Determination of acute toxicity of ammonium in juvenile Patagonian blenny (*Eleginops maclovinus*)

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Abstract

Deionized ammonium (NH₃) acute toxicity (LC₅₀-96h) in Patagonian blenny juveniles (*Eleginops maclovinus*) was assessed. Concentrations of deionized ammonium in salt water were prepared by using 24.09 ± 2.1 g ammonium chloride (NH₄Cl). Fish were exposed in triplicates to different ammonium concentrations: 0.05; 0.094; 0.175; 0.325 and 0.605 mg NH₃ L⁻¹. Additionally, a control group was included. Experimental fish were kept at a photoperiod of 16:8h. Average temperatures were 16.24 ± 1.40 °C. Oxygen concentration was 7.16 ± 0.40 mg L⁻¹. Water pH was 7.89 ± 0.2. LC₅₀-96h was estimated by using Probit statistical method (95% intervals) using EPA software (1993). Juveniles of *E. maclovinus* showed a LC₅₀-96h of 0.413 mg NH₃ L⁻¹ value, different from most marine species. This study presents the first record of ammonium toxicity in marine species of Chile.

Keywords: acute toxicity, *Eleginops maclovinus*, LC₅₀-96h.

Determination da toxicidade aguda do amônio em Babosas-da-Patagônia (*Eleginops maclovinus*) juvenis

Resumo

A toxicidade aguda (LC₅₀-96h) de amônia deionizada (NH₃) em Babosas-da-Patagônia (*Eleginops maclovinus*) juvenis foi avaliada. As concentrações de sal de amônia deionizada em água foram preparados com 24.09 ± 2.1 g de cloreto de amônio (NH₄Cl). Os peixes foram expostos em triplicata a diferentes concentrações de amônia: 0.05; 0.094; 0.175; 0.325 e 0.605 mg NH₃ L⁻¹. O pH da água foi de 7.89 ± 0.2. LC₅₀-96h foi estimada usando o método estatístico de Probit (intervalos de 95%) usando software EPA (1993). Juvenis de *E. maclovinus* apresentaram uma LC₅₀-96h de 0.413 mg NH₃ L⁻¹ valor diferente da maioria das espécies marinhas. Este estudo apresenta o primeiro registro de toxicidade amônia em espécies marinhas do Chile.

Palavras-chave: toxicidade aguda, *Eleginops maclovinus*, LC₅₀-96h.

1. Introduction

Although the salmon and trouts are the main exportation products from Chilean aquaculture, the urgent aquaculture diversification of Chilean fish farming is necessary. Currently, the Chilean aquaculture production has a 98% FOB in salmon farming, and only the 3.2% includes other species. The “Patagonian blenny” (*Eleginops maclovinus*, Georges Cuvier, 1830) is an endemic species with high potential for aquaculture production (Sa et al., 2014).

The *E. maclovinus* belongs to Perciformes order and it is distributed in Pacific Ocean from Valparaiso (33° S)
to Tierra del Fuego island (54° S) and in the Atlantic Ocean until Buenos Aires province (39° S) (Calvo et al., 1992), and was reported around Falkland islands. It is a prothandric hermaphrodite with sexual reversion in function of its corporal size and age, under intraspecific competence (Licandeo et al., 2006). It is an omnivorous species and its diet varies according to geography, substrate and season (Pavez et al., 2005).

An intensive production system requires adequate environmental conditions that promote growth and development of all organisms and in this context it is necessary for each species such as dissolved oxygen, carbon dioxide and total ammonium (TAN) that determine the success of the fish farming activities (Rakocy et al., 1992; Gerber et al., 2015; Zhou et al., 2017; Silva et al., 2018). The total ammonium is the total sum of ammonium ($NH_4^+$) and ammonia ($NH_3$), the latter is considered a product of body protein and nucleic acid biochemical degradation (Verbeet et al., 1999; Shrivastava et al., 2017; Xiang et al., 2016). Eddy (2005) informed that the ammonia tolerance in freshwater fishes varied between 0.068 and 2.00 mg $NH_4^+$ L$^{-1}$ and between 0.09 and 3.35 mg $NH_3$ L$^{-1}$ in marine fishes. In the least fishes the nitrogen is normally eliminated through gills and urine (Carter et al., 1998). The pH is affected by the presence of ammonium ion or ammonia (Randall and Tsui, 2002).

The previous turbot studies ($Scophthalmus maximus$) showed that this species is less tolerant to normoxia condition (80-85% oxygen saturation), in comparison to tolerance to under hyperoxia condition (110-115% oxygen saturation) (Foss et al., 2007). These studies agree with Carter et al. (1998) who reported that low oxygen environmental level might enhance the ammonia toxic effects. An evidence of this factor is the depolarization that is produced in gills under ammoniac presence, simultaneously an excessive ATP consumption by the glutamate receptor activation (NMDA). As a result of intoxication, the individuals have convulsions and dead (Beaumont et al., 1995; Randall and Tsui, 2002). Other ammoniac effects are (Miranda-Filho et al., 1995), alterations in cerebellum tissue (Wajsbrot et al., 1991), increment of respiratory and cardiopulmonary function (Adams et al., 2001), swimming behaviour (Wicks et al., 2002) and loss of fish reproductive capacity (Russo and Thurston, 1998).

The studies of ammonium have been carried out with marine and freshwater fishes. The toxic effects are notoriously different for both groups. Data was obtained from determination of lethal media concentration ($LC_{50}$) that corresponds to the level of 50% of the population; this is the main procedure (Franson, 2002).

The fish sensibility to ammoniac depends on the species, for example, the $LC_{50}$ for $S. maximus$ juveniles is from 1.7 to 2.7 mg L$^{-1}$ of ammoniac, and is different compared to 0.2 to 0.7 mg L$^{-1}$ in salmonid juveniles (Person et al., 2007). There is not available information about ammoniac tolerance for “Patagonian blenny” $E. maclovinus$. The aim of the present study is to determine the first ecotoxicological parameters, specifically the acute ammoniac toxicity $LC_{50-96h}$ in $E. maclovinus$ under farming conditions.

2. Material and Methods

“Patagonian blenny” $E. maclovinus$ juveniles were obtained from Quillaipe marine station of Fundacion Chile (41°31’S; 72°44’W). Fish were acclimated during 14 days period prior the experiment. During this period the fishes were fed ad libitum with salmon pellet, this feeding was suspended 48 hours before the experiment. The procedure used was suggested by Franson (2002). For the $LC_{50-96h}$ determination fish specimens of an average weight 24.09 ± 2.1 g were selected and separated in triplicates in a control group, and five experimental groups with five individuals for each experimental units, and these were exposed to starvation during 48 hours. The acute toxicity assay was carried out in a close recirculating system with 0.015 m$^3$ tanks. Each 12 hour the temperature, pH and oxygen values were measured. The photoperiod used was of 18 hours light: 6 hours dark. The ammoniac and ammonium chloride concentrations were specified in Table 1. Mortality and swimming fish were recorded twice daily and classified as a normal (N), lethargic swimming (L) and dead (†), when no movement in their gills showing no reaction to mechanical stimuli (Weirich and Riche, 2006). Ammonia was monitored at 0; 24; 48; 72 and 96 h in according to Solorzano (1969) fide Bayne, 1985). To determine $LC_{50-96h}$, Probit statistical method was used with a range of 95% by the program EPA.

3. Results

During the experimental period (96 hr) the temperature varied between 16.24 ± 1.4 °C, the dissolved oxygen concentration in 7.16 ± 0.40 mg L$^{-1}$, pH between 7.89 ± 0.20. The utilized fish individuals in the experiment did not show abnormal behaviour and mortality during acclimation. There was no mortality in T1 group (fish reared in 0.05 mg L$^{-1}$ ammoniac concentration). However at 72 hr after the experimental period, the fish from T1 group showed lethargic swim (Table 2); 48 hr after the experiment beginning, fish showed lethargic swim (Table 2). The group exposed to high ammoniac concentration (0.605 mg L$^{-1}$) did not show mortality during the experimental period (Table 2). 48 hours after the beginning of experiment, the fish showed lethargic swim (Table 2). 48 hours after the beginning of experiment, both groups showed intoxication effects. The first group T1

| Treatments | NH3 (mg$^+$L$^{-1}$) | NH4CL (gL$^{-1}$) |
|------------|------------------|------------------|
| T 1        | 0.050            | 0.500            |
| T 2        | 0.094            | 0.931            |
| T 3        | 0.175            | 1.732            |
| T 4        | 0.325            | 3.224            |
| T 5        | 0.605            | 6.000            |
Table 2. Swim behaviour of fish individuals during a 96 h period.

| mgNH₃ L⁻¹ | Treatment | 0  | 24 | 48  | 72 | 96 |
|-----------|-----------|----|----|-----|----|----|
| Control   | Control   | N  | N  | N   | N  | N  |
| 0.050     | T1        | N  | N  | N   | L  | L  |
| 0.094     | T2        | N  | N  | N   | L  | L  |
| 0.175     | T3        | N  | N  | N   | L  | L  |
| 0.325     | T4        | N  | L  | N   | L  | L  |
| 0.605     | T5        | N  | L  | ↑   | ↑  | ↑  |

Normal (N), 100% lethargic (L) y 100% mortality (†).

Table 3. Survival of E. maclovinus juveniles in five deionized ammonium concentration (mgNH₃ L⁻¹) during 96 h.

| mgNH₃ L⁻¹ | Treatment | n  | Survival (%) |
|-----------|-----------|----|---------------|
| Control   | Control   | 5  | 100 ± 0.0     |
| 0.050     | T1        | 5  | 100 ± 0.0     |
| 0.094     | T2        | 5  | 80 ± 8.0      |
| 0.175     | T3        | 5  | 32 ± 7.0      |
| 0.325     | T4        | 5  | 12 ± 5.0      |
| 0.605     | T5        | 5  | 0.0 ± 0.0     |

exposed to low ammoniac concentration (0.050 mgNH₃ L⁻¹) was not mortality, whereas the T2 (0.094 mgNH₃ L⁻¹) group had 80% survival, T3 (0.175 mgNH₃ L⁻¹) had 32% survival, T4 (0.325 mgNH₃ L⁻¹) had 12% survival, and finally T5 (0.605 mgNH₃ L⁻¹) had 100% mortality after 96 hours (Table 3). Direct correlation between survival time and NH₃ was observed and it showed a low survival under high ammoniac concentration. The LC₅₀,₉₀th ammoniac calculation, for E. maclovinus juveniles was of 0.413 mgNH₃ L⁻¹ (the limit at 95% was between 0.233 to 0.611 mgNH₃ L⁻¹), that indicated that the same species has low tolerance to ammoniac concentration in comparison to other marine species.

4. Discussion

The LC₅₀,₉₀th for E. maclovinus obtained of 0.413 mgNH₃ L⁻¹ (limited at 95% between 0.233 to 0.611 mgNH₃ L⁻¹) is comparable with the results obtained for Menidia beryllina that has a LC₅₀ of 0.61 mgNH₃ L⁻¹. The tolerance level for other marine species are of 2.29 mgNH₃ L⁻¹ for Cyprinodon variegatus and 3.35 for Gateropus acaletus (Handy and Poxton, 1993). On the basis of these results it can be considered that E. maclovinus has tolerance ranks similar to inland water fishes, such as Oncorhynchus mykiss in example it observed a tolerance level of 0.291 mgNH₃ L⁻¹ (Thurston and Russo, 1983), Salmo trutta was of 0.592 mgNH₃ L⁻¹, Prosopium williamsoni was of 0.358 mgNH₃ L⁻¹ (Thurston and Meyn, 1984), and Ictalurus punctatus was of 0.50 mgNH₃ L⁻¹.

The ammoniac is considered as the most toxic fraction of TAN that is defined by the sum of ammonium ion and ammoniac (EPA, 1998). The ammoniac effect is related to temperature, salinity, oxygen and pH (Randall and Tsui, 2002; Fang et al., 2017), on this basis, the temperature observed in the present study was, within the optimal tolerance level (Randall and Tsui, 2002), and whereas at salinity level at high NaCl concentration the ammoniac toxic effect is lower (Randall and Tsui, 2002; Rodrigues et al., 2007; Fang et al., 2017). Also, in increment condition of sodium and calcium in oxygen saturated water the ammoniac effect decreased (Foss et al., 2007). The TAN toxic effect in water is regulated mainly by pH, temperature, salinity and oxygen (Randall and Tsui, 2002; Gerber et al., 2015; Xiang et al., 2016). One pH variation of a 25% can affect significantly the ammoniac concentration (EPA, 1998).

Other important topic that has effect on ammonium concentration is the feeding activity. Wicks and Randall (2002) showed that in fish feed, it increases the ammoniac level in plasm, in comparison to the observed level in water, nevertheless in this experiments the fish survived. These results can explain that the fish has glutamine synthesis capacity. Other important factor is the fish capacity for resist the ammoniac concentration under stress conditions (Popovic et al., 2016), then in this scenario Randall and Tsui (2002), showed that the fish during exhaustive exercise and stress incremented its ammonium production and are more sensible to external ammoniac. This can be explained by the cortisol increment that is positively related to the glutamine synthesis.

They reported that in Rachycentron canadum juveniles erratic and lethargic swim arised when the fish were exposed to ammoniac concentration of 0.80 NH₃ L⁻¹. Similar results to this study were observed in the fifth experimental group exposed to 0.605 NH₃ L⁻¹, that has lethargic swim after 24 h. This experimental group was followed by fourth treatment (exposed to 0.325 NH₃ L⁻¹) and third (exposed to 0.175 NH₃ L⁻¹) after 48 and 72 h respectively. The E. maclovinus acute toxicity observed value was of 0.413 NH₃ L⁻¹, in conclusion these results would serve of basis for the intensive culture of this species.

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