ACUTE TOXICITY OF AMMONIA IN JUVENILE BRAZILIAN SARDINE
*Sardinella brasiliensis**

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ABSTRACT

The Brazilian sardine (*Sardinella brasiliensis*) is the main fishery resource in Brazil which holds directly the canning industry, as raw material, and indirectly the tuna fishing because juveniles are used as live bait. The UFSC through Live-Bait Project is developing production techniques of Brazilian sardine in captive aimed at the sustainable use of the resource, however there is no information about the toxicity of nitrogenous compounds in the culture of this species. This study was conducted to estimate the acute toxicity of ammonia (NH_3-N) on the juvenile Brazilian sardine. Groups of five fish (1.04 ± 0.20 g) were exposed to five concentrations of NH_3-N (0.60 to 1.47 mg L^{-1}) for 96 h plus control group in three replicates. Tests were run using a standard semi-static system with 100% daily renewal of water and toxicants. The results were based on mortality data registered for the different concentrations tested, using the software Trimmed Spearman Karber method. The median lethal concentration (LC_{50}) after 96 h of exposure to NH_3-N was estimated at 0.74 (0.61-0.90) mg L^{-1}. The results indicates that the juvenile Brazilian sardine is relatively sensitive to ammonia compared to other marine fish and particular attention must be given to this compound in culture systems.

Key words: ammonia; LC_{50}; toxicity; marine fish; Brazilian sardine

TOXICIDADE AGUDA DA AMÔNIA EM JUVENIS DE SARDINHA-VERDADEIRA
Sardinella brasiliensis

ABSTRACT

A sardinha-verdadeira (*Sardinella brasiliensis*) é o principal recurso pesqueiro do Brasil que sustenta diretamente a indústria de enlatados, como matéria prima, e indiretamente a pesca dos tunídeos, pois juvenis são utilizados como isca-viva. A UFSC através do Projeto Isca-Viva vem desenvolvendo técnicas de produção de sardinha-verdadeira em cativeiro com vistas ao uso sustentável do recurso, entretanto não existem informações sobre a toxicidade dos compostos nitrogenados no cultivo desta espécie. O objetivo deste estudo foi de estimar a toxicidade aguda de amônia (NH_3-N) em juvenis de sardinha-verdadeira. Grupo de cinco peixes (1,04 ± 0,20 g) foram expostos a cinco concentrações de NH_3-N (0,60 a 1,47 mg L^{-1}) durante 96 h mais um grupo controle em triplicata. Os testes foram conduzidos em um sistema semi-estático com renovação diária total dos meios experimentais. Os resultados foram apresentados como dados de mortalidade, registrados nas diferentes concentrações testadas, utilizando o software Trimmed Spearman Karber Method. A concentração letal (LC_{50}) após 96 h de exposição a NH_3-N foi estimada em 0,74 (0,61-0,90) mg L^{-1}. Os resultados indicam que juvenis de sardinha-verdadeira são relativamente sensíveis à amônia em relação a outros peixes marinhos e atenção especial deve ser dada a este composto em sistemas de cultivo.

Palavras-chave: amônia; LC_{50}; toxicidade; peixes marinhos; sardinha-verdadeira

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INTRODUCTION

Ammonia is the main nitrogenous excrement of teleost fish and is produced from the catabolism of dietary protein (EDDY, 2005; IP and CHEW, 2010). In the aqueous solution, ammonia exists in two forms: un-ionized ammonia (NH_3-N) and ionized ammonium (NH_4+-N), and their sum composes the total ammonia nitrogen (TAN). Ammonia is excreted through the gill epithelium either by passive diffusion as NH_3-N along a gas partial pressure gradient between fish blood and water, or through active transport as NH_4+-N in exchange for sodium (HARGREAVES and KUCUK, 2001, BARBIERI, 2010). The NH_3-N/NH_4+-N equilibrium is determined by pH and temperature (IP and CHEW, 2010).

Ammonia toxicity is expressed by and depends mainly on the presence of NH_3-N which is able to easily diffuse across gill membranes due to a lack of charge and its lipid solubility (TOMASSO 1994; BENLI and KÖKSAL 2005). Sub-lethal exposure to high levels of NH_3-N can induce changes in behavioral, physiological and biological responses in fish, thus suppressing immunocompetence followed by hyperventilation, hyperexcitability, coma, convulsions and finally death (HARGREAVES and KUCUK, 2001; EDDY, 2005).

Ammonia toxicity can be a crucial factor that leads to mass mortality under unfavorable aquacultural conditions, it is therefore important to determine the level supported by any species. Despite the existence of many studies on different fish species concerning acute ammonia toxicity (WEIRICH and RICHE, 2006a; WEIRICH and RICHE, 2006b; COSTA *et al.*, 2008; BARBIERI and DOI, 2012; RODRIGUES *et al.*, 2014), no studies were found on the effects of ammonia exposure on sardine species.

The Brazilian sardine *Sardinella brasiliensis* is a marine pelagic and schooling species that is the largest commercial fish resource along the southeastern Brazilian Bight (CERGOLE *et al.*, 2002). It is currently captured by various fleets for use in the canning industry and extensively as live bait for skipjack tuna fishery, and its availability varies seasonally due to over-fishing and environmental changes (SANTOS and RODRIGUES-RIBEIRO, 2000). Commercial catches of the Brazilian sardine began in the late 1950s, and had quick growth in the 1960s, reaching a peak of 228 000 tons in 1973 (CERGOLE *et al.*, 2002). However, in 2011, production was only 75 223 tons (MPA, 2014). Considering its economic importance, one of the efforts that could avoid collapse of the Brazilian sardine population is its production in captivity (CERGOLE and DIAS-NETO, 2011). The "Universidade Federal de Santa Catarina", through the Live-Bait Project is developing actions for the production of Brazilian sardine in captivity. Research has been conducted on reproduction, nutrition and feed management (BALOI *et al.*, 2016); however, there is no information about toxicity of nitrogenous compounds to Brazilian sardine. Therefore, this study was conducted to estimate the acute toxicity of ammonia (NH_3-N) on juvenile Brazilian sardine.

METHODS

Source of experimental fish

The study was carried out at the "Laboratorio de Piscicultura Marinha" (LAPMAR) of the "Universidade Federal de Santa Catarina" (UFSC, Brazil). Juvenile Brazilian sardine were produced at LAPMAR using a methodology described by BALOI *et al.* (2016). All animal handling procedures were approved by UFSC's Animal Ethics Committees (PP00861).

Acute toxicity assay

Groups of 5 fish (1.04 ± 0.20 g) were exposed to five concentrations of NH_3-N ranging from 0.60 to 1.47 $mg.L^{-1}$ for 96 h, plus a control group where no toxicant was added. The experiments were run in triplicate and test solutions were obtained with reagent grade ammonium chloride (Synth, Brazil). NH_3-N concentrations were calculated using the equations of OSTRENSKY *et al.* (1992) adapted from WHITFIELD (1974) and BOWER and BIDWELL (1978) using the values of TAN, pH, temperature and salinity. The toxicity trial was conducted in 20 L plastic tanks, using a semi-static system where water and test solutions were fully exchanged daily (at 8 am), and food was withheld for 24 hours before and during the trial.

The water quality parameters in each experimental tank were measured daily (8:30 am). Temperature and dissolved oxygen were measured using an

YSI Pro20 dissolved oxygen meter (Yellow Springs Instruments, Yellow Springs, USA), the pH was measured with a pH10A meter (Yellow Springs Instruments, Yellow Springs, USA) and salinity was measured using a portable refractometer (Instrutherm RTS-101ATC-03137, Sao Paulo, Brazil). TAN was determined 20 minutes after collection, following the method described by STRICKLAND and PARSONS (1972). Temperature was maintained at 19.2 ± 0.8°C, dissolved oxygen at 7.53 ± 0.04 mg L^{-1}, salinity at 33.2 ± 0.3 g L^{-1} and pH at 8.01 ± 0.06.

Fish behavior was observed during the trial. Mortality was evaluated twice a day (8 am and 5 pm) and was presumed when fish were motionless on the bottom, exhibited no opercular movement and failed to respond to tactile stimuli. Dead fish were immediately removed from the tanks and cumulative mortalities were recorded every 24 h.

Statistical analysis

Cumulative mortality data were used to calculate median lethal concentration (LC_{50} with 95% confidence limits) and their respective confidence intervals (95%) using the Trimmed Spearman–Karber method (HAMILTON *et al.*, 1977). Safe levels were obtained by multiplying 96-h LC_{50} values by a factor of 0.1 (SPRAGUE, 1971). Comparisons among median toxic lethal concentrations for NH_3-N were made using one-way ANOVA followed by a Tukey Test with a significance level of 95%.

RESULTS AND DISCUSSION

The percentage of mortality, median lethal concentrations and the respective confidence intervals of TAN and NH_3-N are presented in Table 1. No mortality, abnormal behavior or clinical signs were detected in the control group. The cumulative mortality increased with increasing ammonia concentrations. At the end of 96 h, all fish exposed to concentrations equal to or higher than 1.31 mgL^{-1} NH_3-N died.

A significant increase in ammonia toxicity was observed with exposure time. Lethal concentration (LC_{50}) values ranged from 1.06 mgL^{-1} of NH_3-N (35.00 mg L^{-1} TAN) at 24 h of exposure to 0.74 mg L^{-1} NH_3-N (25.62 mg L^{-1} TAN) at 96 h of exposure.

Table 1. Mortality (%) of *Sardinella brasiliensis* exposed to different concentrations of NH_3-N for 96h and median lethal concentrations (LC_{50} with 95% confidence limits).

	Concentrations (mg L^{-1})							
	Nominal TAN	0	21.00	28.00	35.00	42.00	49.00	
	Real TAN	0	19.95	27.01	34.77	40.88	48.89	
	NH_3-N	0	0.60	0.81	1.07	1.31	1.47	LC_{50} of TAN (mg L^{-1}) / LC_{50} of NH_3-N (mg L^{-1})
Exposure time (h)	24	0	13.3	26.7	33.3	80.0	100	35.00 (31.17-39.31)[a] / 1.06 (0.93-1.21)[a]
	48	0	13.3	46.7	53.3	93.3	-	35.00 (26.86-34.14)[b] / 0.90 (0.79-1.03)[b]
	72	0	26.7	66.7	66.7	100	-	25.62 (21.65-30.32)[c] / 0.74 (0.61-0.90)[c]
	96	0	26.7	66.7	66.7	-	-	25.62 (21.65-30.32)[c] / 0.74 (0.61-0.90)[c]

Different letters at each column indicate significant difference ($P < 0.05$)

The toxicity of ammonia on fish have been studied in numerous fish species, however it is difficult to compare tolerance among species because it is dependent on the environmental conditions the trials were run (temperature, pH) and also on biological characteristics (weight, age, nutritional condition). Ammonia is acutely toxic for marine fish in the range of 0.54 mg L^{-1} for black sea bass *Centropristis striata* (WEIRICH and RICHE, 2006b) and at 2.93 mg L^{-1} of NH_3-N for threespine stickleback *Gasterosteus aculeatus* (HAZEL *et al.*, 1971), the most tolerant known species.

Results of our study indicate that, in comparison to other marine fish, Brazilian sardine is relatively sensitive to NH_3-N (LC_{50}-96h of 0.74 mg L^{-1} NH_3-N). Similar results were observed in pompano *Trachinotus marginatus* (LC_{50}-96h 1.06 mg L^{-1} NH_3-N) (COSTA *et al.*, 2008), cobia *Rachycentron canadum* (LC_{50}-96h 0.68 mg L^{-1} NH_3-N) (BARBIERI and DOI, 2012), maroon clownfish *Premnas biaculeatus* (LC_{50}-96h 0.89 mgL^{-1} NH_3-N) (RODRIGUES *et al.*, 2014) and false clownfish *Amphiprion ocellaris* (LC_{50}-96h 0.75 mg L^{-1} NH_3-N) (MEDEIROS *et al.*, 2015).

At concentrations lower than lethal concentrations, ammonia can have so-called "sub-lethal" effects, such as reduced growth, poor feed conversion and reduced disease resistance. SPRAGUE (1971) suggested that keeping the concentration of toxicant under 10% of the estimated acute toxicity should keep fish from sub-lethal symptoms. The safe level calculated for Brazilian sardine was estimated to be 2.53 and 0.07 mg L^{-1} for TAN and NH_3-N respectively.

In our study, we observed that swimming activity reduced with increased ammonia concentrations and exposure time. Lethargic and decreased swimming activities, increased ventilation rate, loss of equilibrium, increased mucous secretion on the skin and gills and continuous swimming at the bottom of the tank are the first signs noticed as abnormal behavior caused by the exposure to nitrogenous compounds (HAMLIN, 2006; RODRIGUES *et al.*, 2007; DAMATO and BARBIERI, 2011).

Ammonia is an environmental toxicant that is especially problematic for aquatic organism and it should be kept below the toxic levels for fish. The maintenance of water quality and knowledge of a species' tolerance limits for water quality are indispensable requirements for any cultivation system. Our results indicate that juvenile Brazilian sardine is relatively sensitive to ammonia compared to other marine fish and the safe levels presented here have important implications for the management of this specie in captivity.

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