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Toxicity of Nitrogenous Compounds to Juveniles of Flatfish *Paralichthys orbignyanus*

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The flatfishes are very appreciated by consumers and greatly commercialized in the world. They generally inhabit shallow waters and are sedentary. Thus, they present good characteristics for cultivation. However, several factors are extremely limiting in aquaculture, among them the species tolerance to water physico-chemical parameters. So, before introducing a new species in aquaculture, its tolerance to those parameters must be studied. In this context, nitrogenous compounds, mainly ammonia and nitrite, are actually considered as an important limiting factor in fish cultivation.

In cultivation tanks, ammonia can largely accumulate due to excretion, mineralization of organic compounds by heterotrophic bacteria and mainly by use of eutrophic waters. This compound is considered one of the most common reasons of mortality in fish hatcheries. So, the determination of the lethal and chronic concentrations for fishes of commercial interest is the aim of several studies (Russo and Thurston 1978; Anderson 1979).

Nitrite, the other important nitrogenous compound cited, is very toxic to aquatic animals (Russo and Thurston 1978). It penetrates fish via gills and reaches the blood. At this level, nitrite oxidizes the iron of the hemoglobin transforming it in methemoglobin. Thus, it largely reduces the oxygen transport capacity of blood, and fishes die by hypoxia (Weirich and Tomasso 1993).

Hence, with the future perspective of introducing the flatfish paralichthys *orbiqnyanus* in aquaculture, several studies have been performed to analyze its tolerance to water physico-chemical parameters. In this study, we have analyzed the tolerance of juveniles to ammonia and nitrite at different temperature and salinity conditions.

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MATERIAL AND METHODS

Experiments were performed at the Marine Aquaculture Station "Msc. Marcos Alberto Marchiori" of the Rio Grande University Foundation (Brazil). Juveniles of the flatfish *P. orbignyanus* were captured at the Cassino beach or at the estuary of the Patos Lagoon (32°10'S/52°5'W) in Southern Brazil. They were transferred to the Marine Station and acclimated in 1,000-L tanks to 30‰ and 20°C for 15 d. Water was continuosly aerated and the photoperiod was fixed at 12L:12D. Flatfishes were fed *ad libitum* with fry of mullet *Mugil platanus* or silverside *Odonthestes sp.* and small crustaceans like the shrimps *Penaeus paulensis* and *Artemesia longinaris.* The acclimation medium was 50% renewed daily.

According to the frequent seasonal extremes of temperature and salinity of the estuarine and coastal water where flatfishes were captured (Baumgarten and Niencheski 1990), two experimental conditions were established: the "winter condition" (0‰ and 12°C) and the "summer condition" (30‰ and 25°C).

First, 96-hr range finding tests were performed employing from 2 to 4 flatfishes. They were done in 20-L plastic trays under controlled temperature and salinity. Ammonia and nitrite concentrations employed ranged between 0 and 300 $mg.L^{-1}$ (winter condition) or 0 and 100 $mg.l^{-1}$ (summer condition). Test solutions were prepared from stock solutions, which were made with ammonium chloride p.a. (Merck) or sodium nitrite p.a. (Merck) dissolved in distilled water. During tests, flatfishes were not fed and the water was continuously aerated. The experimental media were 100% renewed daily. The photoperiod was maintained at 12L:12D. Every 24 hr, salinity, temperature, pH and flatfish mortality were monitored. The criterion of death adopted was absence of locomotory responses, even after mild mechanical stimulation.

Based on the results from such 96-hr range finding tests, experiments were performed in 30-L plastic tanks employing 10 juveniles of flatfish for each experimental condition, to determine ammonia and nitrite toxicities. The general procedure employed in these experiments was the same described for the 96-hr range finding tests.

In the ammonia toxicity experiments, the concentrations tested were: (1) "winter condition" = 0; 60; 80; 90; 100; 110; 120; 160; 200 and 300 $mg.L^{-1}$; (2) "summer condition"= 0; 20; 30; 40; 50; 55; 65; 70 and 80 $mg.L^{-1}$ of N as total ammonia. The ammonia concentration was monitored following the method described by Solorzano (1969). The mean proportions of un-ionized ammonia in relation to total ammonia were calculated employing the mean data of temperature, salinity and pH registered in each experimental condition. The mean weight (± SD) of all fishes employed in these experiments was 120 ± 21 g (N=190).

In the nitrite toxicity experiments, the concentrations tested were: (1) "winter condition" = 0; 15; 20; 25; 30; 40; 60; 90 and 120 $mg.L^{-1}$; (2) "summer condition" = 0; 10; 20; 25; 30; 40; 50 and 60 $mg.L^{-1}$ of N as nitrite. The nitrite concentration was monitored following the method described by Bendschneider and Robinson (1952). The mean weight (± SD) of all flatfishes employed was 88 ± 17 g (N=170).

The LC_{50} of ammonia and nitrite for 24, 48, 72 and 96 hr of test were estimated from the survival data by means of the Trimmed Spearman Karber Method developed by Hamilton et al. (1977). Comparisons between LC_{50} were performed based on the 95% confidence limits employing the statistical package developed by Rodriguez (1991). The safe concentrations of ammonia and nitrite were calculated according to Sprague (1971).

RESULTS AND DISCUSSION

The ammonia toxicity was tested in "winter" and "summer" conditions. In the former, no mortality was observed in concentrations equal or lower than 80 $mg.L^{-1}$ even after 96 hr of test, while total mortality was registered in concentrations equal or higher than 200 $mg.L^{-1}$. In the latter condition, no mortality was observed in concentrations equal or lower than 40 $mg.L^{-1}$ even after 96 hr of test, while total mortality was registered only at 80 $mg.L^{-1}$.

Based on mortality data, the LC_{50} of total ammonia was estimated for 24, 48, 72, and 96 hr of test. The values estimated for "winter condition" are significantly higher than those for "summer condition". The LC_{50} for gaseous ammonia was also estimated (Table 1). It is interesting to note that the LC_{50} values obtained in the "summer condition" are higher than those obtained in the "winter condition". The value estimated for 96 hr of test in the "summer condition" (0.67 $mg.L^{-1}$) is very similar to those estimated for other marine fishes. For example, the LC_{50} (9 (hr) for juveniles of silverside *Odontesthes argentinensis* was estimated as 0.80 $mg.L^{-1}$ (Ostrensky and Brugger 1992); for the sunshine bass *Morone sp.* as 0.7 $mg.L^{-1}$ (Weirich and Tomasso 1993) and for fry of mullet *Mugil platanus* as 0.84 $mg.L^{-1}$ of N as NH_3 (Miranda 1993). Data of LC_{50} (96 hr) for others flatfishes are not available. The safe concentration of gaseous ammonia for *P. orbignyanus* in saline water (0.067 $mg.L^{-1}$ - Table 2) is very similar to that proposed by Haywood (1983) for

Table 1. LC_{50} (96 hr) of total and gaseous ammonia for juveniles of the flatfish *Paralichthys orbignyanus* in "winter" and "summer" conditions. Data are expressed in $mg.L^{-1}$. Data in brackets represent the 95% confidence limits of the LC_{50}.

Time (hr)	Total ammonia Winter	Total ammonia Summer	Gaseous ammonia Winter	Gaseous ammonia Summer
24	237.37 * (223-251)	67.45 (62-73)	0.46 * (0.43-0.50)	0.98 (0.84-0.99)
48	142.34 * (126-160)	66.14 (59-74)	0.27 * (0.24-0.30)	0.90 (0.80-1.01)
72	109.92 * (99-121)	55.96 (50-63)	0.21 * (0.20-0.23)	0.76 (0.68-0.86)
96	97.14 * (89-105)	49.63 (46-54)	0.19 * (0.17-0.20)	0.67 (0.62-0.73)

* Represents different LC_{50} ($P<0.05$) when data from "winter" and "summer" conditions were compared.

Table 2. Safe concentrations of total and gaseous ammonia and nitrite for juveniles of the flatfish *Paralichthys orbignyanus* in the "winter" and "summer" conditions. Data are expressed in $mg.L^{-1}$. Data in brackets represent the 95% confidence limits of the safe level.

Nitrogenous compound	Experimental condition Winter	Experimental condition Summer
total ammonia	9.71 (8.98-10.51)	4.96 (4.58 - 5.37)
gaseous ammonia	0.019 (0.017-0.020)	0.067 (0.062-0.073)
nitrite	2.40 (2.13 - 2.71)	3.06 (2.73 - 3.42)

Table 3. LC_{50} (96 hr) of nitrite for juveniles of the flatfish *Paralichthys orbignyanus* in "winter" and "summer" conditions. Data are expressed in $mg.L^{-1}$. Data in brackets represent the 95% confidence limits of the LC_{50}.

Time (hr)	Experimental condition Winter	Summer
24	107.86 * (89.3 - 130.4)	55.78 * (51.8 - 60.0)
48	52.98 * (44.5 - 63.1)	38.52 * (34.9 - 42.5)
72	33.69 * (27.8 - 40.8)	31.30 * (28.0 - 34.9)
96	24.01 * (21.3 - 27.1)	30.57 * (27.3 - 34.2)

* Represents different LC_{50} ($P<0.05$) when data from "winter" and "summer" conditions were compared.

marine fishes (0.05 $mg.L^{-1}$). Further, it is very close to those presented by Anderson (1979) as those having no effect on the growth of the flatfishes *Scophthalmus maximus* and *Solea solea* (0.066 and 0.11 $mg.L^{-1}$, respectively).

However, the gaseous ammonia toxicity was significantly higher in freshwater (LC_{50}=0.19 $mg.L^{-1}$) than in saline water (LC_{50}=0.67 $mg.L^{-1}$), despite the lower temperature in the first condition. Higher ammonia toxicity in freshwater was also observed for other fish species. For example, the LC_{50}(96 hr) for the catfish *Ictalurus punctatus* was estimated as 0.263 $mg.L^{-1}$ (Bader and Grizzle 1992); for fry of cutthroat trout *Salmo clarki* as 0.5 $mg.L^{-1}$ (Thurston et al. 1978) and for fry of mullet *Mugil platanus* as 0.58 $mg.L^{-1}$ of N as NH_3 (Miranda 1993). Compared with these species, *P. orbignyanus* is more susceptible to ammonia. Despite this, considering that gaseous ammonia concentrations as high as the LC_{50} (96 hr) estimated in this study will seldom be registered in cultivation tanks or in environment, the flatfish *P. orbignyanus* can be considered as being very tolerant to ammonia both in freshwater and saline water.

Considering the nitrite toxicity tests, in the "winter condition", no mortality was registered at 15 $mg.L^{-1}$ after 96 hr of test, while total mortality was observed at concentrations equal or higher than 60 $mg.L^{-1}$. In the "summer condition", no mortality was registered at 10 $mg.L^{-1}$ after 96 hr of test, while total mortality was observed at concentrations equal or higher than 50$mg.L^{-1}$. From mortality data, nitrite LC_{50} values were estimated for 24, 48, 72 and 96 hr of test. In the first hours of experiment, the tolerance of *P. orbignyanus* to nitrite was higher in the "winter condition" than in the "summer condition". But, after 96 hr of test, it was higher in the later (Table 3). The safe concentrations of nitrite to juveniles of *P. orbignyanus* for the "winter" and "summer" conditions are presented in Table 2. Compared with other fish species, *P. orbignyanus* seems to be very tolerant, both in the "winter" (LC_{50}-96 hr = 24.0 $mg.L^{-1}$) and "summer" conditions (LC_{50}-96 hr = 30.5 $mg.L^{-1}$). Weirich and Tomasso (1993) estimated the LC_{50}(96 hr) for the sunshine bass *Morone sp.* as 12.8 $mg.L^{-1}$ while Miranda (1993) estimated it as 1.51 $mg.L^{-1}$ for fry of the mullet *Mugil platanus.* Further, the salmonides *Salmo clarki, Oncorhynchus mykiss* and *Oncorhynchus tshawytscha* are more susceptible to nitrite than *P. orbignyanus* because LC_{50}(96 hr) for those species does not exceed 0.9 $mg.L^{-1}$ of N as nitrite (Thurston et al. 1978). Data available for marine fish are very scanty. The LC_{50}(96 hr) for fry of the mullet *Mugil platanus* in sea water is 35.89 $mg.L^{-1}$ of N as nitrite (Miranda 1993).

It is interesting to note that the results obtained after 96 hr of test are in accordance with the reviews of Lewis and Mooris (1986) and Wise and Tomasso (1989), who established an inverse relationship between the toxic effect of nitrite and salinity, for several freshwater and marine fishes. The cause of the higher toxicity of nitrite in freshwater is not well understood. Thurston et al. (1978) showed that this toxicity is reduced with increased chloride concentrations.

We can conclude that juveniles of the flatfish *P. orbignyanus* are very tolerant to ammonia and nitrite, both in fresh and saline waters. Despite this, in cultivation tanks their concentrations must be monitored, because they can eventually reach values as high as those proposed as safe levels. Further, only the mortality was observed in this study. Growth inhibition, for example, can occur at lower concentrations than those established as lethal for the species. We also must take into account the possible synergism between the different nitrogenous compounds and/or other aquatic pollutants.

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