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Acute toxicity of nitrite on juveniles of common snook *Centropomus undecimalis* (Perciformes, Centropomidae)

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Abstract. The tolerance of common snook *Centropomus undecimalis* juveniles to acute nitrite exposure was evaluated at 30 g L^{-1} salinity. Lethal effects on fish were observed. An increase in nitrite toxicity was observed during exposure time and LC_{50} NO_2 96 h was 166.89 mg L^{-1}.

Key words: Marine fish; mariculture; $LC_{50;}$ nitrogen compound.

Resumo: Toxicidade aguda de nitrito em juvenis de robalo flecha *Centropomus undecimalis* (Perciformes, Centropomidae). Avaliou-se a tolerância de juvenis de robalo flecha *Centropomus undecimalis* a exposição aguda de nitrito em salinidade 30 g L^{-1}. Houve efeito letal nos peixes com o aumento da toxicidade do nitrito ao longo do tempo de exposição e a CL_{50} 96 h foi estimada em 166,89 mg L^{-1} NO_2.

Palavras-chave: peixe marinho; maricultura; $CL_{50;}$ composto nitrogenado

The concentration of nitrite in natural water is typically low (Kroupová *et al.* 2005) but it can build up in intensive fish culture systems such as recirculation aquaculture systems (RAS) (Doleželová *et al.* 2011). Elevated nitrite concentrations cause great problems in intensive culture of commercial fish species (Jensen 2003, Svobodová *et al.* 2005, Kroupová *et al*. 2005, Park *et al.* 2007, Jia *et al.* 2015). Among Centropomidae fish, the common snook *Centropomus undecimalis* is the most promising species in terms of aquaculture (Souza-Filho & Cerqueira 2003, Alvarez-Lajonchère & Tsuzuki 2008) and it can be cultured intensively in earthen-ponds and net cages, or extensively (Silva & Pereira 1997). Regarding the importance to maintain safety levels of nitrite in rearing water and to avoid economic losses in its culture, this study aimed to evaluate the acute toxicity of nitrite in common snook juveniles.

Fish were produced using a methodology described by Passini *et al*. (2016) and procedures approved by UFSC's Animal Ethics Committees (PP00861/CEUA/PROPESQ/2013). Before trial, larvae were fed with commercial diet (55% crude protein) four times a day and kept in captivity at 25°C in a 5,000 L fiber tank with seawater, with daily renewal (100%) and constant aeration. After 24 h of starvation, the fish were transferred to experimental units and kept in starvation during trial. Groups of 10 juveniles (1.26±0.39 g and 5.17±0.62 cm) were exposed to five nitrite concentrations ranged from 132 to 426 mg L^{-1} with addition of sodium nitrite (Dinâmica, São Paulo, Brazil) plus one control group (0 mg L^{-1}), in three replicates. Values of nitrite concentrations for treatments were determined based on previous tests regarding fish mortalities. The five nitrite concentrations tested were achieved by stoichiometric calculation of nitrite from sodium nitrite and were measured in water according to the diazotization method for nitrite (Strickland & Parsons, 1972), every day during the previous tests and at the first day of experiment. The same group of individuals were monitored during 24, 48, 72 and 96 h. Toxicity trial was carried out in semi-static system where water and test solutions were fully

Table I. Mortality (%) of common snook *Centropomus undecimalis* juveniles exposed to different concentrations of nitrite (NO_2^-) and median lethal concentration (LC_{50}) after 24, 48, 72 and 96 h of exposure to NO_2 (n=10).

Exposure time (h)	NO_2^- (mg L^{-1})						LC_{50} of NO_2^- (mg L^{-1})
	0	132	211	280	337	426	
24	0.00	0.00	0.00	0.00	6.67	20.00	*
48	0.00	0.00	26.67	40.00	66.67	86.67	285.00 (256.67-316.46)[a]
72	0.00	6.67	46.67	86.67	66.67	100.00	210.49 (191.80-231.00)[b]
96	0.00	13.33	86.67	100.00	100.00	-	166.89 (157.78-176.53)[c]

Mean values (confidence interval). Different letters indicate significant difference among exposure times by Tukey´s test ($P < 0.05$). *Value not calculable by the program.

exchanged. Toxicity trial was carried out in semi-static system where water and test solutions were fully exchanged daily, and natural photoperiod of 12 h light, using 2 L glass vessels filled with 1.5 L of filtered sea water. Twice a day fish were observed regarding mortalities and behavior. Water was constantly aerated through air stones at temperature 14.9±1.01 °C, salinity 30.0±0.2 g L^{-1}, pH 8.0±0.17, dissolved oxygen 8.5±0.35 mg L^{-1}, alkalinity 141.80±7.68 mg L^{-1} $CaCO_3$ and total ammonia 0.13±0.06 mg L^{-1}, measured daily.

Data were first evaluated for homogeneity of variances using Bartlett test and for normal distribution using Kolmogorov–Smirnov test. 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). Comparisons among median lethal concentrations of NO_2 (285.00, 210.49 and 166.89 mg L^{-1} for 48, 72 and 96 h, respectively) were performed using one-way ANOVA followed by Tukey´s test with a significance level of 95%.

The percentage of mortality, median lethal concentrations and the respective confidence interval of NO_2 are presented in Table I. No mortality, abnormal behavior or clinical signs were detected in the control group. The cumulative mortality increased with increasing nitrite concentrations and exposure time. Moreover, at the end of 96 h, all fish exposed to 426 mg L^{-1} NO_2 died. Dying fish presented clear symptoms of hypoxia, such as gasping behavior and hyperventilation.

Although it is difficult to compare nitrite toxicity, juveniles of common snook appear to be less tolerant than other fish. Regarding marine species, LC_{50} 96 h NO_2 usually varies from 30 mg L^{-1} for *Paralichthys orbignyanus* (Bianchini *et al.* 1996) to 675 mg L^{-1} for *Chanos chanos* (Almendras 1987), while other species show intermediary toxicity as 85 mg L^{-1} for *Sciaenops ocellatus* (Wise & Tomasso 1989) and 199 mg L^{-1} for *Odontesthes argentinensis* larvae (Sampaio *et al.* 2006). Hence, *C. undecimalis* was classified in this group, showing LC_{50} 96 h 166 mg L^{-1} NO_2 in the present study. At 210 mg L^{-1}, only 30 % of cobia (*Rachycentron canadum*) juveniles died after 96 h (Rodrigues *et al.* 2007).

Nitrite toxicity to fish varies considerably and depends on physicochemical parameters and biological factors, as salinity, water pH, temperature and oxygen concentrations; length of exposure; fish species, size and age; as well as individual fish susceptibility, respectively (Kroupová *et al.* 2005, Wang *et al.* 2015). Sublethal and lethal effects can be caused by the oxidation of hemoglobin to methemoglobin, which reduces the total oxygen-carrying capacity of blood (Jia *et al*. 2015). Accordingly, mortalities observed in common snook could be caused by the compromised oxygen transport, since dying fish presented clear symptoms of hypoxia. Hypoxia symptoms due to nitrite included gasping behavior and hyperventilation, followed by death, with brownish color of gills and blood, besides opened mouth, as reviewed by Kroupová *et al.* (2005) in nitrite toxicity on fish.

In conclusion, acute nitrite concentrations from 132 mg L^{-1} caused mortalities in common snook juveniles, demonstrating lethal effects. The increase in nitrite toxicity to fish was observed during exposure time and LC_{50} NO_2 96 h was 166.89 mg L^{-1}.

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