

**EFFECTS OF EXOGENOUS AMMONIA ON
BRAIN MONOAMINE TRANSMITTER LEVELS IN
FATHEAD MINNOWS**

PATRICK J. RONAN
Department of Biology
University of South Dakota
Vermillion, South Dakota 57069
telephone: (605)677-6541
fax: (605)677-6557
email: PRonan@Charlie.USD.EDU

MARK P. GAIKOWSKI¹, JOHN M. MATTER,
STEVEN J. HAMILTON², KEVIN J. BUHL² AND CLIFF H. SUMMERS

¹National Biological Service
Upper Mississippi Science Center
2630 Fanta Reed Road
La Crosse, Wisconsin, USA

²Midwest Science Center
Field Research Station
R.R. 1, Box 295
Yankton, SD 57078

Introduction

Aquatic contaminants have been shown to affect stress hormones and central neurotransmitter levels in fish after exposure to sublethal levels (fluorine analogs, Thomas *et al.* 1981; endosulphan, Gopal *et al.* 1985; lindane, Rozados *et al.* 1991; lead, Weber *et al.* 1991; PAHs, PCBs, mercury, Hontela *et al.* 1992; copper, De Boeck *et al.* 1995). Ammonia is a common aquatic toxicant. It is generally agreed that a primary mode of toxicity of ammonia is on the nervous system (Viseck, 1968; Smart, 1981; Cooper and Plum, 1987) via mechanisms which are not well understood. The purpose of this study was to determine if exposure to sublethal concentrations of ammonia results in changes in central monoamine synthesis, metabolism, or release in adult male, fathead minnows (*Pimephales promelas*).

Both acute and chronic toxic effects of ammonia in fish are well documented (Singh *et al.* 1985; Sheehan and Lewis, 1986; Solbé and Shurben, 1989; Thurston and Russo, 1983; Thurston *et al.* 1983; Thurston *et al.* 1984; Thurston *et al.* 1986). Secondary toxic effects of ammonia exposure in tissues of fish include connective tissue lesions (Thurston *et al.* 1986), gill lamellae aneurysms (Bullock, 1972; Larmoyeux and Piper, 1973; Smart, 1976), gill epithelium hyperplasia, and renal tissue histopathologies (Larmoyeux and Piper, 1973; Thurston *et al.* 1984). In rainbow trout acclimated to freshwater, increased exogenous ammonia causes blood acidemia (Wilson and

Taylor, 1992). Ammonia also decreases plasma oxygen carrying capacity in fish (Brockway, 1950; Sousa and Meade, 1977).

Un-ionized ammonia (NH_3) has been demonstrated to be much more toxic than the ionized state (NH_4^+) (Wuhrmann and Woker, 1948; Downing and Merkens, 1955; Alabaster and Lloyd, 1980; Sheehan and Lewis, 1986). As a result of this selective toxicity, the toxicity of aqueous ammonia ($\text{NH}_3 + \text{NH}_4^+$) solutions is generally expressed in terms of un-ionized ammonia. Marine teleosts may have enhanced permeability to NH_4^+ (Clairborne and Evans, 1988). Thurston and colleagues (1981) have suggested that NH_4^+ exerts some measure of toxicity in freshwater fish. However, based on acute toxicity tests that account for osmotic effects, even extremely high exogenous levels of NH_4^+ have been shown to be essentially nontoxic in at least one freshwater species and concentrations up to 1,787 mg $\text{NH}_4^+\text{-N/L}$ were not lethal (Sheehan and Lewis, 1986).

In this study the effects of increasing exogenous ammonia on monoamine levels in brains of fathead minnows were measured. Eight monoamines and associated metabolites were quantified: the catecholamines dopamine (DA), norepinephrine (NE) and epinephrine (Epi); the indoleamine serotonin (5-HT); associated metabolites 3-methoxy-4-hydroxyphenylglycol (MHPG), 5-hydroxytryptophan (5-HT), 3,4-dihydroxybenzeneacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA). Serotonergic activity (as determined by the 5-HIAA/5-HT ratio), dopaminergic activity (DOPAC/DA) and noradrenergic activity (MHPG/NE) were also measured.

Materials and Methods

Ammonia exposure

A 48-hour flow-through exposure was conducted. Ammonia concentrations were chosen to bracket the 96-hr LC_{50} values reported by Thurston et al. (1983) for fathead minnows 57 mm total length, 47 μM - 79 μM (0.796 - 1.34 mg/L NH_3). Serial dilution of stock solution by the diluter system resulted in nominal un-ionized ammonia concentrations of 0.44 μM (0.0 mg/L control), 7 μM (0.12 mg/L), 15 μM (0.25 mg/L), 31 μM (0.52 mg/L), 51 μM (0.86 mg/L), and 113 μM (1.9 mg/L).

High performance liquid chromatography (HPLC) analysis

Frozen whole brain samples were homogenized in 10 w/v (wet weight/volume) cold 25 mM sodium acetate extraction buffer at pH 5.1 (Barbato, 1990). Diluted supernatant (1:100) was analyzed using an ESA 5200 Coulochem II liquid chromatograph employing two electrodes at reducing then oxidizing potentials of -40mV and +320mV.

Statistical analyses

Differences among transmitters or metabolite levels and catabolite/transmitter quotients in fish exposed to various ammonia concentrations were analyzed using one-way ANOVA followed by Duncan's Multiple Range Test.

Results

Brain levels of neurotransmitters and metabolites

Catecholamines: Dopamine

There was a significant ($F_{5,32}=13.92$, $P<0.001$) dose-dependent reduction in brain dopamine (DA) levels over the range of ammonia concentrations tested (Fig. 1). The lowest ammonia concentration (0.44 μM , control), equivalent to unpolluted stream water, was coincident with the highest DA concentration and was not significantly different from the next lowest ammonia concentration (7 μM). All other concentrations tested (15 μM - 113 μM) had significantly lower DA levels compared to the control and low test concentrations. The lowest DA concentrations

were concomitant with the two highest ammonia concentrations and were significantly lower than all other concentrations tested.

There were no significant differences ($F_{5,35}=0.99, P<0.45$) in DOPAC concentrations, the major dopaminergic catabolite measured, among any ammonia concentration treatment groups (Table 1). There was also no significant difference ($F_{5,24}=1.42, P<0.30$) in the ratio DOPAC/DA (used as an estimate of DA activity) among ammonia treatment groups.

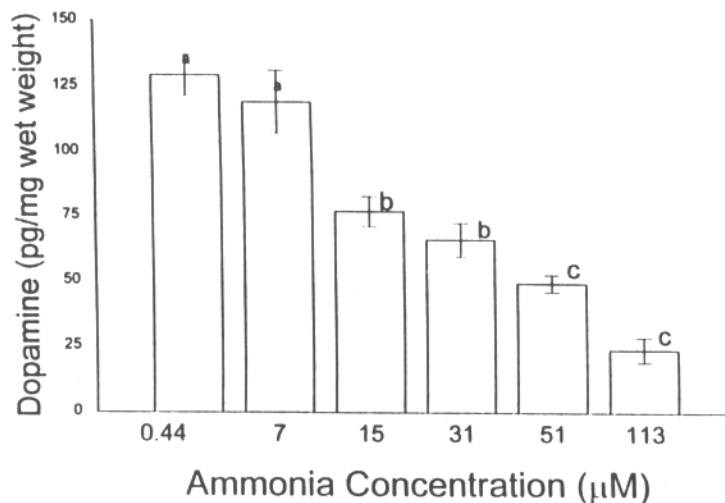


Figure 1. Mean DA levels (pg/mg wet weight + SEM) in *P. promelas* brain by exogenous un-ionized ammonia (NH_3) molarity. Means with different superscripts are significantly different (Duncan's $p < 0.05$).

Norepinephrine

Norepinephrine (NE) levels, like those of DA, decreased significantly ($F_{5,51}=15.90, P<0.005$) in a dose-dependent manner with increasing ammonia concentrations (Fig. 2). The highest concentration of NE was found in brains from the group exposed to the lowest concentration of ammonia (0.44 µM) and was significantly higher than NE concentrations in the group exposed to the next lowest concentration (7 µM). The lowest concentrations of NE were associated with the two highest concentrations of ammonia and were significantly different from all other groups

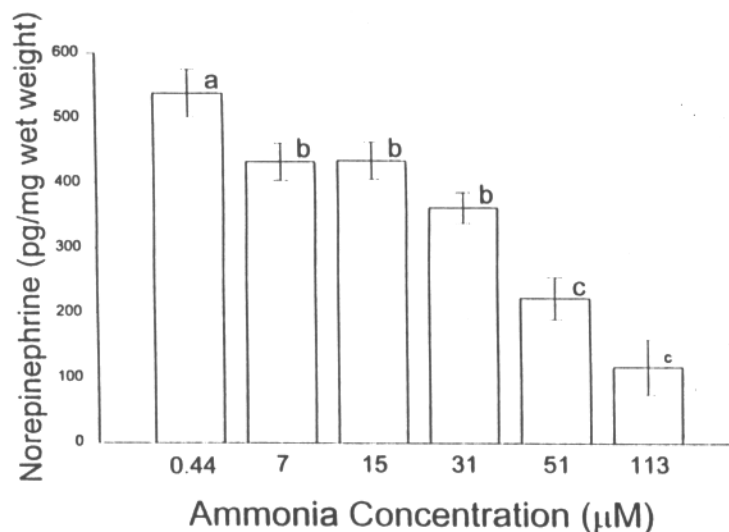


Figure 2. Mean NE levels (pg/mg wet weight + SEM) in *P. promelas* brain by exogenous un-ionized ammonia (NH_3) molarity. Means with different superscripts are significantly different (Duncan's $p < 0.05$).

No significant differences were measured between groups ($F_{5,33}=0.8312$, $P<0.55$) among MHPG levels, a major catabolite of NE, (Table 1) nor in the ratio of MHPG/NE ($F_{5,33}=1.1993$, $P<0.35$).

Epinephrine

There were no significant differences ($F_{5,50}=0.4031$, $P<0.85$) measured among Epi levels from the ammonia concentrations tested (Fig. 3).

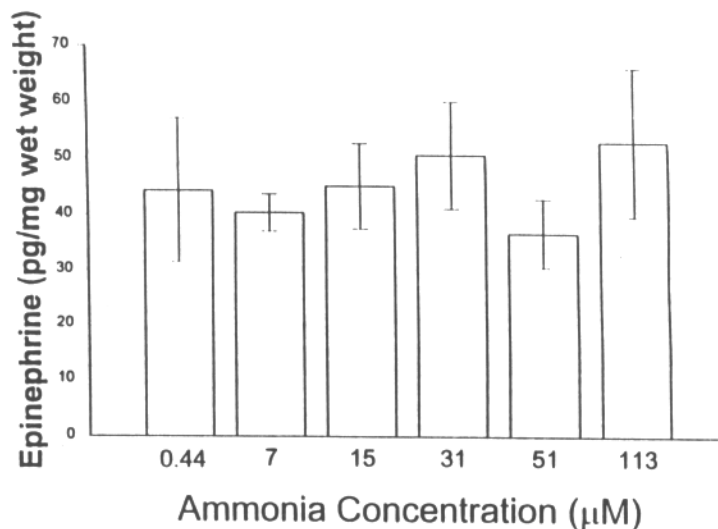


Figure 3. Mean Epi levels (pg/mg wet weight + SEM) in *P. promelas* brain by exogenous un-ionized ammonia (NH_3) molarity.

Indoleamine: Serotonin

Levels of 5-hydroxytryptamine (5-HT) decreased significantly ($F_{5,50}=18.00$, $P<0.005$) with exposure to increasing ammonia concentrations (Fig. 4). The highest level of 5-HT was found in fish exposed to the lowest ammonia concentration (0.44 μM) and was significantly higher than 5-HT concentrations determined in the next lowest concentration of ammonia (7 μM). The three highest ammonia exposure groups had 5-HT concentrations significantly lower than the three lowest concentrations of ammonia.

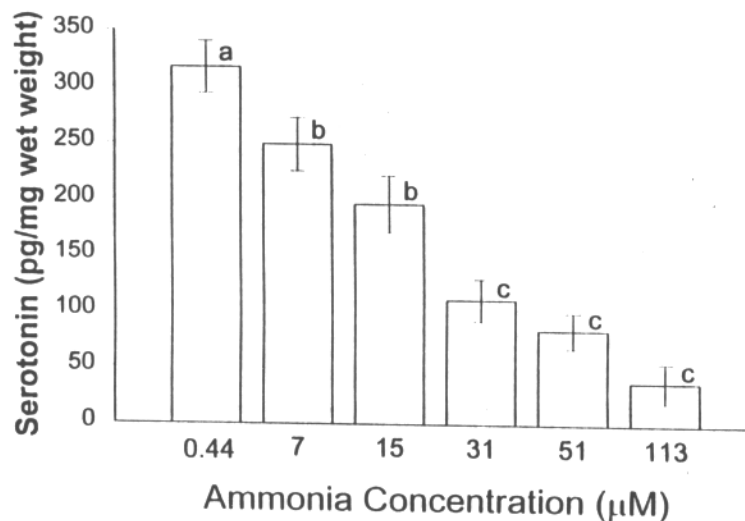


Figure 4. Mean 5-HT levels (pg/mg wet weight + SEM) in *P. promelas* brain by exogenous un-ionized ammonia (NH_3) molarity. Means with different superscripts are significantly different (Duncan's $p < 0.05$)

Concentrations of 5-hydroxytryptophan (5-HTP), the precursor for 5-HT, also exhibited a significant dose-dependent decrease with increasing ammonia concentration (Fig. 5). The 5-HTP

concentration in fish exposed to the second lowest ammonia concentration was significantly higher ($F_{5,39}=2.8163$, $P<0.05$) from the 5-HTP levels present in brains of fish exposed to the two highest concentrations.

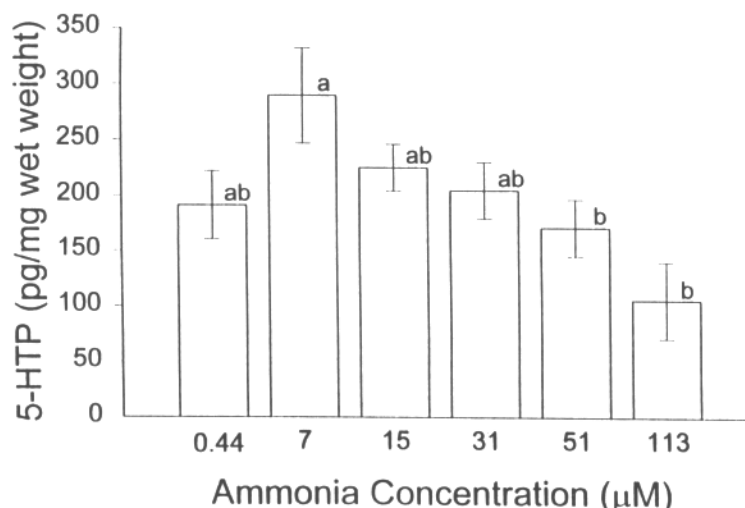


Figure 5. Mean 5-HTP levels (pg/mg wet weight + SEM) in *P. promelas* brain by exogenous un-ionized ammonia (NH_3) molarity. Means with different superscripts are significantly different (Duncan's $p < 0.05$).

No significant differences ($F_{5,51}=0.6044$ $P<0.7$) were measured for 5-hydroxyindoleacetic acid (5-HIAA) concentrations, the major catabolite of 5-HT, between any ammonia concentrations tested (Table 1). However, there was a significant increase in the 5-HIAA/5-HT ratio ($F_{5,50}=24.49$, $P<0.005$) between groups exposed to the lower ammonia concentrations when compared to the highest ammonia concentration group.

Table 1. There are no significant differences between mean levels of the catabolites 3,4-dihydroxybenzeneacetic acid (DOPAC), 3-methoxy-4-hydroxyphenylglycol (MHPG), or 5-hydroxyindoleacetic acid (5-HIAA). Data are means plus or minus SEM.

	0.44 µM	7 µM	15 µM	31 µM	51 µM	113 µM
DOPAC	14.4±2.2	12.5±1.5	15.2±2.0	31.9±17.0	9.2±2.9	14.0±11.9
MHPG	943±186	434±97.9	746±122	305±46.0	785±307	260±61.1
5-HIAA	56.1±6.5	56.8±6.3	56.4±3.2	54.2±5.2	44.1±3.8	50.7±24.3

Discussion

Monoamine transmitter levels, rather than levels of their catabolites, were affected by increasing ammonia concentrations in fathead minnows. Catecholamines and serotonin were diminished sequentially with increasing concentrations of ammonia. This dose-dependent response was characteristic of all neurotransmitters measured with the exception of Epi.

In conclusion, exposure to increasing concentrations of exogenous ammonia decreased levels of monoamine transmitters whose anabolic and catabolic pathways are directly dependent on molecular oxygen. Decreased transmitter levels are likely the result of decreased synthesis due to the effects of depleted levels of molecular oxygen on two oxygen-dependent rate-limiting anabolic enzymes. Even a small reduction in the amount of oxygen available may reduce the synthesis of monoamines (Gibson *et al.* 1981). Brain energy metabolism may be disrupted as

well. An increase in release and activity may have been masked by inhibitory effects of depleted oxygen levels on catabolic enzymes. Toxic effects of ammonia on metabolism and concentrations of brain monoamines will have detrimental ramifications for the organism as a whole. Changing monoamine system activation will affect efficient neural response and consequently, cause behavioral and physiological changes via alterations in neural-endocrine interactions, sympathetic and parasympathetic activation, as well as metabolism of peripheral catecholamines and indoleamines. Environmental disrupters of neuronal function could negatively affect overall fitness and reproductive success.

References

- ALABASTER, J. S. AND LLOYD, R. (1980). *Water Quality Criteria for Freshwater Fish*, 2nd Edition. London, Butterworths.
- BARBATO, G.F. (1990). A fast hplc analysis of catecholamines and indoleamines in avian brain tissue. *J. Liquid Chrom.* **13(13)**, 2553-2560.
- BROCKWAY, D.R. (1950). *Prog. Fish Cult.* **12**, 126.
- BULLOCK, G. L. (1972). Studies on selected myxobacteria pathogenic for fishes and on bacterial gill disease in hatchery-reared fish. *Tech. Pap. Bur. Sport Fish. Wildl.* **60**, 30 pp.
- CLAIBORNE, J. B. AND EVANS, D. H. (1988). Ammonia and acid-base balance during high ammonia exposure in a marine teleost (*Myoxocephalus octodecimspinosus*). *J. exp. Biol.* **140**, 89-105.
- COOPER, A.J.L. AND PLUM, F. (1987). Biochemistry and Physiology of brain ammonia. *Phys. Rev.* **67(2)**, 440-519.
- DE BOECK, G., NILSSON, G.E., ELOFSSON, U., VLAEMINCK, A. AND BLUST, R. (1995). Brain monoamine levels and energy status in common carp (*Cyprinus carpio*) after exposure to sublethal levels of copper. *Aquat. Toxicol.* **33**, 265-277.
- DOWNING, K. M. AND MERKENS, J. C. (1955). The influence of dissolved oxygen concentration on the toxicity of un-ionized ammonia to rainbow trout (*Salmo gairdneri* Richardson). *Ann. Appl. Biol.* **42**, 243-246.
- GIBSON, G., PULSINELLI, W., BLASS, J. AND DUFFY., T. (1981). Brain dysfunction in mild to moderate hypoxia. *Am. J. Med.* **70**, 1247-1254.
- GOPAL, K., ANAND, M., MERHROTRA AND RAY, P.K. (1985). Neurobehavioral changes in freshwater fish *Channa punctatus* exposed to endosulphan. *J. Adv. Zool.* **6(2)**, 74-80.
- HONTELA, A., RASMUSSEN, J.B., AUDET, C. AND CHEVALIER, G. (1992). Impaired cortisol stress response in fish from environments polluted by PAHs, PCBs, and mercury. *Arch. Env. Contam. and Tox.* **22**, 278-283.
- LARMOYEUX, J.D. AND PIPER, R.G. (1973). Effects of water re-use on rainbow trout in hatcheries. *Prog. Fish Cult.* **35**, 2-8.
- ROZADOS, M.V., ANDRES, M.D., AND ALDEGUNDE, M.A. (1991). Preliminary studies on the acute effect of lindane (γ -HCH) on brain serotonergic systems in rainbow trout *Oncorhynchus mykiss*. *Aquat. Toxicol.* **19(1)**, 33-40.
- SAYER, M. D. J. AND DAVENPORT, J. (1987). The relative importance of the gills to ammonia and urea excretion in five seawater and one freshwater teleost species. *J. Fish. Biol.* **31**, 561-570.
- SHEEHAN, R.J. AND LEWIS, W.M. (1986). Influence of pH and ammonia salts on ammonia toxicity and water balance in young channel catfish. *Trans. Am. Fish. Soc.* **115**, 819-899.
- SMART, G.R. (1981). Aspects of water quality producing stress in intensive fish culture. In *Stress and Fish* (Pickering, A.D. ed.). pp. 277-93. New York. Academic Press.
- SMART, G. R. (1976). The effect of ammonia exposure on gill structure of the rainbow trout *Salmo gairdneri*. *J. Fish. Biol.* **8**, 471-475.
- SOLBÉ, J.F. DE L.G. AND SHURBEN, D.G. (1989). Toxicity of ammonia to early life stages of rainbow trout (*Salmo gairdneri*). *Water Res.* **23**, 127-129.

- SOUSA, R. J. AND MEADE, T.L. (1977). The influence of ammonia on the oxygen delivery system of coho salmon hemoglobin. *Comp. Biochem. Physiol.* **58A**, 23-28
- THOMAS, P., WOFFORD, H.W. AND NEFF, J.M. (1981). Biochemical stress responses of striped mullet (*Mugil cephalus* L.) to fluorine analogs. *Aquat. Toxicol.* **1**, 329-342.
- THURSTON, R.V., RUSSO, R. C., MEYN, E. L., ZAJDEL, R. K. AND SMITH, C. E. (1986). Chronic toxicity of ammonia to fathead minnows. *Trans. Am. Fish. Soc.* **115(2)**, 196-207.
- THURSTON, R.V., RUSSO, R. C., LUEDTKE, J., SMITH, C. E., MEYN, E. L., WANG, K. AND BROWN, C. J. D. (1984). Chronic toxicity of ammonia to rainbow trout. *Trans. Am. Fish. Soc.* **113(1)**, 56-73.
- THURSTON, R.V. AND RUSSO, R. C. (1983). Acute toxicity of ammonia to rainbow trout. *Trans. Am. Fish. Soc.* **112**, 696-704.
- THURSTON, R.V., RUSSO, R. AND PHILLIPS, G. (1983). Acute toxicity of ammonia to fathead minnows. *Trans. Am. Fish. Soc.* **112**, 705-711.
- THURSTON, R.V., RUSSO, R. AND VINOGRADOV, G. (1981). Ammonia toxicity to fishes. Effect of pH on the toxicity of the un-ionized ammonia species. *Env. Sci. and Tech.* **15**, 837-840.
- VISECK, W. J. (1968). Some aspects of ammonia toxicity in animal cells. *J. Dairy Sci.* **51**, 286-295.
- WEBER, D.N., RUSSO, A., SEALE, D.B. AND SPIELER, R.E. (1991). Waterborne lead affects feeding abilities and neurotransmitter levels of juvenile fathead minnows (*Pimephales promelas*). *Aquat. Toxicol.* **21**, 71-80.
- WILSON, R. W. AND TAYLOR, E. W. (1992). Transbranchial ammonia gradients and acid-base responses to high external ammonia concentration in rainbow trout (*Onchorhynchus mykiss*) acclimated to different salinities. *J. exp. Biol.* **166**, 95-112.
- WUHRMANN, K. AND WOKER, H. (1948). Experimentelle untersushungen über die ammoniak- und Blausäurevergiftung. *Z. Hydrol.* **11**, 210-244.

Introduction

Aquatic contaminants have been shown to affect stress hormones and central neurotransmitter levels in fish after exposure to sublethal levels (fluorine analogs, Thomas *et al.* 1981; endosulphan, Gopal *et al.* 1985; lindane, Rozados *et al.* 1991; lead, Weber *et al.* 1991; PAHs, PCBs, mercury, Hontela *et al.* 1992; copper, De Boeck *et al.* 1995). Ammonia is a common aquatic toxicant. It is generally agreed that a primary mode of toxicity of ammonia is on the nervous system (Viseck, 1968; Smart, 1981; Cooper and Plum, 1987) via mechanisms which are not well understood. The purpose of this study was to determine if exposure to sublethal concentrations of ammonia results in changes in central monoamine synthesis, metabolism, or release in adult male, fathead minnows (*Pimephales promelas*).

Both acute and chronic toxic effects of ammonia in fish are well documented (Singh *et al.* 1985; Sheehan and Lewis, 1986; Solbé and Shurben, 1989; Thurston and Russo, 1983; Thurston *et al.* 1983; Thurston *et al.* 1984; Thurston *et al.* 1986). Secondary toxic effects of ammonia exposure in tissues of fish include connective tissue lesions (Thurston *et al.* 1986), gill lamellae aneurysms (Bullock, 1972; Larmoyeux and Piper, 1973; Smart, 1976), gill epithelium hyperplasia, and renal tissue histopathologies (Larmoyeux and Piper, 1973; Thurston *et al.* 1984). Ammonia also decreases plasma oxygen carrying capacity in fish (Brockway, 1950; Sousa and Meade, 1977).