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Acute Toxicity of Ammonia to *Penaeus semisulcatus* Postlarvae in Relation to Salinity

MEHMET KIR[1]
Faculty of Aquaculture and Fisheries, Mugla University, 48140 Kotekli, Mugla, Turkey

METIN KUMLU
Faculty of Fisheries, Cukurova University, 01330 Balcali, Adana, Turkey

Stocking densities of cultured penaeid shrimp have been intensified because of the limited availability of land for pond construction. In an intensive culture system, ammonia is the most common toxicant resulting from nitrogen excretion by cultured animals and mineralization of unconsumed feed and feces (Lin and Chen 2003). In decapod crustaceans, nitrogen is excreted mainly as ammonia (60–70% of total ammonia) (Lee and Chen 2003). Concentrations of total ammonia-nitrogen (TAN) increase directly with culture period and might reach as high as 46 mg TAN/L in intensive grow-out ponds (Chen and Lin 1992). Accumulation of ammonia-nitrogen in pond water may deteriorate water quality, reduce growth, increase oxygen consumption, alter concentrations of hemolymph protein and free amino acid levels, and even cause high mortality (Chen and Lin 1992; Chen et al. 1994).

TAN is composed of NH_3-N (unionized ammonia) and NH_4^+ (ionized ammonia) (Losordo et al. 1992; Masser et al. 1992). It is the unionized form that is most toxic to aquatic organisms as it can readily diffuse through cell membranes and is highly soluble in lipids (Chin and Chen 1987; Frias-Espericueta et al. 1999). There is an equilibrium between NH_4^+ and NH_3-N in the water. This equilibrium is affected by pH, temperature, and salinity. The higher the pH and temperature, the higher the proportion of NH_3-N, while an increase in salinity will lead to lower proportions of the unionized form, hence the toxicity of the TAN to aquatic animals (Losordo et al. 1992; Masser et al. 1992; Sampaio et al. 2002).

A number of studies have been conducted on the lethal effects of ammonia at various life stages of penaeid shrimps, such as *Penaeus chinensis* (Chen and Lin 1992), *P. monodon* (Chen and Lei 1990), *P. paulensis* (Ostrensky and Wasielesky 1995), *P. penicillatus* (Chen and Lin 1991), *P. semisulcatus* (Wajsbrot et al. 1990), and *Metapenaeus ensis* (Nan and Chen 1991). However, little information is available on the lethal effect of ammonia at different salinity levels for penaeid shrimps. Chen and Lin (1992) and Lin and Chen (2003) studied ammonia toxicity in *P. chinensis* and *Litopenaeus vannamei* at three different salinity levels, but no results have been reported for other penaeid species.

Green tiger shrimp (*P. semisulcatus)* is an Indo-Pacific species, distributed along the coast of the Eastern Mediterranean, and is one of the most important commercial species in this part of the world. This species is less tolerant to low salinity levels (Soyel and Kumlu 2003), and knowledge of the effects of ammonia toxicity at various salinity regimes that may be confronted during the culture and overwintering periods in the subtropical areas is of importance for the farmers. This paper provides information on acute toxicity of ammonia to juveniles of this shrimp species at six different salinity levels (15, 20, 25, 30, 35, and 40 ppt).

Materials and Methods

Penaeus semisulcatus PLs (mean weight 27.5 ± 1.4 mg and total length 17.7 ± 2.1 mm) used in this study were cultured from eggs obtained from the broodstock captured in the northeastern Mediterranean Sea. Trials were conducted at the Yumurtalık Marine Research Station of Çukurova University, Adana, Turkey. Prior to

[1] Corresponding author.

toxicity tests, the animals from water with a salinity of 40 ppt were acclimated to the desired salinity (15, 20, 25, 30, 35, and 40 ppt) over 1 wk.

Ammonia stock solutions were prepared by dissolving required amounts of ammonium chloride (NH_5Cl) (Merck KGaA, Darmstadt, Germany) in seawater as described in Kir et al. (2004). Experimental concentrations of ammonium chloride for test solutions were 0 (control), 20, 40, 60, 80, 100, 120, 160, and 200 mg/L. The actual amount of TAN in seawater was measured with a 4050 UV/visible Ultraspec II brand spectrophotometer (Biochrom Ltd., Cambridge, UK) prior to the test by the phenate method at 640 nm (Parsons et al. 1985). The concentrations of TAN in the test solutions ranged from 6.84 to 67.4 mg/L. The NH_3-N as nitrogen concentrations (ranged from 0.29 to 2.85 mg/L) were calculated according to the equations of Bower and Bidwell (1978) based on a temperature of 25 C and the pH of the test solutions, which remained constant about 8.2 at salinities of 15, 20, 25, 30, 35, and 40 ppt. The pH at each assay solution was regularly measured by a pH 315i pH meter (WTW Co., Weilheim, Germany) throughout the study.

Short-term median lethal concentration (LC_{50}) toxicity tests were carried out according to the methods described by the APHA (1989). Shrimps were randomly sampled from the holding tanks and transferred to the test and control containers. Bioassay experiments to establish tolerance limits were conducted in triplicate 10-L polyethylene containers having 8 L of test solutions. Each container contained 20 shrimps, and continuous aeration was provided through air stones connected to a blower. In accordance with the "static renewal method" for toxicity tests, each test solution was renewed daily. During the experiment, the shrimps were not fed. Dissolved oxygen was maintained above 5 mg/L.

Survival was checked at 24-h intervals up to 96 h. Death was assumed when shrimps were immobile and showed no response when touched with a glass rod. Dead shrimps were removed daily from the containers. The LC_{50} value of TAN and NH_3-N and their 95% confidence limits were calculated by the Bliss Probit method. The general linear model procedure of the SPSS version 11.0 software was used to compare relationships between survival, salinity, and concentration of ammonia and exposure time.

Results

The relationship between LC_{50} data, salinity, and exposure time are shown in Figure 1. Statistical analysis indicated that the LC_{50} of TAN had a negative exponential relationship with exposure time but a positive exponential relationship with salinity.

At higher salinities of 30, 35, and 40 ppt, in 40 mg/L test solution (13.5 mg/L TAN), mortality started to occur at 36 h (5% mortality), 36 h (10% mortality), and 48 h (5% mortality) after the exposure, respectively. The LC_{50} values for TAN and their 95% confidence intervals at six different salinity levels and exposure times for *P. semisulcatus* juveniles are shown in Table 1. Tolerance of *P. semisulcatus* to TAN and NH_3-N showed a decline with decreasing salinity and increasing exposure time. After 24, 48, 72, and 96 h of exposure, the LC_{50} values for TAN were 4.5, 2.9, 2.9, and 2.6 times higher at 40 ppt than at 15 ppt, respectively. The "safe" levels calculated as described in Sprague (1971) for rearing *P. semisulcatus* PLs were estimated to be 0.7, 0.7, 0.8, 1.4, 1.8, and 1.9 mg/L for TAN and 0.034, 0.035, 0.039, 0.061, 0.075, and 0.082 mg/L for NH_3-N at 15, 20, 25, 30, 35, and 40 ppt salinity levels, respectively. The safe level was 2.5-fold higher for TAN and NH_3-N at 40 ppt in comparison to 15 ppt.

Discussion

The data obtained in this study indicate that salinity affects the tolerance to ammonia of *P. semisulcatus* PLs. Tolerance to ammonia increases as water salinity increases. The higher the salinity, the higher was the survival rate. The mortality rate increased with increasing ammonia concentrations and exposure time. Similar results were also reported for other shrimp species by Chin and Chen (1987), Frias-Espericueta et al. (1999), and Lin and Chen (2001). Low levels of ammonia had no effects, but higher concentrations were toxic to the PLs at even 24-h exposure time.

The toxicity experiments carried out on *P. semisulcatus* PLs in this study revealed that

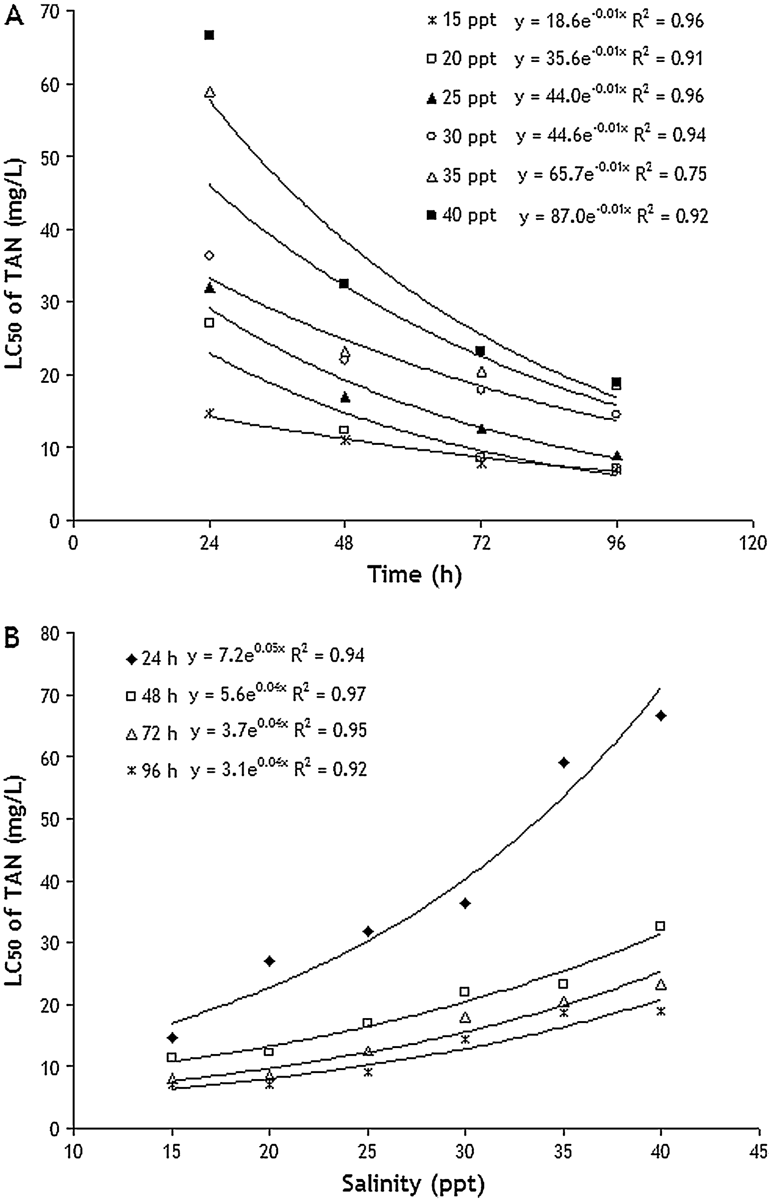

FIGURE 1. *Relationship between median lethal concentration (LC_{50}) of total ammonia-nitrogen (TAN) and exposure time at various salinity levels (A) and between LC_{50} of TAN and salinity at various exposure times (B).*

96-h LC_{50} values at 30 and 35 ppt for TAN were 14.51 and 18.72 mg/L, respectively. The 96-h LC_{50} values for other shrimp species ranged between 23.7 mg/L for *P. semisulcatus* (Wojsbrot et al. 1990) and 70.9 mg/L for *P. monodon* (Chen and Lei 1990). The 96-h LC_{50} found for *P. semisulcatus* in this study appears to be lower than those reported for other species. We found similar results in another study in which the 96-h LC_{50} value for *P. semisulcatus* juveniles (1.6 g) was 11.44 mg/L at 26 C and 39 ppt (Kir et al. 2004). Different responses of the juveniles of various species to this toxicant might be species specific or might be affected by pH, temperature, or size of animals. In any case, our results and those of Wojsbrot et al.

TABLE 1. *Median lethal concentration (mg/L) of total ammonia-nitrogen (TAN) and NH_3-nitrogen for* Penaeus semisulcatus *and their 95% confidence limits (in parentheses) at six different salinity and various exposure times.*

Salinity (ppt)		Time (h) 24	48	72	96
15	TAN[a]	14.81 (13.17–16.46)	11.18 (9.54–12.81)	7.92 (6.24–9.59)	7.07 (5.50–8.63)
	NH_3-N[b]	0.73 (0.51–0.94)	0.55 (0.33–0.76)	0.39 (0.16–0.61)	0.34 (0.15–0.54)
20	TAN[a]	27.06 (25.48–28.64)	12.34 (10.80–13.89)	8.49 (6.70–10.29)	7.11 (5.59–8.62)
	NH_3-N[b]	1.26 (1.06–1.46)	0.57 (0.38–0.76)	0.41 (0.14–0.65)	0.35 (0.15–0.51)
25	TAN[a]	31.95 (30.44–33.45)	16.98 (15.53–18.43)	12.53 (11.23–13.84)	8.94 (7.45–10.42)
	NH_3-N[b]	1.41 (1.23–1.58)	0.75 (0.58–0.91)	0.55 (0.43–0.67)	0.39 (0.22–0.56)
30	TAN[a]	36.24 (35.02–37.46)	22.06 (20.69–23.42)	18.02 (16.66–19.39)	14.51 (12.96–16.05)
	NH_3-N[b]	1.53 (1.44–1.61)	0.93 (0.79–1.06)	0.76 (0.62–0.89)	0.61 (0.42–0.80)
35	TAN[a]	58.88 (57.34–60.42)	23.33 (21.93–24.73)	20.50 (19.21–21.79)	18.72 (17.43–20.01)
	NH_3-N[b]	2.38 (2.23–2.52)	0.94 (0.79–1.09)	0.83 (0.71–0.98)	0.75 (0.64–0.86)
40	TAN[a]	66.65 (64.81–68.49)	32.49 (31.25–33.74)	23.23 (21.89–24.57)	19.06 (17.68–20.44)
	NH_3-N[b]	2.47 (2.37–2.57)	1.26 (1.16–1.35)	0.90 (0.77–1.03)	0.74 (0.60–0.87)

[a] Estimated values based on experimental data.
[b] Calculated from TAN based on pH 8.2, experimental temperatures 25 C, and salinity 15, 20, 25, 30, 35, and 40 ppt.

(1990) may suggest that *P. semisulcatus* might be less tolerant to ammonia as compared to other shrimp species.

Sprague (1971) recommended that a safe-level concentration of pollutant that has no adverse effect on organisms might be obtained by multiplying a 96-h LC_{50} value by a factor of 0.1. By this method, a safe concentration can be suggested in which organisms not only survive but also can actually thrive. The safe level calculated for *P. semisulcatus* PLs at 15, 20, 25, 30, 35, and 40 ppt salinities levels was estimated to be 0.7, 0.7, 0.8, 1.4, 1.8, and 1.9 mg/L for TAN and 0.034, 0.035, 0.039, 0.061, 0.075, and 0.082 mg/L for NH_3-N, respectively. These values for *P. vannamei* juveniles (0.99–3.88 g) were reported to be 6.52 and 7.09 mg/L for TAN and 0.29 and 0.13 mg/L for NH_3-N (Frias-Espericueta et al. 1999). Chen et al. (1990a) stated that the safe level of TAN and NH_3-N for *P. monodon* adolescents were 4.26 and 0.08 mg/L. These levels were reported to be 3.51 and 0.14 mg/L for *P. chinensis*, respectively (Chen et al. 1990b). Chen and Lin (1991) reported a value of 0.24 mg/L NH_3-N for *P. penicillatus* juveniles. It is clear from this data that the safe levels for TAN and NH_3-N at 40 ppt appear to be 2.5-fold higher in comparison to 15 ppt for *P. semisulcatus*.

Penaeids are generally cultured intensively in a semistatic environment with salinity varying from 17 to 34 ppt (Chen and Lin 1992). In this study, at 40 ppt, the 24-, 48-, 72-, and 96-h LC_{50} values for TAN and NH_3-N were 4.5, 2.9, 2.9, and 2.6 times higher than those at 15 ppt. Percent ammonia as NH_3-N increases with pH and temperature but decreases with salinity (Losordo et al. 1992; Masser et al. 1992; Sampaio et al. 2002). Because the pH and temperature were almost constant among test solutions, any difference was assumed to be due to salinity. The percentage of NH_3-N in TAN decreases with salinity, and this can explain the higher tolerance displayed by *P. semisulcatus* PLs to TAN at high salinity level.

The fact that toxicity of ammonia increased as salinity decreased is considered because of higher uptake of ammonia at low salinity. In addition, it is reported that the optimal salinity level is 30–35 ppt for *P. semisulcatus* (Kumlu et al. 1999). Therefore, this study indicated that there is a relationship between better growth and tolerance to ammonia in higher salinity.

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