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Toxicity of Un-ionized Ammonia and High pH to Post-larval and Juvenile Freshwater Shrimp *Macrobrachium rosenbergii*

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Abstract

Post-larval and juvenile *Macrobrachium rosenbergii* were exposed for 72 h at 29 C to four pH levels (8.5, 9.0, 9.5, 10.0) and four concentrations of un-ionized ammonia-nitrogen (0, 1, 2, and 3 mg/L NH_3-N). Results indicated potentiation between NH_3 and high pH. Juveniles were more tolerant of high pH and NH_3 than post-larvae.

For post-larvae, estimates of 72 h LC50 for pH were 9.43, 9.21, and 8.71 at 0, 1, and 2 mg/L NH_3-N, respectively; 72 h LC50 estimates for NH_3-N were 2.18 and 1.45 mg/L at pH levels of 8.5 and 9.0, respectively. For juveniles, estimates of 72 h LC50 for pH were 9.91, 9.56, 9.04, and 8.76 at 0, 1, 2, and 3 mg/L NH_3-N, respectively; 72 h LC50 estimates for NH_3-N were 2.02 and 0.54 mg/L at pH 9.0 and 9.5, respectively.

In pond culture of *M. rosenbergii,* high pH levels can cause mortality at stocking. The 72 h data can be used as an indication of safe stocking levels of pH and ammonia. These data suggest that post-larvae should not be exposed to pH $>$ 9.0 nor to NH_3-N $>$ 1 mg/L in the pH range 8.5–9.0 and juveniles should not be exposed to pH $>$ 9.5 nor to NH_3-N $>$ 0 mg/L at pH 9.5, $>$ 1 mg/L at pH 9.0, or $>$ 2 mg/L at pH 8.5.

Macrobrachium rosenbergii de Man, the giant freshwater shrimp (or prawn) native to the Indo-Pacific region, is an important pond aquaculture species in some tropical areas and could become a seasonal pond crop in warmer temperate-zone areas such as the southern United States. Mortality associated with high afternoon pH levels in aquaculture ponds has been reported for this species (AQUACOP 1979; Sandifer et al. 1983; Clardy et al. 1985). High pH is usually the result of rapid rates of net photosynthesis by phytoplankton, whose growth is stimulated by nutrients, including ammonia, released by the cultured animals and other organisms in the pond.

Acute toxicities of ammonia (Wickins 1976) and high environmental pH (Sarver et al. 1979; Hummel 1986) to *M. rosenbergii* post-larvae have been studied separately, but in practice the two variables interact because pH affects the concentrations of ionized (NH_4^+) and un-ionized (NH_3) ammonia. At higher pH, a greater percentage of the total ammonia in water exists as NH_3, which is more toxic to aquatic animals than NH_4^+ (Colt and Armstrong 1981). The present study was therefore undertaken to determine the acute toxicity of combinations of high pH and un-ionized ammonia to post-larval and juvenile *M. rosenbergii* in fresh water.

Materials and Methods

Static toxicity tests (CMTTAO 1975; APHA 1985) were conducted in 18 tanks with 20 post-larval or 10 juvenile freshwater shrimp per tank. Animals were exposed to pH levels of 8.5, 9.0, 9.5, and 10.0 (range ± 0.1 unit), and NH_3-N concentrations of 0, 1, 2, and 3 mg/L (range ± 0.25 mg/L) were tested at each pH level. There were also two control tanks per trial. Three trials were conducted for each size of shrimp. Treatments were randomized among tanks.

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Tanks were 72 L glass aquaria with plastic covers. A sloped section of white plastic light louver (51 × 21 cm, with 13 mm grid squares) was provided in each tank to allow shrimp to distribute themselves better throughout the water column, in order to reduce cannibalism associated with crowding. An airstone in each tank provided aeration at a low rate to minimize ammonia stripping while maintaining dissolved oxygen levels greater than 75% saturation. Water temperatures were maintained at 29 ± 0.5 C with electric immersion heaters and were measured daily with a mercury thermometer. Control tanks (NH_3-N < 0.01 mg/L) and tanks intended to have zero NH_3-N concentrations were provided with outside biological filters, containing conditioned non-carbonate gravel, to oxidize waste ammonia. A 16 h light/8 h dark light cycle was employed to simulate a summer photoperiod.

Shrimp were obtained as post-larvae from Blue Lobster Farms, Madera, California; some were subsequently reared to juvenile size in nursery tanks in a greenhouse. Post-larvae averaged 12.9 ± 0.4 mg and 9.6 ± 0.1 mm, while juveniles averaged 836.0 ± 23.0 mg and 34.5 ± 0.3 mm ($\bar{x}$ ± SE, blotted wet weights, postorbital lengths, N = 90). Prior to stocking experimental tanks, shrimp of each size were held in an acclimation tank with a biofilter for 48 h without feeding under the above conditions of dissolved oxygen, temperature, and light cycle. Shrimp were not fed during an experiment in order to minimize variability due to nutritional and metabolic condition (CMTTAO 1975; APHA 1985).

Tanks were filled with 60 L of non-chlorinated well water which had been aerated for at least 24 h prior to use. The water was analyzed at the beginning and at the end of the 7 month study and had the following characteristics, respectively: total dissolved solids 113, 117 mg/L; total alkalinity 84, 88 mg/L as $CaCO_3$; calcium 8, 12 mg/L; total hardness 55, 28 mg/L as $CaCO_3$; chloride 10, 10 mg/L. Experimental pH values were achieved and maintained using 0.5 M NaOH or HCl. The pH was monitored in each tank several times daily with a pH meter and adjusted as required. Control tanks did not receive any pH adjustment (pH 8.3–8.5). Un-ionized ammonia nitrogen concentrations were achieved by adding calculated aliquots of a 1,000 mg/L NH_4Cl solution. They were monitored and adjusted at 24 h intervals by measuring total ammonia nitrogen (TAN) (phenate method, APHA 1985), pH (Fisher Accumet pH meter), and temperature, and were calculated as percentages of TAN by means of the table of Thurston et al. (1979). Nitrite-nitrogen concentrations were also measured every 24 h (sulfanilamide method, APHA 1985) to ensure that nitrite would not be a source of stress or mortality.

Molted exoskeletons and dead animals were recorded and removed when observed. Mortality was determined by a lack of movement after gentle prodding. Survivors were counted at 1, 3, 6, 9, 12, 24, 48, and 72 h. A 72 h test was used instead of the standard 96 h exposure period because by 96 h, tanks without biofilters often had unstable levels of pH and NH_3-N, increased nitrite concentrations, and dissolved oxygen below 75% saturation. Replicate trials were rejected if survival in the control tanks averaged less than 90%. Estimates of LC50 were determined for each pH-ammonia combination and shrimp size using the mean mortality of shrimp in the three replicates and the trimmed Spearman-Karber method (Hamilton et al. 1977).

Results

Survival among replicate trials was generally consistent and was usually lowest at high pH and NH_3-N levels (Figs. 1, 2). Observations indicated that molting was rarely associated with mortality. Additional information can be found in Straus (1988).

Post-larval survival at 72 h was good (97%) at pH 8.5 and 0–1 mg/L NH_3-N, but poor (≤75%) at higher NH_3-N concentrations. At pH 9.0, survival was also good

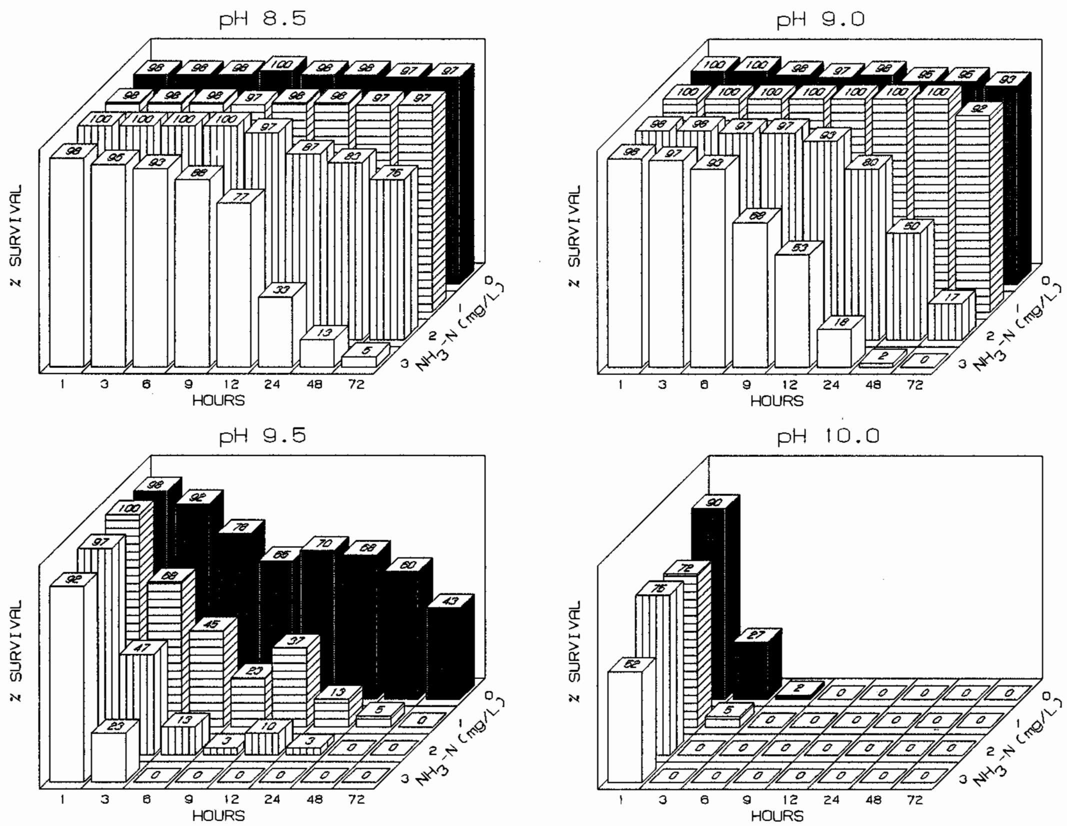

FIGURE 1. *Mean survival of post-larval freshwater shrimp exposed to 4 concentrations of un-ionized ammonia-nitrogen for 72 h (3 replications). Survival at 9 h was averaged over only 2 replications because data were not recorded at 9 h during the first trial.*

($\geq$92%) at 0–1 NH_3-N, but poor ($\leq$17%) at higher NH_3-N concentrations. At pH 9.5, post-larvae survived only at 0 mg/L NH_3-N, and their survival was poor (43%). No post-larvae survived at pH 10.0. At higher pH, toxicity of a given concentration of NH_3-N was greater, i.e., there was potentiation. For example, at pH 8.5, 72 h survival decreased from 97% at 0 mg/L NH_3-N to 75% at 2 mg/L NH_3-N, while at pH 9.0, survival decreased from 93% at 0 mg/L NH_3-N to 17% at 2 mg/L NH_3-N.

Juvenile freshwater shrimp were more tolerant than post-larvae to both NH_3 and high pH. Juvenile 72 h survival at pH 8.5 was good ($\geq$93%) at all NH_3-N concentrations. At pH 9.0, survival was good (100%) at 0–1 mg/L NH_3-N and poor ($\leq$57%) at higher NH_3-N concentrations. At pH 9.5, survival at 0 mg/L NH_3-N was good (100%), but poor ($\leq$63%) at higher NH_3-N concentrations. At pH 10.0 survival was poor ($\leq$40%) at all NH_3-N concentrations. Un-ionized ammonia and pH also exhibited apparent potentiation, similar to that observed with post-larvae.

Estimates of pH LC50 for post-larvae varied with exposure time and NH_3-N concentration, and ranged from 8.71 at 72 h and 2 mg/L NH_3-N to 9.82 at 3 h and 0 mg/L NH_3-N; for juveniles, pH LC50 estimates at similar exposure periods and NH_3-N concentrations were higher and ranged from 8.76 at 72 h and 3 mg/L NH_3-N to 10.00 at 9 h and 1 mg/L NH_3-N (Table 1). Un-ionized ammonia-nitrogen LC50 estimates for post-larvae ranged from 0.02 mg/L NH_3-N at pH 9.5 and 48 h to 2.64 mg/L NH_3-N at pH 8.5 and 24 h; NH_3-N LC50 estimates for juveniles ranged from

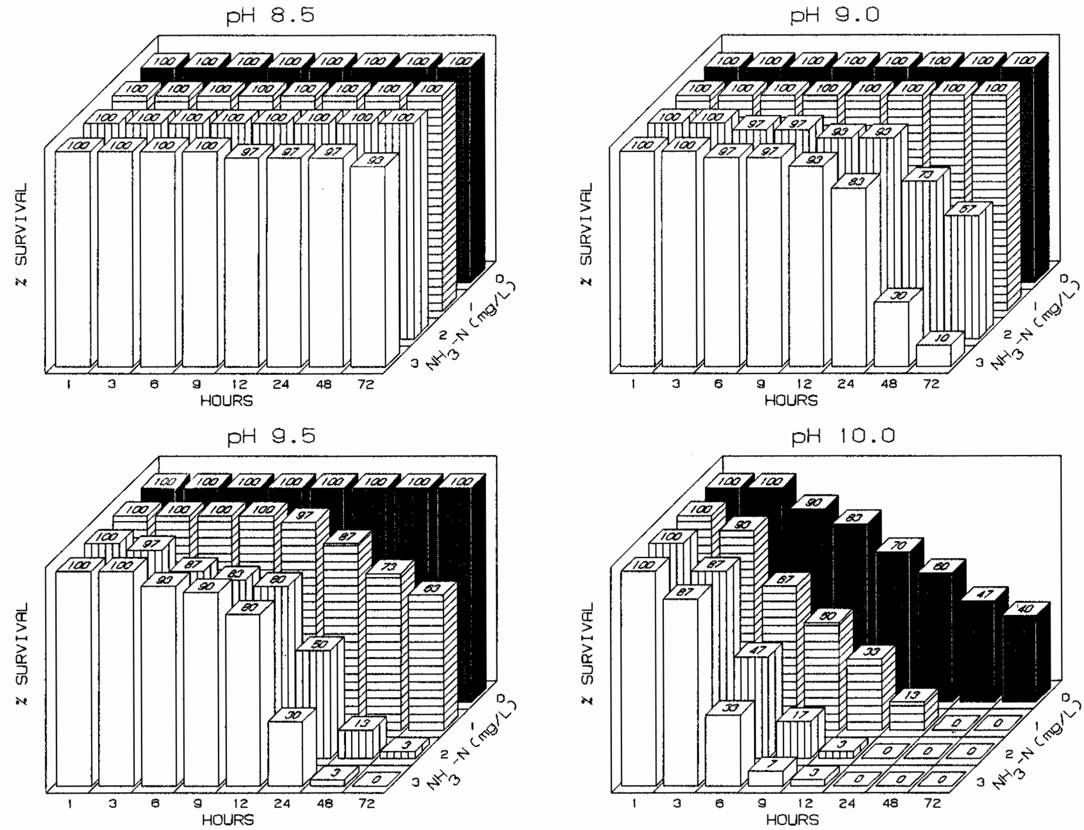

FIGURE 2. *Mean survival of juvenile freshwater shrimp exposed to 4 concentrations of un-ionized ammonia-nitrogen for 72 h (3 replications).*

0.03 mg/L NH_3-N at pH 10.0 and 24 h to 2.48 mg/L NH_3-N at pH 9.0 and 48 h (Table 2).

Discussion

Wickins (1976) tested the toxicity of un-ionized ammonia to post-larval *M. rosenbergii* at pH 7.0, 29 C, and a salinity of 3 ppt. Animals were exposed to three NH_3 concentrations: 1.43, 2.21, and 2.81 mg/L. The data collected could not be used to calculate LC50 estimates, but LT50 (exposure times resulting in 50% mortality) estimates were 1,700, 1,400, and 560 minutes, respectively. The pH of Wickins' experiments was below those in the present study, but he demonstrated that toxicity of NH_3 increased with increasing concentration.

Sarver et al. (1979) tested 72 h tolerances of *M. rosenbergii* post-larvae of three ages (newly-settled, 2 weeks post-settling, and 1 month post-settling) to three pH levels (9.0, 9.5, and 10.0). Temperature was not specified but was probably about 29 C, based on other information in the article. No animals died at pH 9.0, 40–80% per age group died at pH 9.5, and all died at pH 10.0. Results of the present study at 0 mg/L NH_3-N are similar to these. Although Sarver et al. (1979) did not report ammonia concentrations, comparison with results of the present study suggests that little ammonia was present.

Hummel (1986) investigated 96 h tolerances of post-larval *M. rosenbergii* of unspecified size and age to ten pH levels ranging from 7.5 to 12.0 in 0.5 unit increments at 28–29 C. All animals exposed to pH levels of 7.5 and 8.0 survived, whereas 1% died at pH 8.5 and 40% died at pH 9.0. In con-

TABLE 1. *Trimmed Spearman-Karber LC50 pH estimates (and 95% confidence intervals) for post-larval and juvenile freshwater shrimp at eight exposure times and four NH_3-N concentrations.*

Time (h)	NH_3-N (mg/L) 0	1	2	3	0	1	2	3
	Post-larvae				Juveniles			
1	a	a	a	a	a	a	a	a
3	9.82 (9.77–9.87)	9.60 (9.55–9.66)	9.47 (9.42–9.52)	9.34 (9.29–9.38)	a	a	a	a
6	9.64 (9.60–9.69)	9.47 (9.41–9.52)	9.29 (9.26–9.33)	9.23 b	a	a	9.96 (9.84–10.08)	9.86 (9.80–9.91)
9	9.55 (9.50–9.60)	9.35 (9.31–9.39)	9.25 (9.22–9.27)	9.08 (9.02–9.15)	a	10.00 b	9.75 (9.71–9.79)	9.73 (9.69–9.78)
12	9.59 (9.54–9.64)	9.42 (9.38–9.47)	9.26 (9.21–9.30)	8.96 (8.87–9.06)	a	9.86 (9.81–9.92)	9.64 (9.59–9.69)	9.63 (9.58–9.69)
24	9.57 (9.51–9.62)	9.30 (9.27–9.34)	9.17 (9.11–9.23)	a	a	9.75 (9.71–9.78)	9.46 (9.40–9.51)	9.30 (9.24–9.37)
48	9.53 (9.47–9.59)	9.26 (9.24–9.28)	8.95 (8.88–9.03)	a	9.97 b	9.61 (9.56–9.65)	9.17 (9.12–9.23)	8.89 (8.84–8.94)
72	9.43 (9.37–9.49)	9.21 (9.18–9.24)	8.71 (8.66–8.76)	a	9.91 b	9.56 (9.51–9.61)	9.04 (8.99–9.09)	8.76 (8.73–8.79)

[a] Insufficient mortality to meet the conditions of the trimmed Spearman-Karber method for estimating LC50.
[b] The 95% confidence interval could not be calculated.

trast, there was low mortality (7%) at pH 9.0 and 0 mg/L NH_3-N in the present study, and Sarver et al. (1979) had no mortality at pH 9.0. In Hummel's study, all animals died within 96 h at pH values higher than 9.0, and the 8 h LC50 for pH was estimated to be between 9.0 and 9.5. Ammonia was monitored at the conclusion of several experiments, and only trace amounts were found (<0.01 mg/L TAN). Hummel's greater mortality at pH 9.0 could possibly have been due to a longer testing period (96 vs. 72 h) or to higher ammonia or nitrite levels during experiments, but neither was monitored then.

The present study indicates that the toxicity of NH_3 to *M. rosenbergii* decreases as animals increase in size and age. This is consistent with general toxicological principles. As aquatic (gill-breathing) animals grow, they become more tolerant to toxicants because they develop physiological detoxication mechanisms and reduce the ratio of gill surface area to body weight; differing rates of excretion of toxic chemicals may also be involved in age-dependent toxicity effects (Rand and Petrocelli 1985).

In pond culture of *M. rosenbergii,* high pH levels can cause mortality at stocking. The 72 h results of the present study can be used as an indication of safe stocking levels of pH and ammonia. These data suggest post-larvae should not be exposed to pH >9.0 or to NH_3-N >1 mg/L in the pH range 8.5–9.0, and juveniles should not be exposed to pH >9.5 nor to NH_3-N >0 mg/L at pH 9.5, >1 mg/L at pH 9.0, or >2 mg/L at pH 8.5. Strategies to minimize pH-related mortality might include stocking early in the morning (when pH is lowest), stocking immediately after filling the ponds (before a dense phytoplankton bloom can develop), or stocking juveniles rather than post-larvae (because of greater tolerance to high pH and ammonia). The data of this study cannot be used to predict safe chronic levels of pH and ammonia for culture of post-larval or juvenile *M. rosenbergii,* but it would obviously be prudent to avoid levels which resulted in less than 90% survival at 72 h.

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TABLE 2. *Trimmed Spearman-Karber LC50 NH_3-N estimates (and 95% confidence intervals) for post-larval and juvenile freshwater shrimp at eight exposure times and four pH levels.*

Time	pH							
(h)	8.5	9.0	9.5	10.0	8.5	9.0	9.5	10.0
	Post-larvae				Juveniles			
1	a	a	a	a	a	a	a	a
3	a	a	1.48 (1.07–2.04)	a	a	a	a	a
6	a	a	0.29 (0.16–0.51)	a	a	a	a	1.77 (1.46–2.16)
9	a	a	0.05 (0.02–0.11)	a	a	a	a	0.38 (0.23–0.61)
12	a	a	0.15 (0.07–0.34)	a	a	a	a	0.12 (0.06–0.26)
24	2.64 (2.51–2.78)	2.43 (2.19–2.70)	0.05 (0.03–0.08)	a	a	a	2.02 (1.71–2.37)	0.03 (0.01–0.06)
48	2.41 (2.23–2.60)	1.86 (1.75–1.97)	0.02 (0.01–0.05)	a	a	2.48 (2.34–2.64)	0.81 (0.63–1.05)	a
72	2.18 (2.07–2.31)	1.45 (1.16–1.80)	a	a	a	2.02 (1.88–2.17)	0.54 (0.42–0.70)	a

[a] Insufficient mortality to meet the conditions of the trimmed Spearman-Karber method for estimating LC50.

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