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Chronic toxicity of nitrate to Pacific white shrimp, *Litopenaeus vannamei*: Impacts on survival, growth, antennae length, and pathology

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ABSTRACT

Chronic toxicity of nitrate (NO_3^-) has not been well documented in the culture of penaeid shrimp. To interpret this problem, lab-scale research was conducted in recirculating aquaculture systems (RAS) to determine the long-term impacts of nitrate on shrimp growth, survival, total mass of shrimp per system (shrimp biomass), antennae length, and tissue pathology. The first experiment, Trial (A), was performed over a six week period at 11 (ppt) salinity and consisted of a Control A (35 ppm nitrate-N), Treatment A1 (220 ppm nitrate-N), Treatment A2 (435 ppm nitrate-N), and Treatment A3 (910 ppm nitrate-N). No differences were observed between control A and treatment A1 in terms of shrimp survival, growth, shrimp biomass, and antennae length. Treatment A2 exhibited no significant differences compared to Control A in terms of survival and growth, but did exhibit significant negative impacts ($P<0.05$) on shrimp biomass and antennae length. Lastly, treatment A3 significantly and negatively impacted ($P<0.05$) survival, growth, total mass, and antennae length. Histopathology of shrimp from Trial A and all three groups of Treatments A1 through A3 demonstrated lesions in the hepatopancreas, and a few individuals from each of these three groups had gill abnormalities (e.g. fouling). A second experiment, Trial (B), was conducted over a five week period to evaluate the effects of elevated nitrate at various salinities. This trial consisted of Control B (9 ppt salinity, 18 ppm nitrate-N), Treatment B1 (9 ppt salinity, 440 ppm nitrate-N), Treatment B2 (2 ppt salinity, 440 ppm nitrate-N), and Treatment B3 (18 ppt salinity, 440 ppm nitrate-N). When compared to Control B, Treatments B1 through B3 exhibited significantly negative effects ($P<0.05$) on shrimp survival, growth, and shrimp biomass, irrespective of salinity. Even though all treatment groups with 440 ppm nitrate-N exhibited negative responses to elevated nitrate, there was evidence ($P<0.05$) that an increase in salinity significantly ($P<0.05$) improved survival and shrimp biomass.

1. Introduction

Inland marine shrimp production has great potential for expansion, but can be limited by the scarcity of salt and water resources. Inland expansion of aquaculture is especially important for countries, like the United States, seeking to expand aquatic production. The inland production model holds great promise, largely due to the prohibitive expense of real estate and stringent environmental regulations along U.S. coasts (Samocha et al., 2004). Reducing water exchange rates in recirculating aquaculture systems (RAS) could minimize environmental impacts by conserving resources, and could allow shrimp farmers to move inland. As this strategy is implemented, farmers need to be aware of how best to manage the system in terms of water quality and waste disposal. A key element of water quality management is control of nitrogenous wastes, including ammonia, nitrite, and nitrate.

In aquaculture systems, ammonia is generated directly from animal excreta and metabolic waste products, as well as from decomposing organic solids, such as excess feed (Maillard et al., 2005). Removal of ammonia and nitrite can be accomplished through the use of autotrophic bacteria (Montoya et al., 2002) using nitrifying biological reactors (Sandu et al., 2002) or within intensive shrimp production raceways (autotrophic-based bioflocs) (Samocha et al., 2010). If systems are designed and managed properly, nitrogen species can be maintained at low levels. However, without water exchange or adequate treatment, nitrate will often accumulate in production systems. Nitrate levels in excess of 450 ppm have been reported in RAS (Otte and Rosenthal, 1979; Kuhn et al., 2009; Samocha et al., 2010). Numerous studies have been conducted to determine acute (short term) toxicity of nitrogenous wastes in shrimp (Wickins, 1976; Frias-Espericueta et al., 1999; Lin and Chen, 2001, 2003;

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Tsai and Chen, 2002; Gross et al., 2004; Sowers et al., 2004; Kir and Kumlu, 2006; Schuler et al., 2010); however, little data have been published regarding the long-term toxicological effects of nitrate to marine shrimp.

The objectives of this research was to determine the effects of salinity and nitrate concentrations on shrimp growth and survival in RAS.

The goal of this research was to define a safe level of nitrate for commercial shrimp culture. Two trials (A and B) were conducted to test the chronic effects of nitrate (NO_3^-) on Pacific White Shrimp, *Litopenaeus vannamei*. This information will help aquaculturists understand how best to manage their systems and remove nitrate, as needed, either through the use of anaerobic cycling bioreactors (Sharrer et al., 2007; Morrison et al., 2008) or water renewal (Timmons et al., 2002).

2. Materials and methods

2.1. Shrimp

Juvenile Pacific white shrimp (less than 2.5 g) were collected from production raceways at Virginia Shrimp Farms (Martinsville, Virginia, US) and transferred to the 150 L test aquaria (30 shrimp/aquarium). The shrimp were subsequently acclimated for 10 to 14 days before the experimental trials commenced. Shrimp were then culled to 18 shrimp per tank, resulting in a stocking density of 45 shrimp/m^2 (0.12 shrimp/L). During the culling process, the largest and smallest shrimp were removed, resulting in a uniform shrimp size.

2.2. Experimental systems

Twelve independent experimental systems were used during each of the toxicity trials, allowing four levels of independent variables to be tested in triplicate and randomly assigned. Each system consisted of a 150 L (0.4 m^2 footprint) acrylic aquarium (Glass Cages, Nashville, Tennessee, US), an air diffuser, a submersible 300 watt heater (Visitherm® Delux Submersibible Heater, Marineland, Blacksburg, Virginia, US), KMT media (Kaldnes Inc., Providence, Rhode Island, US), and a 1890 L/h hang-on waterfall filter (110/500 AquaClear Power Filter, Hagen, Montreal, Quebec, Can). The culture units were maintained as clear water systems with few to no algae.

2.3. Experimental design

Each of the two trials consisted of four treatment levels in triplicate. In Trial A the effects of elevated nitrate levels on shrimp over 42 days at a constant salinity of 11 ppt were studied. This trial consisted of Control A (35 ppm nitrate-N), Treatment A1 (220 ppm nitrate-N), Treatment A2 (435 ppm nitrate-N), and Treatment A3 (910 ppm nitrate-N). In trial B over 35 days, the effects of varying salinities at a constant, elevated nitrate level were compared to a control with low nitrate on shrimp. The variables in this trial consisted of Control B (9 ppt salinity, 18 ppm nitrate-N), Treatment B1 (9 ppt salinity, 440 ppm nitrate-N), Treatment B2 (2 ppt salinity, 440 ppm nitrate-N), and Treatment B3 (18 ppt salinity, 440 ppm nitrate-N).

2.4. Water quality

Culture water was formulated from well water and synthetic sea salt (Crystal Sea® Marinemix, Marine Enterprise International, Baltimore, Maryland, US). Nitrate levels for treatments other than the control were adjusted with lab-grade sodium nitrate (Fisher Chemical, Pittsburgh, Pennsylvania, US). Some nitrate accumulated naturally in all experimental systems. Evaporative loss of water will concentrate nitrate levels. Therefore, water addition was equally added to all experimental systems to account for evaporative losses. Each of the twelve experimental systems were rigorously monitored using the water quality methods and number of sampling events presented in Table 1. Water samples were analyzed using a HACH DR/2800 spectrophotometer (HACH Co., Loveland, Colorado, US). Unionized ammonia levels were calculated using equations presented by Emerson et al. (1975).

2.5. Feeding regime

Twice a day, shrimp were fed a commercially-available shrimp feed (2.4 mm pellet, Ziegler Bros. Inc., Gardners, Pennsylvania, US) with 43% crude protein and 12% lipid, at a rate of 5% body weight per day. Shrimp weights were determined weekly on a per-tank basis to monitor growth and adjust feed rations accordingly. At this feeding rate, excessive feed was not observed in the control tanks. However, in tanks where shrimp health was compromised, excessive and uneaten feed was removed every 24 h before the morning feeding.

2.6. Shrimp histology

Histology was performed on shrimp at the end of Trial A using methods presented in Bell and Lightner (1988). Three live shrimp were sampled from each tank resulting in 9 shrimp from each treatment. These shrimp were transported live to the Aquatic Medicine Laboratory (VMRCVM) where they were killed and preserved by injecting 3–5 ml of Davidson's fixative into the hepatopancreas and midgut region of the 2nd–4th abdominal segment. The cuticle over the cephlathorax and abdomen was then opened along the dorsal midline with a pair of dissecting scissors. Shrimp were then placed in Davidson's fixative for a minimum of 72 h before being placed in 70% ethyl alcohol for storage. For processing, shrimp were trimmed sagittally just off midline, sectioned at 5 μm and stained with hematoxylin and eosin. Photographic images were captured with a Vanox microscope fitted with an Olympus digital imaging program.

2.7. Shrimp performance indicators

Survival rates were recorded daily and moribund/dead shrimp were removed each day. Shrimp were weighed on a weekly basis in groups of three, until all shrimp in each tank had been weighed. Survival, growth, total mass of shrimp per system (shrimp biomass), and antennae length were used to assess the effects of nitrate.

2.8. Analysis of data

Statistical analysis was performed using SAS v9.1.3 for Windows (Cary, North Carolina, US). Differences in mean water quality parameters among treatments (4 levels in triplicate) were evaluated using an analysis of variance (ANOVA). ANOVA with repeated measures over time was also used for shrimp performance data to reveal the quantitative effects of nitrate (4 levels) during Trial A and salinity at

Table 1
Methods and number (n) of sampling events used to determine water quality during Trial A and B.

Parameter	Trial A (n)	Trial B (n)	Method
Ammonia-N, total	16	19	Nessler method, HACH method 8038[a,b,c]
Dissolved oxygen	22	24	YSI model 85 (Yellow Springs, Ohio, US)
Nitrite-N	28	23	Diazotization method, HACH method 8507[a,c]
Nitrate-N	28	23	Cadmium reduction method, HACH method 8039[a,b]
pH	7	11	HI 9024 pH meter (HANNA Instruments, Woonsocket, Rhode Island, US)
Salinity	26	24	YSI model 85 (Yellow Springs, Ohio, US)
Temperature	26	24	YSI model 85 (Yellow Springs, Ohio, US)

[a] HACH Co., Loveland, Colorado, US.
[b] Method developed/adapted from APHA (2005).
[c] USEPA approved for wastewater analysis.

elevated nitrate concentrations (4 levels) during Trial B. A Tukey's adjustment was applied to multiple comparison tests to look for differences amonget means at a given time. Differences were considered to be significant when $P<0.05$.

3. Results

3.1. Trial A: effects of nitrate at varying levels

As expected, nitrate levels varied significantly ($P<0.05$) among all treatments (Table 2). No differences in other water quality parameters were observed. This allowed for direct comparisons to be made for the various treatments.

Survival was impacted with nitrate levels greater than 220 ppm (Fig. 1). More specifically, final survival rates for Control A, Treatment A1, Treatment A2, and Treatment A3 were 87.1 ± 4.0, 87.4 ± 9.5, 64.2 ± 10.7, and 14.6 ± 4.2%, respectively. On and after day 14, Treatment A3 had significantly ($P<0.05$) lower survival rates compared to the Control A, Treatment A1, and Treatment A2.

Shrimp growth was negatively correlated with increasing nitrate levels and significant differences ($P<0.05$) were noted among the various treatments from day 21 onward (Fig. 2). At the end of the study, Treatment A3 shrimp were significantly smaller than those in the rest of the treatment groups. Final growth rates were 1.00 ± 0.12, 0.84 ± 0.06, 0.70 ± 0.13, and 0.24 ± 0.18 g/wk, respectively, for the Control A, Treatment A1, Treatment A2, and Treatment A3.

Shrimp biomass is shown in Fig. 3. Beginning on day 14, there were significant differences ($P<0.05$) in shrimp biomass, which was negatively correlated with increased nitrate levels. Final shrimp biomass for the control A, treatment A1, treatment A2, and treatment A3 were 158 ± 7, 138 ± 24, 86 ± 14, and 41 ± 25 g/system, respectively.

Antennae lengths of shrimp were negatively impacted by elevated nitrate levels (Table 3). Control A animals had significantly ($P<0.05$) longer antennae compared to treatments A2 and A3. Antennae of Treatment A3 shrimp were significantly ($P<0.05$) shorter than all other treatments.

All three groups of treatments A1 through A3 had lesions in the hepatopancreas (Fig. 4). Tubules of the hepatopancreas showed a variety of lesions. Some tubules had a reduction in the number and size of stored lipid vacuoles, while other tubules were completely devoid of epithelial cells and dilated. Both types of tubules contained sloughed epithelial cells and cellular debris. A smaller number of tubules demonstrated a granulomatous appearance with a necrotic, bacteria-filled center. A few individuals from treatments A1 through A3 had gill abnormalities (Fig. 5). There was evidence of bacteria and amorphous debris accumulating to the surface of the secondary gills filaments of numerous individuals, and in several individuals there was a loss of the regular structure of the columnar epithelium of the gill.

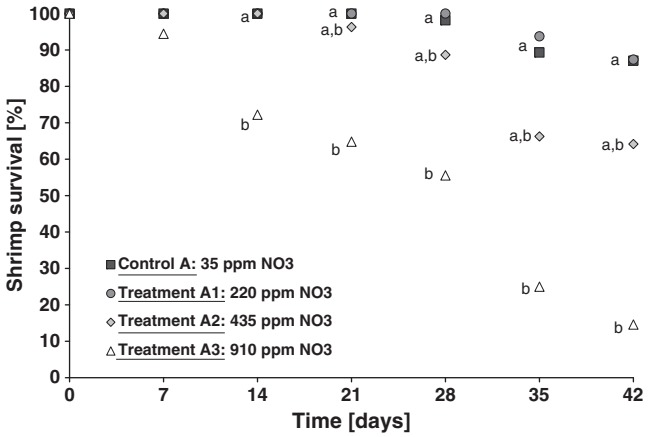

Fig. 1. Shrimp survival rates during Trial A, alphas denote significant differences at time, t ($P<0.05$).

3.2. Trial B: effects of elevated nitrate at varying salinities

As anticipated, salinity levels varied significantly ($P<0.05$) among all treatments with elevated nitrate levels (Table 2). Even though dissolved oxygen and nitrite varied significantly ($P<0.05$) among treatments, mean levels of dissolved oxygen and nitrite were consistently greater than 5.75 ppm and less than 0.26 ppm, respectively.

After the first week, survival rates during Trial B (Fig. 6) exhibited significant differences ($P<0.05$) among treatments. Treatment B2 exhibited 100% mortality between 14 and 21 days. By the 28th day, all treatments (B1–B3) exhibited significantly lower ($P<0.05$) survival rates, as compared to the Control. Survival was negatively correlated with salinity at elevated nitrate levels. Final survival rates for Control B, Treatment B1, Treatment B2, and Treatment B3, were 81.5 ± 4.9, 16.7 ± 11.1, 0, and 44.4 ± 5.6%, respectively.

Shrimp growth was negatively correlated with salinity at elevated nitrate levels and significant differences ($P<0.05$) among treatments were observed from day 14 until the end of the trial (Fig. 7). Control B exhibited the fastest growth rates. Shrimp exposed to elevated levels of nitrate at 9 and 18 ppt salinity (treatments B1 and B3) did not vary significantly between each other in regards to growth. Shrimp in Treatment B2 did not grow over the first 14 days and died (100% mortality) by day 20. Final growth for Control B, Treatment B1, and Treatment B3 were 1.07 ± 0.06, 0.51 ± 0.08, and 0.59 ± 0.11 g/wk, respectively.

Table 2
Water quality results and mean values with ANOVA output for Trial A and B. Alphas denote significant differences.

	Dissolved oxygen [ppm]	Nitrate-N [ppm]	Nitrite-N [ppm]	pH	Salinity [ppt]	Total ammonia-N [ppm]	Temperature [°C]
Trial A							
Control A	5.72	34.5[a]	0.43	7.60	11.3	0.57	29.3
Treatment A1	5.85	220[b]	0.76	7.63	11.2	0.69	29.0
Treatment A2	5.86	437[c]	0.72	7.61	11.4	0.55	29.3
Treatment A3	6.05	912[d]	0.50	7.62	11.3	0.63	29.3
Pooled error	0.1367	25.12	0.2582	0.0361	0.2586	0.0852	0.2614
P>F	0.0954	0.0001	0.3886	0.7686	0.6889	0.2451	0.4709
Trial B							
Control B	6.09[a,b]	18.3[a]	0.05[a]	8.08[a]	9.40[b]	0.38	29.3
Treatment B1	6.19[b,c]	442[b]	0.22[b,c]	8.11[a,b]	9.13[b]	0.39	29.4
Treatment B2	6.53[c]	441[b]	0.16[b]	8.21[b]	2.26[a]	0.40	29.5
Treatment B3	5.76[a]	439[b]	0.25[c]	8.10[a,b]	17.6[c]	0.38	29.5
Pooled error	0.1423	19.15	0.0267	0.0451	0.9338	0.0287	0.3606
P>F	0.0012	<0.0001	<0.0001	0.0270	<0.0001	0.8365	0.8783

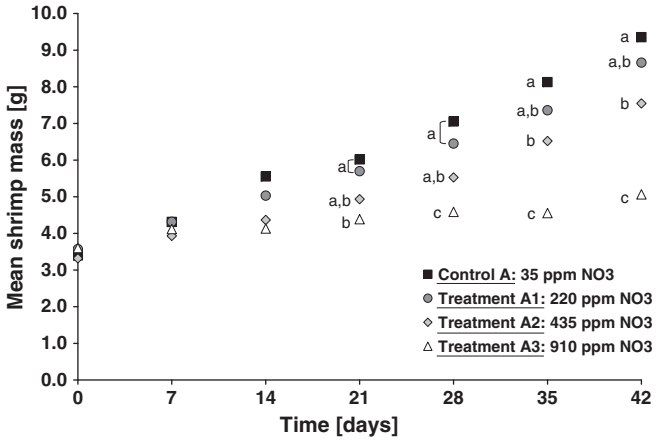

Fig. 2. Individual mean shrimp mass during Trial A, alphas denote significant differences at time, t ($P<0.05$).

Shrimp biomass can be observed in Fig. 8 for trial B. Significant differences ($P<0.05$) among treatments began on day 7, and shrimp biomass was negatively correlated with decreased salinity levels at elevated nitrate levels. Final shrimp biomass for control B, treatment B1, and treatment B3 were 114 ± 2, 15 ± 24, and 41 ± 4 g/system, respectively.

4. Discussion

Water quality parameters remained within desired ranges for shrimp health (Van Wyk et al., 1999). Even though dissolved oxygen varied significantly among treatments, values were consistently greater than 5.75 ppm and exceeded minimum requirements of 3.0 ppm for shrimp culture (Seidman and Lawrence, 1986). Temperature ranged between 29 and 30 °C, which are considered optimum for promoting rapid growth (Wyban et al., 1995). Mean unionized ammonia concentrations did not exceed 0.045 ppm; this is less than 1.5% of the 24 hour lethal concentration that kills 50% of the exposed population (24 h LC50) as reported by Lin and Chen (2001). Nitrite concentrations varied significantly during Trial B; however, mean levels did not exceed 0.76 ppm NO_2-N, which is significantly less than 0.1% of the reported 48 h LC50 of 143 ppm (Lin and Chen, 2003). Similar results were noted in Schuler et al. (2010) for combined effects of ammonia and nitrite.

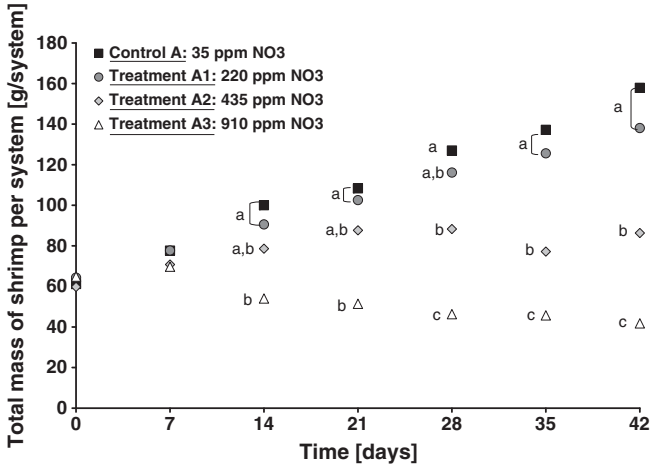

Fig. 3. Shrimp biomass during Trial A, alphas denote significant differences at a time, t ($P<0.05$).

Table 3
Mean final antennal length of shrimp under various nitrate levels during Trial A. Alphas denote significant differences.

	Antennae length (cm)
Control A	8.46[a]
Treatment A1	7.17[a,b]
Treatment A2	5.09[b]
Treatment A3	2.02[c]
Pooled error	1.875
P>F	0.0271

This study demonstrated that shrimp could be cultured at 220 ppm nitrate at 11 ppt salinity over a six week period. Meanwhile, nitrate levels of 435 ppm were found to be unsafe for shrimp culture. Tsai and Chen (2002) conducted an acute nitrate toxicity trial on *Penaeus monodon*. These authors used an empirical application factor presented by Sprague (1971) to estimate safe chronic levels from determined acute levels. The safe level for long-term culture of shrimp was estimated to be 145 ppm nitrate at 15 ppt salinity. Results from the current study are similar despite the fact that a different species of shrimp was used.

Even though no published studies have directly tested the chronic toxicity of nitrate, suggestions for safe levels of nitrate have been made for *Litopenaues vannamei*. Van Wyk et al. (1999) reported that shrimp can be safely cultured at nitrate levels less than 60 ppm, however, no scientific references were provided to support this statement. Data from the current study supports this assertion, but also suggest that more than three and a half times that level (220 ppm nitrate) may not reduce production over a few week period. This difference is significant when considering the potential water and other resource conservation aspects (such as, energy use, salt, water discharge, etc.) of an RAS operation; e.g., shrimp farmers could theoretically reduce their water exchange rates in half, if nitrate level was the primary variable of concern.

Antennal lengths of shrimp were evaluated because they are an indication of animal well-being and overall health. Shrimp antennae function as both tactile and sensory perception (New et al., 2010). Moreover, if live shrimp markets are to be considered, shrimp with short antennae may not be acceptable to ethnic consumers, who have expressed a preference for long antennae (Gardner, 2010). Shrimp antennae lengths were considered to be of unacceptable length at the 435 ppm nitrate levels and higher because they were significantly shorter. Broken and shortened antennae are early signs of compromised health shrimp (FAO, 2001). Perhaps, in this study, elevated levels of nitrate disrupted normal endocrinological control of molting. In crustaceans, it has been documented that molting and regeneration

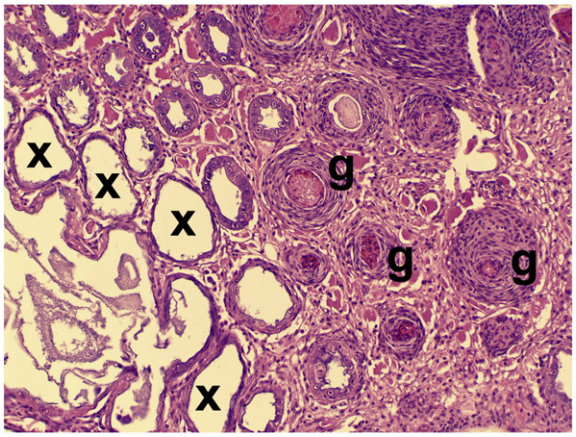

Fig. 4. Representative lesions in hepatopancreas of shrimp exposed to elevated nitrate levels during Trial A. Magnification at 250 times. Some tubules of the hepatopancreas demonstrated a granulomatous reaction with a bacteria-filled center (g), while other tubules were dilated and devoid of epithelial cells lining the tubule (x). Both types of tubules contained varying degrees of sloughed, necrotic epithelial cells and cellular debris.

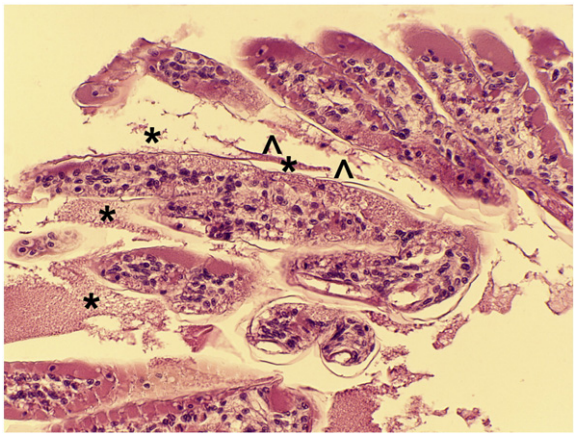

Fig. 5. Representative gill fouling in shrimp exposed to elevated nitrate levels in Trial A. Magnification at 500 times. There is evidence of bacteria and amorphous debris accumulating to the surface of the secondary gills filaments (*) and a loss of the regular structure of the columnar epithelium of the gill (arrows).

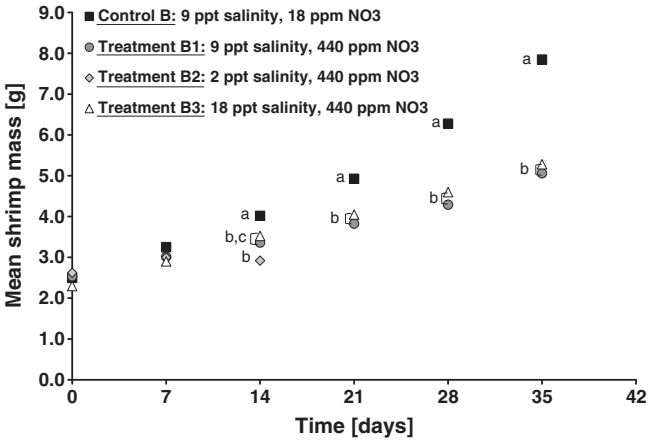

Fig. 7. Individual mean shrimp mass during Trial B, alphas denote significant differences at time, t ($P<0.05$).

of appendages are interrelated (Madhavan and Madhavan, 1981). Even though the exact mechanism is not known, it was clear that shrimp did not have the ability to regenerate healthy antennae when exposed to high levels of nitrate over a sustained period of time.

Shrimp from all three treatment groups in Trial A with elevated nitrate levels displayed varying degrees of tissue pathology, most notably in the hepatopancreas and gill. Numerous tubules of the hepatopancreas had a reduced number of stored lipid vacuoles, while other tubules were dilated and devoid of epithelial cells. These changes suggest that the shrimp were probably not eating well or metabolizing feeds normally (Villalon, 1993). A smaller number of tubules also demonstrated a granulomatous appearance indicating that the host was producing an inflammatory response to the injured or insulted epithelial cells of the hepatopancreas in an attempt to destroy or wall off the injured tissues (Jiravanichpasal and Miyazaki, 1994; Estevea and Herrerab, 2000). The gills of many individuals showed evidence of bacteria and amorphous debris accumulating to the surface of the secondary gills filaments. This may have been due to decreased cleaning activity by the shrimp or increased suspended material in the water column (Bauer, 1977, 2002; Martin et al., 2000). There was also a loss of the regular surface structure of the gill in several individuals possibly the result of the gill fouling. Gill fouling is reported to impair water movement across the gill tissue which would ultimately negatively effect the health of the affected shrimp (Bauer, 1979). All of these changes suggest that elevated nitrate levels negatively effect the health of the shrimp even if outward signs are not evident.

Trial B demonstrated that nitrate was less toxic at higher salinities. These results are consistent with toxicity studies focused on salinity and the toxic effects of nitrogenous wastes other than nitrate (Lin and Chen, 2001; Kir and Kumlu, 2006; Li et al., 2007). In regards to nitrate, Tsai and Chen (2002) determined that acute toxicity of nitrate to *Penaeus monodon* increased as the salinity decreased from 35 to 15 ppt. Even though the mechanism whereby higher salinities dampen the toxic effects of nitrogenous wastes has not been well documented, it is understood that in low salinity environments marine shrimp are under additional stress and metabolic and physical processes can be inhibited. The iso-osmotic point for shrimp is approximately 18.5 ppt, the mean of the reported 24.6 ppt by Castille and Lawrence (1981) and 10 to 15 ppt as reported by Wyban and Sweeney (1991). The primary site for ion regulation, including nitrate, is through the gills (Pequeux, 1995). Shrimp in low salinity waters need to maintain a higher osmotic pressure in their hemolymph compared to the environment. This hypo-osmotic state requires additional metabolic energy (Pequeux, 1995). Consequently, less energy is available for dealing with toxic chemicals this is especially relevent for long-term stresses compared to short term. Also, at lower salinities, more nitrate would be drawn into the body as part of osmoregulation under hypo-osmotic conditions (Mantel and Farmer, 1983; Kir and Kumlu, 2006).

Possible causes of suppression of growth and shrimp mortalities include metabolic depression, reduced feeding efficiencies, and impaired endocrine function (Kurihara et al., 2008). In aquaculture, it is

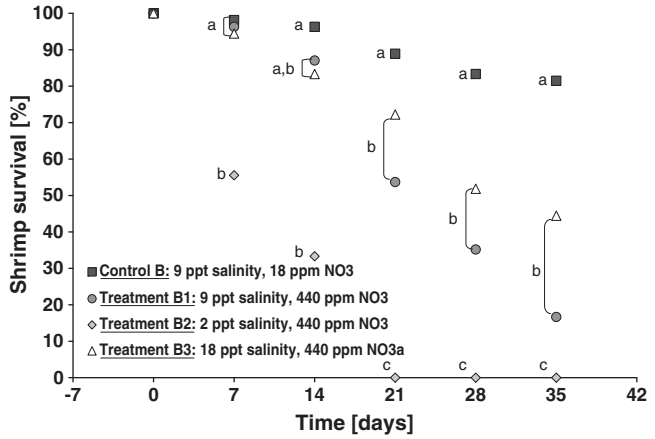

Fig. 6. Shrimp survival rates during Trial B, alphas denote significant differences at time, t ($P<0.05$).

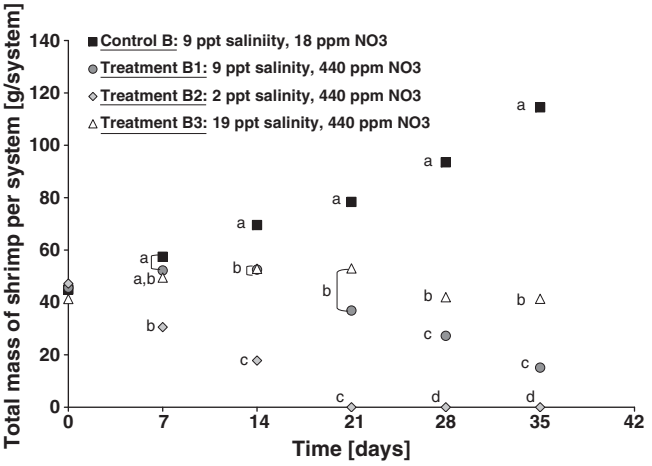

Fig. 8. Shrimp biomass during Trial B, alphas denote significant differences at a time, t ($P<0.05$).

commonly thought that a toxicant suppresses growth before it results in mortality. However, in this study, there was evidence that survival rates were impacted to a greater extent than growth rates in elevated nitrate treatments. This is particularly noteworthy because shrimp farmers generally rely on accurate growth data, but cannot accurately determine the population, due to difficulties in collecting information about every single shrimp in a system.

5. Conclusion

Overall, results from this chronic toxicity study demonstrated that nitrate had negative impacts on survival, growth, and shrimp biomass at levels greater than 220 ppm nitrate-N at 11.0 ppt salinity. Levels of nitrate-N have been reported to exceed 450 ppm in RAS (Kuhn et al., 2009; Samocha et al., 2010). Nitrate inhibited survival rates more than growth. An increase in nitrate toxicity was also observed at lower salinities. Chronic effects of elevated nitrate on shrimp physiology included suppressed antennal lengths, lesions in the hepatopancreas, and a few individuals had gill abnormalities (e.g. fouling). Results from this study provides information that will help aquaculturists and shrimp farmers understand how to manage their system in terms of avoiding dangerous nitrate levels.

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