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Acute toxicity and sublethal effects of ammonia and nitrite for juvenile cobia *Rachycentron canadum*

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Abstract

Nitrogenous compounds can be toxic to aquatic animals especially when they are reared at high stocking densities. Cobia (*Rachycentron canadum*) is a fast growing fish currently reared in cages, but with expanding production in intensive recirculating aquaculture systems (RAS). Therefore, the objective of this study was to evaluate the acute toxicity of ammonia and nitrite to juvenile cobia. Juveniles (1.74±0.11 g for ammonia and 0.88±0.06 g for nitrite toxicity evaluation) were acclimated to test conditions (temperature 26 °C and salinity 22‰) and acutely exposed to ammonia (0.25–1.30 ppm NH_3-N) and nitrite (30–210 ppm NO_2-N) at 0.2 fish L^{-1}. Tests were run in 50 L semi-static tanks, experimental water was fully renewed daily, and all test concentrations plus the controls were run in triplicate. Mortality, feeding and swimming behavior were observed during 96 h, toxic concentrations for 50% the population and the respective 95% confidence intervals for these three end points were estimated using the Trimmed Spearman Karber Method. Cobia ceased to eat at 0.62 (0.56–0.70) ppm NH_3-N and 76.1 (73.2–79.0) ppm NO_2-N. Swimming behavior was affected at higher concentrations: 0.80 (0.74–0.85) ppm NH_3-N and 88.8 (82.6–95.5) ppm NO_2-N. Even higher concentrations were necessary to kill juvenile cobia, LC50–96 h for ammonia was estimated at 1.13 (1.06–1.19) ppm NH_3-N, and within the range of concentrations tested for nitrite it was not possible to estimate the LC50–96 h, as only 30% of the individuals died at the highest concentration after 96 h (210 ppm NO_2-N). The results of the present experiments demonstrate that ammonia could be problematic at relatively low levels for the intensive rearing of juvenile cobia; however, it is unlikely that the high levels of nitrite needed to harm juvenile cobia would be reached in a well designed and properly operating RAS.

Keywords: Cobia; Ammonia; Nitrite; Toxicity; Swimming; Feeding

1. Introduction

Cobia *Rachycentron canadum* is widely distributed in tropical and subtropical waters of Atlantic, Pacific and Indian Oceans (Shaffer and Nakamura, 1989). This is a fast growing fish, reaching from egg over 6 kg in one year, and once hatchery technology was developed in the 90's, its culture became popular in Asia (Liao et al., 2001).

Cage culture of cobia comprises around 80% of marine fish cultured in cages in Taiwan, where land and freshwater resources are limited (Liao et al., 2004). However, in the United States, environmental regulations limit marine fish culture in cages, and recirculating

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aquaculture systems (RAS) are therefore considered an option (Weirich et al., 2003).

Cobia is a carnivorous species, thus requiring a protein rich diet (Chou et al., 2001; Craig et al., 2006). Fish meal can be replaced by soybean meal, but for optimum growth only 17% of fish meal can be replaced (Chou et al., 2004). Protein rich diets lead to high rates of ammonia excretion (Carter et al., 1998), and as a consequence, there might be a build up of nitrogenous compounds in a RAS. Ammonia and nitrite are known to be toxic to fish, and special attention must be paid to their toxicity in intensive culture systems, where high concentrations of both products can be reached (Ruyet et al., 1997). Cobia is tolerant to nitrite toxicity, juvenile survives to concentrations up to 32 ppm NO_2-N (Atwood et al., 2004), but nothing is known on ammonia toxicity to this species.

Most studies dealing with ammonia and nitrite toxicity on fish consider acute exposure (Sampaio et al., 2002; Weirich and Riche, 2006a,b). Nevertheless, there are also evaluation of their chronic effects, mainly on growth of larvae and fingerlings (Foss et al., 2004; Lemarié et al., 2004; Siikavuopio and Sæther, 2006). However, other endpoints of importance for aquaculture must be considered, including those related to fish behavior. Swimming and feeding are crucial for a cultured individual, and once these traits are compromised, negative effects upon growth and overall performance will quickly follow. The purpose of the present work was to evaluate the levels for acute toxicity of ammonia and nitrite to juvenile cobia, and also to observe the toxic effects of both products on swimming and feeding behavior.

2. Materials and methods

Fertilized cobia eggs were air transported from the University of Texas Marine Science Institute's Fisheries and Mariculture Laboratory (FAML) in Port Aransas, Texas to Virginia Tech's Virginia Seafood Agricultural Research and Extension Center located in Hampton, Virginia (USA). Larvae and juveniles were produced at the VSAREC laboratory according to standard larviculture production protocols (Faulk and Holt, 2005) that were further optimized for cobia production (Schwarz et al., 2006).

Prior to the experiments fish were acclimated to experimental conditions for 2 weeks in a 300 L tank, fed a commercial diet (Otohime, Japan) 6 times per day, where continuous light was provided (350 lx at the water surface). Water quality was determined to be: temperature 27±1 °C; pH 7.7±0.2; minimum dissolved oxygen 5.5 mg L^{-1}; maximum total ammonia nitrogen (TA-N) 0.3 ppm; maximum nitrite nitrogen (NO_2-N) 0.18 ppm; and salinity was maintained at 22±1‰.

Juvenile cobia (1.74±0.11 g; 76.6±1.5 mm for ammonia and 0.88±0.06 g; 61.3±1.4 mm for nitrite; n=30) were acutely exposed to seven concentrations of either ammonia (0.25–1.30 ppm NH_3-N) or nitrite (30–210 ppm NO_2-N) during 96 h (Table 1). The experiments were run separately with different groups of fish from the same acclimation tank, with both experiments having controls where no toxicant was added. The test concentrations and the controls were all conducted with three replicates each. The desired concentrations were obtained from stock solutions made with reagent grade ammonium chloride and sodium nitrite (Mallinckrodt, Phillipsburg, USA).

Toxicity trials were run in a semi-static system, where water and the toxicant were fully replaced every day. Tank volume was 50 L, stocking density was equal to 10 fish per test tank and water was constantly aerated through air stones. Temperature was maintained at 26 °C and salinity 22‰. Constant illumination was provided, and light intensity at the water surface of tank ranged from 200 to 350 lx (Digital light meter, Greenlee Textron, Taiwan). During the trials, fish were hand fed two times per day to satiation on a dry diet (Otohime, Japan). Within 30 min after food was offered any remaining diet pellets and feces were siphoned out.

Table 1
Mean (±SD) concentration and respective standard error for total ammonia (ppm NH_3+NH_4-N), gaseous ammonia (ppm NH_3-N) and nitrite (ppm NO_2-N) during 96 h

Product	Concentration								
Total ammonia	Nominal	0	6	12	18	24	30	36	42
	Real	0.25±0.0	6.9±0.16	13.5±0.20	19.1±0.12	26.9±0.21	33.7±0.17	38.5±0.14	43.9±0.21
Gaseous ammonia	Nominal	0	0.20	0.40	0.60	0.80	1.00	1.20	1.40
	Real	0.01±0.0	0.26±0.01	0.44±0.01	0.72±0.01	0.92±0.02	1.03±0.03	1.16±0.03	1.31±0.02
Nitrite	Nominal	0	30	60	90	120	150	180	210
	Real	0.7±0.4	31.5±1.3	59.7±1.6	89.7±2.2	115.7±2.2	146.3±2.4	174.4±2.3	210.3±2.0

Table 2
Mean (±SD) water quality parameters during the evaluation of toxicity of ammonia and nitrite for juvenile cobia *Rachycentron canadum*

Parameter	Toxicant	
	Ammonia	Nitrite
Temperature (°C)	25.9±0.0	25.9±0.0
Salinity (‰)	22.2±0.0	22.1±0.1
Dissolved oxygen (mg L^{-1})	6.5±0.0	6.6±0.0
pH	7.80±0.01	7.92±0.01
Calcium (mg L^{-1})	262.6±3.6	252.8±4.1
Chloride (g L^{-1})	14.8±0.2	14.9±0.2
Alkalinity (mg L^{-1} as $CaCO_3$)	126.7±6.7	126.7±6.7

Mortality, feeding and swimming behavior were evaluated twice a day. Fish were considered dead when they were motionless on the bottom, exhibited no opercular movement, and presented no response to mechanical stimuli. To evaluate toxicity on feeding and swimming behavior, the number of fish actively eating and the number of fish swimming erratically (circular swimming and loss of equilibrium) were visually observed and their number registered at the moment fish were fed.

Median lethal concentration (LC50) and their respective confidence intervals (95%), plus the concentrations at which 50% of the fish ceased feeding (FC50) and showed erratic swimming (SC50) after being exposed to either ammonia or nitrite during 24, 48, 72, and 96 h were calculated using the software Trimmed Spearman Karber Method (Hamilton et al., 1977). Comparisons among median toxic concentrations for ammonia and nitrite in each parameter observed were made by one-way ANOVA followed by the Test of Tukey with significance level of 95%, using the software Statistica 6.0.

Temperature, salinity, pH, dissolved oxygen, total ammonia–nitrogen, nitrite and nitrate of each experimental tank were measured daily at 9:00 and 21:00 h in each experiment. One sample per day of water was collected in the reservoir tank before being transferred to the experimental tanks for determination of alkalinity, calcium and chloride. These parameters were determined using Hach digital titration methods. Temperature and salinity were measured with a YSI Model 30 m (Yellow Springs Instruments, Yellow Springs, USA). Dissolved oxygen concentration was measured with a YSI Model 550A meter (Yellow Springs Instruments, Yellow Springs, USA) and the pH was measured with a YSI Model pH100 meter (Yellow Springs Instruments, Yellow Springs, China). TA-N, NO_2-N and NO_3-N were determined via colorimetric assays, methods 10031, 8153 and 8039, using a D/R 2010 spectrophotometer (Hach, USA). NH_3-N levels were determined according TA-N, temperature, salinity and pH values using the equations of Ostrensky et al. (1992) adapted from Whitfield (1974) and Bower and Bidwell (1978). Water quality parameters during the toxicity tests are presented in Table 2.

3. Results and discussion

Ammonia and nitrite concentrations measured every day did not differ from nominal concentrations (Table 1) due to the low stocking density used in these trials, approximately 0.18 g L^{-1} and 0.34 g L^{-1} for nitrite and ammonia respectively.

Within the range of concentrations of ammonia and nitrite tested, it was not possible to estimate their toxic effects to juvenile cobia during the first 24 h of exposure. Median lethal concentrations were estimated only after 96 h of exposure, but FC50 and SC50 were estimated after 48, 72, and 96 h. The effect of ammonia and nitrite on feeding was stabilized after 48 h, while the effect of nitrite on swimming behavior of juvenile cobia was significantly higher ($P<0.05$) after 96 h than at 72 h of exposure (Table 3).

The concentrations of ammonia and nitrite at which toxic effects for 50% of the population were observed were significantly different for feeding, swimming, and mortality ($P<0.05$) (Figs. 1 and 2). Feeding was the first parameter compromised among those observed in this study, both for ammonia and nitrite. Next, fish lost

Table 3
Median concentrations and respective confidence intervals for gaseous ammonia (ppm NH_3-N) and nitrite (ppm NO_2-N) regarding feeding and swimming behavior for 96 h after exposure to the toxicants

	Gaseous ammonia		Nitrite	
	FC50	SC50	FC50	SC50
24 h	–	–	–	–
48 h	0.81(0.73–0.89)^{a}	0.98(0.93–1.04)^{a}	142.6(137.8–147.5)^{a}	–
72 h	0.62(0.56–0.69)^{b}	0.88(0.81–0.94)^{ab}	80.8(76.3–85.6)^{b}	142.3(128.4–157.7)^{a}
96 h	0.62(0.57–0.70)^{b}	0.80(0.74–0.85)^{b}	76.1(73.2–79.0)^{b}	88.8(82.6–95.5)^{b}

Values of FC50 and SC50 followed by different letters in the same column are significantly different ($P<0.05$) after one-way ANOVA followed by the Test of Tukey.

equilibrium and their swimming behavior became erratic. At the end, higher concentrations of ammonia were necessary to kill 50% of the cobia population. LC50–96 h for nitrite could not be estimated for cobia, since only 30% of the exposed fish died at 210 ppm NO_2-N, the highest concentration tested (Fig. 2). However, this concentration is already 2.4 times higher than the amount of nitrite necessary to reach SC50–96 h. This is contrasting to the effects of ammonia, where the LC50–96 h is only 1.4 times higher than what is observed for SC50–96 h. When comparing the rate of lethal toxicity to feeding, the most sensitive parameter observed, the results are even higher. LC50–96 h for ammonia is 1.8 times higher than FC50–96 h, while for nitrite, the concentration resulting in 30% mortality is 2.8 times than FC50–96 h.

Toxicity of ammonia to cobia is on the range of ammonia toxicity for other marine fish species. However, regarding nitrite, this seems to be a very resistant species, as Atwood et al. (2004) have already observed.

Ammonia is acutely toxic for marine fish on the range of 0.54 ppm NH_3-N for *Centropristis striata* (Weirich and Riche, 2006b) to 1.77 ppm NH_3-N for *Menidia beryllina* (Miller et al., 1990). Comparatively to ammonia, nitrite is less toxic to fish. The acute toxicity of nitrite to marine fish varies between 30 ppm NO_2-N for *Paralichthys orbignyanus* (Bianchini et al., 1996) and 675 ppm NO_2-N for *Chanos chanos* (Almendras, 1987). Other species show intermediary toxicity: 85 ppm NO_2-N for *Sciaenops ocellatus* (Wise and Tomasso, 1989) and 199 ppm NO_2-N for *Odontesthes argentinensis* (Sampaio et al., 2006).

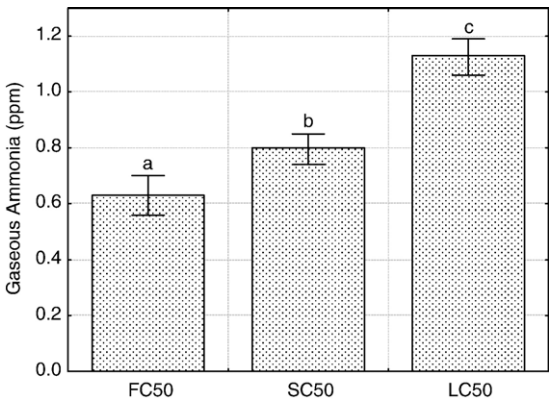

Fig. 1. Median concentrations and respective confidence intervals for gaseous ammonia (ppm NH_3-N) regarding feeding (FC50–96 h), swimming (SC50–96 h), and acute mortality (LC50–96 h) after exposure during 96 h. Different letters among columns indicate significant difference ($P<0.05$) after one-way ANOVA followed by the Test of Tukey.

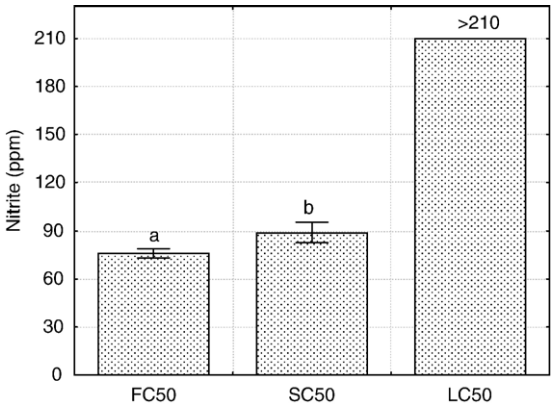

Fig. 2. Median concentrations and respective confidence intervals for nitrite (ppm NO_2-N) regarding feeding (FC50–96 h), swimming (SC50–96 h), and acute mortality (LC50–96 h) after exposure during 96 h. Different letters among columns indicate significant difference ($P<0.05$) after one-way ANOVA followed by the Test of Tukey.

Atwood et al. (2004) determined that juvenile cobia can survive at salinities lower than full strength sea water, but mortality is observed when salinity drops to 8‰. Later Resley et al. (2006) demonstrated the viability to rear juvenile cobia at a salinity as low as 5‰. The possibility to grow cobia in low-salinity water in a RAS is an important feature. However, special attention should be given to the interaction of low salinity and the toxicity of nitrogenous compounds, which are known to be more intense in fresh water, as it has already been observed for *Mugil platanus* (Sampaio et al., 2002) and *C. striata* (Weirich and Riche, 2006b).

The tolerance to high levels of nitrogenous compounds is a positive feature for intensive culture of cobia, especially in RAS. The build up of ammonia and nitrite concentrations can be harmful to several fish species, but according to the results of the present work, it is not likely that culture of cobia could be compromised by nitrite toxicity. Despite showing good tolerance to high ammonia concentration, the level at which ammonia hampers swimming and feeding can be easily achieved on intensive rearing facilities, and it must be monitored closely, as reduced feeding will lead to lower growth rates, increased food conversion rates and higher susceptibility to diseases.

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