Effect of hypoxia, normoxia and hyperoxia conditions on gill histopathology in two weight groups of beluga (*Huso huso*)

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ABSTRACT

The influence of dissolved oxygen concentration on gill histopathology of great sturgeon (*Huso huso*) was evaluated in two weight classes (initial weight 280.9±49.2 g and 1217.9±138.1 g respectively). Oxygen treatments included hypoxia (2-3 mg/l), normoxia (5-6 mg/l) and hyperoxia (9-10 mg/l). The fish were acclimated to experimental tanks for one week then randomly distributed into 9 tanks in each of the initial weight classes (3 and 6 fish per tank in higher and lower initial weight classes respectively) for 8 weeks. In order to find the histopathological changes, gill samples were collected, dehydrated through ethanol series, embedded in paraffin, sectioned at 7 µm thickness using a Leitz microtome and stained with H & E. No mortality was observed over the 8 weeks of the experimental period. There were significant differences in weight and feed intake between treatments in the both weight classes (P<0.05). Fork length showed significant differences in lower initial weight class (P<0.05). The main histopathological changes were observed in gills including: Hyperplasia, loss of secondary lamellae, hemorrhage and congestion in primary and secondary lamellae, lamellar fusion, epithelial lifting in secondary lamellae, clubbing of secondary lamellae, telangiectases, increase in melanin pigments and numerous vacuoles in primary and secondary lamellae (in hyperoxia treatment). All these lesions may reduce gill functional surface of gaseous exchange, impairing respiratory function.

Keywords: Fish, sturgeon, hypoxia, normoxia, hyperoxia, gill

INTRODUCTION

The internal environment of fish is separated from external environment by a delicate gill epithelium (Cengiz, 2006). Fish gill come into immediate contact with the environment and it is generally considered as a good indicator of water quality, being a model for studies of environmental impact (Velmurugan et al., 2007).

Exposure to both hyperoxia (high levels of dissolved oxygen, here 9-10 mg/l) and hypoxia (low levels of dissolved oxygen, here 2-3 mg/l) may be damaging, resulting in suboptimal growth and hence lower biomass production and in fish, exposure to hyperoxia can induce a reduction in gill ventilation and elevate the partial pressure of CO₂ in the blood, resulting in a respiratory acidosis and chloremia (Heisler, 1993). The respiratory acidosis may be compensated within days, but short-term exposure to hyperoxia may cause gill oxidative cell damage (Brauner et al., 2000).

Great sturgeon is a valuable fish in terms of meat and caviar production and it is one of the most common species of sturgeon cultured in Russia, Eastern Europe, Japan, and Iran (Rafatnezhad and Falahatkar, 2011).

Long-term effects of exposure to hyperoxia and hyperoxia are less known. So, the
objective of the present study was determination of response of great sturgeon \textit{(Huso huso)} to chronic hypoxia, normoxia and hyperoxia by examining effects on gill histology.

**MATERIALS AND METHODS**

Fish of two weight classes were studied in the International Sturgeon Research Institute (Guilan province, Iran) including 54 fish with an average weight of 280.9 ± 49.2 g and fork length of 35.7 ± 0.3 cm (lower initial weight class) and 27 fish with an average weight of 1217.9 ± 138.1 g and fork length of 55.8 ± 0.4 cm (higher initial weight class). The fish were kept in 18 fiberglass tanks (diameter= 1m, water depth = 30 cm, surface area= 0.8 m² and volume=500L) at a water temperature of 18 ± 0.7 ºC, Nitrite = 0.02 ± 0.01mg/l, Fe=0.025 ± 0.001 ppm, NH4=0, pH=6.4 and controlled photoperiod (6D: 18L). Fish were fed at a rate of 1-2% of their body weight per day (44.8% crude protein). Oxygen treatments including hypoxia (2-3 mg/l), normoxia (5-6 mg/l) and hyperoxia (9-10 mg/l) were set up by controlling inflowing water and using systems equipped with oxygen enriching for Hyperoxygenation (pure oxygen injected). Hypoxic conditions were obtained by reducing the water inflow (without reducing water volume in the tank) to a level by which the fish oxygen consumption exceeded the oxygen supply. Oxygen saturation decreased gradually from 6 mg O₂/l to 2-3 mg O₂/l. The inflowing water in all treatments were the same as in hypoxia. In normoxia treatment the oxygen saturation set up by controlling aeration system. Fish were acclimated to experimental tank for one week then randomly were distributed into 9 tanks for each initial weight class and kept for 8 weeks in this situation. 3 specimens per tank were removed for biometric measurements and then were necropsied for sampling. Gill tissues were collected and fixed by immersing in Bouin’s fluid for 24 h, then were dehydrated through ethanol series, cleaned with xyline and embedded in paraffin. Samples were serially sectioned at 7 µm thickness using a Leitz microtome and mounted on glass slides. Sections were dried for 24 h then stained with haematoxylin and eosin (H&E) and with Periodic Acid Schiff (PAS) (Lillie, 1954). Histopathological changes in the gill tissues were observed under light microscope (Olympus DX51) and then photographed by camera (Olympus DP12).

**RESULTS**

No mortality was observed over the eight weeks of the experimental period (Table 1). Oxygen levels influenced growth parameters of beluga. Mean weight of the fish showed significant differences at the end of experiment (P<0.05, Table 1). There were significant differences in feed intake between treatments in both groups (P<0.05, Table 1). In the lower initial weight class, oxygen treatments showed significant differences in fork length (p<0.05, Table 1). Fig. 1 shows gill structure at pre trial period in both weight classes. At the end of experiment fish showed several histological alterations (Figs. 2-3).

![Fig.1. Gill structure at pre trial period. A. Lower initial weight class (H&E, 400X). B. Higher initial weight](image)
Fig. 2. Gill structure in higher initial weight class. A. Hypoxia treatment: clubbing of secondary lamellae (a) - hemorrhage (b) edema in the basement membrane of secondary lamellae (c) – hyperplasia and cell proliferation with thickening of gill primary lamella epithelium (d) - increase of melanin pigments in primary lamellae (e) curling of secondary lamellae (f); (H&E, 400X). B. Normoxia treatment- congestion in primary lamellae (a) - congestion in secondary lamellae (b) proliferation of cartilage in primary lamellae (c). C. Hyperoxia treatment - edema in the basement membrane of secondary lamellae (a) – hyperplasia (b) – Onset of clubbing of secondary lamellae (c) - increase of melanin pigments (d) – severe congestion in primary lamellae (e)- congestion in secondary lamellae (f) – shortening of secondary lamellae (g), telangiectases (h); (H&E, 750X).

Fig. 3. Gill structure in lower initial weight class. A. Hypoxia treatment - severe congestion of secondary lamella (a) - hemorrhage (b) - hyperplasia (c) - increase of melanin pigments in primary lamellae (d). Loss of secondary lamellae (e) shortening of secondary lamellae (f). B. Normoxia treatment - congestion in gill primary lamellae (a) - congestion in secondary lamellae (b) - epithelial lifting in secondary lamellae (c). C. Representative light micrographs of gills in hyperoxia treatment - lower initial weight - vacuoles (a) - clubbing of secondary lamellae (b) - congestion in primary lamellae (c) - congestion in secondary lamellae (d) - hyperplasia (e); (H&E, 750X).
Table 1- Some growth parameters in beluga (*Huso huso*) after 8 weeks rearing under different oxygen levels.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>Initial fork length (cm)</th>
<th>Final fork length (cm)</th>
<th>Feed intake (g)</th>
<th>Survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower initial weight group</td>
<td>Hypoxia</td>
<td>282.9 ± 12.8</td>
<td>396.8 ± 14.7 *</td>
<td>35.2 ± 0.5</td>
<td>40.2 ± 0.6 *</td>
<td>1004.7 ± 10 b</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Normoxia</td>
<td>276.9 ± 11.6</td>
<td>400.6 ± 28.4 *</td>
<td>35.9 ± 0.5</td>
<td>42.6 ± 0.8 *</td>
<td>1493.8 ± 18.9 a</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Hyperoxia</td>
<td>282.9 ± 11.0</td>
<td>522 ± 23.3 *</td>
<td>36.0 ± 0.4</td>
<td>42.2 ± 0.4 *</td>
<td>1541.8 ± 70.1 a</td>
<td>100</td>
</tr>
<tr>
<td>Higher initial weight group</td>
<td>Hypoxia</td>
<td>1177.2 ± 43.1</td>
<td>1272.8 ± 55.2 *</td>
<td>55.7 ± 0.8</td>
<td>58.8 ± 0.7</td>
<td>634.1 ± 44.3 c</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Normoxia</td>
<td>1194.7 ± 51.4</td>
<td>1420.2 ± 67.2 *</td>
<td>56.2 ± 0.9</td>
<td>59.9 ± 1.0</td>
<td>1695.7 ± 68.9 a</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Hyperoxia</td>
<td>1292.9 ± 38.0</td>
<td>1721.4 ± 75.8 *</td>
<td>56.1 ± 0.7</td>
<td>58.2 ± 1.5</td>
<td>2203.9 ± 145.5 b</td>
<td>100</td>
</tr>
</tbody>
</table>

Means identified by a different superscript in the columns (a, ab, b and c) were significantly different (P < 0.05) as determined by ANOVA and Tukey’s test for each parameter; Values are mean ± SE.

DISCUSSION

In the present study fish attained the highest length and maximum weight in hyperoxia treatment. Similar results were reported by Thorarensen, *et al.*, 2010 who found that the growth of halibut, *Hippoglossus hippoglossus* progressively increased when the oxygen saturation increased from 57% to 100%.

There is some evidence that moderate hyperoxia may improve the growth of fish (Foss *et al.*, 2003; Dabrowski *et al.*, 2004; Hosfeld *et al.*, 2008), while Person-Le Ruyet *et al.* (2002) reported that after exposure to O₂-supersaturation for 30 days juvenile turbot showed no significant differences in feed intake and growth. On the other study Ritola *et al.* (2002) reported that none of the episodic hyperoxia treatment or continuous hyperoxia caused mortality or resulted in better growth in rainbow trout, *Oncorhynchus mykiss*.

The results of the present study are in agreement with those of several other studies, which indicate that oxygen saturation close to 100% or even higher required to support the maximum growth (Buentello *et al.*, 2000; Crampton *et al.*, 2003). The results from the present study clearly showed reduced feed intake and growth in *H. huso* in hypoxia treatment. This is in agreement with previous findings that hypoxia affects both the appetite and growth rate of fish (Wang *et al.*, 2009). Decreased feed utilization might be an indicator of the higher levels of stress (Rafatnezhad and Falahatkar, 2011) and it could be an indirect mechanism by which prolonged hypoxia reduces growth and may be a way to reduce energy and thus oxygen demand (Pichavant *et al.*, 2001).

In this study the most common morphological anomalies was hyperplasia. Several other authors reported hyperplasia (e.g., Korai *et al.*, 2010; Good *et al.*, 2010; Barillet *et al.*, 2010 and Sharifpour *et al.*, 2011). Gill hyperplasia might serve as a defensive mechanism leading to a decrease in the respiratory surface and an increase in the toxicant-blood diffusion distance (Cengiz, 2006). Physiological effects of gill hyperplasia may be related to hypoxia.

Numerous vacuoles were observed in the hyperoxia treatment in primary and secondary lamellae. In the other studies Olson *et al.* (1973) reported appearance of vacuolated epithelial cells after exposure to either mercuric chloride or methyl mercury in rainbow trout and De Silva and Samayawardhena (2002) reported increased vacuolation after exposure to chlorpyrifos in guppy, *Poecilia reticulata*.

Accidents and poor control of oxygenation and poor management of ponds may result in extreme saturations of oxygen, and gas bubble disease may be a consequence (Edsall and Smith, 1991; Lygren *et al.*, 2000; Salas-Leiton *et al.*, 2009).

The edema, epithelial lifting as well as lamellar fusion are defense mechanisms that reduce the branchial superficial area in contact with the external milieu. These mechanisms also increase the diffusion barrier to the pollutant (Laurén & McDonald 1985, Van Heerden *et al.* 2004). Arellano *et al.* (1999) demonstrated that
separation of epithelium on the lamellae increases the distance across which waterborne pollutants must diffuse to reach the bloodstream thus edema with lifting of lamellar epithelium could serve as a mechanism of defense. Epithelial lifting may be a consequence of a decrease in the gill surface area imposed by the need to maintain osmotic stability while sustaining the progressive loss of epithelial cells (Abel & Skidmore, 1975) or probably induced by the incidence of severe edema (Arellano et al. 1999, Pane et al. 2004, Schwaiger et al. 2004).

In the present study, the gill secondary lamellae were shortened, reducing the functional surface of the organ. Shortening of secondary lamellae was reported after exposure to zeta cypermethrin in Lebistes reticulatus (Caliskan et al., 2003) and sub-lethal nitrate concentrations in Cobia, Rachycentron canadum (Rodrigues et al., 2011). Melano-macrophage centers are distinctive groupings of pigment-containing cells (Agius & Roberts, 2003) and there are some reports of their occasional occurrence in gills, brain and gonads (Macchi et al., 1992).

In the present study the increase of melanin pigments were observed in primary and secondary lamellae in hypoxia and hyperoxia treatments. In the present study, secondary lamellar clubbing was observed in hypoxia and hyperoxia treatments. It has been reported after acute exposure to environmental nitrite in 96 h in Siberian sturgeon (Gisberta et al., 2004), juvenile turbot (Psetta maxima, L.) and after sublethal concentrations of ozone-produced oxidants in ozonated seawater (Reiser et al., 2010, 2011).

Nonspecificity of branchial alterations suggest the primarily represent stereotyped physiological reactions of gills to stress, and many of them are logically considered defense responses (Mallatt, J., 1985). All these lesions may reduce gill functional surface for gaseous exchange, impairing respiratory function.

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اثر آسیب شناسی سطوح مختلف اکسیژن بر بافت آبشش در دو گروه وزنی از فیل ماهیان (Huso huso) پوئرتشی ف. باقرزاده لاکاتی، م. ستاری، ع. شریف پور، ر. کاظمی

چکیده
اثر سطوح مختلف اکسیژن بر روی بافت آبشش فیل ماهیان (Huso huso) در دو گروه وزنی (وزن اولیه 20-3 mg/l) Hypoxia (5-6 mg/l) و Hyperoxia (6-5 mg/l) نمود. ماهیان به مدت پیک هفته با تانک های آزمایش سازگار شدند و سپس به صورت تصادفی در 9 تانک در هر گروه وزنی توزیع (برای گروه وزنی بالا 3 ماهی و برای گروه وزنی پایین 6 ماهی در هر تانک) و به مدت 8 هفته در این شرایط تکیه‌گاهی شدند. برای یافتن تغییرات هیستوپاتولوژیک نمونه‌برداری از آبشش صوتی گرفت. نمونه‌های بافتی با روش‌های معمول آگئیری شده و در پارافین قابل گیری شدند. سپس یا در روش همراه کسی منظور شدند. در طول 8 هفته پوئرتشی هیچ تلفاتی مشاهده نشد. اختلاف معنی‌داری در وزن و غنای مصرفی در تیم‌ها در هر دو گروه وزنی مشاهده شد (P<0.05). طول چنگالی اختلاف معنی‌داری در گروه وزنی پایین نشان داد (P<0.05). پیشرفتی عارضه مشاهده شده در نمونه‌ها شامل هیپیریزی از بين رفت و نیش نارسایی، خونریزی و پرحوری در نیش های اولیه و ثانویه، جسدگی اسید و رنگ‌های مشکوک نشان داده رنگ‌های ثانویه و رنگ‌های اولیه نشان داد. (Hypoxia، Hyperoxia، تلاکترکاری، تورم، تغییر رنگ‌های ملایم و واکنش‌های متعدد در نیش‌های اولیه و ثانویه در تیم‌ها در تمام این آسیب‌ها ممکن است کارایی سطح تبادل گازی آبشش را کاهش داده و عملکرد تنفسی آبشش را مختل کند.

*مولف مسئول