Histopathological study of gill, kidney and liver of Persian Sturgeon (*Acipenser persicus* Borodin, 1897) and Stellate (*Acipenser stellatus* Pallas, 1811) exposed to sublethal concentration of un-ionised ammonia UAN

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Abstract This study was carried out to investigate the histopathological changes of gill, kidney and liver of Persian Sturgeon and Stellate in response to various sublethal concentration of ammonia. The sublethal doses were determined after a lethal pre-test and then the experiment was done in six treatments with three replicates for both species. In Persian sturgeon, the treatments were including 5, 7, 9.7, 13.2, 18 and 25 mg/l ammonia. In stellate, the concentrations of 5, 6.23, 7.76, 9.66, 12.04 and 15 mg/l ammonia were considered as treatments. Also, one group without ammonia was considered as control for each experiment. The histopathological investigations were done after 96 h exposure. According to our observations, a range of histopathological alternations were found in gill, liver and kidney of both examined fish. The alternations of gill tissue were lamellar necrosis, hyperplasia, lamellar adhesion, haemorrhage, swelling of gill lamellae. Severity of these alternations was dose-dependent and was significantly higher in Persian sturgeon than in stellate. The degree of histopathological alternations of liver did not show a regular pattern between treatments in both species. These alternations were hyperemia, bile record, cell necrosis, cell atrophy, fatty degeneration and cloudy swelling. The signs of kidney alternations intensified with increasing of ammonia concentration in both species. The signs such as hyperemia,

vision tissue degeneration, cell necrosis, dilatation of Bowman's space. In conclusion, our results showed that ammonia in concentration of more than 5 mg/l have more adverse impacts on gill, liver and kidney tissues.

Keywords Ammonia · Histopathology · Gill · Liver · Kidney · Persian sturgeon · Stellate

Introduction

In aquaculture, the control of water quality is necessary to produce sustainable crop. Among aquatic pollutants, ammonia is a common aquatic pollutant which in un-ionized form is toxic to a variety of aquatic organisms including fish (Harris et al. 1998). Ammonia is the principal nitrogenous waste product of fishes and is normally oxidized first to nitrite then to nitrate. It is also, the main nitrogenous waste material excreted by gills beside urea and amines. Un-ionized form of ammonia can readily diffuse through cell membranes and is highly soluble in liquids. It can cause impairment of cerebral energy metabolism, damage to gill, liver, kidney, spleen and thyroid tissue in fish, crustaceans and mollusks (Smart 1978). Knowledge on the concentrations of ammonia that restrict growth, survival and impair respiration is useful for aquaculture management and may assist in the maximization of production (Colt and Armstrong 1981). Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory (Wester and Canton 1991; Thophon et al. 2003; Arellano et al. 2004; Evans et al. 2005; Oliveira Ribeiro et al. 2005; Vigliano et al. 2006; Nascimento et al. 2012) and field studies (Zeeman and Brindley 1981; Hinton et al. 1992; Schwaiger et al. 1997; Teh et al. 1997). The present study was undertaken to determine the sublethal effects of ammonia on

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histology of gill, kidney and liver of Persian sturgeon and stellate. Sturgeons are economically and culturally important in the world and the management of culturing media especially ammonia concentration provides a key to their successful production in aquaculture systems.

Materials and methods

The experiment was conducted in Shahid Beheshti Artificial Sturgeon Propagation and Rearing Center, Iran, Rasht. A total of 760 Persian sturgeon ($n = 360$, TW: 1.6 ± 0.4 g,

TL: 7.3 ± 0.8 cm) and stellate ($n = 360$, TW: 2.4 ± 0.3 g, TL: 10.5 ± 0.6 cm) were considered for the experiment. Before the experiment, a pre-test was done to determine the sublethal doses of ammonia. In this regard, the values of LC_{10} , LC_{50} and LC_{90} were measured after 24, 48, 72 and 96 h expose of fish with various concentrations of ammonia. After pre-test, ranges of 5–25 mg/l ammonia for Persian sturgeon and 5–15 mg/l ammonia were considered as sublethal concentrations and used for the experiment. In the present study, ammonia solution 25 % (Merck Company) and Aqualytic Al250 set were used to regulate and record ammonia concentration in each treatment respectively.

Fig. 1 Cross section (H.E. $\times 40$) of gill of persian sturgeon exposed to various doses of ammonia (a, b: 5 and 7 mg/l, c 9.7 mg/l, d 13.2 mg/l, e 18 mg/l, f 25 mg/l). (la lamellar adhesion, hp

hyperplasia, he haemorrhage, Hm hyperemia, nc lamellar necrosis, lat lamellar atrophy, sw swelling of gill lamellae)

Fig. 2 Cross section (H.E. $\times 40$) of gill of stellate exposed to various doses of ammonia (**a**, **b** 5 and 6.23 mg/l, **c** 7.76 mg/l, **d** 9.66 mg/l, **e** 12.04 mg/l, **f** 15 mg/l). (*la* lamellar adhesion, *hp* hyperplasia, *he*

haemorrhage, *Hm* hyperemia, *nc* lamellar necrosis, *lat* lamellar atrophy, *sw* swelling of gill lamellae, *cl* lamellar clubbing)

The experiment was conducted in 42 tanks (21 tank for Persian sturgeon and 21 tanks for stellate) containing 25 lit of dechlorinated and gentle aerated water in each of them. Both species were treated with various concentrations of ammonia as 6 experimental treatments and one control group with three replicates. The experimental treatments were: for Persian sturgeon: T₁: 5, T₂: 7, T₃: 9.7, T₄: 13.2, T₅: 18, T₆: 25 mg/l ammonia and for stellate: T₁: 5, T₂: 6.23, T₃: 7.76, T₄: 9.66, T₅: 12.04 and T₆: 15 mg/l ammonia. The control group was water without

ammonia. To each experimental tank, a set of 10 fish were added. Also, no feeding was done during the experiment to avoid production of endogenous ammonia by fish samples.

The histology of gill, kidney and liver was investigated after 96 h experiment. The tissue samples were fixed in Bouin's solution, embedded in paraffin wax, sectioned at 5–7 mm thick, and stained with hematoxylin and eosin. At the end, the slides of tissue samples were investigated under a light microscope at $\times 200$ magnification.

Fig. 3 Cross section (H.E. $\times 40$) of liver of persian sturgeon exposed to various doses of ammonia (**a, b** 5 and 7 mg/l, **c** 9.7 mg/l, **d** 13.2 mg/l, **e** 18 mg/l, **f** 25 mg/l). (*br* bile record, *hm* hyperemia, *he* haemorrhage, *nc* cell necrosis, *cat* cell atrophy)

Results

Light microscopic examination of the gills shows the arrangement of damages in response to ammonia concentrations in Persian sturgeon (Fig. 1) and stellate (Fig. 2). These damages were: lamellar necrosis, hyperemia, hyperplasia, lamellar atrophy, lamellar adhesion, haemorrhage, swelling of gill lamellae. Severity of these histological alternations was intensified with increasing of ammonia concentration. As well as, the degree of histological damages was higher in Persian sturgeon than in

stellate. In liver level, some alternations including hyperemia, haemorrhage, bile record, cell necrosis and cell atrophy were observed in both of stellate (Fig. 4) and Persian sturgeon (Fig. 3), although the fatty degeneration and cloudy swelling was observed in stellate as well. In kidney level (Figs. 5, 6), the damages were very little compared to gill. The most alternations were: hyperemia, tissue degeneration, cell necrosis, dilatation of Bowman's space and homosidrinin. The signs of kidney alternations intensified with increasing of ammonia concentration in both species.

Fig. 4 Cross section (H.E. $\times 40$) of liver of stellate exposed to various doses of ammonia (a,b: 5 mg/l and 7 mg/l; c: 9.7 mg/l; d: 13.2 mg/l; e: 18 mg/l; f: 25 mg/l). (br bile record, he haemorrhage, hm hyperemia, nc cell necrosis, cat cell atrophy, ft fatty degeneration, cw cloudy swelling)

Dissection

Ammonia is widely recognized as a common pollutant in aquatic environments. In aquaculture, the high levels of ammonia limits growth and impair respiration and damages gill, liver, kidney, spleen and thyroid tissue. Therefore, the control of ammonia concentration and reorganization of its adverse effects on fish is necessary.

In the present study, fish showed various histopathological changes in gill, liver and kidney tissues dependent on the concentration of water ammonia. In both examined fish, the severity of histopathological alternations increased with elevation of ammonia concentration. Histological study of the gills shows some alternations including lamellar necrosis, hyperplasia, lamellar adhesion, haemorrhage and Oedema of gill lamellae. Oedema of gill

Fig. 5 Cross section (H.E. $\times 40$) of kidney of persian sturgeon exposed to various doses of ammonia (**a**, **b**: 5 and 7 mg/l, **c** 9.7 mg/l, **d** 13.2 mg/l, **e** 18 mg/l, **f** 25 mg/l). (*bd* dilatation of Bowman's space,

he haemorrhage, *hm* hyperemia, *nc* cell necrosis, *ng* glomerular necrosis, *id* degeneration of interregional tissue, *hc* hemosiderin)

lamellae is very common gill changes in all studied species and can be considered as defense responses to toxic agents.

The increased number of mucous cells in the epithelium of lamellae (hyperplasia) is also very common and has been associated with an increase in the surfactant layer that protects the gill lamellae (Temmink et al. 1983). Necrosis of the gill epithelium observed in this study reflects the direct effect of ammonia (Temmink et al. 1983; Garcia-Santos et al. 2007). Generally, this tissue alternation occurs in high levels of ammonia. The Lamellar blood congestion was another alternation that observed in our study. This alteration can harm the gas exchange function of the gill tissue causing destruction of the pillar cell system, with loss of their support capacity and consequently structural disorder of the lamellae.

In kidney level, some damages were observed in response ammonia toxicity including hyperemia, tissue degeneration, cell necrosis, dilatation of Bowman's space, hemosiderin. Likely, hemosiderin is a result of hemorrhage and phagocytose of died red blood cells by white blood cells. In liver level, some alternations including hyperemia, bile record, cell necrosis and cell atrophy were observed in

both of stellate and Persian sturgeon, although the fatty degeneration and cloudy swelling was observed in stellate as well. The increase in blood flow in liver tissue (hyperemia) may be due to the presence of metabolites arising from ammonia impacts.

According to our results, the severity of histological damages was higher in Persian sturgeon than in stellate. This probably shows that the Persian sturgeon is more sensitive to ammonia than stellate. Overall, histological alterations in response to environmental changes such as ammonia can represent adaptive strategies for conservation of some physiological functions in the face of a stressor. This can trigger a series of endocrine responses, together called stress response, a mechanism that permits the fish to preserve its health when faced with threats from stressors.

In conclusion, our results showed that Un-ionized form of ammonia has various damaging impacts on gill, kidney and liver tissue depending on its concentration. As well as, the Persian sturgeon is more sensitive to ammonia toxicity than stellate. Thus, the control of ammonia concentration is very important in sturgeon nursery pounds especially for Persian sturgeon.

Fig. 6 Cross section (H.E. $\times 40$) of kidney of stellate exposed to various doses of ammonia (**a, b**: 5 and 7 mg/l, **c** 9.7 mg/l, **d** 13.2 mg/l, **e** 18 mg/l, **f** 25 mg/l). (*bd* dilatation of Bowman's space, *he* haemorrhage, *hm* hyperemia, *nc* cell necrosis, *ng* glomerular necrosis, *hc* homosidrinin)

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