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Determination of acute toxicity of unionized ammonia in juvenile longfin yellowtail (*Seriola rivoliana*)

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Abstract

The median lethal concentration (LC_{50}) of unionized ammonia (NH_3-N) in longfin yellowtail, *Seriola rivoliana* juveniles was assessed after 96 h of exposure using a semistatic water system. Experimental fish were exposed in triplicate to different ammonia concentrations: 0.55 ± 0.00; 0.94 ± 0.02; 1.18 ± 0.00; 1.72 ± 0.02, and 1.97 ± 0.09 NH_3-N mg/L. Additionally, a control group (0.00 ± 0.00 NH_3-N mg/L) was included. The 96 h LC_{50} of unionized ammonia in *S. rivoliana* was 0.58 mg/L. Fish exposed to different ammonia concentrations for 96 h displayed several lesions on gill tissues, that is, hyperplasia, epithelial lifting, fusion of the secondary lamellae, and some irreversible damage. Erratic behavior, such as swimming in circles and hyperventilation was observed before the fish died. The safe concentration of unionized ammonia in this study was estimated at 0.06 and 1.68 mg/L of total ammonia nitrogen (TAN). The outcomes observed in this study provide valuable insights into unionized ammonia effects for better water quality management in semi-intensive and intensive systems for *S. rivoliana* culture.

1 | INTRODUCTION

Ammonia is a nitrogenous by-product of amino acid catabolism that is excreted by fish (fish alternatively excrete ammonia as urea) via the gill membranes (Franklin & Edward, 2019). Total ammonia nitrogen (TAN) exists in aquatic environments in ionized (NH_4^+) and unionized (NH_3-N) forms. The latter form is considered toxic to aquatic organisms because NH_3-N diffuses readily through the gills and is highly soluble in lipids (Shin et al., 2016). Elevated ammonia levels in the environment either impair ammonia excretion or cause a net uptake of ammonia from the environment (van der Meeren & Mangor-Jensen, 2020). Therefore, a negative gradient caused by a high ambient ammonia concentration causes NH_3-N to accumulate within fish blood and tissues, causing toxic effects (Franklin & Edward, 2019; Nerici et al., 2012). In aquaculture operations, the accumulation of excreted ammonia can lead to decreased growth, increased vulnerability to disease, pathological changes in gill structure, physiological and behavioral responses, and mortality (Pedrotti et al., 2018; Wilkie, 1997; Zuffo et al., 2021), all of which contribute to a significant drop in productivity of the aquaculture system. Therefore, the determination of ammonia toxicity is necessary to obtain adequate rearing conditions, especially in semi-intensive and intensive cultures and recirculating aquaculture systems (RAS), in which degradation of the water quality by uneaten food and waste products usually occurs (Cobo et al., 2014; Ip & Chew, 2010). A validated analytical method, of known accuracy, precision, and sensitivity, for acute short-term test (48 or 96 h exposures) to determine ammonia toxicity is the median lethal concentration (LC_{50}), that is, the concentration at which 50% of the exposed fish die (Anderson & Phillips, 2016). The acute and chronic toxicity of ammonia has been widely reviewed for freshwater species. However, there is a scarce amount of literature relating to the impacts of ammonia levels on brackish and marine fish (Franklin & Edward, 2019; Maltez et al., 2019; van der Meeren & Mangor-Jensen, 2020). The toxicity of ammonia (96 h LC_{50}) in freshwater fish species ranges between 0.068 and 2.0 NH_3-N mg/L, similar to that of marine species (0.09–3.35 NH_3-N mg/L) (Eddy, 2005). These values are dependent on ambient temperature, pH, salinity, age, and species (Lemarié et al., 2004).

Seriola rivoliana (Carangidae, Valenciennes, 1833) is a relevant species distributed globally throughout the subtropical to warm water seas, commonly known as longfin yellowtail. This species is currently recognized as a potential species for aquaculture diversification as a result of its fast growth, a high market value, and growing consumer acceptance (Kissinger et al., 2016; Reinoso et al., 2020; Viader-Guerrero et al., 2021). The genus *Seriola* are carnivorous fish farmed mainly in cages, and several studies have provided basic information about the development of larviculture techniques (Kissinger et al., 2016; Reinoso et al., 2020; Roo et al., 2014). *S. rivoliana* adapts well to farming conditions and feeds on commercial dry pellet feeds (Roo et al., 2014). Therefore, it is considered a prospect for intensive aquaculture. However, there are very few studies investigating the toxicity of unionized ammonia in the *Seriola* species. Consequently, the purpose of this research was to evaluate the effect of acute toxicity of unionized ammonia (NH_3-N) on welfare conditions and histopathological alterations in gills of longfin yellowtail *S. rivoliana* juveniles exposed for 96 h, in order to contribute to a better understanding of the aquaculture management and upcoming productivity of this species.

2 | MATERIALS AND METHODS

2.1 | Experimental fish

The present study was carried out at the National Aquaculture and Marine Research Center of ESPOL Polytechnic University "CENAIM-ESPOL." Juveniles of *S. rivoliana* at 50 days post-hatch (DPH) and an initial weight of 4.8

± 0.2 g were obtained following the protocol for larval production from CENAIM-ESPOL, which included the addition of live food initiated at 2 DPH and adjusted according to the stage of larval development (2–10 rotifers/mL; 0.5–1 *Artemia* nauplii/mL; 0.5–3 *Artemia*-enriched metanauplii/mL). Co-feeding (live and dry food) began at 21 DPH with complete weaning at 26 DPH. The photoperiod was 12 L:12 D, and water was replaced daily, starting with 25% at 5 DPH and up to 200% from the *Artemia* feeding period onward. Selected fish (50 DPH) were kept in starvation for 15 h before the trial. Afterward, eight fish were randomly allocated into each of eighteen 30-L fiberglass tanks with ambient seawater. The dissolved oxygen (DO) concentration was maintained with constant aeration. All applicable institutional guidelines for the care and use of animals were followed by the authors.

The fish species used in this study are included in the list of allowed species for aquaculture activities in Ecuador (MAGAP-INP-2015-0606-M and MAP-SUBACUA-2017-5899-M). Captivity and maintenance conditions were in accordance with permission given by Ministerio del Ambiente, Santa Elena, Ecuador (Resolution No. 006-2018-IC-FAU-DPSE-MA).

2.2 | Acute ammonia toxicity test

The experimental system was semistatic with 75% of the water renewed every 24 h with water at the same temperature, pH, and ammonia concentration. Test solutions of ammonia were prepared by dissolving 100 g/L ammonium chloride (NH$_4$Cl; VWR Chemicals, Solon, OH, USA) in 1 L of distilled water (Zuffo et al., 2021) as a stock solution and then diluting it to the desired concentration (Table 1). An LC$_{50}$ test was conducted with six NH$_3$-N concentrations: 0.00 (control), 0.55 ± 0.00, 0.94 ± 0.02, 1.18 ± 0.00, 1.72 ± 0.02, and 1.97 ± 0.09 NH$_3$-N mg/L. The bioassay experiment was carried out in triplicate to determine the LC$_{50}$ at 12, 24, 36, 48, 60, 72, 84, and 96 h. TAN and alkalinity were determined immediately after water sample collection according to APHA (1998), and only measured concentrations were used in determining ammonia tolerance. The variability of TAN concentration was checked by taking samples in triplicate every 24 h. The resulting NH$_3$-N concentration was determined based on the measured TAN, pH, salinity, and water temperature according to Whitfield (1974). The fish were not fed throughout the experiment (96 h).

2.3 | Water quality and sample collection

Water quality parameters were monitored daily throughout the experiment. These parameters varied as follows: temperature: 24.6–25.3°C; salinity: 31–33 g/L; DO concentration: 5.5–5.9 mg/L; pH: 7.80–8.14 and alkalinity: 78.5–94.1 as CaCO$_3$ mg/L. The temperature and DO were measured using a multiparameter EcoSense DO200A (YSI Incorporated, Yellow Spring, OH, USA), salinity was measured with a portable refractometer (Vital SineTM SR6, Pentair Aquatic Eco Systems, Inc., Apopka, FL, USA), and pH was measured with a pH55 meter (Milwaukee Instrument, Rocky Mount, NC, USA).

Fish were externally observed by two persons every 3 h for mortality and swimming behavior such as erratic swimming (in circles), lethargic behavior with the head pointed to the tank bottom, and loss of equilibrium. Juveniles without opercular movement and without response to mechanical stimuli were considered dead and removed from

TABLE 1 Total ammonia nitrogen (TAN) and unionized ammonia (NH$_3$-N) levels in the exposure experiment.

Nominal TAN, mg/L	0.00	10.00	18.00	32.00	56.00	100.00
Measured TAN, mg/L	0.00	12.78 ± 0.15	22.82 ± 1.37	35.00 ± 2.05	66.73 ± 1.30	103.40 ± 1.10
Calculated NH$_3$-N, mg/L	0.00	0.55 ± 0.00	0.94 ± 0.02	1.18 ± 0.00	1.72 ± 0.02	1.97 ± 0.09

the tanks (Medeiros et al., 2016). At the end of the experimental period, three randomly live fish from each replicate were anesthetized in Eugenol's solution (25 mg/L), and blood was taken by cardiac puncture using a 1 mL sterile plastic syringe and then mixed well in a vial containing EDTA (Blaxhall & Daisley, 1973). The total red blood cell (RBC) count and hematocrit (Ht) value were determined immediately. RBCs were counted using an optical microscope (OLYMPUS CX 31, Olympus America Inc., Center Valley, PA, USA) with an improved Neubauer hemocytometer after dilution 1:200 with Dacie's solution. The Ht value was determined by centrifuging (Sorvall ST 8/R Centrifuge; Thermo Fisher Scientific®, Waltham, MA, USA) the blood in a capillary tube at 10,000g for 10 min (Pedrotti et al., 2018) and was expressed as the total volume percentage. The mean corpuscular volume (MCV) was calculated as follows:

$$MCV\,(fL) = \frac{Ht\,(\%) \times 10}{RBC\left(10^6/\mu L\right)}.$$

After blood collection, three juveniles from each treatment were euthanized for collection of the second-gill arch, fixed in Davidson solution for 24 h, and then transferred to a 70% ethanol solution. Except for those treated with higher ammonia concentrations (0.94 ± 0.02, 1.18 ± 0.00, 1.72 ± 0.02, and 1.97 ± 0.09 NH$_3$-N mg/L), gills from fish were removed immediately after the last fish died. Once fixed, samples were dehydrated through a graded series of ethanol solutions, followed by incubation in xylene prior to embedding in paraffin. Microtome sections (4 μm thick) were stained with hematoxylin and eosin (H&E). The slides were examined under an optical microscope (OLYMPUS CX31) equipped with a camera (LANOPTIK MDX503, Lanoptik Technologies, China). Histological changes were analyzed in a semiquantitative manner using the degree of tissue change method as described by Poleksić and Mitrović-Tutundžić (1994), with three stages based on the severity of each lesion. The degree of change in a single fish gill (I value) was calculated using the following formula:

$$I\,value = 1 \times \Sigma I + 10 \times \Sigma II + 100 \times \Sigma III.$$

where I, II, and III correspond to the number of lesion types within each of the three stages.

The scale of the I value and the associated effects were as follows: functionally normal gills (0%–10%); slightly to moderately damaged gills (11%–20%); moderately to heavily damaged gills (21%–50%) and irreparably damaged gills (>100%, Poleksić & Mitrović-Tutundžić, 1994).

Finally, each fish was sampled for total length (LT) and weighed (body weight, BW) to the nearest 0.2 g using a WBW 5a scale (ADAM, Adam Equipment Inc., Oxford, MS, USA) to calculate the condition factor (K_F) as follows:

$$K_F\,(g/cm) = 100 \times \frac{BW}{LT^3}.$$

2.4 | Statistical analysis

The LC$_{50}$ of NH$_3$-N and its confidence interval (95%) were estimated at 12, 24, 36, 48, 60, 72, 84, and 96 h using the probit transformation method (Finney, 1971) and regression analyses. The statistical analysis of this study was conducted using XLSTAT® January 1, 2017 (Addinsoft, Paris, France). The normality and variance homogeneity of the data were checked using the Kolmogorov–Smirnov and Bartlett's test. If non homogeneity of variances was detected (e.g., K_F), values were transformed using $y = 1/x$ to comply with parametric test assumptions. Data were compared using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference test (significance level $p < 0.05$). All data are expressed as the mean ± standard error (SE).

3 | RESULTS

As expected, fish mortality increased as the NH_3-N concentration increased. Treatments of 1.72 and 1.97 NH_3-N mg/L had a lethal effect at even <12 h of exposure (100% mortality). On the other hand, the control and 0.55 NH_3-N mg/L treatments showed no mortality after 96 h of exposure.

The LC_{50} values for NH_3-N and TAN and their calculated 95% confidence limits at different exposure times are summarized in Table 2. The LC_{50} values for NH_3-N were 0.99 and 0.58 mg/L for 12 and 96 h (Figure 1), equivalent to 30.58 and 16.76 mg/L of TAN, respectively. No significant differences ($p > 0.05$) were registered in the hematological parameters (RBC, Ht, MCV) of the surviving fish after 96 h at 0.55 NH_3-N mg/L exposure compared to control fish (Table 3).

The gills of the control fish showed a normal appearance (Figure 2a). Meanwhile, exposed fish gills revealed lamellar deformation characterized by irregular hyperplasia of the secondary lamella (Figure 2b,c), hemorrhages with rupture of the epithelium (Figure 2c), fusion of the secondary lamella and telangiectasia (Figure 2d), shortening of the secondary lamellae and lifting of respiratory epithelial cells (Figure 2e), and severe destruction of the lamellar epithelium (Figure 2f). Gill lesions of each stage (I, II, III) are shown in Table 4. Shortening of the secondary lamellae, hemorrhages with rupture of the epithelium and lamellar thickening showed the highest occurrence in stages I (14.8%), II (4.6%) and III (8.3%), respectively. According to the I value, irreparably damaged gills were reached in exposed fish to 0.94 NH_3-N mg/L (108%); 1.18 NH_3-N mg/L (104%); 1.72 NH_3-N mg/L (114%); 1.97 NH_3-N mg/L (180%).

TABLE 2 Lethal concentration (LC_{50} at 95% confidence interval) of unionized ammonia (NH_3-N) and total ammonia nitrogen (TAN) after different exposure periods.

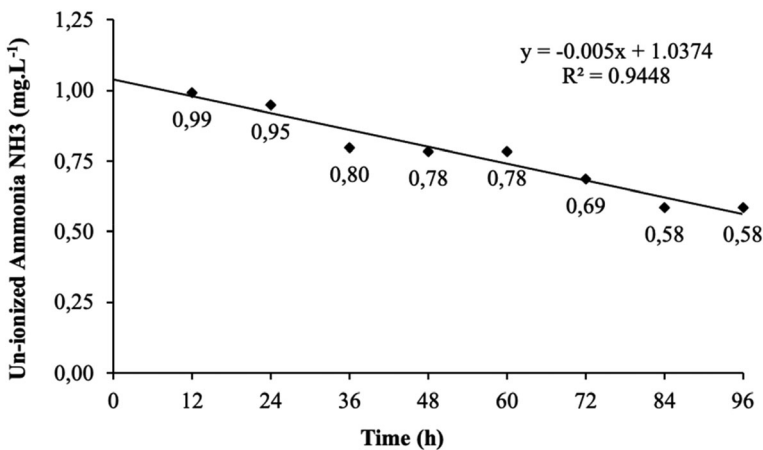| LC_{50}, mg/L	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h
NH_3-N	0.99	0.95	0.80	0.78	0.78	0.69	0.58	0.58
Lower limit	0.94	0.90	0.76	0.74	0.74	0.65	0.55	0.55
Upper limit	1.04	1.00	0.84	0.82	0.82	0.72	0.61	0.61
TAN	30.58	29.15	24.11	23.56	23.56	20.34	16.76	16.76
Lower limit	29.05	27.69	22.90	22.38	22.38	19.32	15.92	15.92
Upper limit	32.11	30.61	25.32	24.74	24.74	21.36	17.59	17.59

$$y = -0.005x + 1.0374$$
$$R^2 = 0.9448$$

FIGURE 1 Lethal concentration of unionized ammonia (LC_{50} NH_3-N) for longfin yellowtail juveniles after different times of exposure.

The final BW and condition factor (K_F) showed significant differences ($p < 0.05$) among the treatments. The K_F at 0.55 and 0.94 NH_3-N mg/L were the highest and lowest, respectively (Table 3). Regarding external observations and swimming behavior, the fish in the control treatment were apparently healthy with respect to coloration and swimming behavior during the bioassay. At 0.55 NH_3-N mg/L, no mortality was registered, however, the fish showed a darker coloration from 72 h onward until the end of the experiment. At 0.94 NH_3-N mg/L lethargic behavior was observed after 15 h of exposure, while at 1.18 NH_3-N mg/L, the same behavior was observed at 6 h. Erratic swimming and hyperventilation were observed before the fish died (0.94 NH_3-N mg/L after 36 h; 1.18 NH_3-N mg/L at 15 h; both 1.72 and 1.97 NH_3-N mg/L at 3 h).

4 | DISCUSSION

Ammonia is toxic to aquatic animals, especially when they are reared for production purposes at high stocking densities (Nerici et al., 2012). The toxic fraction of ammonia (NH_3-N) exists in equilibrium with NH_4^+, and its concentration depends on pH, temperature, and salinity (Zuffo et al., 2021). In this study, the water quality parameters observed in all treatments were within the range considered safe for *S. rivoliana* farming (Reinoso et al., 2020; Viader-Guerrero et al., 2021). As environmental conditions were consistent among the treatments, the ammonia level was considered the only factor that could have affected fish health. However, it has been suggested that ammonia may interact with other environmental variables, such as nitrite (NO_2-N), and oxygen, causing fish to die, as a result of nitrite spreading into red blood cells and oxidizes hemoglobin to methemoglobin that is unable to carry oxygen, resulting in hypoxia and, ultimately, death in fish (Kir & Sunar, 2018). The buildup of nitrite concentrations can be harmful to several fish, but according to previous studies, higher nitrite levels are needed to compromising marine fish health (190 NO_2-N mg/L, *Centropristis striata*, Weirich & Richie, 2006; 210 NO_2-N mg/L, *Rachycentron canadum*, Rodrigues et al., 2007; 108 NO_2-N mg/L, *Amphiprion ocellaris*, Medeiros et al., 2016). Although the purpose of this study was not related to acute nitrite toxicity, it can be a potential threat to the health of this species, and further research is needed to prevent the accumulation of this nitrogenous compound in marine production systems.

In the current study, the LC_{50} of NH_3-N was inversely related to the exposure time. The concentration of unionized ammonia that caused 50% mortality after 96 h of exposure in longfin yellowtail juveniles was 0.58 NH_3-N mg/L (lower level = 0.55 NH_3-N mg/L, and upper level = 0.61 NH_3-N mg/L), equivalent to 16.76 mg/L of TAN (lower level = 15.92 mg/L and upper level = 17.59 mg/L of TAN). Similar results to those found in the present study have been reported for *Centropristis striata* juveniles (BW = 9.9 ± 0.9 g; LC_{50} 96 h = 0.54 NH_3-N mg/L; Weirich &

TABLE 3 Final biometrics and welfare indices of the longfin yellowtail juveniles immediately after exposure to unionized ammonia (NH_3-N).

NH_3-N, mg/L	BW_F, g	TL, cm	K_F, %	Ht, %	RBC $\times 10^6$, cel/μL	MCV, fL
0.00	4.9 ± 0.4[ab]	7.2 ± 0.1	1.32 ± 0.08[ab]	18 ± 3	0.70 ± 0.16	293 ± 78
0.55	5.0 ± 0.2[a]	6.8 ± 0.2	1.62 ± 0.22[a]	17 ± 3	0.68 ± 0.16	275 ± 65
0.94	4.3 ± 0.2[ab]	7.2 ± 0.2	1.15 ± 0.04[b]			
1.18	3.9 ± 0.3[b]	6.9 ± 0.2	1.21 ± 0.06[ab]			
1.72	4.4 ± 0.1[ab]	7.0 ± 0.1	1.29 ± 0.01[ab]			
1.97	4.4 ± 0.2[ab]	7.0 ± 0.2	1.29 ± 0.02[ab]			

Note: Values (mean ± SE) in the same column with different superscript letters are significantly different (Tukey's test, $p < 0.05$).

Abbreviations: BW_F, final body weight; Ht, hematocrit; K_F, condition factor; MCV, mean corpuscular volume; RBC, red blood cells; TL, total length.
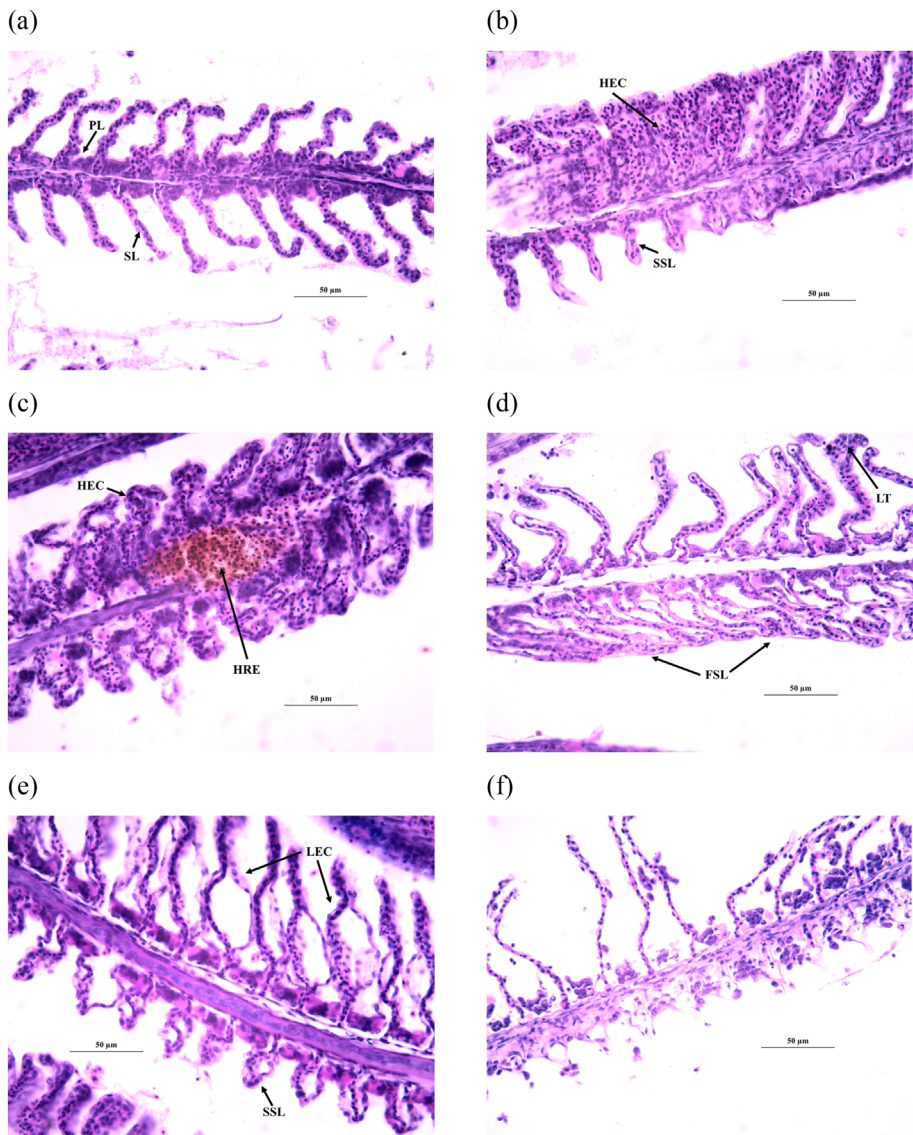
FIGURE 2 Histological sections of gills of *Seriola rivoliana* juveniles (×400). (a) Normal; (b–e) gill alterations in fish exposed to different NH$_3$-N concentrations; (f) severe destruction of the lamellar epithelium (scar tissue). FSL, fusion of the tips of the secondary lamellae; HEC, irregular hyperplasia of epithelial cells with filling of interlamellar spaces; HRE, hemorrhages with rupture of the epithelium; LEC, lifting of respiratory epithelial cells; LT, lamellar telangiectasis; PL, primary lamellae; SL, secondary lamellae; SSL, shortening of the secondary lamella.

Richie, 2006). On the other hand, this species was more sensitive to NH$_3$-N than *Centropomus undecimalis* (BW = 20.35 ± 6.10 g; LC$_{50}$ 96 h = 3.52 NH$_3$-N mg/L; Pedrotti et al., 2018) and *Dicentrarchus labrax* (BW = 1.1 ± 0.3 g; LC$_{50}$ 96 h = 0.73 NH$_3$-N mg/L; Kir et al., 2019). However, there are some marine species that are less tolerant to NH$_3$-N, such as *Argyrosomus regius* (BW = 3.0 ± 0.9 g; LC$_{50}$ 96 h = 0.44 NH$_3$-N mg/L; Kir et al., 2016) and *Paralichthys orbignyanus* (BW = 107.3 ± 1.9 g; LC$_{50}$ 96 h = 0.12 NH$_3$-N mg/L; Maltez et al., 2019). The comparisons described above were performed under similar environmental parameters (salinity 30–32 g/L; pH8; temperature 22–26°C).

TABLE 4 Gill lesions in stages I, II and III and occurrence (%) for each treatment of the exposed juveniles of *Seriola rivoliana* to different concentrations of NH_3-N.

Stage	Gill lesions	NH_3-N, mg/L					
		0.00	0.55	0.94	1.18	1.72	1.97
I	Irregular hyperplasia and lifting of epithelial cells	11.1	16.7	0.0	16.7	16.7	16.7
	Shortening of the secondary lamellae	16.7	5.6	16.7	16.7	16.7	16.7
	Fusion of the tips of the secondary lamellae	5.6	5.6	5.6	16.7	16.7	5.6
	Fusion of several secondary lamellae	0.0	11.1	0.0	0.0	5.6	0.0
	Lamellar telangiectasis	0.0	0.0	0.0	11.1	5.6	11.1
II	Hemorrhages with rupture of epithelium	0.0	5.6	11.1	0.0	11.1	0.0
	Complete fusion of all the secondary lamellae	0.0	0.0	0.0	0.0	5.6	16.7
III	Scar tissue-lamellar thickening	0.0	0.0	16.7	16.7	5.6	11.1
	Scar tissue-destruction of the lamellar epithelium	0.0	0.0	0.0	0.0	11.1	16.7

Note: $n = 18$ fish.

The LC$_{50}$ of ammonia to juvenile *S. rivoliana* revealed that toxicity was augmented by increased concentrations of NH_3-N and manifested by massive convulsions, abnormal swimming, and mortality. In acute NH_3-N exposure cases, where rapid toxic levels of ammonia are reached, there is an inhibition of Na^+ influx, and the fish experience convulsions of white muscles and ultimately death (Ip & Chew, 2010). In addition, because ammonia toxicity interferes with nervous function, there may be impairment of activity and behavior inducing hyperactivity, convulsions, and coma, leading to death (Eddy, 2005; Wilkie, 1997). Behavioral experiments may reveal how fish are able to detect and avoid polluted areas.

Histological alterations in target organs (i.e., gills) of fish exposed to pollutants can easily be recognized and provide crucial information on the health status of the fish (Maltez et al., 2017). It is well known that gills are the first organ to react to adverse environmental conditions. Some studies have shown that acute ammonia exposure can cause osmotic dysfunction and severe histopathological damage to the gills, which in turn may compromise gas exchange and ion and acid–base regulation (Gao et al., 2021; Mangang & Pandey, 2021). In the present study, histological alterations of the gills, such as cell hyperplasia, epithelial lifting, fusion of the secondary lamellae, blood congestion with hemorrhage, and destruction of lamellar epithelium, were observed. Similar changes in the gills of various freshwater and marine fish species exposed to unionized ammonia have been observed by several authors (Mangang & Pandey, 2021; Zuffo et al., 2021). Changes in gill structure, such as those mentioned above, could be examples of defensive responses against the prevailing stress because these changes act as a barrier between the external environment and the blood and thus prevent the entry of pollutants (Mangang & Pandey, 2021; Poleksić & Mitrović-Tutundžić, 1994). Epithelial lifting is although to be an initial response of the branchial apparatus and can be induced by low doses of toxicants, while the fusion of the secondary lamellae is induced by higher doses of toxicants or as a final result of hyperplasia in long-term sublethal poisoning (Poleksić & Mitrović-Tutundžić, 1994). Ammonia can enter through the fish gills, and once in the blood, it can alter hematological parameters (Gao et al., 2021). According to Shin et al. (2016), in many fish, hematological parameters can be a sensitive and reliable indicator to assess the toxicity of pollutants in fish. In the present study, no blood samples were taken at higher NH_3-N concentrations because no surviving fish were observed after 96 h of exposure. In both the control and 0.55 NH_3-N mg/L treatments, no significant differences in RBC, Ht or MCV were observed. However, lower values of these blood indicators were observed at 0.55 NH_3-N mg/L. Previous studies have reported that hematological parameters were reduced in response to ammonia exposure (Gao et al., 2021; Mangang & Pandey, 2021). Although the final body weight and condition factor were significantly different among the treatments, we cannot precisely ensure that these differences were related to the unionized ammonia concentration used in the present survey.

However, a previous study showed a significant reduction in growth when fish were reared for 60 days under suble-thal unionized ammonia concentrations (Vaage & Myrick, 2021). Thus, more research into growth performance and NH_3-N effects in marine fish is needed.

According to Sprague (1971) and Kir and Sunar (2018) a safe level (no observed effect concentration, NOEC) is defined as the amount of toxic substance that has no adverse effect on aquatic organisms, and it is calculated by dividing the LC_{50} 96 h value by a factor of 10. The safe levels estimated in this study were 0.06 mg/L of NH_3-N and 1.68 mg/L of TAN. A similar safe level of NH_3-N has been reported for euryhaline species such as *D. labrax* (0.07 mg/L, Kir et al., 2019). In addition, a higher safe level of TAN was reported for *Sparus aurata* (1.94 mg/L, Kir & Sunar, 2018). These safe levels of NH_3-N and TAN are important to be considered for the adequate management of these nitrogenous compounds in *S. rivoliana* production systems.

5 | CONCLUSIONS

This study establishes first-hand the available data on the effects of unionized ammonia in this particular fish species for better water quality management in aquaculture systems. To our knowledge, there are no published ammonia toxicity studies on any of the life stages of *S. rivoliana*. The findings of this study suggest that the TAN concentration should not exceed 1.68 mg/L at a salinity of 32 g/L, pH8, and 25°C water temperature during *S. rivoliana* juvenile rearing.

CONFLICT OF INTEREST STATEMENT
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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