS-Not significant

Discussion

The p^H plays a vital role in the growth and development of shrimp. In the present study, an attempt was made to know the impact of the change in the p^H media on the growth and development of Litopenaeus Vannamei. It has been observed from the results and understood that no mortality was recorded in p^H range of 5.5 to 9.5 during the entire rearing period. The LC50 value was found to be 5.5p^H on acidic medium and 9.5p^H on basic medium. Moreover, 75 percent mortality was found at 4.5p^H. Impact of altered p^H media on aquatic animals was carried out by several investigators (Lloyd & Jordon, 1964; Murthy, Reddanna & Govindappa, 1981).

All the studies confirmed the impact of altered p^H on the metabolism and the growth and development. The severe morphological changes have been observed in the shrimp in altered p^H media, wherein they changed their colour. A large amount of mucus has been accumulated on the body and gills, wherein, their body got irritated due to extreme p^H media. Occasionally, the movements of the animal were irregular due to irritation of the eye. Bhaskar in 1982 carried out a research study on the effect of altered p^H media on different aquatic animals and their metabolism, wherein they found that the main reason for mortality of shrimp in extreme p^H ranges was due to the accumulation of mucus in the gill and decreased oxygen supply to the animal. This hypoxia brings changes in the p^H at hemolymph. It is

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fairly evident that oxygen carrying capacity decreases due to changes in the altered p^H of hemolymph, which is known as Bohr Effect, wherein researchers have exhaustively studied this phenomenon. This decreased haemolymph p^H has been due to the entry of H^+ ions into gill from outside of the environment, particularly at gill surface. Moreover, the increased mucus secretion over the gill decreases the air diffusion across the gill membrane has been observed by various research studies. The present study recorded that survival time of Litopenaeus Vannameis has been very less (almost an hour) at extreme p^H such as 3.5 and 10.5. This result has been supported by a study conducted by Packer and Dunson in 1972. The tolerance of p^H is not exactly same in the acidic or basic environment, wherein the tolerance of shrimp was little more on alkaline side than on acidic side. Furthermore, it shows that stress is more on the acidic side than at alkaline p^H. Total shrimp survived at 7.5 and 8.5p^H. Therefore, the researchers continued to study the effect of p^H stress in these animals during their entire life period. At the two p^H ranges, the animals survived only for 90 days as per the experimental study at different p^H ranges.

The data also showed that the animals survived better at 7.5p^H when compared to 8.5p^H, wherein the food consumption was more at 8.5p^H.However, the animal growth was lesser and productivity has been low at 8.6p^H. The important economic parameter of food conversion ratio has been more than7.5p^H when compared to 8.5p^H. The increased intake of food and decreased productivity may be due to increased basal metabolic rate. During stress conditions, the metabolic rate will be increased to compensate the stress, wherein energy consumption may be high. This may be the reason for low productivity 8.5p^H. Apart from these p^H interferences with microbial environment of the surrounding media changes the nutrition of animals. The gut microbial flora might have changed. Moreover, at increased alkaline p^H the oxygen consumption was more, which might have burned the excess calories in the body.

Suggestions

The p^H can be maintained properly through appropriate control mechanisms for consistent growth and production of Litopenaeus Vannamei, which ultimately lead to high economy.

Conclusions

The optimal p^H is highly essential for shrimp growth and productivity. Short term and long-term pH modifications influence the economy of shrimp crop. However, during severe acidic and alkaline conditions metabolic rate will be increased to compensate the stress, wherein the energy consumption may be high. This might be the reason for low productivity. Hence, perfect water quality management system is essential for Litopenaeus Vannamei culture.

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