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Effect of Temperature on Acute Toxicity of Nitrite to Meagre, *Argyrosomus regius* (Asso, 1801)

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Abstract

Meagre, *Argyrosomus regius*, is a candidate marine fish species for aquaculture diversification, presenting a high economic value in the Mediterranean. Tolerance of juvenile meagre to nitrite (NO_2-N) was determined relating to temperature. Fish (3.2 ± 0.6 g and 5.4 ± 0.9 cm) were exposed to different NO_2-N concentrations in a series of acute toxicity tests by the static renewal method at three temperatures (18, 22, and 26 C) at a pH of 8.0. Low temperature clearly increased tolerance to NO_2-N ($P < 0.05$). The 96-h median lethal concentration (LC_{50}) values of NO_2-N were 177.63, 139.55, and 49.61 mg/L, at 18, 22, and 26 C, respectively. The safe levels of NO_2-N for juvenile meagre were estimated to be 17.7, 13.9, and 4.9 mg/L at 18, 22, and 26 C, respectively ($P < 0.05$). This study indicates *A. regius* is more sensitive to nitrite than other marine fish species cultured in the Mediterranean.

Meagre, *Argyrosomus regius*, is considered a candidate species for marine finfish aquaculture in the Mediterranean region, as it presents a high economic value, high growth rate, and excellent flesh quality (Poli et al. 2003; Cardeira et al. 2012; Valles and Estevez 2013). Meagre aquaculture has been carried out since the mid 1990s. Meagre production has increased in recent years with significant production beginning in 2006, a result of captive reproduction (Martinez-Llorens et al. 2011). However, optimal environmental conditions for meagre production are relatively unknown. One important environmental concern is the presence of nitrite in culture systems. Nitrite, a natural component of the nitrogen cycle in ecosystems, is toxic to fish at elevated concentrations (Lewis and Morris 1986; Jensen 2003; Svobodova et al. 2005)

Nitrite can be actively taken up across the gill epithelium and can accumulate in body fluids (Jensen 1995). One critical consequence of nitrite accumulation is the oxidation of hemoglobin to methaemoglobin, which does not carry oxygen; nitrite may thus cause anoxia in fish (Lewis and Morris 1986). Nitrite toxicity to fish varies considerably by species and depends on a number of factors. The most important factors are temperature, pH, salinity, and oxygen concentration (Brownell 1980; Lewis and Morris 1986; Sampaio et al. 2002; Jensen 2003)

Although nitrite toxicity has been well documented for several species of freshwater finfish, little information is available on toxicity of nitrite to marine fish species. Previously studied marine fish species include European seabass, *Dicentrarchus labrax* (Saroglia et al. 1981), gilthead seabream, *Sparus aurata* (Parra and Yufera 1999), European eel, *Anguilla anguilla* (Saroglia et al. 1981; Kamstra 1996), hybrid striped bass, *Morone chrysops* × *Morone saxatilis* (Weirich et al. 1993), red drum, *Sciaenops ocellatus* (Wise and Tomasso 1989), and mullet, *Mugil platanus* (Sampaio et al. 2002). There is no information about nitrite toxicity in meagre, a potential new species for marine fish diversification of commercial aquaculture. The purpose of the study was to determine tolerance of juvenile meagre to nitrite at different temperatures.

Material and Methods

Juvenile meagre (3.2 ± 0.6 g and 5.4 ± 0.9 cm) were obtained from Kilic Holding near Bafa Lake in Turkey. Toxicity experiments were

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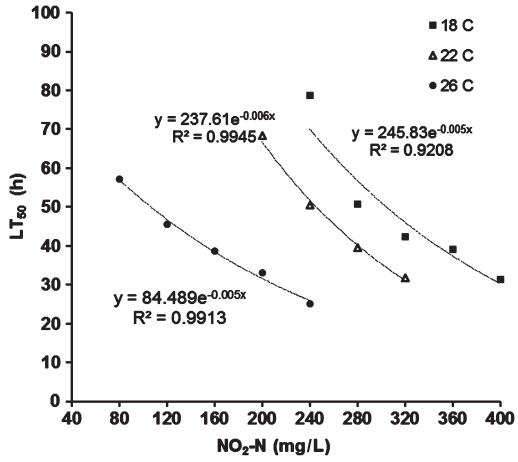

FIGURE 1. *LT_{50} (h) values for juvenile meagre,* Argyrosomus regius*, exposed to different concentrations of NO_2-N at 18, 22, and 26 C.*

carried out at Mugla Sitki Kocman University, Faculty of Fisheries. Ambient water temperature and salinity were 28 C and 30 ppt when fish were transferred. Before the study, fish were acclimated to experimental conditions. Ambient water temperature was gradually decreased from 28 to 22 C. A total of 300 fish, which had been held at a water temperature of 22 C, were distributed randomly into three groups. Each group was than acclimated to 18, 22, and 26 C in different thermostatic aquariums at a rate of 1 C/d for 1 wk.

Nitrite stock solution (30 g/L) was prepared with sodium nitrite ($NaNO_2$) (Merck, Damrstadt, Germany) as reported by Chen and Lei (1990) and diluted to the desired concentrations of NO_2-N with 18, 22, and 26 C seawater. Nominal experimental concentrations of NO_2-N in the test solutions were 80, 120, 160, 200, 240, 280, 320, 360, and 400 mg/L. The actual concentrations of NO_2-N in test solution were measured using the method described by Bendschneider and Robinson (1952). The pH at each assay solution was regularly measured by a pH 3210 pH meter (WTW, Weilheim, Germany) throughout the study. Salinity levels were checked using a salinity meter (YSI model Y30, Yellow Springs Instruments, Yellow Springs, OH, USA).

Short-term median lethal concentration (LC_{50}) toxicity tests were carried out according to the methods described by APHA (1989). Fish were sampled randomly from holding tanks and transferred to the test and control solutions. Bioassay experiments to establish tolerance limits were conducted in two replicates in polyethylene containers containing 10 L test solutions. Each container contained 10 fish, and air stones supplied continuous aeration. Each test solution was renewed daily, in accordance with the static renewal method for toxicity tests (Buikema et al. 1982). Feeding of fish was discontinued 12 h before experimental trials. During the experiment (96 h), the fish were not fed. Dissolved oxygen was maintained above 6 mg/L.

Observations were made at 12 h intervals up to 96 h. Death was assumed when fish were immobile, exhibited no opercular movement, and did not respond to mechanical stimulation. Dead fish were removed daily from containers. The LT_{50} (median lethal time to kill half of the population) and LC_{50} (median lethal concentration to kill half of the population) of NO_2-N and their 95% confidence limits were calculated by the Bliss Probit method (Sprague 1969). The estimated probit line and results of a chi-square test for goodness of fit were computed. Two-way analysis of variance of the general linear model procedure was used to compare differences between survival, temperature, or NO_2-N concentration and exposure time. Regression analyses were used to determine relationship between NO_2-N concentration and LT_{50} values or LC_{50} values and exposure time.

Results and Discussion

During the toxicity experiment, no fish died in the control solutions at any temperature. While 10% mortality was observed for fish exposed for 96 h to 80 mg/L NO_2-N at 26 C, no mortality was observed in the same test solution at a water temperature of 18 and 22 C during the toxicity experiment. The LT_{50} values calculated at different concentrations of NO_2-N in the study are shown in Figure 1. Statistical analysis indicated the LT_{50} had a negative exponential relationship

TABLE 1. *Relationship between probit mortality (Y) and log NO_2-N as mg/L (x) at various exposure times and temperature for juvenile meagre,* Argyrosomus regius.

Temperature	$Y = ax + b$	R^2	χ^2	n^a	χ^2 df	0.95
18 C						
24 h	$Y = 4.308x - 6.478$	0.9737	0.073	4	2	5.991
48 h	$Y = 4.481x - 6.542$	0.9229	0.289	3	1	3.841
72 h	$Y = 10.163x - 19.24$	0.9787	0.090	3	1	3.841
96 h	$Y = 6.428x - 9.457$	0.9998	0.002	3	1	3.841
22 C						
24 h	$Y = 6.143x - 10.895$	0.9979	0.002	3	1	3.841
48 h	$Y = 9.620x - 18.494$	0.9340	0.835	3	1	3.841
72 h	$Y = 8.244x - 14.359$	0.9983	0.004	3	1	3.841
96 h	$Y = 4.0374x - 3.672$	0.9715	0.065	3	1	3.841
26 C						
24 h	$Y = 3.766x - 4.179$	0.8313	0.802	3	1	3.841
48 h	$Y = 3.302x - 2.398$	0.9094	0.851	4	2	5.991
72 h	$Y = 3.359x - 1.841$	0.9803	0.109	3	1	3.841
96 h	$Y = 2.458x + 0.811$	0.9642	0.046	3	1	3.841

[a]Number of concentrations used for calculation of *NO_2-N*.

TABLE 2. *Mean ± SE LC_{50} (mg/L) of NO_2-N for juvenile meagre,* Argyrosomus regius *at four different temperatures and various exposure times.*[a]

	Time (h)			
Temperature (C)	24	48	72	96
18	463.50 ± 1.27	376.22 ± 1.14	242.26 ± 1.08	177.63 ± 1.15
22	387.57 ± 1.33	278.59 ± 1.06	223.20 ± 1.15	139.55 ± 1.34
26	268.19 ± 1.26	174.08 ± 1.18	108.58 ± 1.18	49.61 ± 2.12

[a]Mean values are significantly different from each other.

with concentrations of NO_2-N. The LT_{50} varied from 25.2 to 57.2 h and from 31.7 to 68.3 h depending on concentration of NO_2-N (240–80 and 320–200 mg/L) at 26 and 22 C, respectively, whereas LT_{50} values ranged between 31.4 and 78.7 h depending on concentration of NO_2-N (400–240 mg/L) at a water temperature of 18 C in the study.

The probit mortalities as a function of log NO_2-N concentration are shown in Table 1. Chi-square analysis showed the probit mortality had a positive linear relationship with log NO_2-N concentration, and all estimated lines were satisfactory at all temperature levels. The LC_{50} values of NO_2-N at different exposure times for juvenile meagre and their 95% confidence limits are summarized in Table 2. The data obtained in this study clearly indicated tolerance of fish to NO_2-N increased with decreasing temperature ($P < 0.05$). The mortality rate increased with increased NO_2-N concentrations and exposure times ($P < 0.05$). Similar results were also reported for other marine fish species, including European seabass (Saroglia et al. 1981) and gilthead seabream (Parra and Yufera 1999).

The relationship between LC_{50} data, temperature and exposure time are illustrated in Figure 2. Statistical analysis indicated that the LC_{50} of NO_2-N had a negative exponential relationship with exposure time. Low levels of NO_2-N had no effects but higher concentrations were toxic to the fish at even with as little as 24-h exposure time. After 24, 48, 72, and 96 h of exposure, the LC_{50} values for NO_2-N were 1.7, 2.1, 2.2, and 3.5 times higher at 18 C than at 26 C, respectively.

In this study, at which the acute toxicity experiments were carried out at a salinity of 30 ppt and a pH of 8.0, the 96-h LC_{50} values of NO_2-N for juvenile meagre (5.4 cm total length) were calculated as 177.6, 139.5, and 49.61 mg/L at

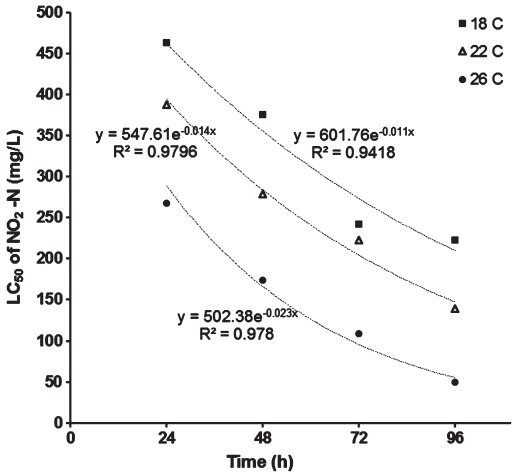

FIGURE 2. *Relationship between LC_{50} of NO_2-N exposure time at 18, 22, and 26 C.*

a temperature of 18, 22, and 26 C, respectively. Saroglia et al. (1981) reported 96-h LC_{50} values of NO_2-N for European seabass (5 cm total length) were 274, 220, and 154 mg/L at 17, 23, and 27 C, respectively. In the same work performed at salinity of 30 ppt and pH of 8.0–8.2, 96-h LC50 value was reported as 812 mg/L for European eel (7 cm in length) (Saroglia et al. 1981). In another study dealing with mullet, the 96-h LC50 value of NO_2-N was reported as 35.89 mg/L at a salinity of 30 ppt and a pH of 7.76 (Sampaio et al. 2002). Such large differences in nitrite toxicity demonstrate that among marine fish, there are species-specific mechanisms which regulate the toxic effects of nitrite (Wise and Tomasso 1989). In all circumstances, this study indicated meagre appear to be more sensitive to NO_2-N than European seabass and European eel but more tolerant than mullet. The 24-h LC_{50} was reported as 1997 mg/L for 12 d old larval gilthead seabream (Parra and Yufera 1999). In this study, 24-h LC_{50} values of this toxicant were calculated as 463, 387, and 268 mg/L for juvenile meagre at 18, 22, and 26 C, respectively. It is reported small fish, even larvae, are unlikely to be more sensitive to nitrite than larger fish of the same species (Lewis and Morris 1986).When the fish size or stage was considered not to be significant, it is clearly evident that meagre is also less tolerant to nitrite than gilthead seabream.

Sprague (1971) recommended a "safe" level (a concentration of pollutant which has no adverse effect on organisms) might be obtained by multiplying a 96-h LC_{50} value by a factor of 0.1. In this manner, a safe concentration can be suggested in which organisms can not only survive but thrive. The safe levels of NO_2-N calculated for meagre were estimated to be 17.7, 13.9, and 4.9 mg/L at 18, 22, and 26 C, respectively. From the present data, the toxic effect of NO_2-N to meagre appears to be 3.5-fold higher at 26 C compared with 18 C.

From this study, it can be concluded the toxicity of nitrite to juvenile meagre increases with increasing temperature. Considering previous studies and the results of this work, meagre should be stocked at lower densities, due to reduced tolerance of nitrite, compared with other fish species cultured in the Mediterranean such as European seabass, gilthead seabream, and European eel.

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