Archives of Razi Institute, Vol. 74, No. 1 (2019) 83-89

Short Communication

Assessment of Gill Pathological Responses in Yellowfin Sea Bream (*Acanthopagrus Latus*) Under *Aeromonas Hydrophila* Exposure

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Received 24 July 2017; Accepted 11 November 2017
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ABSTRACT

Bacterial diseases in cultured fish are considered the main problem with aquaculture system in Iran. The gills are multifunctional organs responsible for respiration, osmoregulation, nitrogenous waste excretion, and acid-base balance. Moreover, they are very sensitive to water contamination. *Aeromonas hydrophila* (*A. hydrophila*) is an opportunist pathogen responsible for a wide range of diseases in different species of fish. The gill histological alterations were used to assess the effects of *A. hydrophila* exposure on yellowfin sea bream, *Acanthopagrus latus* (*A. latus*). In this regard, 90 *A. latus* were exposed to the concentrations of *A. hydrophila* (10^3 and 10^6 CFU/ml) for three weeks. The most histopathological alterations in the gill of the exposed fish included hypertrophy and hyperplasia of the epithelial cells, lamellar fusion, club shaping of gill lamellae, lifting of the epithelium and edema of lamellae with large sub-epithelial space, blood congestion, and hypertrophy and hyperplasia of the mucosal cells. The histopathological alterations were observed in the gill of fish exposed to higher levels of *A. hydrophila* (10^6 CFU/ml) consisted of aneurysm and hemorrhage with blood congestion. According to the obtained results of this study, *A. hydrophila* could cause severe histopathological changes in the gill of *A. latus* and decrease gas change capability in yellowfin sea bream. Furthermore, the findings of the present study suggested that histopathological changes of the gill provide helpful information about the environmental conditions and as particular biomarkers may help the evaluation of fish general health.

Keywords: Histopathological, Gill, *Acanthopagrus latus*, *Aeromonas hydrophila*

Évaluation des réponses pathologiques des branchies chez la pagre à nageoires jaunes (*Acanthopagrus latus*) exposée à *Aeromonas hydrophila*

Résumé: Les maladies bactériennes chez les poissons d'élevage sont considérées comme le principal problème du système d'aquaculture en Iran. Les branchies sont des organes multifonctionnels responsables de la respiration, de l'osmorégulation, de l'excrétion des déchets azotés et de l'équilibre acido-basique chez le poissonet sont très sensibles à la contamination de l'eau. *Aeromonas hydrophila* (*A. hydrophila*) est un agent pathogène opportuniste responsable d'un large éventail de maladies chez différentes espèces de poissons. Les modifications histologiques des branchies ont été utilisées pour évaluer les effets de l'exposition à *A. hydrophila* sur le pagre à nageoires jaunes, *Acanthopagrus latus* (*A. latus*). À cet égard, 90 poissons de l'espèce *A. latus* ont été exposés à diférenres concentrations d'*A. Hydrophila* (10^3 et 10^6 CFU/ml) pendant trois semaines. Les altérations histopathologiques les plus importantes au niveai deses branchies des poissons exposés incluaient

l'hypertrophie et l'hyperplasie des cellules épithéliales, la fusion lamellaire, la formation de lamelles de branchies, la levée de l'épithélium et l'œdème des lamelles avec un grand espace sous-épithélial, la congestion sanguine et l'hypertrophie et l'hyperplasie des cellules muqueuses. D'importantes altérations histopathologiques ont été observées dans les branchies de poissons exposés à des taux plus élevés d'*A. hydrophila* (10^6 CFU/ml), qui consistaient en un anévrisme et une hémorragie avec congestion sanguine. Selon les résultats de cette étude, *A. hydrophila* pourrait provoquer de graves changements histopathologiques dans les branchies de l'*A. latus* et diminuer la capacité de changement de gaz chez le pagre à nageoires jaunes. En outre, les résultats de la présente étude suggèrent que les modifications histopathologiques des branchies fournissent des informations utiles sur les conditions environnementales et que des biomarqueurs particuliers peuvent aider à évaluer la santé générale des poissons.
Mots-clés: Histopathologie, branchie, *Acanthopagrus latus*, *Aeromonas hydrophila*

INTRODUCTION

Bacterial diseases in fish farming are considered the main problem with aquaculture system in Iran. Fish farming have been facing great problems due to bacterial fish diseases that cause severe damage and mortality in Iran. *Aeromonas hydrophila* (*A. hydrophila*) is an opportunist pathogen in different species of fish. The bacterium widely distributes to aquaculture and diseases in warm water fish in Iran (Abdelhamed et al., 2017). Motile aeromonas septicemia is a more dramatic bacterial disease affecting various species of fish in both freshwater and seawater and causes serious problems for fish farming industry in Iran, as well as in other countries (Arunkumar et al., 2000). *A. hydrophila* produces many extracellular proteins associated with its pathogenicity and environment adaptability. The main virulence factors that affect the pathogenicity are enterotoxins, hemolysin, aerolysin, as well as adhesion and mucinase (Austin, 2006). One of the most important benefits of using histopathological biomarkers in the environmental screening is a possibility of examination of the specific target organs, including the skin, liver, and gills, which are responsible for vital functions, such as respiration, excretion, and biotransformation in different species of fish (Jiraungkoorskul et al., 2002). The gills are multifunctional organs responsible for respiration, osmoregulation, nitrogenous waste excretion, and acid-base balance that are also very sensitive to water contamination (Boas et al., 2006). The exposure to bacteria, such as *A. hydrophila* compounds can cause a number of damages in different fish organs and the gill represents an important target organ suitable for histopathological assessment in searching for cells and tissue damages (Brown et al., 2004). In the present study, the histopathological structure was examined in the gill of *A. latus* after the exposure to different concentrations of *A. hydrophila* over a period of three weeks. The aim of this investigation was to report lesions and damages in the gill after an experimental exposure to *A. hydrophila* in one of the most ecologically and commercially important species of the Persian Gulf, yellowfin sea bream (*A. latus*).

MATERIAL AND METHODS

Fish maintenance. A total of 90 immature male *A. latus* were randomly obtained from Naseri pond (Khorramshahr, Iran) and acclimated for two weeks in the wet lab of Khorramshahr Marine Science and Technology University in Khouzestan, Iran, in nine 300-liter indoor tanks containing filtered aerated pond water treated with ultra violet water purification system.

Experimental design. Following acclimation, the fish was randomly placed in nine 300-liter tanks (10 fish in each tank). Then, the tanks were divided into one control and two experimental groups (each group in triplicate). The experimental groups were exposed to four concentrations of *A. hydrophila* 10^3 CFU/ml (group 1) and 10^6 CFU/ml (group 2) for three weeks.

The average temperature of water was 23◦ C, with 7.8 pH and 14ppt salinity. At the end of the experiment, five fish from each tank in every group was chosen, the right opercula were taken away, and the gills were removed carefully and quickly to prevent the damage to the tissues. The fish was anesthetized with a 2-phenoxyethanol solution (0.2%). In addition, the gill histological changes were classified using the method of Poleksić and Mitrović-Tutundžić (Poleksić and Mitrović-Tutundžić, 1994).

Tissue processing. The tissue samples of the gills were taken from the second gill arch on the right side of the fish and were fixed at 10% neutral buffered formalin. The samples were routinely processed and 5-6 µm paraffin sections were prepared. The tissue sections were stained with hematoxylin and eosin, and then histological evaluation was performed using Dino lite lens (with Dino capture software, FDP2, Taiwan) (Suvarna et al., 2013).

RESULTS AND DISCUSSION

Control group. The gill had a normal tissue structure in control fish and did not represent any abnormality in the cell and tissue structure. A series of separate and regular lamellae are sited on the upper and lower surface of each gill filament in control fish .Normal epithelial cells were situated along the entire length of the filaments and lamellae. The chloride and mucous cells were distributed in the filament epithelium (Figure 1).

Experimental groups. Table 1 tabulates the histopathological changes detected in the gills of the *A. hydrophila* exposed fish. The degree of tissue change of the gills within the treated groups is determined as stages I and II according to the method of Poleksić and Mitrović-Tutundžić. The most common detected abnormalities included the lifting of the lamellar epithelium and edema, appearance of chloride cells, hypertrophy and hyperplasia of mucosal cells, hypertrophy and hyperplasia of epithelial cell, hemorrhage, as well as telangiectasia (lamellar capillary aneurysm) (Figure 2). Nowadays, histopathology is known as an important tool for the assessment of the disease effects in vital process and detection of early changes in cells, tissues, and organs.

Table 1. Histopathological changes in the gills of *Acanthopagrus latus* treated by *Aeromonas hydrophila*

Stage	Group	Histological alternations
I	Group 1 (10^3 CFU/ml)	Hyperplasia and hypertrophy of the lamellar fusion Edema of the lamellae Epithelial lifting of lamellae Hypertrophy and hyperplasia of the mucosal cells
II	Group 2 (10^6 CFU/ml)	Blood congestion Epithelial lifting of lamellae Club shaping of gill lamellae Hyperplasia of epithelial cells and lamellar fusion Lamellar aneurysm and hemorrhage

Histopathological biomarkers have been used in fish to identify and evaluate the toxic effects of exposure to pollutants (Austin, 2006). Due to the harmful effects of bacteria in aquatic environments, the histocytological responses of fish to a variety of xenobiotics necessitated to be determined and characterized (Carletta et al., 2002). The fish gill is an important organ that participates in many important functions, such as respiration, acid-base balance, osmoregulation, and nitrogenous waste excretion. The gill structure provides a large surface area for direct and constant contact with water pollutants. Therefore, this organ is too sensitive to microorganisms in water and is considered as the primary target organ to contaminants (Harikrishnan and Balasundaram, 2005). Consequently, the pathologic assay of the gill in fish exposed to environmental pollutants has been used as a useful biomarker for the evaluation of the quality of aquatic ecosystems. Several types of the gill impairment have been documented in the fish experimentally exposed to contaminants or in those sampled from unhygienic ecosystems (Schlenk and Benson, 2003). It seems that the biological characteristics of fish (e.g., age and sex) or seasonal factors do not affect the response of the fish

gill to bacterial diseases. Generally, the gill histopathology appears to be a promising biomarker for general environmental contamination, although tissue preparation for histopathological study of the gill is time-consuming (Hesp et al., 2004).

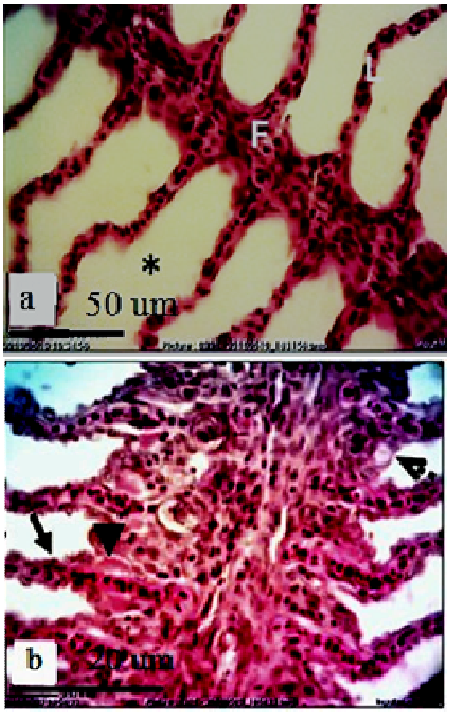

Figure 1. Normal histological structure of gill within the control group of *Acanthopagrus latus*; (a) light micrograph showing normal aspect of filament with possession of distinct regular lamellae: the filament (F), the lamellae (L), the water channel (black*) (hematoxylin and eosin;×750); (b) different cell types with common numbers and thickness, epithelial cell (black arrow), chloride cell (black arrow head), mucous cell (dashed arrow) (hematoxylin and eosin;×2900).

According to the obtained results of the present study, *A. hydrophila* can cause histophathological changes in the gill of *Acanthopagrus latus* decreasing its gas interchange capability. These changes ranged from mild to severe in fish depending on the concentration of *A. hydrophila.* As the obtained results showed, although *A. latus* is one of the most resistant fish species, even the lower concentrations of *A. hydrophila* influenced the normal structure of the gill in this fish. Most of the histopathological alterations of the gill described in the present study are in agreement with those reported in other fish species under a broad range of exposure conditions. Then it seemed that these effects revealed physiological modification to stress rather than special and restricted toxic responses to the concentrations of *A. hydrophila.* considered in this study. The changes, such as edema with epithelial lifting and telangiectasia, the hyperplasia and hypertrophy of the lamellae, besides the partial fusion of some lamellae recognized in the present investigation are usual gill lacerations in response to many other bacteria (Harikrishnan et al., 2003). These are the examples of protection mechanisms that generally result in the distance increase between water and the blood to act as a barrier to the entrance of contamination (Kumar et al., 2016). The major alternations in the gill of *A. latus* exposed to various concentrations of *A. hydrophila* in the present study were a thickening of the filament epithelium that filled the space between lamellae in some cases; therefore, the lamellae appeared to shorten and separate from the lamellar epithelium or vast edema, as it has been reported upon the exposure of mosquitofish (*Gambusia holbrooki*) to *A. hydrophila* (Rauta et al., 2017). Lamellar fusion and epithelial lifting in Neotropical fish species *Prochilodus lineatus* were reported after the exposure to *Aeromonas hydrophila* for 14 days (Khalil and Mansour, 1997). According to (Carletta et al. 2002), these alterations could be due to contaminants or infectious conditions. Similar histophatological alterations in the gill have also been reported in the fish exposed to infection by *A. hydrophila* (Hesp et al., 2004). When fish have a more severe type of stress some alterations in blood vessels may also occur. In similar situations, the injured pillar cell can cause an increased blood flow inside the lamella and influence the dilation of the marginal channel, blood congestion, or even an aneurysm (Brown et al., 2004). This is a severe type of damage, the recovery from which is possible; however, it is more difficult than the lamellae epithelial changes. The rupture of pillar cells as a result of bigger blood flow or the direct effects of bacteria on these cells leads to the

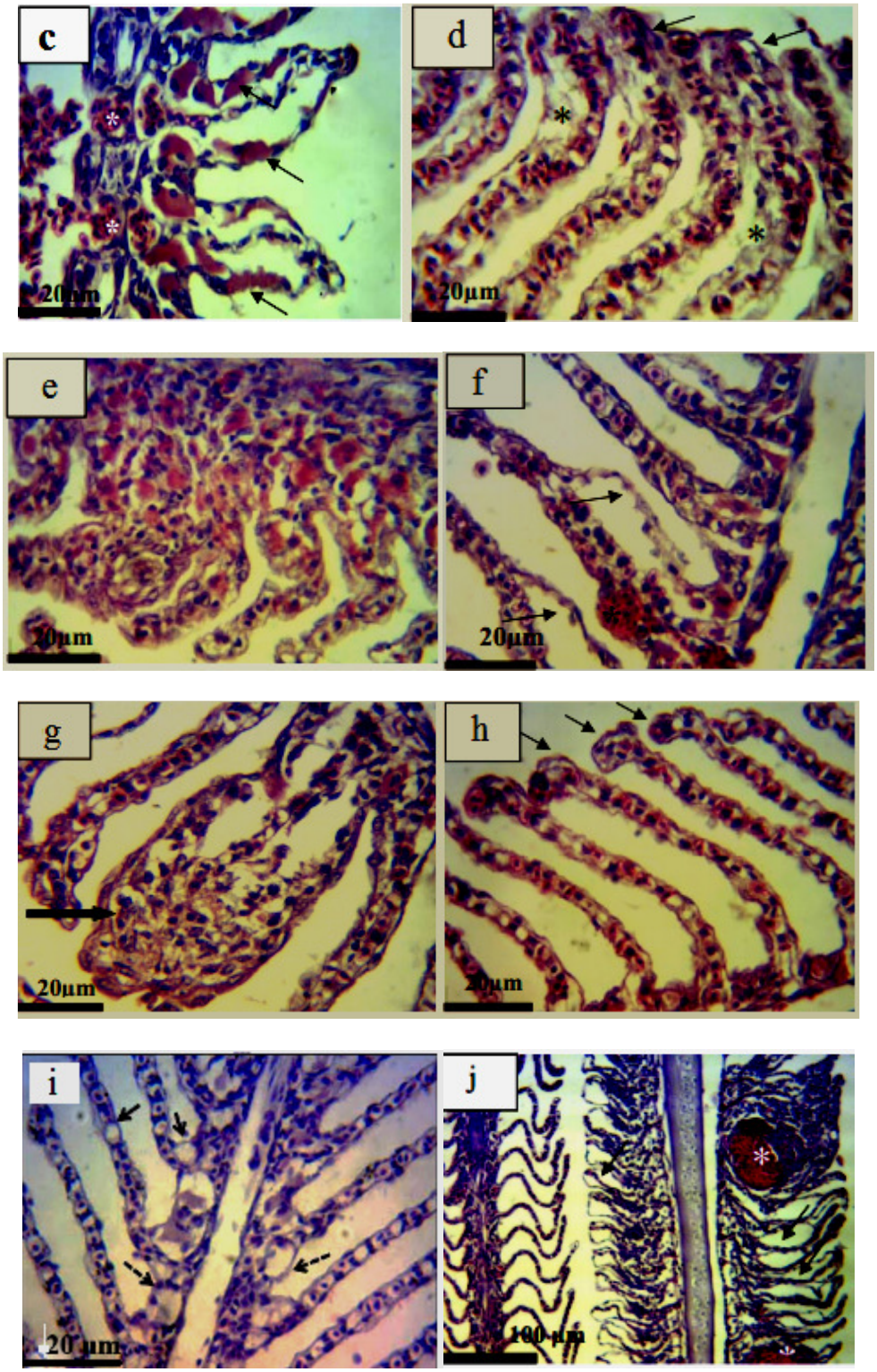

Figure 2. Photomicrographs of histopathological alternations of gills within *Acanthopagrus latus* groups exposed to *Aeromonas hydrophila*; (c) epithelial lifting and edema of the lamellae (black arrow head), appearance of chloride cells (black arrow), hemorrhage (white*); (d) hypertrophy of the lamellae (black arrow) and edema (black*); (e) hyperplasia of the lamellae; (f) epithelial lifting (black arrow) and hemorrhage (black*); (g) hyperplasia of the lamellae; (h) club shaping of gill lamellae (black arrow); (i) hypertrophy (dashed arrow) and hyperplasia of the mucosal cells (black arrow); (j) epithelial lifting (black arrow) and lamellar aneurysm (black*) (hematoxylin and eosin;×2900).

formation of an aneurysm (Schlenk and Benson, 2003). Aneurysms as severe pathological alteration resulting from disturbance in blood flow could be considered as a specific reaction of the gill to the toxic substances. In such conditions, increased blood flow in the lamellae that occur through pillar cells damage is followed by the dilation of marginal canal, blood congestion, and aneurysm. This alteration occurs when fish suffer from a more severe type of stress (Brown et al., 2004). The abnormal increase of chloride cells in the interlamellar spaces is consistent with altered ion flux in the gills, as previously reported by Kumar et al. (2016). Several studies reported that the increase of these cells in fish is caused by a number of pollutants, such as mercury, cadmium, and microorganisms (Rodríguez et al., 2008). The increase of mucus secretion, as observed in the gills of *A. latus*, can act as a barrier to reduce the absorption of toxicants; however, it can also result in the increase of blood-gas diffusion distance and gas exchange reduction (Sahoo et al., 2008). Similar changes were also reported by Mohamed (2009) in the gills of Tilapia (*Oreochromis mossambicus*). In this study, the fish treated with the higher concentrations of *A. hydrophila* (group 2) showed vascular alterations, such as blood congestion, and in some cases aneurysm which indicated the critical condition of the water in these groups. The shortening and progressive disappearance of lamellae observed in the present study were similar to the responses produced by other bacteria (Schlenk and Benson, 2003; Sahoo et al., 2007; Rauta et al., 2017). Yun et al. reported the hyperplasia of epithelial cells of the gill filaments and fusion of secondary lamellae giving a club shaped appearance of filaments, along with contraction and sloughing of the respiratory epithelium in *Heteropneustes fossilis* treated with *A. hydrophila* at sublethal dose for 16 days (Yun et al., 2017). The epithelial lifting, as well as hyperplasia and hypertrophy of the epithelial cells, that may occur due to the partial fusion of lamellae are defense mechanisms resulting in the increased distance between the aquatic environment and blood acting as a barrier to the pollutants entrance. However, these changes in the gill structure lead to oxygen uptake impairing, and fish has to increase the respiration rate through compensating (Rauta et al., 2017). The same findings following 72 h of exposure of carps *(Cyprinus carpio)* to sublethal doses of *A. hydrophila* has been reported. Similar histopathological changes in the gills are also reported by Oliveira et al. (1996), Harikrishnan et al. (2003) and Brown et al., (2004) indicating that such alterations in gills are non-specific and could occur as a consequence of different types of pollutants, such as inorganic mercury and other contaminants. The findings of the present study revealed that the gills of *A. latus* were structurally affected by the exposure to *A. hydrophila* the concentration of which influenced the severity of the alterations to the tissue and extent of damages. In addition, the obtained results in this study suggested that the histopathological changes of the gill provide helpful information about the environmental conditions. Furthermore, these changes can act as particular biomarkers for the evaluation of the general health and stress status of fish.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors would like to thank Khorramshahr Marine Science and Technology University for financial support.

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