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Effect of Salinity on Acute Toxicity of Ammonia and Nitrite to Juvenile *Mugil platanus*

L. A. Sampaio, W. Wasielesky, K. Campos Miranda-Filho

University of Rio Grande, Department of Oceanography, Laboratory of Mariculture, CP 474, Rio Grande RS, 96201-900, Brazil

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Ammonia originating from natural and anthropogenic sources can reach toxic levels in intensive fish culture tanks (Bader and Grizzle, 1992). However, due to the nitrifying action of aerobic bacteria upon ammonia, nitrite can also build up to harmful levels for fishes (Kamstra et al., 1996).

There is an equilibrium between ionized (NH_4^+) and gaseous (NH_3) ammonia in the water. This equilibrium is affected by pH, temperature and salinity in decreasing order of importance (Spotte and Adams, 1983). The higher the pH, the higher is the proportion of NH_3, while an increase in salinity will lead to lower proportion of NH_3. Due to the seawater buffering system, the more alkaline pH favors the equilibrium towards NH_3 in comparison to lower salinity waters.

NH_3 is considered to be the most toxic form of ammonia, because it can readily diffuse across gill membranes, while the passage of ionized ammonia through gill membranes is more difficult (Fromm and Gillete, 1968). Ammonia causes impairment of cerebral energy metabolism and nerve function (Randall and Wright, 1987). Nitrite also reaches fish blood by diffusion across the gills, and its toxicity is due to the oxidation of haemoglobin into methahaemoglobin. The haemoglobin oxidation reduces blood's oxygen-carrying capacity, leading to fish death by hypoxia (Weirich et al., 1993). The toxicity of these nitrogenous compounds is influenced by salinity. According to Soderberg and Meade (1991) and Bianchini et al. (1996), higher salinity may have mitigating effects on the toxicity of ammonia and nitrite to fishes.

The chronic effects of ammonia and nitrite have already been studied for juvenile mullet *Mugil platanus* (Miranda-Filho et al., 1995), but surprisingly there is no information regarding the acute toxicity of either compound to this species. Considering the euryhalinity of *M. platanus*, and its potential to aquaculture, the objective of this work was to study the acute toxicity of ammonia and nitrite in different salinities. Results are also discussed in terms of the stabilization of LC_{50} values over time, due to genetic variability of test organisms. A comparison of the safe levels: 10% of LC_{50}, proposed by Sprague (1971); and acute:chronic ratio, proposed by Wise and Tomasso (1989), was performed in order to establish the best approach for *M. platanus*.

Correspondence to: L. A. Sampaio

MATERIALS AND METHODS

The acute tests were conducted at the Laboratory of Mariculture from the Federal University of Rio Grande, Brazil. Juveniles of *M. platanus* were collected at "Lagoa dos Patos" estuary (32 °S 52 °W), and immediately transported to the laboratory, where they were placed in a 1,000 L tank filled with water with the same salinity as in the collection site (0 ‰).

One week later, fish were transferred to three tanks (60 L) where they were acclimated to the experimental salinities (0, 15 and 30 ‰) during 15 days before being exposed to ammonia and nitrite. During the acclimation period, temperature for all tanks was equal to 25 ± 1 °C, and average pH was equal to 7.25, 7.69, and 7.71 for salinities 0, 15, and 30 respectively. The intermediate salinity was obtained by dilution of seawater (30 ‰) with dechlorinated tapwater (0 ‰). Feeding was *ad libitum*, using a commercial trout diet supplemented with a mixture of *Callinectes sapidus* ova and *Artemia* biomass.

Based on the results of range finding tests, juvenile mullets were exposed to five concentrations of ammonia and nitrite in triplicate, plus three controls (no ammonia or nitrite added) for each salinity (Table 1). The desired concentrations were obtained from stock solutions made with ammonium chloride or sodium nitrite.

Mullets (190 ± 20 mg, 28 ± 2 mm) were transferred in groups of 15 fishes to 2 L beakers one day before the tests. During the trials (96 h) water was continuously aerated, mullets were not fed and test solutions were fully exchanged daily. Before renewing the experimental media, pH, temperature and salinity were measured. Dead fish were removed and counted, immobile fish were considered dead if they did not respond to mechanical stimuli. Temperature was controlled with thermostat at 25 ± 1 °C in a water bath and did not vary among salinities. Average pH values were 7.34, 7.72, and 7.76 for salinities 0, 15, and 30‰ respectively.

Total ammonia nitrogen concentrations were transformed into NH_3-N using the equations of Ostrensky et al. (1991) adapted from Whitfield (1974) and Bower and Bidwell (1978) according to the values of pH, temperature and salinity measured during the experiment.

Median lethal concentrations (LC_{50} 24, 48, 72 and 96 h) and their respective confidence intervals (95%) for total and gaseous ammonia, and nitrite were calculated using the software Trimmed Spearman Karber Method developed by Hamilton et al. (1977). The program includes Abbot's correction for control mortality. The safe levels were calculated following Sprague (1971) and Wise and Tomasso (1989). Comparisons among LC_{50} values and safe levels for total ammonia, gaseous ammonia, and nitrite in each salinity were made by examining confidence limits for overlap as recommended by APHA (1989).

Table 1. Experimental design showing the concentrations of total ammonia nitrogen and nitrite nitrogen used in the acute toxicity tests with juvenile *Mugil platanus*.

Product	Salinity (‰)	Concentrations ($mg.L^{-1}$)					
Total ammonia-N	0	0	10	20	30	40	50
	15	0	15	20	25	30	35
	30	0	15	20	25	30	35
Nitrite-N	0	0	1	3	6	9	12
	15	0	15	30	45	60	90
	30	0	10	25	50	100	200

RESULTS AND DISCUSSION

The effects of salinity on the pattern of acute toxicity of ammonia to juvenile *M. platanus* are different when considering total ammonia or NH_3. *M. platanus* is significantly more sensitive to total ammonia when acclimated to salinity 15 or 30 ($P<0.05$), but if NH_3 is considered, the LC_{50}-96h to fish acclimated to freshwater is significantly lower ($P<0.05$) (Table 2). These results emphasize the importance of reporting ammonia toxicity in terms of NH_3, otherwise misleading conclusions might be reached.

Weirich et al. (1993) did not find an influence of salinity on the acute toxicity of ammonia to sunshine bass (female *Morone chrysops* x male *Morone saxatilis*). On the other hand, *Salmo salar* (Alabaster et al., 1979) and *Oncorhynchus tshawytscha* (Harader and Allen, 1983) kept in freshwater are more susceptible to ammonia toxicity than when they are acclimated to saltwater.

Nitrite toxicity was also influenced by salinity, *M. platanus* acclimated to freshwater were significantly more sensitive to nitrite ($P<0.05$) than those kept at higher salinities (Table 3). Nitrite was also found to be more toxic in freshwater for *Anguilla anguilla*, *Sciaenops ocellata*, and sunshine bass (female *Morone chrysops* x male *Morone saxatilis*) respectively by Saroglia et al. (1981), Wise and Tomasso (1989), and Weirich et al. (1993) .

The difference found for nitrite toxicity in different salinities was much more pronounced than that observed for ammonia. The 96h-LC_{50} for NH_3 in freshwater was not even half of that in salt water, but for nitrite it was 23 times lower in freshwater than saltwater. The same trend was observed for other species. While ammonia was 3 times more toxic in freshwater for *Oncorhynchus tshawytscha* (Harader and Allen, 1983), nitrite toxicity for *Anguilla anguilla* increases by a factor of 13 in freshwater when compared to saltwater (Saroglia et al., 1981), and by a factor of 30 for *Sciaenops ocellata* (Wise and Tomasso, 1989).

Table 2. Median lethal concentrations (LC_{50}) of total ammonia nitrogen ($mg.L^{-1}$) and gaseous ammonia nitrogen (NH_3-N $mg.L^{-1}$) to juvenile mullet (*Mugil platanus*) in different salinities. Data in brackets represent the 95% confidence limits of the LC_{50}.

Time (h)	Total ammonia-N			NH_3-N		
	0 ‰	15 ‰	30 ‰	0 ‰	15 ‰	30 ‰
24	41.2^{a}	23.5^{b}	26.2^{b}	0.78^{x}	0.95^{y}	1.03^{y}
	(37.1-45.8)	(21.8-25.3)	(24.7-27.8)	(0.71-0.88)	(0.89-1.03)	(0.97-1.09)
48	35.1^{a}	22.6^{b}	23.8^{b}	0.67^{x}	0.92^{y}	0.94^{y}
	(30.7-40.3)	(20.7-24.6)	(22.1-25.6)	(0.59-0.79)	(0.84-1.00)	(0.87-1.01)
72	32.3^{a}	21.6^{b}	22.5^{b}	0.62^{x}	0.88^{y}	0.89^{y}
	(25.8-40.4)	(19.8-23.6)	(20.5-24.7)	(0.49-0.77)	(0.81-0.96)	(0.81-0.97)
96	30.1^{a}	20.7^{b}	21.3^{b}	0.58^{x}	0.84^{y}	0.84^{y}
	(24.1-37.7)	(17.1-25.2)	(19.3-23.5)	(0.45-0.71)	(0.69-1.02)	(0.70-0.92)

Values of LC_{50} followed by different letters in the same row (within total ammonia-N or NH_3 -N) are significantly different ($P<0.05$).

Ashe et al. (1996) reported small changes for LC_{50} values over time after 24 h of exposure of *Morone chrysops* to ammonia. It is attributed to a narrow range of tolerance within the test population, or to the acclimation of the fishes to the toxin. A similar pattern was observed for *M. platanus*, but instead, LC_{50} values stabilized only after 48 h. This result might suggest that the response should, in a first moment, be more related to the homogenization of the tested population. The individuals analyzed by Ashe et al. (1996) were produced in the laboratory, while those used in this study came from the field, probably holding a higher genetic variation. This pattern seems to be consistent for other species routinely spawned in captivity as is the case of juvenile *Scophthalmus maximus* where LC_{50} stabilizes after approximately 16 h (Person-Le Ruyet et al., 1994), while organisms captured in nature like *O. argentinensis*, the LC_{50} stabilizes after 48 h (Sampaio and Minillo, 1995).

There are some controversy when comparing the safe levels of Sprague (1971) estimated in the present work (Table 4), with the chronic toxicity values reported by Miranda- Filho et al. (1995). While growth was reduced at 0.04 $mg.L^{-1}$ NH_3-N, the safe level for NH_3-N was estimated at 0.08 $mg.L^{-1}$. The opposite was observed for nitrite, where a more conservative estimate was found for the safe level (3.59 $mg.L^{-1}$ NO_2^--N) than for the chronic toxicity test (8.00 $mg.L^{-1}$ NO_2^-- N).

Wise and Tomasso (1989) suggest a different approach to estimate the safe level for rearing fishes. They suggest that acute:chronic ratios should be calculated where the information is available, Thurston et al. (1986) calculated acute:chronic ratios as high as 17 for fathead minnows, using the results from Wajsbrot et al. (1993) the acute:chronic rate for *Sparus aurata* was estimated to be equal to 4. Acute:chronic ratio for *M. platanus* in salt water was calculated as 20, suggesting that the correct safe level for this species actually is around 5% (0.042 $mg.L^{-1}$ NH_3-N) of the acute toxicity.

Table 3. Median lethal concentrations (LC_{50}) of nitrite ($mg.L^{-1}$) to juvenile mullet (*Mugil platanus*) in different salinities. Data in brackets represent the 95% confidence limits of the LC_{50}.

Time (h)	Nitrite-N		
	0 ‰	15 ‰	30 ‰
24	11.75^a	115.66^b	182.24^c
	(8.98-15.38)	(101.79-131.43)	(170.36-194.95)
48	3.70^a	61.37^b	65.61^b
	(2.79-4.90)	(53.72-70.11)	(55.92-76.99)
72	1.58^a	39.31^b	37.64^b
	(1.24-2.02)	(34.14-45.26)	(28.72-49.33)
96	1.51^a	36.17^b	35.89^b
	(1.21-1.87)	(31.58-41.43)	(32.55-39.56)

Values of LC_{50} followed by different letters in the same row are significantly different ($P<0.05$).

Ammonia and nitrite are known to be toxic to fishes, especially when high stocking densities are coupled to recirculation systems (Tomasso, 1994). Wajsbrot et al. (1993) mention that the high buffering capacity of seawater should lead to a higher proportion of NH_3, making this ambient potentially dangerous to fishes due to ammonia toxicity. However, the interaction between biological and physical-chemical data must be considered together. As a general rule, as it was verified for *M. platanus*, fish kept in freshwater are more sensitive to ammonia and nitrite when compared to those acclimated in saltwater (Harader and Allen, 1983; Bianchini et al., 1996). This higher sensitivity in freshwater should not be considered a problem of acclimation, since *M. platanus* occurs naturally in freshwater bodies.

Special care should be taken when fish are being raised in estuaries, where fluctuating salinity is commonly observed. As a consequence, water pH will also vary, altering the proportion of NH_3 to total ammonia. The potential changes in water pH make water quality management a difficult task in intensive fish culture, since carrying capacity of the system will vary with time. One could say that the higher concentration of NH_3 in salt water will result in a lower carrying capacity of the salt water pond, but it should also be considered that *M. platanus* are more tolerant to NH_3 toxicity in salt water. As fish are also much more resistant to nitrite in salt water, there should be a lower carrying capacity for freshwater ponds.

The reasons for the increased toxicity of ammonia and nitrite in freshwater are not clear. Cameron and Heisler (1983) suggest that the mechanism for ammonia exchange at the gills counts on the exchange of Na^+ for NH_4^+, so the reduced availability of Na^+ in freshwater might compromise the ammonia excretion rate, allowing blood ammonia concentration to reach lethal levels. Regarding nitrite, it is possible to accumulate this compound into fish blood through the branchial chloride cells in freshwater, while fish kept in saltwater have this process

Table 4. Safe levels of total ammonia nitrogen, gaseous ammonia nitrogen (NH_3-N) and nitrite ($mg.L^{-1}$) for juvenile mullet (*Mugil platanus*) in different salinities. Data in brackets represent the 95% confidence limits of the LC_{50}.

Nitrogenous products	Salinity (‰)		
	0	15	30
Total ammonia-N	3.01[a]	2.07[b]	2.13[b]
	(2.41-3.77)	(1.71-2.52)	(1.93-2.35)
NH_3-N	0.06[a]	0.08[b]	0.08[b]
	(0.05-0.07)	(0.07-0.10)	(0.07-0.09)
Nitrite-N	0.15[a]	3.62[b]	3.59[b]
	(0.12-0.19)	(3.16-4.14)	(3.26-3.97)

Safe levels followed by different letters in the same row are significantly different ($P<0.05$). Values were estimated following Sprague (1971).

hampered by the presence of chloride ions, because they competitively exclude nitrite uptake on the gill membrane (Tomasso, 1994). Wise and Tomasso (1989) demonstrated experimentally the possibility to reduce the toxicity of nitrite increasing the ambient chloride concentration.

From the results obtained in this study it can be concluded that the toxicity of ammonia and nitrite to juvenile *M. platanus* increases in freshwater in comparison to higher salinities. Special care should be taken when raising juvenile *M. platanus* in estuaries, because the carrying capacity of a given pond can be lowered during the rainy season, when freshwater dominates the environment.

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