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# Effect of Ammonia at Different pH Levels on *Penaeus monodon* Postlarvae

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## Abstract

The effect of ammonia on *Penaeus monodon* postlarvae (5.7 ± 0.9 mg, 12.0 ± 1.4 mm) varied with pH level. As the postlarvae were exposed to 250 $mg \cdot l^{-1}$ ammonia-N, the $LT_{50}$ and $LT_{100}$ values decreased with pH increase. Postlarvae exposed to 60 $mg \cdot l^{-1}$ ammonia-N and a pH of 9.10 had less tolerance than those exposed to 250 $mg \cdot l^{-1}$ ammonia-N and a pH of 8.31. The $LT_{50}$ value of postlarvae exposed to 0.07 $mg \cdot l^{-1}$ ammonia-N and pH of 9.10 was less than half of the $LT_{50}$ value of postlarvae exposed to the same concentration of ammonia-N but a pH of 8.31. Increasing pH level in a given ammonia solution increased the proportion of $NH_3$ and increased the ammonia toxicity on *P. monodon* postlarvae. Monitoring the ammonia level and preventing an increase of pH are suggested for shrimp culture.

## Introduction

Ammonia, the most common toxicant of the shrimp *Penaeus monodon* in culture ponds in southern Taiwan, can increase to 0.808 $mg \cdot l^{-1}$ ammonia-N in the hatchery, and 6.497 $mg \cdot l^{-1}$ ammonia-N in intensive growout ponds even with frequent water replacement (Chen et al. 1986, 1989).

Ammonia originates from the ammonification of organic matter and the deamination or excretion of the aquatic animals as a principal end product of nitrogenous compounds in a cultured system (Spotte 1979). The accumulation of ammonia is a potential threat to cultured aquatic organisms (Wickins 1976; Mevel and Chamroux 1981).

In water, ammonia is comprised of an un-ionized ($NH_3$) and ionized ($NH_4$+) form. The former is more toxic due to its ability to diffuse readily across cell membranes (Fromm and Gillette 1968; Emerson et al. 1975). $NH_4^+$ is considered nontoxic, or considerably less toxic (Tabata 1962). The relative proportions of $NH_3$ and $NH_4^+$ in a given solution are dependent principally on pH and temperature and to a lesser extent on salinity (Bower and Bidwell 1978).

Since larvae of the shrimp *P. monodon* are generally reared in semistatic water systems in Taiwan (Chwang et al. 1986), the accumulation of toxic forms of ammonia and the effects on the larvae are major concerns. This paper defines the toxicity of ammonia at various pH levels on *P. monodon* postlarvae in the laboratory.

## Materials and Methods

*P. monodon* postlarvae (PL12-15) obtained from a private hatchery located at Tungkang were acclimated for three days in the laboratory before beginning the test. The postlarvae had an average length of 12.0 ± 1.4 mm and weighed 5.7 ± 0.9 mg. Seawater was pumped from the Keelung coast through a sand and gravel filter and aerated one day before use. The chemical characteristics of the aerated seawater have been described by Chin and Chen (1987).

Ammonia test solutions were prepared by dissolving ammonium chloride (Merck reagent grade) in seawater (25 ppt). The pH was adjusted with 1N HCl or 1N NaOH. Eight treatments were used in the experiment (Table 1): three for 250 mg/l ammonia-N (with three pH levels), one for 60 mg/l ammonia-N (with pH level of 9.10) and four 0.07 mg/l ammonia-N (with four pH levels). Each treatment had four replicates.

Pyrex glass bottles (300 ml) were used for the bioassay study. Six postlarvae were randomly collected from the stock tank and exposed to each test solution. All bottles were capped and placed in a water bath (30°C). Observations were made every 10 minutes. The median lethal time (the length of time required to kill 50% of the test population, $LT_{50}$) and the lethal time (the length of time required to kill all of the test population, $LT_{100}$) was recorded in each bottle. Death was assumed when larvae were immobile and showed no response when culture bottles were shaken gently. Dissolved oxygen (DO) was measured with Delta 2110 DO meter (Delta company) at the beginning and end of the test.

Table 1. Median lethal time ($LT_{50}$) and lethal time ($LT_{100}$) of *Penaeus monodon* postlarvae ($PL_{12\text{-}15}$) exposed to various ammonia and pH values in sealed bottles at 25 ppt and 30°C. Initial and residual DO concentration are reported.

| Group | pH | Concentration Ammonia-N | $NH_4^+$-N | $NH_4^+$-N ($mg \cdot l^{-1}$) | $LT_{50}$* (min) | $LT_{100}$* (min) | Initial DO ($mg \cdot l^{-1}$) | Residual DO ($mg \cdot l^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| A | 6.62 | 250.00 | 249.277 | 0.723 | 1,743 | 1,828 | 7.60 | 2.23 |
| | | | | | (404) | (83) | (0.06) | (0.53) |
| B | 7.64 | 250.00 | 242.531 | 7.469 | 146 | 437 | 7.63 | 6.10 |
| | | | | | (13) | (94) | (0.06) | (0.33) |
| C | 8.31 | 250.00 | 216.922 | 33.078 | 25 | 66 | 7.66 | 6.70 |
| | | | | | (9) | (12) | (0.06) | (0.23) |
| D | 9.10 | 60.00 | 23.722 | 36.728 | 16 | 45 | 7.59 | 7.25 |
| | | | | | (4) | (13) | (0.06) | (0.06) |
| E | 6.62 | 0.07 | 0.07 | 0 | 1,474 | 1,596 | 7.62 | 0.70 |
| | | | | | (92) | (81) | (0.02) | (0.31) |
| F | 7.64 | 0.07 | 0.068 | 0.002 | 1,533 | 1,625 | 7.67 | 0.54 |
| | | | | | (81) | (105) | (0.06) | (0.29) |
| G | 8.31 | 0.07 | 0.061 | 0.009 | 1,572 | 1,963 | 7.69 | 0.63 |
| | | | | | (61) | (373) | (0.05) | (0.31) |
| H | 9.10 | 0.07 | 0.028 | 0.042 | 760 | 1,496 | 7.66 | 2.84 |
| | | | | | (186) | (332) | (0.06) | (0.86) |

* Mean value of four replicates with standard deviation in parentheses.

The concentrations of $NH_3N$ and $NH_4^+$-N were calculated from ammonia-N, pH, water temperature and salinity according to the formulae described by Bower and Bidwell (1978) and Whitfield (1978). The terms used in this study are:

| | | |
|---|---|---|
| Ammonia | : | Total inorganic ammonia (un-ionized ammonia + ionized ammonia) |
| Ammonia-N | : | Total inorganic ammonia as nitrogen |
| $NH_3$-N | : | Un-ionized ammonia as nitrogen |
| $NH_4^+$-N | : | Ionized ammonia as nitrogen |

## Results and Discussion

When postlarvae were exposed to 250 $mg \cdot l^{-1}$ ammonia-N, the $LT_{50}$ and $LT_{100}$ values decreased with increase of pH, and the residual DO increased with increase of pH (Table 1). This suggested that high $NH_3$-N and pH level killed more animals than low $NH_3$-N and pH level, thereby less DO was consumed. The $LT_{50}$ values of the A, B and C groups also showed that ammonia toxicity on *P. monodon* postlarvae increased as pH increased. When the postlarvae were exposed to 0.07 $mg \cdot l^{-1}$ ammonia-N and pH levels of 6.62, 7.64, 8.31 and 9.10 (E, F, G and H group), the $LT_{50}$ and $LT_{100}$ values and residual DO also revealed that higher pH level increased the toxicity of ammonia on postlarvae. Duncan's Multiple Range Test on residual

DO versus test solution indicated that the group A (250 mg·l-1 ammonia-N and 6.62 pH) was highly significant different ($P < 0.01$) from group B (250 mg·l-1 ammonia-N and 7.64 pH) and group C (250 mg·l-1 ammonia-N and 8.31 pH) (Table 2).

Armstrong et al. (1978) reported that the 24-hour $LC_{50}$ values of ammonia on *Macrobrachium rosenbergii* larvae were 200 mg·l-1 (pH 6.83), 115 mg·l-1 (pH 7.60) and 37 mg.l-1 ammonia-N (pH 8.34), which were equivalent to 0.54 mg.l-1, 1.73 mg.l-1 and 2.95 mg.l-1 $NH_3$-N, respectively. They also indicated that ammonia toxicity increased due to increasing proportion of $NH_3$ in a given solution. Wickins (1976) reported that the 48-hour $LC_{50}$ value of ammonia on larvae of seven penaeid species (50-250 mg) combined together was 1.29 mg·l-1 $NH_3$-N which corresponded to 24 mg·l-1 ammonia-N (pH 8.0, 28°C, 33 ppt) or 227 mg·l-1 ammonia-N (pH 7.0, 28°C, 33 ppt).

Table 2. Duncan's Multiple Range Test on residual dissolved oxygen (DO) versus different groups of the test solutions for *Penaeus monodon* postlarvae ($PL_{12\text{-}15}$).

| Group | Mean D | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| D | 7.25 | | | | | | | | |
| C | 6.70 | 0.55 | C | | | | | | |
| B | 6.10 | 1.15 | 0.60 | B | | | | | |
| H | 2.84 | 4.41** | 3.86** | 3.26** | H | | | | |
| A | 2.23 | 5.02** | 4.47** | 3.87** | 0.61 | A | | | |
| E | 0.70 | 6.55** | 6.00** | 5.40** | 2.14** | 1.53* | E | | |
| G | 0.63 | 6.62** | 6.07** | 5.47** | 2.21** | 1.60* | 0.07 | G | |
| F | 0.54 | 6.71** | 6.16** | 5.56** | 2.30** | 1.69* | 0.16 | 0.09 | F |

*: $P < 0.05$  **: $P < 0.01$

The $LT_{50}$ and $LT_{100}$ values of group D (60 mg·l-1 ammonia-N, pH 9.10, 36.278 mg·l-1 $NH_3$-N) were lower than those of group C (250 mg·l-1 ammonia-N, pH 8.31, 33.078 mg·l-1 $NH_3$-N). These results demonstrated that increasing pH value even in a low ammonia-N will also increase the proportion of $NH_3$-N and increase the toxicity of ammonia.

Studies on rainbow trout *Salmo gairdneri* demonstrated that ammonia toxicity increased with increasing pH level due to an increase of $NH_3$; there was a positive correlation between pH and acute toxicity of $NH_3$ over the pH range of 6.5-9.0 (Thurston et al. 1981).

The 24-hour $LC_{50}$ values of ammonia-N on *P. monodon* nauplius, zoea, mysis and postlarva ($PL_6$) were 6.00 mg·l-1, 8.48 mg·l-1, 24.04 mg·l-1 and 52.11 mg·l-1 ammonia-N at pH 8.2, 29.5°C, 34 ppt, respectively (Chin and Chen 1987). The $LT_{50}$ of group G was 1,572 minutes, I.C. 26-hour at 0.07 mg·l-1 ammonia-N (pH 8.31, 30°C, 25 ppt). This suggested that the toxicity of ammonia on *P. monodon* postlarvae increased with elevated temperature and pH level and decreased with increasing salinity (Bower and Bidwell 1978). Similar results on increased toxicity with a decrease in salinity have been obtained with salmon smolts (Alabaster et al. 1979).

In the hatchery, *P. monodon* larvae from nauplius to the 10-12th day of postlarva stage were regularly reared in a dark semistatic seawater system (Chwang et al. 1986). Chen et al. (1986) monitored the water quality of hatchery ponds and found that ammonia-N ranged from 7.5 to 808.4 $\mu g \cdot l^{-1}$, $NH_3$-N ranged from 0.7 to 79.7 $\mu g \cdot l^{-1}$ and pH from 7.88 to 8.42. In this bioassay, even in the solutions having 0.07 $mg \cdot l^{-1}$ ammonia-N, the residual DO indicated that group H (pH 9.10, 0.042 $mg \cdot l^{-1}$ $NH_3$-N) was highly significantly different ($P < 0.01$) from group E (pH 6.62, 0 $mg \cdot l^{-1}$ $NH_3$-N), group F (pH 7.64, 0.002 $mg \cdot l^{-1}$ $NH_3$-N) and group G (pH 8.31, 0.009 $mg \cdot l^{-1}$ $NH_3$-N). The $LT_{50}$ and $LT_{100}$ values of the group H solution were less than half those of groups E, F and G indicating the higher toxicity of ammonia at higher pH level. Thus, for *P. monodon* postlarvae in a saltwater environment with a salinity of 25 ppt and pH level of 9.10, half of the population may be killed in less than 13 hours even at an ammonia-N concentration as low as 0.07 $mg \cdot l^{-1}$.

The slightly lower $LT_{50}$ and $LT_{100}$ of group E than groups F and G may be due to nitrite rather than ammonia concentrations. Low pH level favors the proportion of nitrous acid ($HNO_2$) in a given nitrite solution. Toxicity in terms of $HNO_2$ increases in freshwater fish over the range of 6.5-9.0 (Wedemeyer and Yasutake 1978; Russo et al. 1981). However, no information is available on nitrite toxicity to marine shrimps at various pH levels. Wickins (1976) reported that seawater having a pH level lower than 6.4 would decrease the growth rate and cause death of *P. monodon* larvae.

It is concluded that the toxicity of ammonia is pH-dependent and careful consideration to monitor $NH_3$ and $NH_4$+ and pH values is suggested for good shrimp rearing.

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