Bull. Environ. Contam. Toxicol. (1993) 51:122–129

Environmental Contamination and Toxicology

Effects of Ammonia on Oxygen Consumption and Ammonia-N Excretion of *Penaeus chinensis* after Prolonged Exposure to Ammonia

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Fleshy prawn, Penaeus chinensis Osbeck (also known as P. orientalis Kishinouye) which was originally distributed in Po Hai and the Yellow Sea between the west of Korea and east of mainland China (Liu 1983), was introduced into Taiwan, and their offsprings were reared and propagated successfully (Tzeng et al. 1990).

Because P. chinensis possesses the characteristic of cold tolerance and rapid growth in the warm subtropical temperate region, an attempt has been made to culture this species as a winter alternative to P. monodon in Taiwan.

Ammonia, a common toxicant in intensive culture systems, increased sharply after a period of cultivation, and could jump to 6.50 mg/L for ammonia-N (un-ionized plus ionized ammonia as nitrogen) and 0.35 mg/L for NH_3-N (un-ionized ammonia as nitrogen) after 115 days of cultivation, even with frequent water replacement (Chen et al. 1989). Accumulation of ammonia to certain level may cause growth retardation and even death of penaeid (Wickins 1976; Chen et al. 1990).

Physiological information such as optimal oxygen demand, oxygen consumption and ammonia-N excretion of P. chinensis was provided by Liu (1983) and Chen et al. (1991). This study was designed to determine the effect of ambient ammonia on oxygen consumption and ammonia-N excretion of P. chinensis juveniles to ascertain whether this species could adjust its physiological situation to resist ammonia following exposure to sublethal levels of ammonia in the laboratory.

MATERIALS AND METHODS

P. chinensis juveniles (0.17 ± 0.05 g) were shipped to the laboratory from Tainan Branch, Taiwan Fisheries Research Institute and acclimated at seawater of 30 ppt

in 25 C for two weeks.

There were two treatment periods. The first period was acclimation treatments and second period was experimental treatments. For acclimation treatments, the shrimp (no= 210) were treated with control solution (no added ammonia), 5 and 10 mg/L ammonia-N (un-ionized plus ionized ammonia as nitrogen) solutions which were renewed every day (American Public Health Association 1985) at seawater of 30 ppt and 25 C for 10 days. During the acclimation period, shrimp were fed a commercial shrimp diet (39 % crude protein) designed for P. monodon by Tairoun Products (Taipei, Taiwan) three times a day based on 15 % body weight per day. In all test solutions, dissolved oxygen was 5.7-6.3 mg/L; pH varies from 7.78 to 8.02 during the experiment.

For experimental treatments, shrimp (no= 105) which were fasted for two days after 10 days of acclimation treatment, were distributed to seven different levels of ammonia-N (0.015, 0.678, 1.103, 2.087, 2.941, 4.279 and 5.167 mg/L). The average weight of the intermolt shrimps was 0.196 $\pm$ 0.041 g, 0.214 $\pm$ 0.065 g, and 0.184 $\pm$ 0.050 g for the control group (the shrimp previously not exposed to ammonia), 5 and 10 mg/L ammonia-N group (the shrimp previously exposed to 5 and 10 mg/L ammonia-N), respectively.

Seawater pumped from the Keelung coast adjacent to the University was diluted with municipal water which was dechlorinated with sodium thiosulfate to 30 ppt, and was filtered through gravel and sand bed by air-lifting and aerated for two days before use. Ammonia test solutions were prepared by dissolving 3.82 g of ammonium chloride (Merck reagent grade) with distilled water to make 1000 mg/L ammonia-N and then diluted to expected level. The nominal concentration of ammonia-N used was based on a short-term toxicity test which indicated that no mortality of P. chinensis juveniles exposed to 5 and 10 ammonia-N at 30 ppt and 25 C (Chen and Lin 1992). The chemical characteristics of seawater are the same as those reported previously (Salinity= 33 ppt, Total alkalinity= 108 mg/L as $CaCO_3$, Ammonia-N= 0.041 mg/L, Nitrite-N= 0.014 mg/L, Nitrate-N= 0.032 mg/L, Orthophosphate-N= 0.019 mg/L, Silica= 0.79 mg/L, COD= 1.22 mg/L; Chen and Nan 1991).

For experimental treatments, shrimp (no= 105) were distributed to seven different levels of ammonia-N, 0.015, 0.678, 1.103, 2.087, 2.941, 4.279 and 5.167 mg/L ammonia-N solutions. Each test solution was conducted in five replicates. Pyrex glass BOD bottles (308 $\pm$ 1.5 mL volume) were filled with test solution. One shrimp was placed in each bottle, and the bottle was capped

and placed in a water bath (25 ± 1 C). The experiment was started at 9:00 am and terminated at 9:00 am next day with renewal of each test solution every 6 hr (15:00, 21:00 and 3:00). Dissolved oxygen (DO) was measured with a Delta Scientific Model 2110 DO meter and electrode probe (Delta Company, USA) attached together with a battery powered stirrer following air calibration with salinity compensation. Prior to each DO reading, the stirrer was operated for 20 sec. Ammonia-N was determined by the phenolhypochlorite method (Solorzano 1969) using standards of 0, 0.01, 0.02, 0.04, 0.08, 0.12, 0.20 and 0.50 mg/L ammonia-N, when test solution was renewed.

Dissolved oxygen and ammonia-N were recorded at every water replacement, and converted to weight specific oxygen consumption and ammonia-N excretion rates by multiplying the water volume of each bottle, and then dividing by body weight (g) and time lapse (hr). The difference of dissolved oxygen and ammonia-N between, before and after water replacement was expressed as oxygen consumption (O_2 mg/g/hr) and ammonia-N excretion (mg/g/hr). All data were subjected to one-way analysis of variance (Steel and Torrie 1980). If the significant differences were indicated at the $p< 0.05$ level, the Duncan's Multiple Range test was used to identify significant differences between treatments (Duncan 1955).

RESULTS AND DISCUSSION

Oxygen consumption and ammonia-N excretion of the shrimp increased when they were exposed to increasing ambient ammonia. In the control group of acclimation treatment, oxygen consumption of the shrimp exposed to 4.279, 5.167 mg/L ammonia-N was significantly higher ($P< 0.05$) than those exposed to 0.015, 0.678, 1.103 mg/L ammonia-N after 24 h. In the 5 mg/L ammonia-N group of acclimation treatment, oxygen consumption of the shrimp exposed to 2.941 mg/L ammonia-N was significantly higher ($P< 0.05$) than those exposed to 0.015 mg/L ammonia-N after 24 hr. In the 10 mg/L ammonia-N group of acclimation treatment, oxygen consumption of the shrimp exposed to 2.087 mg/L ammonia-N was significantly higher ($P< 0.05$) than those exposed to 0.015 mg/L ammonia-N after 24 hr.

Oxygen consumption of the shrimp previously treated with 5 and 10 mg/L ammonia-N in acclimation period and then to 0.015 mg/L ammonia-N averaged 0.575 and 0.724 O_2 mg/g/h, respectively. However, oxygen consumption of those previously treated with 5 and 10 mg/L ammonia-N in acclimation period and then to 4.279 mg/L ammonia-N averaged 0.890 and 0.985 O_2 mg/g/hr, respectively. The percentage of mean oxygen consumption of the shrimp

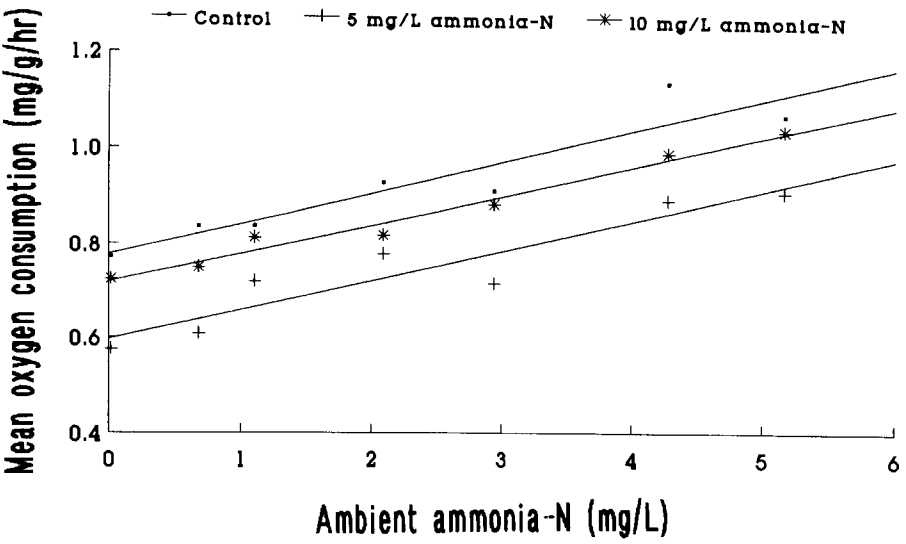

Figure 1. Realtionship between mean oxygen consumption (O_2 mg/g/hr) and different concentrations of ambient ammonia-N (mg/L) after 6, 12, 18 and 24 hr, after Penaeus chinensis juveniles were exposed to control, 5 and 10 mg/L ammonia-N with static renewal for 10 days at 30 ppt and 25 C.

previously exposed to 5 and 10 mg/L ammonia-N during acclimation period and then exposed to different concentrations of ambient ammonia-N after 6, 12, 18 and 24 hr ranged from 73-86% and 87-97% of those previously exposed to control solution during acclimation period and then exposed to treatment group (Fig. 1). MOC= 0.778 + 0.064 C (r= 0.937), MOC= 0.597 + 0.062 C (r= 0.934) and MOC= 0.719 + 0.060 C (r= 0.988) in the control, 5 and 10 mg/L group, respectively, whereas MOC is mean oxygen consumption (O_2 mg/g/hr) and C is ambient ammonia-N (mg/L).

In the control group of acclimation treatment, ammonia-N excretion of the shrimp exposed to 0.678 mg/L ammonia-N was significantly higher ($P< 0.05$) than those exposed to 0.015 mg/L ammonia-N after 6 hr. In the 5 and 10 mg/L ammonia-N group of acclimation treatment, ammonia-N excretion of the shrimp exposed to 1.103 mg/L ammonia-N was significantly higher ($P< 0.05$) than those exposed to 0.015 mg/L ammonia-N after 6 and 18 hr, respectively.

Ammonia-N excretion of the shrimp previously exposed to 5 and 10 mg/L ammonia-N during acclimation period and

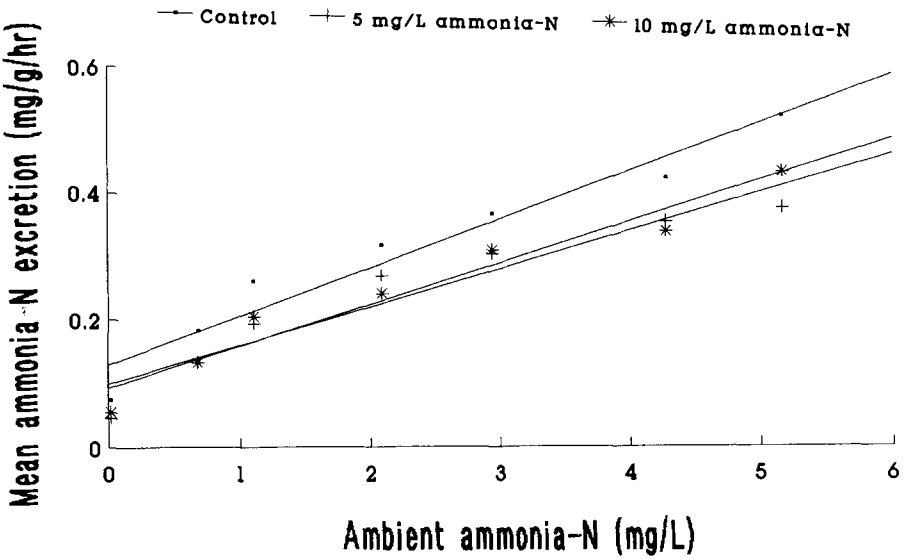

Figure 2. Relationship between mean ammonia-N excretion (mg/g/hr) and different concentrations of ambient ammonia-N (mg/L) after 6, 12, 18 and 24 hr, after Penaeus chinensis juveniles were exposed to control, 5 and 10 mg/L ammonia-N with static renewal for 10 days at 30 ppt and 25 C.

then to 0.015 mg/L ammonia-N averaged 0.046 and 0.055 mg/g/hr, respectively. However, ammonia-N excretion of those previously exposed to 5 and 10 mg/L ammonia-N for 10 days and then to 1.103 mg/L ammonia-N averaged 0.193 and 0.204 mg/g/hr, respectively. The percentage of mean ammonia-N excretion of the shrimp previously exposed to 5 and 10 mg/L ammonia-N during acclimation period for 10 days and then exposed to different concentrations of ambient ammonia-N after 6, 12, 18 and 24 hr ranged from 62-85% and 73-84% of those previously exposed to control solution during acclimation period and then exposed to treatment group (Fig. 2). MAE= 0.129 + 0.076 C (r= 0.972), MAE= 0.099 + 0.060 C (r= 0.954) and MAE= 0.093 + 0.065 C (r= 0.974) in the control, 5 and 10 mg/L group, respectively, whereas MAE is mean ammonia-N excretion (mg/g/hr) and C is ambient ammonia-N (mg/L).

The shrimp previously exposed to 5 and 10 mg/L ammonia-N during acclimation period and then to 2.941 and 2.087 mg/L ammonia-N was reduced in the percentage of mean oxygen consumption to the control by 21.4% and 11.8%. The shrimp previously exposed to 5 and 10 mg/L ammonia-N during the acclimation period and then to 0.678 and

1.103 mg/l ammonia-N was reduced in the percentage of mean ammonia-N excretion to the control by 27.5% and 21.5%.

In an intensive culture system for penaeids, ammonia-N increased exponentially with time elapsed, increased to 0.81 mg/L after 20 days and 0.35, 0.95 mg/L after 32, 61 days of cultivation in the hatchery and grow-out farm even with frequent water replacement (Chen et al. 1986, 1989). Chen et al. (1991) indicated that ammonia-N as low as 0.678 mg/L would cause increasing oxygen consumption and ammonia-N excretion of P. chinensis juveniles (0.079 $\pm$ 0.004 g) in 20 hr. The present study indicated that water with 4.279 mg/L ammonia-N would cause increasing oxygen consumption of P. chinensis (0.198 $\pm$ 0.054 g) in 12 hr, and 0.678 mg/L ammonia-N in the water would cause increasing ammonia-N excretion in 6 hr. Therefore, in an intensive culture system, a little increase of water ammonia could affect physiological function of cultured penaeid.

Redner and Stickney (1979) stated that 48-hr LC50 of NH_3-N on Tilapia aurea not exposed to ammonia prior to acute testing was 2.40 mg/L, however, concentration of NH_3-N at 3.40 mg/L caused no mortality of the fish within 48 hr, after they were previously exposed to 0.43-0.53 mg/L NH_3-N for 35 days. This fact indicated susceptibility to ammonia decreased as the fish were previously exposed to sublethal level of ammonia for prolonged time.

The present study indicated that ammonia-N excretion increased as P. chinensis juveniles exposed to ambient ammonia-N in the range of 0.015-5.167 mg/L in a closed system. Conversely, addition of ammonia to 8 mg/L would cause blood level rise of rainbow trout (Salmo gairdneri) from 40 to 70 mg/L with a greater concomitant decrease in ammonia excretion over total nitrogen excretion (Fromm and Gillette 1968).

The present study indicated that both oxygen consumption and ammonia-N excretion of the shrimp previously to 5 and 10 mg/L ammonia-N during acclimation period, and then to different concentrations of ammonia were lower than those previously exposed without ammonia water. Shrimp exposed to water containing ammonia for long term may cause metabolic disorder. Ambient ammonia inhibiting ammonia-N excretion through surface epithelium membrane causes increase of ammonia in the blood to toxic levels. Chen and Kou (1991) reported that if P. japonicus (13.91 $\pm$ 0.65 g) were exposed to water with ammonia-N higher than 10 mg/L ammonia-N, diffusion of ammonia from hemolymph to water was inhibited and diffusion of ammonia from ambient water to hemolymph

occurred.

Diffusion of NH_3 from blood to water is the main route for fish and crustacean to excrete metabolic ammonia, since blood levels are normally much higher than ambient concentration (Kinne 1976). Ammonia in the blood of S. gairdneri was reported to be 9-40 times greater than that in ambient water (Fromm and Gillette 1968). Ammonia in the hemolymph of P. japonicus was 66-80 times greater than that in the ambient water (Chen and Kou 1991). The fact that the previous studies (Chen et al. 1991) and the present study indicated that ammonia-N excretion increased as ambient ammmonia-N increased suggested that ammonia-N diffusion via hemolymph to ambient water was greater than that via ambient water to hemolymph in the range of 0-5 mg/L ammonia-N in the ambient.

Lloyd and Orr (1969) observed that urine excretion increased as much as six times when S. gairdneri were exposed initially to sublethal levels of ammonia as compared to those exposed for controls, while, excretion rate declined after exposure to sublethal level of ammonia after 48 hr. Lloyd and Orr (1969) concluded that animals were able to acclimate to sublethal levels of ammonia, when they were previously exposed to ammonia after 48 hr. The present study indicated that ammonia-N excretion by P. chinensis decreased as they were previously exposed to 5 and 10 mg/L ammonia-N for 10 days. This suggested that prolonged exposure to ammonia-N at 5 and 10 mg/L might damage the respiratory and excretion system of the shrimp. However, we do not know whether prolonged exposure to ammonia causes shift from ammonotelic to ureotilic excretory pattern in penaeids as that documented in freshwater teleosts (Olson abd Fromm 1971).

Acknowledgments. This work was supported by National Science Council of the Republic of China (Project number: NSC 81-0409-B019-09). We would like to thank Dr. Y.Y. Ting and Dr. M.N. Lin, Tainan Branch, Taiwan Fisheries Research Institute for providing the animals. Thanks are also due to Dr. S. S. Sheen for his advice and review on this paper.

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Received May 16, 1992; accepted January 20, 1993.