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CONTRIBUTION ON ACUTE TOXICITY OF AMMONIA TO FRY OF TWO MUGILID SPECIES (CHELON LABROSUS AND LIZA AURATA)

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ABSTRACT

The purpose of the study was to determine the acute toxicity of un-ionized ammonia to Chelon labrosus and Liza aurata fry. A static bioassay for ammonia toxicity was applied. Our results show that C. labrosus fry can tolerate high un-ionized ammonia concentrations (96 h LC_{50} =1.45 mg/l). The fry of L. aurata were much more sensitive (24 h LC_{50} = 0.805 mg/l) to un-ionized ammonia than C. labrosus fry. C. labrosus fry can be classified among fishes fairly tolerant to dissolved ammonia, while L. aurata fry most likely belong to susceptible group.

Key words: Ammonia; Acute toxicity; *Chelon labrosus*; *Liza aurata*

INTRODUCTION

Although artificial propagation has been achieved, mullet (Mugilidae) farming still relies on the capture of fry (<60 mm) and juveniles (>60 mm) from the wild. In mullet farming, a rather common practice is to keep captured fry in nursery ponds before they are introduced into grow-out ponds (Ben-Yami, 1981; Crosetti & Cataudella, 1994).

In the coastal region of Montenegro, several locations are particularly attractive for development of mullet farming (Morović, 1974;

Kinne & Rosenthal, 1977). The recommended approach consists of capturing fry in their natural habitats, culturing them in closed recirculation systems for intensive aquaculture up to an appropriate stage, and transferring them to open fish ponds for final growth, up to the commercially acceptable size (Rosenthal & Merker, 1978).

The design of recycling systems for fry culturing is based on the knowledge of water quality standards that must be maintained in the presence of a continuous load of metabolic wastes produced by the fish. Ammonia represents the main end product of protein metabolism in teleost fishes (Forster & Goldstein, 1969). It is known to be toxic to fish, and special attention must be paid to its toxicity in intensive culture systems, where high concentrations of this metabolic product can be reached (Person-Le Ruyet *et al.,* 1995). The acute and chronic toxic effects of ammonia have been extensively reviewed for freshwater fishes (Alabaster & Lloyd, 1980; Haywood, 1983). Ammonia toxicity data for marine fishes are still relatively scarce and mainly concern species whose intensive farming is in expansion (Person-Le Ruyet *et al.,* 1995; Lemarié *et al.,* 2004; Weirich & Riche, 2006; Rodrigues *et al.,* 2007; Costa *et al.,* 2008). When tolerance limits to the ammonia toxicity are not known, it is of paramount importance to estimate levels that can be considered lethal. Knowledge of concentrations of ammonia that may limit survival is particularly important in closed recycling systems, which are used for nursing fry for stocking purposes, and where it is advantageous to maintain the greatest density of fish per water volume. The purpose of this study was to determine the acute toxicity of ammonia to *Chelon labrosus* and *Liza aurata* fry, both representing promising candidates for development of mullet lagoon culture.

MATERIALS AND METHODS

Chelon labrosus fry were captured in the South Adriatic coastal waters (mouth of the Jaška River near the town of Budva). They were transported to the laboratory of the Institute for Multidisciplinary Research in Belgrade, where they were maintained during 4 months in a small recycling system for experimental aquaculture (180 l total volume; salinity ≈ 20‰; T = 18°C), and fed on a commercial trout feed (daily ration: 2-4% body weight). During two weeks preceding the toxicity experiment, the fry were gradually acclimated (1°C daily temperature increments) to a constant water temperature of 23°C. A static bioassay for ammonia toxicity was then applied to 70 *C. labrosus* fry divided into 7 equal groups (mean body weights ranging from 1.17 to 1.5 g), each accommodated in a 15 l glass container (synthetic sea water S = 20‰), supplied with an air-lift device. One group served as control, while the remaining six groups were exposed to concentrations of un-ionized ammonia ranging from 1.753 to 3.602 mg/l. Concentrations of un-ionized ammonia were established by the addition of predetermined amounts of NH_4Cl to the containers, and controlled daily by means of the hypochlorite/phenol assay for dissolved ammonia (Anon., 1984). Bower's formula (Bower & Bidwell, 1978) was used for calculating the percentage of un-ionized ammonia. During the four day test, feeding was discontinued. Temperature, pH, and oxygen concentrations were controlled four times per day. Water pH varied between 7.98 and 8.06, and water temperature between 23.2 and 23.7°C, while the concentration of dissolved oxygen remained near the saturation level throughout the test period (93 to 96%). Fish were observed continuously and dead individuals (cessation of opercular movements) were immediately removed. Data analyses were performed by probit analysis (Wardlaw, 1985) and by linear regression, using the program package STATISTICA 6.0.

The described experimental procedure was repeated in the experiment on *Liza aurata* fry (caught at the same locality; transported to Belgrade and maintained for two months in the experimental recycling system). Preceding the toxicity experiment, the fry were acclimated to a constant water temperature of 20°C. Six experimental groups were established (mean body weights ranging from 0.562 to 0.678 g). One group served as the control, while the remaining five groups were exposed to concentrations of un-ionized ammonia ranging from 1.617 to 3.892 mg/l. Water pH varied between 8.01 and 8.26, and water temperature between 20.2 and 20.8°C, with the concentration of dissolved oxygen remaining near the saturation level throughout the test period (92 to 96%).

RESULTS

No mortalities were observed in the control groups. At the end of the 4-day test period, 100% mortality was recorded in all treatment groups for the acute toxicity bioassay carried out on *C. labrosus* fry. In the experiment on *L. aurata* fry, all fish exposed to the test concentrations of un-ionized ammonia died within 24 h interval.

For each treatment group, the mean survival time (ST_{50}), as a function of the log survival time, and probit transformed mortality data were determined by linear regression (Figs. 1, 2). The resulting linear regressions were highly significant ($P < 0.05$). Summary statistics for parameter estimation of the regression equations are given in Tables 1 and 2. As regards the *C. labrosus* fry, the ST_{50} were 84.72, 64.27, 46.40, 39.97, 33.91, and 29.79 h for concentrations of 1.753, 2.132, 2.651, 3.048, 3.149, and 3.602 mg/l un-ionized ammonia, respectively. For *L. aurata* fry the ST_{50} were 17.88, 13.37, 13.41, 11.54, and 9.26 h for concentrations of 1.617, 2.256, 2.306, 3.025, and 3.892 mg/l un-ionized ammonia, respectively.

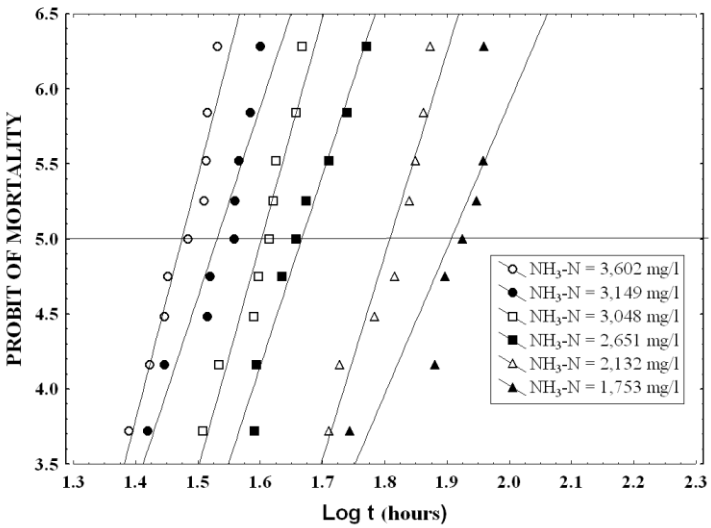

Figure 1. Probit of mortality versus log survival time for C. labrosus fry. The straight lines have been fitted by linear regression.

Table 1. Linear regression results for probit of mortality versus log survival time relation for the acute toxicity bioassay carried out on C. labrosus fry.

Concentration (mg/l)	Intercept	Slope	r
1.753	-13.512	9.710	0.863
2.132	-19.554	13.581	0.959
2.651	-16.204	12.723	0.982
3.048	-19.060	15.021	0.967
3.149	-14.346	12.641	0.951
3.602	-18.942	16.243	0.970

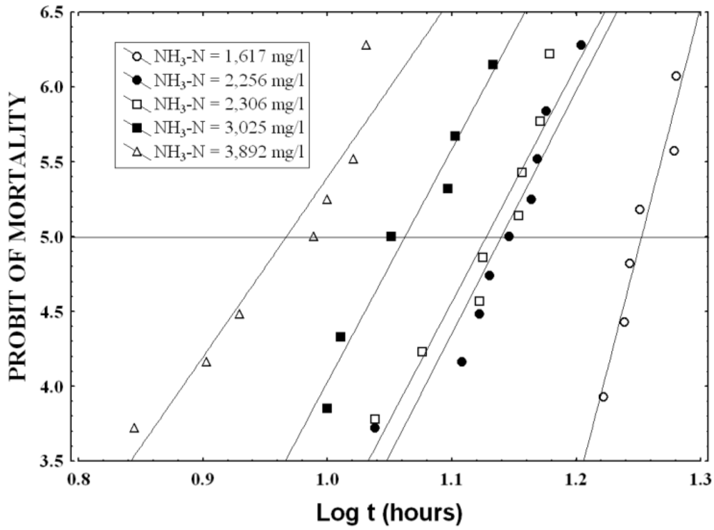

Figure 2. Probit of mortality versus log survival time for *L. aurata* fry. The straight lines have been fitted by linear regression.

Table 2. Linear regression results for probit of mortality versus log survival time relation for the acute toxicity bioassay carried out on *L. aurata* fry.

Concentration (mg/l)	Intercept	Slope	r
1.617	-35.484	32.326	0.967
2.256	-13.486	16.218	0.956
2.306	-12.802	15.787	0.953
3.025	-11.543	15.573	0.982
3.892	-6.603	12.000	0.954

In Fig. 3, the ST_{50} of both species as a function of the concentration of un-ionized ammonia are compared. As it is shown, the mean survival times decreased linearly with increasing the log concentrations of un-ionized ammonia.

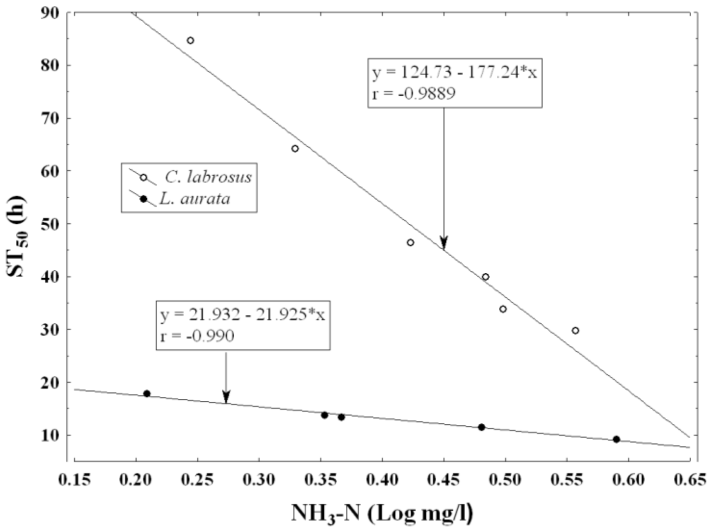

Figure 3. Comparison of ST_{50} of *C. labrosus* and *L. aurata* fry as a function of the concentration of un-ionized ammonia in the water.

For *C. labrosus* fry, according to the linear equation presented in Fig. 3, un-ionized ammonia concentrations of 3.70, 2.71, 1.98, and 1.45 mg/l are expected to cause the death of 50% of individuals in the course of 24, 48, 72, and 96 hours, respectively. Fry of *L. aurata* were less tolerant to ammonia and, according to the corresponding linear equation, un-ionized ammonia concentration of 0.805 mg/l is expected to kill 50% of population in the course of the 24-hour period.

DISCUSSION

In water, ammonia occurs in un-ionized (NH_3) and ionized (NH_4^+) form. Since the un-ionized ammonia is some 300-400 times more toxic than the ionized form, by convention, the ammonia toxicity is expressed in terms of the un-ionized form (Person-Le Ruyet *et al.,* 1995). The ratio between the two forms of ammonia is mainly related to the temperature and pH, while the influence of salinity is of lesser extent. In both experiments, the ambient ammonia concentrations and other environmental conditions such as temperature, pH, and salinity were stable. Furthermore, no mortality was recorded in the control groups.

Our results show that *C. labrosus* fry can tolerate high un-ionized ammonia concentrations. The determined 96 h LC_{50} (1.45 mg/l) was in the range of values reported for other marine species (Person-Le Ruyet *et al.,* 1995; Weirich & Riche, 2006; Rodriges *et al.,* 2007; Costa *et al.,* 2008). The 96 h LC_{50} dose varies from 0.54 mg/l for *Centropristis striata* (Weirich & Riche, 2006) to 2.6 mg/l for *Scophthalmus maximus* (Person-Le Ruyet *et al.,* 1995). To the best of our knowledge, within the aforesaid range, the only data which is related to mugilids is the data of 96 h LC_{50} = 0.84 mg/l for *Mugil platanus* (Sampaio *et al.,* 2002). The fry of *L. aurata* were much more sensitive to un-ionized ammonia than the fry of *C. labrosus*. Although practically identical concentrations were tested, all *L. aurata* fry died in the course of one day and the 24 h LC_{50} dose was 0.805 mg/l, thus being almost five times lower than the corresponding dose for *C. labrosus* fry (3.70 mg/l). Person-Le Ruyet *et al.* (1995) reported that the 24 h LC_{50} of un-ionized ammonia varied in ranges of 1.19-2.64 mg/l, 2.62-3.45 mg/l, and 1.98-3.44 mg/l for juvenile *Dicentrarchus labrax*, *Sparus aurata*, and *Scophothalmus maximus*, respectively. In general, it is assumed that among the mullet species that inhabit the Adriatic Sea, *C. labrosus* represents the species that is the most tolerant to pollution, while *L. aurata* is a much more susceptible species. This assumption is based on ecological characteristics and distribution of the regarded species. Namely, it is well-known that *C. labrosus* populations are abundant in heavily polluted areas (for example: ports, sewage effluents, and industrial areas). On the other hand, *L. aurata* is either absent from such environments or its presence is very rare.

According to our results, *C. labrosus* fry can be classified among fishes that are fairly tolerant to dissolved ammonia and less susceptible to its effects. However, *L. aurata* fry most likely belong to the susceptible group.

This study represents a preliminary report, and further investigations are still needed. In mullet species, the sublethal effects of ammonia are yet to be experimentally established. The acute toxicity test should be considered only as the first step in ammonia toxicity studies.

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