Int. J. Aquat. Biol. (2018) 6(6): 314-320
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

Acute and chronic toxicity of ammonia in Persian sturgeon, *Acipenser persicus*, fingerlings

Shahram Naghizadeh Raiesi*[1], Mohammad Mazandarani[2], Seyed-Ahmad Shahidi[1], Azade Ghorbani-HasanSaraei[1], , Fatemeh Khani[2], Sjad Fatahi[2]

[1]Department of Food Science and Technology, College of Agriculture and Food Science, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran.
[2]Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Abstract: In the present study, susceptibility of Persian sturgeon, *Acipenser persicus,* to un-ionized ammonia (UIA) was studied. Fish with an average weight of 5.52±0.45 g were exposed to different concentrations of un-ionized ammonia (UIA) to determine median lethal concentration (96-h-LC_{50}). The fish were exposed to six different concentrations of UIA (control, 0.16, 0.31, 0.62, 0.94 and 1.25 mg/l UIA). The control group was not challenged with ammonia (0.0008 mg/l UIA). The results showed that the 96-h-LC_{50} of UIA in Persian sturgeon was 0.46 mg/l. According to 96-h-LC_{50}, mid-term exposure to sub-lethal UIA was examined. In the chronic examination, the fish were exposed to different levels of sub-lethal ammonia; control (0.0008 mg/l UIA), 10% of LC_{50} (0.05 mg/l UIA), 5% of LC_{50} (0.025 mg/l UIA) and 1% of LC_{50} (0.005 mg/l UIA) for 30 days. Final length, final weight, weight gain, specific growth rate, daily weight gain and body weigh index decreased significantly in the fish exposed to 0.05 and 0.025 mg/l UIA. According to the results, conversion ratio and condition factor indices were higher in the fish exposed to 0.005 mg/l UIA and control group compared with the fish exposed to 0.05 and 0.025 mg/l UIA.

Article history:
Received 29 April 2018
Accepted 10 December 2018
Available online 25 December 2018

Keywords:
Sturgeon
Ammonia
LC_{50}
Growth performance
Toxicity

Introduction

Sturgeons' rearing centers have been developing rapidly because catching the sturgeons has dramatically declined in the recent years. These species are reared in intensive and semi-intensive ponds, thus, water ammonia elevation is one of negative factors which can affect growth performance parameters of fish (El-Shafai et al., 2004). Agricultural and industrial run off, and decomposition of organic and biological compounds are the external sources of ammonia (Barbieri and Bondioli, 2015; Benli et al., 2008). However, if the fish rearing water is un-polluted, ammonia elevation in intensive fish culture is related to protein catabolism of fish (Walton and Cowey, 1977; Hasanalipour et al., 2013).

In aquatic environments, there are two forms of ammonia, ionized and un-ionized (UIA), which the un-ionized ammonia is more toxic to fish. Transformation of the ionized and un-ionized ammonia forms depend on water temperature and pH (Emerson et al., 1975). Catabolism of protein in fish leads mostly to un-ionized ammonia production, which is mainly excreted by gills (Wilkie, 1997). Although ammonia has a great affinity to be ionized, there is an equilibrium between ionized and un-ionized ammonia transformation in aqueous environment. For instance, one unit pH elevation lead to 10 folds increase in coefficient of ionized to un-ionized ammonia transformation (Emerson et al., 1975). The ammonia accumulation in intensive aquaculture is particularly unavoidable because of high protein catabolism (Person-Le Ruyet et al., 1995; Handy and Poxton, 1993). Ammonia elevation may affect the fish growth and reproduction performance parameters (Guan et al., 2010; Ip and Chew, 2010).

In aquaculture, it is important to know the concentrations of toxic ammonia under certain water temperature and the other physic-chemical conditions. Also, Fish vulnerability to ammonia is depend on their species, age and size. There are several studies on the ammonia toxicity in fishes (Barbieri and Bondioli, 2015; Benli et al., 2008; Dong et al., 2013; El-Shafai

*Corresponding author: Shahram Naghizadeh Raiesi
E-mail address: shahram9112006@outlook.com
DOI: https://doi.org/10.22034/ijab.v6i6.425

et al., 2004; Lemarie et al., 2004; Person-Le Ruyet et al., 1997; Rodrigues et al., 2014; Wajsbrot et al., 1991). Reporting different range of ammonia safe concentrations in several studies confirm that it is depend on different fish species and physic-chemical conditions. Therefore, having information on UIA susceptibility of each species is essential. Although there are some reports on susceptibility of some sturgeon species to ammonia such as Siberian sturgeon, *Acipenser baeri* (Salin and Williot, 1991) and shortnose sturgeon, *A. brevirostrum* (Isely and Tomasso, 1998), there is a lack of information on susceptibility of Persian sturgeon, *A. persicus* to water ammonia. Considering that Persian sturgeon is cultured in Iran, determining the susceptibility of this species to water ammonia seems crucial.

Materials and Methods

Ammonia preparation: To determine the water UIA concentration, water pH and temperature should be controlled. Water pH was fixed at 8.2 with KOH (0.1 N) and water temperature was fixed at 25°C with heater (Aquent co, USA). NH_4Cl (Merck, Darmstadt, Germany) was used to prepare different concentrations of the ammonia. Stock ammonia solution was prepared for each treatment. Water total ammonia was measured based on Le and Boyd (2012) and the proportion of ionized and UIA was determined according to water temperature and pH (Emerson et al., 1975).

Determination of un-ionized ammonia median lethal concentration (LC_{50}): A total of 300 Persian sturgeon fingerlings (5.52±0.45 g) were provided from the Shahid Marjani Sturgeon Hatchery (Golestan Province, Iran). The fish were stocked in three 900L tanks (1.5×1.5×0.4 m). After one week acclimation, susceptibility of these fish to un-ionized ammonia was studied. 144 fish were distributed into 18 aquaria with 60 L volume (0.5×0.3×0.4 m) and acclimatized to the experimental conditions for another one week. All aquaria were aerated continuously during experimental period (DO: 6.2±1.11 mg/l). During adaptation, the fish were fed with commercial feed (Energy, Mahiran co, Iran). Feeding was performed 3% of the fish biomass twice a day. To determine ammonia median lethal concentration (LC_{50}), the fish were exposed to six concentrations of ammonia (0.16, 0.31, 0.62, 0.94 and 1.25 mg/l UIA and a control group) for 96 hours, which assigned in three replication for each treatment. The fish were exposed to the aforementioned ammonia concentrations in semi-static condition, during which, water was daily renewed and required amounts of ammonia were added to each aquarium to keep the ammonia concentration constant. Feeding was ceased 24 h before ammonia exposure and thereafter. Fish mortality, clinical signs and behaviors were recorded every 12 h (at 8 am and 8 pm). During the trial, the water hardness was 267±5.48 mg/l. Temperature, dissolved oxygen and pH were controlled at 25±1°C, 6.2±1.11 mg/l and 8.2±0.11, respectively.

Mid-term exposure to sub-lethal concentrations of ammonia: Chronic experiment was conducted according to the LC_{50}, and fish were exposed to 1, 5 and 10% of 96-h-LC_{50}. In this regard, 120 fish were randomly divided into 12 aquaria with 45 L water. After one week acclimation period, the aquaria were classified into four groups (triplicate per treatment). No ammonia was added to control group (un-ionized ammonia determined 0.0008 mg/l). The ammonia treated groups were exposed to 0.05 mg/l (10% of 96h LC_{50}), 0.025 mg/l (5% of 96h LC_{50}) and 0.005 mg/l (1% of 96h LC_{50}) un-ionized ammonia for 30 days. During experiment, fish were fed at 3% of biomass twice a day by commercial food (Energy, Mahiran co, Iran). The maintenance conditions are the same as which is mentioned in acute toxicity of the experiment. Fish growth characteristics were recorded every 10 days as follows (El-Husseiny et al., 2008):

Survival rate (SR) = final number of fish ×100 / initial number of fish

Specific growth rate (SGR) = [(Ln final weight - Ln initial weight×100) / days]

Food conversion ratio (FCR) = the total food consumption (g) / weight gain (g)

Weight gain (WG) = final weight - initial weight

Condition factor (CF) = final weight ×100 / (final length)^3

Body weight index (BWI) = (final weight - initial

Table 1. LC_{50}, LOEC and NOEC of un-ionized ammonia in Persian sturgeon fingerlings.

	Concentration (mg/l)	95% confidence limits Upper	Lower	Slope±S.E	Intercept±S.E	LOEC	NOEC
LC_{50}	0.46	0.37	0.50	5.82±0.92	2.17±0.36	0.31	0.16

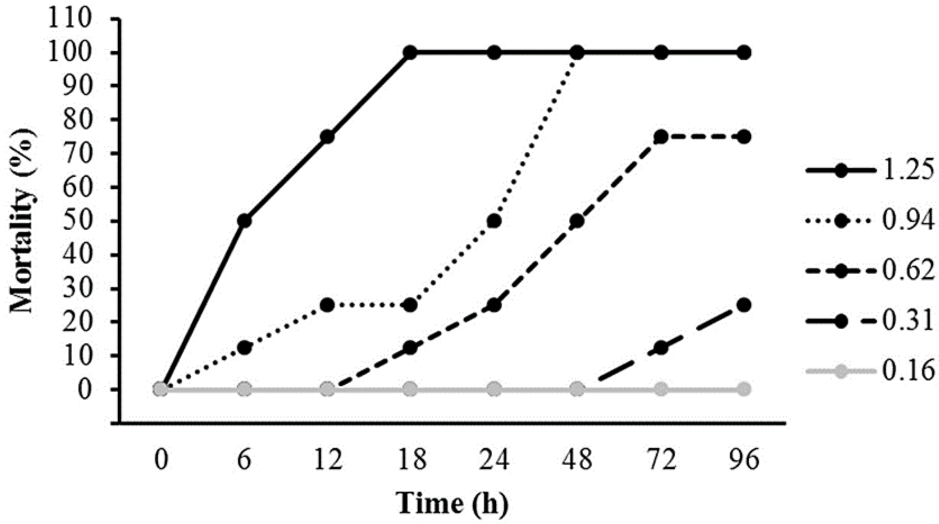

Figure 1. Mortality rate of Persian sturgeon fingerlings during 96 h of exposure to different un-ionized ammonia concentrations (0.16, 0.31, 0.62, 0.94 and 1.25 mg/l).

weight) ×100 / initial weight

Daily weight gain (DWG) = [final body weight (g) - initial body weight (g)] / days

Water physic-chemical characteristics: Water total ammonia was measured by salicylate method (Le and Boyd, 2012). UIA was calculated according to Emerson et al. (1975). Other water physic-chemical characteristics were measured by portable water checker and photometer with commercial kits provided by the manufacturer (Wagtecch Portable Photometer 7100, Berkshire, UK).

Statistical analyses: LC_{50} was determined using SPSS v. 22 software and Probit test. The lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC) were determined according to Rand (1995). Data of Growth parameters were analyzed by using one-way ANOVA and Duncan test to find significant differences. $P<0.05$ was considered as significance. Data are presented as mean ± standard deviation (SD).

Results

The mortality of the fish exposed to different UIA concentrations (0.16, 0.31, 0.62, 0.94 and 1.25 mg/l) during 96 h is presented in Figure 1. There was no mortality in the control group and fish that exposed to 0.16 mg/l UIA. In the 0.31 mg/l UIA treatment, mortality was started at 48 h and reached 25% after 96 h. In the 0.62 mg/l UIA treatment, mortality was started after18 h and it reached 75% up to 72 h post challenge. In the 0.94 mg/l UIA and the highest concentration (1.25 mg/l) treatments, 100% of fish died after 6 hours exposure (Fig. 1). According to the results, 0.46 mg/l (037-0.50 mg/l confidence limits) was calculated as 96-h-LC_{50} of un-ionized ammonia in Persian sturgeon. LOEC and NOEC were 0.31 and 0.16 mg/l, respectively (Table 1).

Behavioral symptoms during acute exposure: The control fish showed normal behavior including normal swimming with no abnormal reactions. The fish exposed to UIA were generally trying to jump out of the aquaria and highly-responsive in the elementary hours of the test. The fish exposed to 1.25 mg/l UIA showed abnormal behaviors such as fast and irregular swimming just after 1 h exposure. Then, they became immobile at the aquaria bottom, followed by loss of equilibrium and imbalance lateral swimming. Fast and irregular swimming was observed in the other

Table 2. Behavioral symptoms of Persian sturgeon fingerlings exposed to different water un-ionized ammonia concentrations.

	Un-ionized ammonia concentration (mg/l)					
Behavioral responses	Control	0.16	0.31	0.62	0.96	1.25
Nervousness	—	+ [5]	+ [5]	+ [5]	+ [5]	+ [1]
Irregular swimming	—	+ [5]	+ [5]	+ [5]	+ [5]	+ [1]
Loss of equilibrium	—	—	—	—	—	+
Imbalance swimming	—	—	—	—	—	+
Fast swimming	—	—	+	+	+	—
Responsive to external stimuli	—	—	+	+	+	—
Bottom sitting	—	+	—	—	—	+ [5]

+: Observed; —: Not observed; + [5]: Observed after 5 h; + [1]: Observed after 1 h.

Table 3. Growth performance of juvenile Persian sturgeon, *Asipenser persicus*, exposed with different level of un-ionized ammonia (UIA) for 30 days.

parameters	**Control**	**0.05 mg/l UIA**	**0.025 mg/l UIA**	**0.005 mg/l UIA**
Initial Weight (g)	6.53±0.81[a]	6.74±0.70[a]	6.71±0.64[a]	6.49±0.61[a]
Final Weight (g)	15.50±0.92[a]	10.19±1.46[d]	10.65±1.91[c]	14.12±1.85[b]
Initial length	12.38±0.82[a]	12.54±0.74[a]	11.98±0.64[a]	12.11±0.72[a]
Final length (mm)	17.21±0.79[a]	13.6±1.08[c]	14.83±1.32[b]	16.74±1.05[a]
Weight gain (g)	8.64±1.22[a]	3.45±1.52[d]	4.60±1.20[c]	7.24±2.91[a]
DWG [1]	0.29±0.04[a]	0.11±0.051[d]	0.15±0.04[c]	0.24±0.16[a]
BWI[2] (%)	125.36±28.57[a]	52.44±2 5.60[c]	66.92±27.41[c]	105.24±43.21[a]
SGR[3] (% day^{-1})	1.18±0.18[a]	0.33±0.28 [c]	0.72±0.29[b]	1.04±0.23[a]
CF [4]	0.31±0.03[c]	0.41±0.0[a]	0.35±0.0[b]	0.30±0.02[c]
FCR [5]	0.94±0.14[c]	2.35±0.34[a]	1.76±0.24[b]	1.11±0.32[c]
Survival rate	100[a]	100[a]	100[a]	100[a]

[a, b, c, d] mean values in the same row with different superscript are significantly different (P<0.05). 1: daily weight gain, 2: body weight index, 3: specific growth rate, 4: condition factor, 5: Feed conversion ratio.

treatments, after 5 h exposure. Also, the fish exposed to 0.31-0.96 mg/l UIA showed fast swimming. In the 0.16 mg/l UIA treatment, the fish showed slow swimming and bottom sitting in the aquaria.

Clinical signs during acute exposure: Opened-mouth and skin petechia was the most frequent clinical signs of the freshly-dead fish. All moribund fish showed moderate hemorrhage at base of pectoral fins and barbels. Great amount of mucus was observed on the skin of fish exposed to ammonia. After 12 h exposure, congestion in intestine and some internal organs was recorded. Accumulation of bloody fluid in the abdominal cavity was also observed in dead fish after 30 h ammonia exposure. Hemorrhage around anus and at the terminal part of the gut was observed in the some moribund fish within 48-96 h post challenge (Table 2).

Growth performance: The growth performance parameters in the Persian sturgeon exposed to different concentrations of unionized ammonia during 30 days are presented in Table 3. No difference was observed in growth performance parameters between control group and the group exposed to 1% 96-h-LC_{50} (fish exposed to 0.005 ppm UIA). Decrease in growth performance parameters was reported by increasing the UIA concentration to 0.025 and 0.05 mg/l. According to the result, most growth parameters (final length, final weight, weight gain, specific growth rate, daily weight gain and body weigh index) decreased significantly (P<0.05) in the fish exposed to 0.05 and 0.025 mg/l UIA. Feed conversion ratio and condition factor were higher in control and 1% 96-h-LC_{50} treatment in comparison with 5% and 10% 96-h-LC_{50} treatments. On the other hand there was significant difference in growth performance parameters between 5% and 10% 96-h-LC_{50} treatments (Table 3).

Discussion

In aquatic environments, dissolved ammonia is presented in two forms, ionized and un-ionized. Un-ionized ammonia has higher permeability in the organism's epitelium, thus, it is more toxic. If it accumulates in high concentrations, it causes severe toxicity in fish (Shingles, 2001; Ip and Chew, 2010). Ammonia concentration can increase to toxic levels in intensive rearing ponds and recirculating water systems. Therefore, exposure to high UIA concentrations in Persian sturgeon is not unlikely. One of the methods to investigate the susceptibility of different fish species to toxicants is the LC_{50} determination. Several studies were conducted on this topic (Ruffier et al., 1981; Wajsbrot et al., 1991; Perso- Le Royet et al., 1995). Different fish species showed different susceptibility to acute un-ionized ammonia exposure. Person-Le Royet et al. (1995) studied on some marine species and reported that 96-h-LC_{50} was 1.79, 2.73 and 3.32 mg/l for sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*) and turbot (*Scophthalmus maximus*), respectively and sea bass was more susceptible than the other species exposed to un-ionized ammonia. 96-h-LC_{50} of ammonia was recorded 0.32 mg/l in fry rainbow trout (*Oncorhynchus mykiss*) (Wajsbrot et al., 1991). Ruffier et al. (1981) found 96-h-LC_{50} of UIA 3.1 mg/l for catfish (*Ichtalarus punctatus*). As mentioned, the sensitivity to UIA is variable depending on the fish species and conditions of fish culture. In the present study, 96-h-LC_{50} of UIA in Persian sturgeon was 0.46 mg/l, and compare to the above studies, it seems that Persian sturgeon fingerlings are high susceptible in exposure to the acute toxicity with UIA.

In the present experiment, after 30 days, 5% and 10% of LC_{50} had no effect on survival rate but they caused the lowest growth performance parameters. Although Persian sturgeon fingerlings can tolerate high concentration of UIA (10% of LC_{50}), their weight gain and growth performance parameters significantly decreased even in lower concentration of UIA (5% of LC_{50}). Several studies show that sub-lethal concentrations of ammonia have negative effects on growth parameters in bony fish. Foss et al. (2003) showed that 96 days exposure to 0.17 mg/l UIA reduced the growth of juvenile Atlantic cod (*Gadus orhua*) significantly. They explained that the growth reduction is attributed to a decrease in food intake. Remen et al. (2007) studied on the interactive effects of oxygen and ammonia on growth and physiological status of juvenile Atlantic cod (*Gadus orhua*). The fish were exposed to nine different combinations of ammonia and oxygen concentrations for 64 days. According to the results, High ammonia concentrations caused a significant decrease in growth throughout the experiment. Although, Foss et al. (2003) showed that 69 days exposure to 0.11 mg/l UIA has no significant effect on growth parameters in juvenile spotted wolffish, *Anarhichas minor*. Because the fish were able to be adapted to the new condition. The effects of chronic and periodic exposure to un-ionised ammonia on growth and food conversion efficiency in juvenile Atlantic halibut, *Hippoglossus hippoglossus* were examined by paust et al. (2011). They reported that chronic medium and high levels of ambient ammonia had a deleterious effect on growth performance but periodic exposure had no significant effect. Alderson (1979) reported the threshold concentration of UIA had no significant effect on juvenile Dover sole (*Solea solea*) and turbot growth. But decreasing pH have a negative effect on the growth of the fish. During last decade sturgeon catch from the Caspian Sea critically has decreased so rearing center for these valuable fish are developing. In this regard, ammonia accumulation in rearing pond is unavoidable and every knowledge abaut its deleterious effect can lead to better culture.

Conclusion

Persian sturgeon fingerlings are sensitive in exposure to UIA moderately and its 96-h-LC_{50} was determined 0.46 mg/l. This fish can tolerate chronic toxicity with sub-lethal concentrations of UIA without any mortality but these concentrations caused decrease in growth performance parameters.

Acknowledgment

The authors gratefully acknowledge Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran, for their financial and commercial support in this project.

References

Alderson R. (1979). The effect of ammonia on the growth of juvenile Dover sole, *Solea solea* (L.) and turbot, *Scophthalmus maximus* (L.). Aquaculture, 17: 291-309.

Barbieri E., Bondioli A.C.V. (2015). Acute toxicity of ammonia in Pacu fish (*Piaractus mesopotamicus*, Holmberg, 1887) at different temperatures levels. Aquaculture Research, 46 (3): 565-571.

Benli A.C.K., Köksal G., Özkul A. (2008). Sublethal ammonia exposure of Nile tilapia (*Oreochromis niloticus* L.): Effects on gill, liver and kidney histology. Chemosphere, 72: 1355-1358..

Dong X., Zhang X., Qin J., Zong S. (2013). Acute ammonia toxicity and gill morphological changes of Japanese flounder Paralichthys olivaceus in normal versus supersaturated oxygen. Aquaculture Research, 44: 1752-1759.

El-Husseiny O.M., El-Din G., Abdul-Aziz M. Mabroke R.S. (2008). Effect of mixed protein schedules combined with choline and betaine on the growth performance of Nile tilapia (*Oreochromis niloticus*). Aquaculture Research, 39(3): 291-300.

El-Shafai S.A., El-Gohary F.A., Nasr F.A., Steen N.P., Gijzen H.J. (2004). Chronic ammonia toxicity to duckweed-fed tilapia (Oreochromis niloticus). Aquaculture, 232: 117-127.

Emerson K., Russo R.C., Lund R.E., Thurston R.V. (1975). Aqueous ammonia equilibrium calculations: effect of pH and temperature. Journal of the Fisheries Research Board of Canada, 32: 2379-2383.

Foss A., Siikavuopio S.I., Sæther B.S., Evensen T.H. (2004). Effect of chronic ammonia exposure on growth in juvenile Atlantic cod. Aquaculture, 237(1-4): 179-189.

Foss A., Evensen T.H., Vollen T., Øiestad V. (2003). Effects of chronic ammonia exposure on growth and food conversion efficiency in juvenile spotted wolfish. Aquaculture, 228: 215-224

Guan B., Hu W., Zhang T.L., Duan M., Li DL., Wang Y.P., Zhu Z.Y. (2010) Acute and chronic un-ionized ammonia toxicity to 'all-fish' growth hormone transgenic common carp (*Cyprinus carpio* L.). Chinese Science Bulletin, 55: 4032-4036.

Handy R.D., Poxton M.G. (1993). Nitrogen pollution in mariculture: toxicity and excretion of nitrogenous compounds by marine fish. Fish Biology, 3: 205-241.

Hasanalipour A., Eagderi S., Poorbagher H., Bahmani M. (2013). Effects of stocking density on blood cortisol, glucose and cholesterol levels of immature Siberian sturgeon (*Acipenser baerii* Brandt, 1869). Turkish Journal of Fisheries and Aquatic Sciences, 13(1): 27-32.

Ip A.Y., Chew S.F. (2010). Ammonia production, excretion, toxicity, and defense in fish: a review. Frontiers in Physiology, 1(1): 134.

Isely J.J., Tomasso J.R. (1998). Acute toxicity of ammonia and nitrite to short nose sturgeon, *Acipenser brevirostrum* fingerlings. North American Journal of Aquaculture, 60(4): 315-318.

Le P.T.T., Boyd C. (2012). Comparison of phenate and salicylate methods for determination of total ammonia nitrogen in freshwater and saline water. Journal of the world aquaculture society, 43: 885-889.

Lemarie G., Dosdat A., Covès D., Dutto G., Gasset E., Person-Le Ruyet J. (2004). Effect of chronic ammonia exposure on growth of European seabass (*Dicentrarchus labrax*) juveniles. Aquaculture, 229: 479-491.

Paust L.O., Foss A., Imsland A.K. (2011). Effects of chronic and periodic exposure to ammonia on growth, food conversion efficiency and blood physiology in juvenile Atlantic halibut (Hippoglossus hippoglossus L.). Aquaculture, 315(3-4): 400-406.

Person-Le Ruyet J., Chartois H., Quemener L. (1995). Comparative acute ammonia toxicity in marine fish and plasma ammonia response. Aquaculture, 136: 181-194.

Person-Le Ruyet J., Delbard C., Chartois H., Le Delliou H. (1997). Toxicity of ammonia to turbot juveniles: effects on survival, growth and food utilisation. Aquatic Living Resources, 10: 307-314.

Person-Le Ruyet J., Galland R., Le Roux A., Chartois H. (1997). Chronic ammonia toxicity in juvenile turbot (*Scophthalmus maximus*). Aquaculture, 154: 155-171.

Rand G.M. (1995 Fundamentals of aquatic toxicology: effects, environmental fate and risk assessment, CRC Press, Washington, DC. 1148 p.

Remen M., Imsland A.K., Stefansson S.O., Jonassen T.M., Foss A. (2008). Interactive effects of ammonia and oxygen on growth and physiological status of juvenile Atlantic cod (*Gadus morhua*). Aquaculture, 274(2-4): 292-299.

Rodrigues R.V., Romano L.A., Schwarz M.H., Delbos B., Sampaio L.A. (2014). Acute tolerance and histopathological effects of ammonia on juvenile maroon clownfish *Premnas biaculeatus* (Block 1790). Aquaculture Research, 45: 1133-1139.

Ruffier P.J., Boyle W.C., Kleinschmidt J.K. (1981) Short-term acute bioassays to evaluate ammonia toxicity and effluent standards. Journal WPCF, Water Pollution Control Fed, 53: 367-377.

Salin D., Williot P. (1991). Acute toxicity of ammonia to Siberian sturgeon, *Acipenser baeri*. Williot, Ed. Acipenser, Cemagref Publ, 153-167.

Shingles A., McKenzie D.J., Taylor E.W., Moretti A., Butler P.J., Ceradini S. (2001). Effects of sublethal ammonia exposure on swimming performance in rainbow trout (*Oncorhynchus mykiss*). Journal of Experimental Biology, 204: 2691-2698

USEPA. (1999). Update of ambient water quality criteria for ammonia- technical version-1999. EPA-823-F-99-024. USEPA, Washington DC, USA.

Wajsbrot N., Gasith A., Krom M.D., Popper D.M. (1991). Acute toxicity of ammonia to juvenile gilthead seabream, *Sparus uuratu* under reduced oxygen levels. Aquaculture, 92: 277-288.

Walton M.J., Cowey C.B. (1977). Aspects of ammoniogenesis in rainbow trout, *Sulmo gairdneri*. Comparative Biochemistry and Physiology, 57: 143-149.

Wilkie M.P. (1997). Mechanisms of ammonia excretion across fish gills. Comparative Biochemistry and Physiology, 118: 39-50.