Toxicology Reports 1 (2014) 621–628

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Assessment of gill pathological responses in the tropical fish yellowfin seabream of Persian Gulf under mercury exposure

Leila Hassaninezhad [a], AliReza Safahieh [a], Negin Salamat [a,*], Ahmad Savari [a], Naeem Erfani Majd [b]

[a] Department of Marine Biology, School of Marine Science, Khorramshahr University of Marine Science and Technology, PO Box: 669, Khorramshahr, Iran
[b] Department of Histology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

ARTICLE INFO

Article history:
Received 10 May 2014
Received in revised form 13 July 2014
Accepted 30 July 2014
Available online 13 August 2014

Keywords:
Histopathology
Gill
Mercuric chloride
Acanthopagrus latus

Chemical compounds studied in this article:
Mercuric chloride
$HgCl_2$
Xylan
Ethanol
Periodic Acid-Schiff

ABSTRACT

Gill histomorphological alterations were used to assess the effects of chronic exposure to $HgCl_2$ on the yellowfin seabream, *Acanthopagrus latus*. In this regard, 90 *A. latus* were exposed to sublethal concentrations of $HgCl_2$ (10, 20, 35 and 50 μg/L) for 3 weeks. Treated fish were erratic and showed respiratory distress. The most common morphological abnormalities included: filaments disorganization, increase of mucus secretion, debris and blood plaques on the filaments, losing or shortening of some filaments. The most frequent histopathological changes detected in the gills included extensive lifting of the lamellar epithelium and edema of lamellae with enlarged sub-epithelial spaces, exfoliated epithelium of lamellae, telangiectasia, hypertrophy and hyperplasia of the epithelial cell resulted in partial fusion of the secondary lamellae and a reduction of the water space, club shaping of gill lamellae, blood congestion. Some more severe alternations found in the gill of fish exposed to higher levels of $HgCl_2$ (35 and 50 μg/L) included lamellar aneurysm and hemorrhages with rupture of the lamellar epithelium. According to the results of the present study, mercuric chloride could cause major histomorphological changes in the gill of *A. latus*, decreasing its gas exchange capability. Two mercury concentrations (10 and 20 μg/L) used in the present study were in agreement with the concentration of mercury in the water of different parts of Mahshahr creeks (the north of Persian Gulf) (3.66 to 15 μg/L). Therefore, based on the results the presence of pathological alteration in *A. latus* inhibited in the natural environment (Mahshahr creeks) seems to be logical.

1. Introduction

Mercury is a hazardous toxic metal that naturally exists in the earth's crust. Natural event such as erosion and volcanic eruptions, as well as anthropogenic activities such as industrial, municipal, or agricultural wastes, may lead to significant environmental contamination [1]. In the aquatic ecosystems, a part of the mercury can be converted into methylmercury by biological processes and readily taken up by aquatic organisms. Mercury finds its way up the trophic levels of the food chain, passing along from phyto- and zooplankton to larger organisms. In this way, its concentration increases in natural populations with the age and/or the trophic level of the organism (biomagnification) [2]. Fish are often at the top of the aquatic food chain and may concentrate large amounts of some metals from the water. Increasing amount of mercury in the aquatic environment lead to greater accumulation of this metal in fish tissues, which consider the major source of mercury in human [3].

* Corresponding author. Tel.: +98 916 616 51 46.
E-mail address: salamatnegin@yahoo.com (N. Salamat).

http://dx.doi.org/10.1016/j.toxrep.2014.07.016

To reveal the biological effects of certain contaminants on aquatic bodies, a variety of biomarkers have been used, both in natural environments and/or experimental conditions [4]. Besides the measurement/evaluation of chemical and physical parameters, histopathological biomarkers can be used as indicators of pollutants effects on organisms [5]. One of the most important benefits of using histopathological biomarkers in the environmental screening is possibility of examining specific target organs, including gills, kidney and liver, which are responsible for vital functions, such as respiration, excretion, accumulation and biotransformation of xenobiotics in the fish [6]. Gills are multifunctional organs; beside of respiration, they are responsible for osmoregulation, acid–base balance and nitrogenous waste excretion, which also make them extremely sensitive to water contamination [5]. Irrespective of the accumulation path, the accumulation amount is partially determined by the rate of metal uptake at the interface between the organism and its environment. For aquatic animals, gill is one of typically biological barriers that the respective permeability and retention ability of which determine the potential uptake of mercury from the water [4]. Exposure to chemical contaminants such as mercury compounds can cause number of damages and injuries in different fish organs and gill represents important target organ suitable for histopathological assessment in searching for cells and tissue damages [7].

In the present study, morphohistopathological features were examined in the gill of *Acanthopagrus latus* after exposure to different concentrations of HgCl2 over a period of 3 weeks. The aim of this investigation was to report lesions and damages in gill after an experimental dietary exposure to HgCl2 in the one of the most ecologically and commercially important species of Persian Gulf, yellowfin sea bream (*A. latus*). Mahshahr creeks, situated in the northwest of Persian Gulf and in the south of Iran, is habitat of yellowfin seabream (*A. latus*). This area is received large amounts of different kinds of pollutants such as Hg, Cd, Zn, Cu and Pb discharge into it by surrounding industries located in the coastlines of some branches [8,9]. According to these researches, the concentration of mercury ranged from 3.66 to 15 μg/L in the water of different parts of Mahshahr creeks.

Our experimental method was closely imitated the contamination conditions of tropical marine ecosystems to increase our biological knowledge about this tropical species and the toxicity of $HgCl_2$ in fish inhabit in such polluted regions.

2. Materials and methods

2.1. Fish maintenance

A total of 90 immature male *A. latus* (190 ± 0.1 mm; 150 ± 0.1 g) were obtained randomly from Mahshahr Creeks and then were acclimated for 2 weeks in Imam Khomeini Mari culture Research Station (Imam Khomeini Port, Iran) in fifteen 300 L indoor tanks containing filtered aerated seawater treated with UV. Fish were fed daily with shrimp but were starved for 48 h prior to the experiment and throughout it.

2.2. Experimental design

Following acclimation, fish were randomly placed in fifteen 300 L tanks. Tanks were then divided into one control and 4 experimental groups (each group run in triplicate). Experimental groups exposed to four concentrations of $HgCl_2$ (10 μg/L (group 1), 20 μg/L (group 2), 35 μg/L (group 3) and 50 μg/L (group 4)) for 3 weeks. These sublethal concentrations were selected on the basis of primary range finding tests and determination of LC50-96h for this species.

The tanks were monitored daily and the concentration of mercuric chloride was determined using a standard cold vapor atomic absorption method. The average temperature = 26.5 °C, ph = 7.8 and salinity = 49 ppt. 70% of the water in the tanks was exchange every two days and the $HgCl_2$ concentrations were replaced.

At the end of the experiment, five fish from per tank per group were sampled and the right operculum was taken away and the gills (only the 2nd and 3rd pairs) were quickly and carefully removed to prevent damaging the tissues.

2.3. Tissue processing

The second gill arch of the right side of each fish was taken for morphological study using stereomicroscope (loop). Also pieces of gills (with diameter of 3–5 mm), always from the second gill arch of the right side of the fish, were fixed in Bouin's fluid for 48 h. Tissue samples were then dehydrated in ascending concentrations of ethanol series, embedded in paraffin and sectioned at 5–6 μm. The tissue sections were stained with hematoxylin and eosin (H&E) and Periodic Acid-Schiff (PAS) and then microscopic evaluation was performed for histological and histometerical study by light microscope using Dino lit lens (with Dino capture software, FDP2, Taiwan).

The gill histological changes were classified based on the severity of the lesions as scores of 0 to 3, where 0 = no alteration, 1 = slight alteration, 2 = moderate alteration and 3 = severe alteration according to Poleksic and Mitrovic-Tutundzic [10]. Slight alteration (1) involves alterations, which do not change the normal function of the gill and healing of gill can occur with improvement of the environmental conditions; Moderate alterations (2) are more severe and harm the normal gill function. These elisions are reparable, but they can lead to severe alterations in the case of chronic pollution; and Severe alteration (3) cause irreversible injure which recovery of them is not possible, even with improvement of the water quality (Table 1).

2.4. Histometrical study

Five individuals per group and five slides per each fish were randomly selected for quantitative histometrical analysis. Five fields per slide were examined and the parameters including lamellae length, lamellae epithelial thickness and the number of the mucus and chloride cells were measured. Data were represented as means ± SEM and the significant difference between values was analyzed using ANOVA test.

Table 1
Histopathological changes in the gills of *A. latus* treated with chronic sublethal levels of $HgCl_2$, indicating stages of damage to the tissue. Stage I: do not alter the normal tissue function; Stage II: more severe and damage the normal function of the tissue.

Groups	Morphological alternations	Histological alternations	Stage
Group 1 (10 μg/L HgCl2)	Fusion of some filaments Losing or shortening of some filaments Increase of mucus	Increase of mucus cells Epithelial lifting of lamellae Edema of the lamellae with enlarged sub-Epithelial spaces Club shaping lamellae	I
Group 2 (20 μg/L HgCl2)	Fusion of some filaments Partial losing or shortening of some filaments More increase of mucus Filament disorganization	Epithelial lifting of lamellae Edema of the lamellae Club shaping lamellae Leucocytes infiltration Hyperplasia of the epithelial cells Lamellar fusion Hypertrophy of the lamellar epithelium Blood congestion	I
Group 3 (35 μg/L HgCl2)	Sever disorganization of filaments Severe increase of mucus partial losing or shortening of some filaments	Leucocytes infiltration Hyperplasia of the epithelial cells Lamellar fusion Hypertrophy of the lamellar epithelium Epithelial lifting of lamellae Lamellar aneurysm Lamellar disorganization	II
Group 4 (50 μg/L HgCl2)	Sever disorganization of filaments Severe increase of mucus Debris and blood plaques on the filaments fusion of some filaments complete or partial losing or shortening of some filaments	Leucocytes infiltration Lamellar aneurysm Lamellar disorganization Hyperplasia of the epithelial cells Lamellar fusion epithelium rupture Hypertrophy of the lamellar epithelium Epithelial lifting of lamellae	II

3. Results

Morphological and histological analysis of the gill in the control and experimental groups was conducted to assess the gill histomorphological alterations resulted from exposure to different concentrations of $HgCl_2$. It should be noted that no mortality occurred during the experiment. Treated fish were erratic and showed respiratory distress, though the control fish exhibited a normal swimming behavior.

3.1. Morphological analysis

3.1.1. Control group

In the control fish, gills had a normal morphological structure. Each gill arch supported perpendicularly many distinct and regular filaments arranged in two rows, without any lesions (Fig. 1).

3.1.2. Experimental groups

The most common morphological changes found in the gill of treated fish included: obvious filaments disorganization in groups treated with lower concentrations of $HgCl_2$ (groups 1 and 2) and severe filaments disorganization in those treated with higher levels of $HgCl_2$ (groups 3 and 4), increase of mucus secretion, debris and blood plaques on the filaments, fusion of some filaments, complete or partial losing or shortening of some filaments and swelling of blood vessels (Fig. 2a–h). Gill morphological alterations in the treated fish are detailed in Table 1.

3.2. Light microscopy analysis

3.2.1. Control group

Gills didn't represent any abnormality in the cell and tissue structure. Series of separate and regular lamellae (where gas exchanges and other physiological events occur) sited on the upper and lower surface of each filament

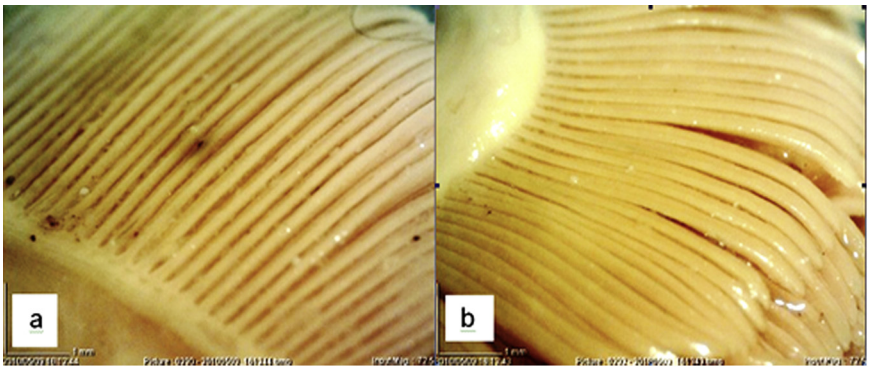

Fig. 1. Morphological structure of the gill within the control group of *Acanthopagrus latus* which possess distinct, regular filaments.

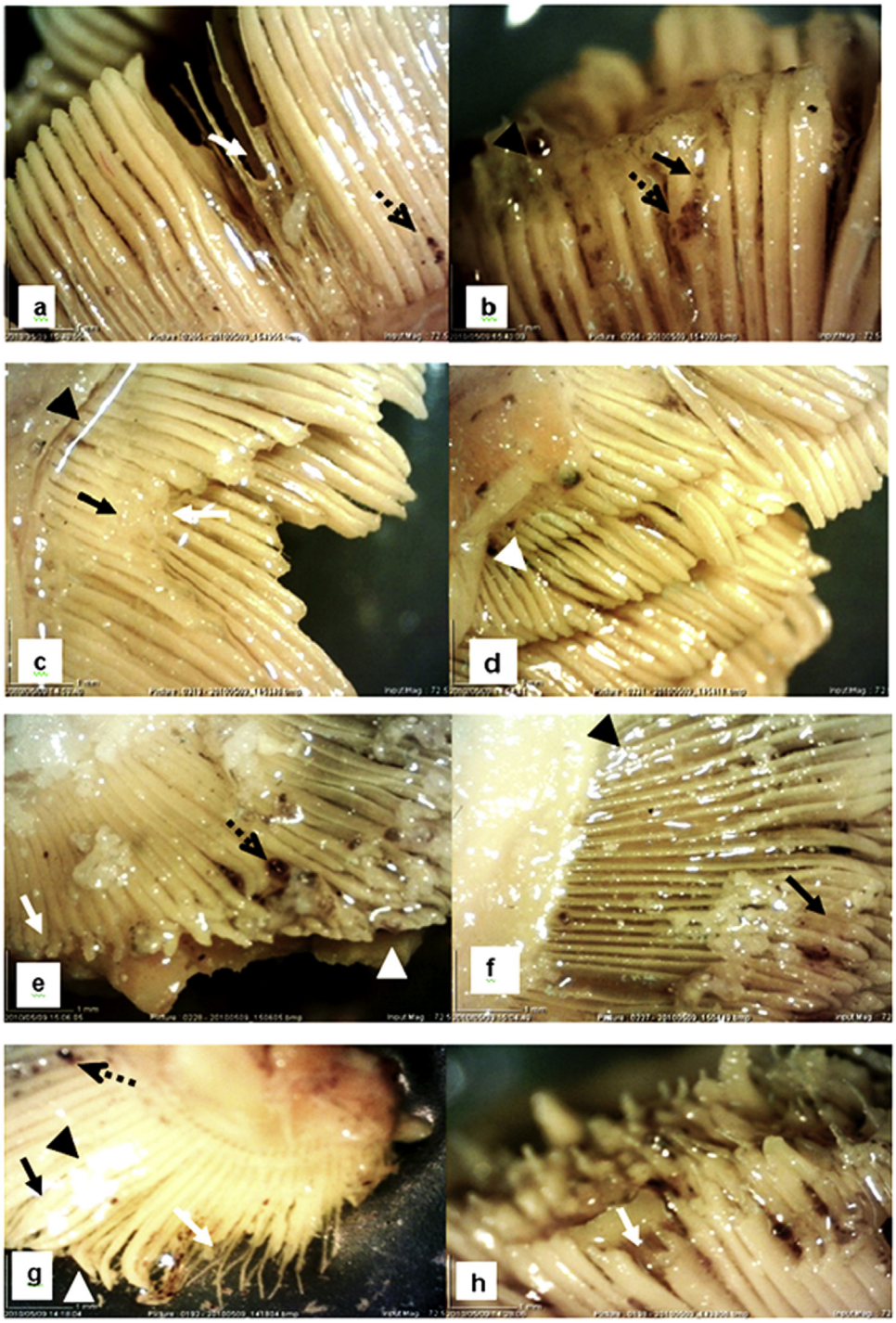

Fig. 2. (a and b) Group 1; fusion of some filaments (black arrow), losing or shortening of some filaments (white arrow), increase of mucus (arrow head), Debris and blood plaques on the filaments (dashed arrow); (c and d) Group 2; fusion of some filaments (black arrow), partial losing or shortening of some filaments (white arrow), more increase of mucus (black arrow head); filament disorganization (white arrow head); (e and f) Group 3; Sever disorganization of filaments (white arrow head), Severe increase of mucus (black arrow head), fusion of some filaments (black arrow), partial losing or shortening of some filaments (white arrow), Debris and blood plaques on the filaments (dashed arrow); (g and h) Group 4; Sever disorganization of filaments (white arrow head), Severe increase of mucus (black arrow head), Debris and blood plaques on the filaments (dashed arrow), fusion of some filaments (black arrow), complete or partial losing or shortening of some filaments (white arrow); (×750).

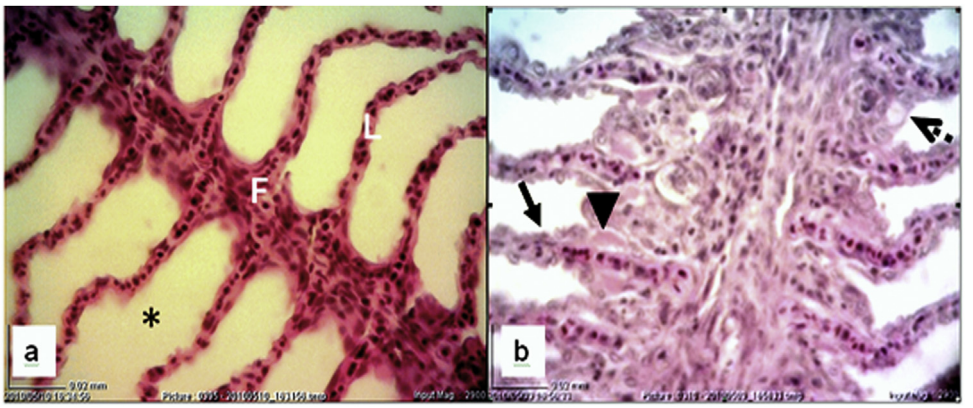

Fig. 3. Normal histological structure of gill within the control group of *Acanthopagrus latus*, (a) Light micrograph showing the normal aspect of the filament which possess distinct regular lamellae: the filament (gray arrow), the lamellae (L), the water channel (*), (H&E; ×750); (b) Different cell types with common numbers and thickness, epithelial cell (black arrow), Chloride cell (black arrow head), mucous cell (dashed arrow). (H&E; ×2900).

(Fig. 3a). The entire length of the filaments and lamellae was lined by normal epithelial cells and chloride cells were large, scattered along the filaments (Fig. 3b). The mucous cells were sparsely distributed in the filament epithelium (Fig. 3b).

3.2.2. Experimental groups

The histopathological changes detected in gills of the $HgCl_2$ exposed fish, detailed in Table 1. The degree of tissue damages of gills within treated groups is determined as stages I and II according to Poleksic and Mitrovic-Tutundzic [10]. The most common abnormalities found included extensive lifting of the lamellar epithelium and edema of the lamellae with enlarged sub-epithelial spaces (Fig. 4a), blood congestion (Fig. 4b), exfoliated epithelium of lamellae (Fig. 4b), dilation of the marginal channel (Fig. 4c), increase of mucosal cells (Fig. 4d), hypertrophy and hyperplasia of the epithelial cell (Fig. 4e and f) resulted in partial fusion of the secondary lamellae (Fig. 4j) and a reduction of the water space, club shaping of gill lamellae (Fig. 4g), Lamellar disorganization (Fig. 4h). Some more severe alternations found in the gill (stage II) were telangiectasia (lamellar capillary aneurism) (Fig. 4k) and hemorrhages (Fig. 4j) with rupture of the lamellar epithelium (Fig. 4b).

4. Discussion

Nowadays, histopathology is known as an important instrument to assess the effects of pollutants in vital processes, detecting early changes in cells, tissues and organs. Histopathological biomarkers have been used in fish to identify and evaluate toxic effects of exposure to pollutants [7]. Due to the harmful effect of pollutants in aquatic environments, the histo-cytological responses of fish to a variety of xenobiotics necessitated to be determined and characterized [11]. The fish gill is a multifunctional organ which participates in many important functions such as respiration, osmoregulation, acid–base balance and nitrogeneous waste excretion. Gill structure provides a large surface area for direct and constant contact with water pollutants. Thus, this organ is too sensitive to chemicals in water [7] and is considered as the primary target organ to the contaminants [6]. Several types of gill impairment have been documented in fish experimentally exposed to contaminants or in those sampled from unhygienic ecosystems [12].

It seems that, the biological characteristics of fish (such as sex and age) or seasonal factors don't affect the response of fish gill to stress/pollutant exposure. Generally, gill histopathology appears to be a promising biomarker for general environmental contamination, although tissue preparation for gill histopathological study is time consuming [7].

According to the results of the present study, mercuric chloride could cause major histomorphological changes in the gill of *A. latus*, decreasing its gas exchange capability. These changes ranged from mild to severe in this fish depending on the concentration of mercuric chloride. As stated by previous studies, the concentration of mercury ranged from 3.66 to 15 μg/L in the water of different parts of Mahshahr creeks (the north of Persian Gulf) [28]. These amounts were in agreement with two mercury concentrations (10 and 20 μg/L) used in the present study. Therefore, based on the results the presence of pathological alteration in *A. latus* inhibited in the natural environment (Mahshahr creeks) seems to be logical.

This compound may result to death in some cases [13]. The death from exposure to $HgCl_2$ is possibly resulted from toxic actions on the biochemical processes associated with cellular metabolic pathways and other inclusions [14]. As the results showed, although *A. latus* is one of the most resistant fish species, even the lower concentrations of $HgCl_2$ influenced the normal structure of gills in this fish. The most of the histopathological alterations of gill described in the present study were in agreement with those reported in other fish species under a broad range of exposure situations, then, it seems that these effects reveal physiological modification to stress rather than as special and restricted toxic responses to the concentrations of $HgCl_2$ considered here.

Changes such as edema with epithelial lifting and desquamation, telangiectasia, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some lamellae as recognized in the present investigation, are usual gill lacerations in response to many other chemicals

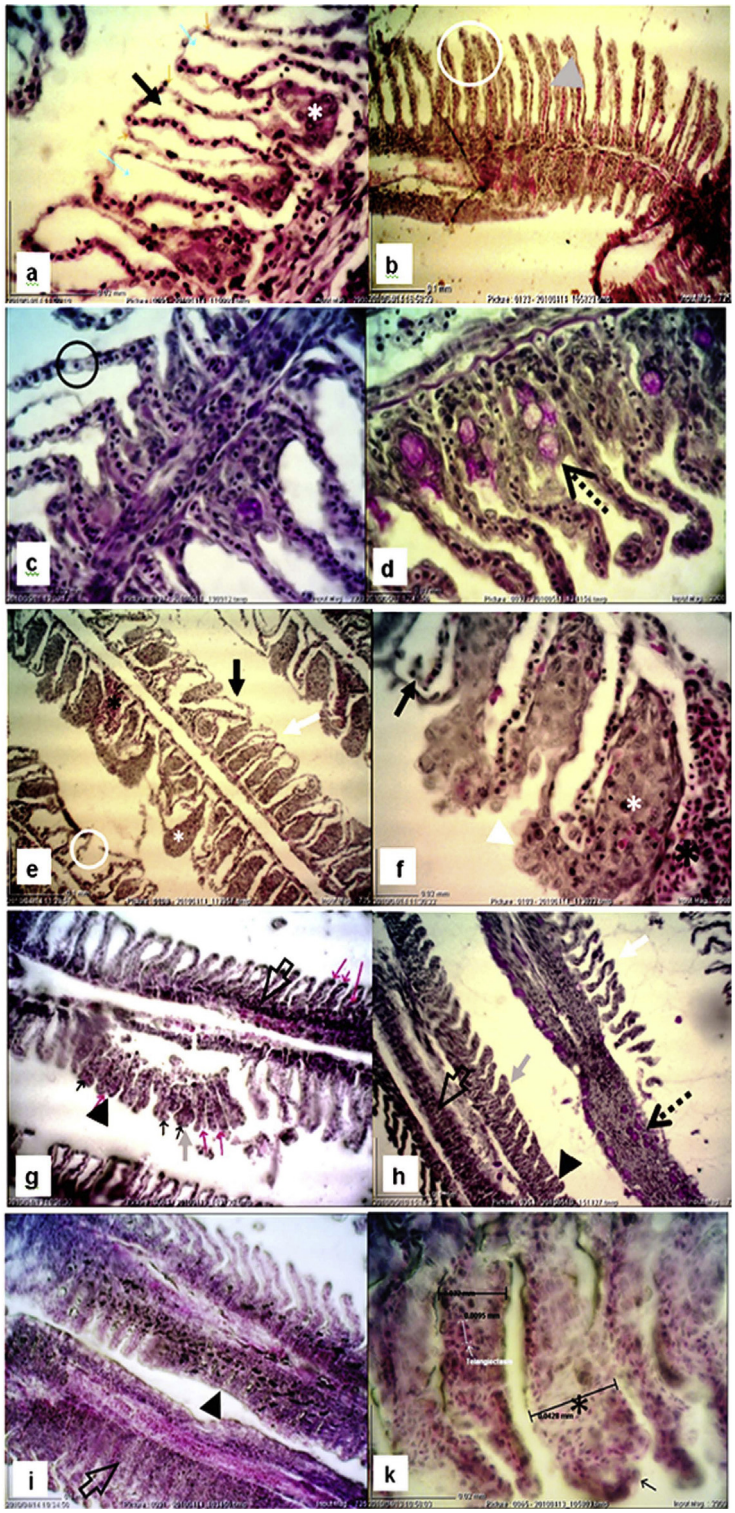

Fig. 4. Photomicrographs of histopathological alternations of gills within the *A. latus* groups exposed to $HgCl_2$; extensive epithelial lifting and edema of the lamellae with enlarged sub-epithelial spaces (black arrows), hyperplasia of the epithelial cells (white *) with partial fusion of the lamellae (black arrowhead), club shaping of gill lamellae (gray arrow), lamellae with the marginal channel dilated (black marked circularly), blood congestion (gray arrowhead), lamellar aneurysm (black *) within the lamellae, lamellar disorganization (white arrow), hypertrophy of the lamellar epithelium (white arrowhead), leukocytes infiltration (hollow arrow), increase of mucosal cells (dashed arrow), epithelium rupture (white marked circularly); (a–c) Sea breams treated with 10 μg/L $HgCl_2$ (group 1); (d–f) Sea breams treated with 20 μg/L $HgCl_2$ (group 2),; (g and h) Sea breams treated with 35 μg/L $HgCl_2$ (group 3); and (j and k) Sea breams treated with 50 μg/L $HgCl_2$ (group 4). (a–c, f and k) (H&E; ×2900); (e, g and i) (H&E; ×725); (d and h) (PAS; ×725).

like petroleum compounds, organophosphates, herbicides and other heavy metals [15]. These are examples of protection mechanisms, which generally result in the increase of the distance between the water and the blood, to act as a barrier to the entry of contaminants [10].

The major alternations in gills of *A. latus* exposed to sublethal concentrations of $HgCl_2$ in the present experiment, were a thickening of the filament epithelium, which filled the space between lamellae in some instances, so lamellae appeared to shorten and disappear and detachment of the lamellar epithelium, or extensive edema, as have been reported upon exposure of mosquito fish (*Gambusia holbrooki*) to mercury(II) [16].

Camargo and Martinez [6], reported epithelial lifting and lamellar fusion in Neotropical fish species *Prochilodus lineatus*, subjected to *in situ* tests for 7 days in a disturbed urban stream. Similar alterations in the gills have also been reported in the fishes exposed to metals [13,17] and organic contaminants [18].

Most of the gill damages resulted from sublethal exposures affected lamellar epithelium [11]; though, when fish bear a more severe type of stress, some alterations in blood vessels may also occur. In situation like this, injured pillar cells can cause an increased blood flow inside the lamellae, leading to dilation of the marginal channel, blood congestion or even an aneurysm [19]. This is a severe type of lesion, recovery from which is possible, but more difficult than the epithelial changes [10]. The rupture of pillar cells in a result of bigger flow of blood or the direct effects of contaminants on these cells, lead to the formation of an aneurysm [16], a severe type of lesion, healing from which is possible, but more difficult than the epithelial changes [10].

In the present study, fishes treated with the higher concentrations of $HgCl_2$ (groups 3 and 4) showed vascular alterations, such as blood congestion and in some cases aneurysm which indicate the critical condition of the water in these groups.

The shortening and progressive disappearance of lamellae observed in the present study, are similar to the responses produced by other metals, including Al [20], Be [16] and Cd [21]. Winkaler et al. [22] described anomalies such as hyperplasia, hypertrophy, dilation of the marginal channel and aneurysm in Neotropical fish, *Astyanax altiparanae*, collected in Cambé stream, the water of which is really polluted and exposure to that causes structural damage to the gill of fish.

Khan et al. [23] reported hyperplasia of epithelial cells of gill filaments, fusion of secondary lamellae giving a club shaped appearance of filaments and contraction and sloughing of respiratory epithelium in fishes treated with $HgCl_2$ at sublethal dose 16 days. Shakoori et al. [24] reported the same results following 48 h of exposure of chinek grass carp to sublethal doses of mercuric chloride. Similar histopathological changes in gills are also reported by Oliveira et al. [25], Anitha and Sree [26] and Hemalatha and Banerjee [27].

Our findings exhibit that, structurally the gills of *A. latus* affect by exposure to $HgCl_2$, the concentration of which influenced the severity of alterations to the tissue and the extent of damages. Also, the results of the present study suggest that morphological and histopathological changes of the gill provide helpful information about the environmental conditions and as particular biomarkers may provide imminent into evaluating the general health and stress status of fish.

Conflict of interest

There is no conflict of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgments

Authors also would like to thank from Khorramshahr University of Marine Science and Technology for practical support.

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