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Research Article

HISTOPATHOLOGICAL STUDIES ON GILLS OF *Leiognathus equulus* (Perciformes) FROM AL-SHABAB LAGOON, IN JEDDAH PROVINCE

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Abstract- The gills of fish are sensitive to chemical and physical changes in the aquatic environment. Common pony fish *Leiognathus equulus* was caught from the Red Sea and Al-Shabab lagoon. This study was carried out to investigate the histopathological alternations induced by the Al-Shabab lagoon pollutants in gills. The common alternations observed were hyperplasia and fusion of gill lamellae, epithelial lifting, desquamation and necrosis of gill epithelial cells

Keywords-Common ponyfish., Histopathology,. Red Sea., Al-Shabab lagoon, Gills.

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Introduction

Leiognathids, commonly known as ponyfishes, are laterally compressed, small to moderate-size silvery fishes characterized by a strongly protrusible mouth, a fin-spine locking mechanism and a bioluminescent organ around the esophagus. The family is distributed in coastal and estuarine waters of the Indo-West Pacific [1] and is consider one of the important commercial wild fish in Asian and introduced as aquaculture fresh fish or processed product [2] .

The assessment of aquatic organisms health status cannot be evaluate directly, so the indicators of heath consider the best way of monitoring tools. Histology and histopathology can be used as assessment tools or indicators of health in toxicity applied as early warning signs of disease [3]. Alterations in histopathological feature are biomarkers effect due to exposure to environmental pollution, effecting on physiological function of organisms [4].

Fish is a good indicator for monitoring environmental stressors because they have an ability to concentrate pollutants in their body directly from water and diet, causing alterations in physiological and biochemical function [5] and [6]. Tissues structural damages in target organs may occur when organisms exposed to pollutants, lead to histological alternation and physiological stress. This stress begins with the changes in the cellular and tissue level affecting on the metabolic functions. Measuring the quantitative data gives better reactions of the organisms to pollutants, although qualitative data are studies in different cases to assess the pathologies of environmental pollutants [7].

Fish are extensively used as indicator to evaluate the health of aquatic ecosystems through histopathological alternation studies serve as biomarkers to monitor the environmental pollution [8,9]. Gills is a site of respiration system transfer of pollution materials around internal organs though blood circulatory system that play an important role in the accumulation, these will lead to an effect on their structure and functions. [10]

In fish the gill surface is more than half of the entire body surface area in length. The branchial function is very sensitive to environmental contamination because the internal environment of fish body is separated from the external environment by a few microns of gill epithelium, therefore gills are the first organs which come in contact with environmental pollutants. Additionally, their large surface area increase and facilitates the interaction with toxicant and absorption [11,12]. Moreover, absorption of toxic material through gills is rapid compare to other internal organs. Many researchers used gills to evaluate the quality of water and the effect of the aquatic pollutants in salt and fresh water habitats [13-15]. Therefore, lesions in gill tissues can be the start of imbalance of the physiological and metabolic processes of fish.

Pollution of the aquatic environment is a serious and growing problem. Increasing number and amount of agricultural and commercial chemicals, industrial, materials discharged into the aquatic environment affected on various deleterious changing on the aquatic organisms and their environment [16]. These may alter the structure and functions of cellular and tissues of Aquatic organisms, including fish and others organisms [17].

Histopathology is the one of biomarker could be used to assessment the effects of various anthropogenic pollutants on different organisms including fish [18].The modifications in varies tissues of fish body appears when fish exposure to chemical contaminants, particularly gills, which histological studies represent a useful tool to monitor the effects of xenobiotics [19,20]. A wide range of histo-cytological alterations in fish have been developed and recommended as biomarkers for assessment the pollution [21,22]. Using of histopathological changes as a biomarker has the benefit of allowing researchers to examine specific target cellular and organs as they are altered by exposure to environmental chemicals polluted [23]. Fish are exposed to aquatic toxicants through their extensive and delicate respiratory surface and consider as commonly the primary target organ for pollution, the structural damage of organs and tissues of fish due to exposure to different chemical material and petroleum derivatives reported by many authors [24-26].

There are different pollution sources parts around the coast of Jeddah city, wastes from factories of petroleum, fish market near the cost of Red sea, untreated sewage wastes and others sources related to desalination factories [27]. Samples of the Red Sea water were collected from 24 important locations near Jeddah and analyzed in the laboratory for various water quality parameters [28].

This study aimed to investigate the impact of the environmental conditions of AL-Shabab lagoon on the histological alternations in gill of *L. equulus*.

It is expected that this study will contribute to our knowledge useful information for providing a basis on which future monitoring of the pollutants or assessment of the fate of environment in this area can based.

Materials and Methods

Fish samples

In this study 178 fish are collected from Red sea around Jeddah city and 226 fish from Al-Shabab lagoon. The fish then were transported in fish ice boxes to the laboratory for examination process.

The fish were weighted to the nearest gram. Total, forked and standard length were measured to the nearest cm. Samples took from gills and placed in 10% neutral formalin fixative for histological examination.

Histological procedures

The gills were fixed in 10% neutral formalin fixative after dissecting the fish. The fixed gills were washed in 70% ethanol prior to dehydration through an ascending series of ethyl alcohol, then cleared in two changes of xylene (15-30 minutes for each change), then infiltrated and embedded in paraffin wax (56-58c°). Serial transverse section were cut at a thickness of 3-4µ. These sections were affixed on albumenized slides, then stained in Eosin-haematoxlyin [29].

Examination of stained sections was done by using Olympus BX 51 Microscope in the central laboratory Faculty of Science King Abdul-Aziz University.

Results and Discussion

Fish are lied under non–target aquatic organisms group, which represent the largest and most distributed group of vertebrates. Fish as aquatic organisms extensively used to evaluate the quality of aquatic ecosystems and their physiological and chemical changes serve as biomarkers to monitor the environmental pollution. A number of characteristics and features make them excellent experimental models for toxicological studies, mainly in the field of the contaminants and aquatic pollution, which are better to exert their impact on aquatic systems [30].

The specimens collected in this study ranged from 84 to 170 mm, which were smaller than the maximum size of 216 and 280 mm recorded in [31]. In most teleost fish, there are four pairs of gill arches, which supported by bone skeleton. Primary lamellae are the extension and branched filaments from the gills arches, and then supported by cartilage, which form the secondary lamellae. Gas exchanges occur through the a simple epithelium of secondary lamellae

The gill surface is very sensitive to environmental contamination compare to other internal organs of fish. Hence, fish serve as excellent bioassay animals for toxicological impact studies done by many researcher and have been widely used for this field research. The study area in this research receives different pollutants from four sources: untreated domestic sewage wastes, oil from the oil refinery, fish wastes from the big fish market (El-Bangalah) and desalination plant effluents which contribute both organic and metallic contaminants [32-34]

In this study, gill structure composed of: primary and secondary lamellae; epithelial cell; pillar cell; and chloride cell. There are four gill arches on each side of the ducal cavity. Each arch supported by bony structure and composed of numerous gill filaments which made up of primary lamellae arranged in double rows, projecting on the lateral sides of which are a series of alternately arranged secondary lamellae and were erector slightly curved [Fig-1].The lamellae are lined by squamous epithelium called pavement cells showing characteristic concentric patterns of micro ridges and which delimited by many pillar cells–separated by a thin layer of basement membrane–which are contractile and separate the capillary channels. One to two erythrocytes are usually recognized within each capillary lumen [Fig-2]. Mucus cells are ovoid and also present in the epithelium of the filament and at the base of lamellae, but they lack the light cytoplasm and are smaller than chloride cells. Chloride cells are spheroid are responsible for ionic regulation. They are identified as large epithelial cells with light cytoplasm, usually present at the base of lamellae [Fig-3].

The gill sections of *L. equulus* sampled from the polluted site of Al-Shabab lagoon showed different histological alternations such as necrosis, hypertrophy and desquamation of lamellar epithelium [Fig-4&5].In addition to these changes, lifting up of epithelium, fusion of adjacent lamellae as a result of epithelial hyperplasia and intraepithelial oedema were also observed [Fig-4, 5, 6, 7 & 9].

Histopathological results indicated that the different polluted material source caused morphological alterations in the gill epithelium and the blood circulatory system and lamellar epithelium lifting. Lamellar lifting, edema and filamentous epithelial proliferation are histopathological alterations, which described by other authors who studies the effects of pesticide contamination on aquatic organisms [35].

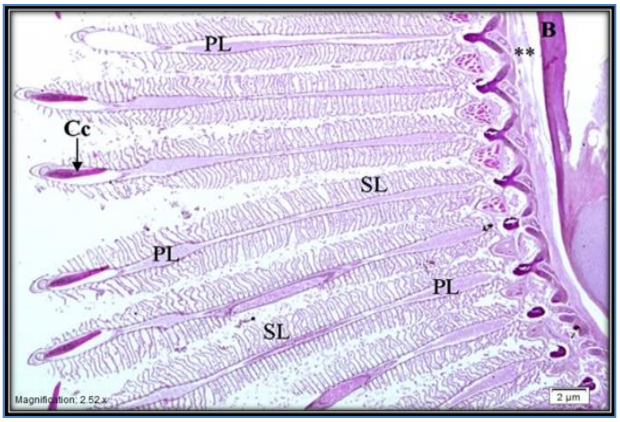

Fig-1 T.S of the gill tissue of non-polluted *L. equulus*: adipose tissue (**); bone (B); chondrocytes (Cc); primary lamella (PL) and secondary lamellae (SL) (H&E, Bar = 2 µm). T.S= testing sample

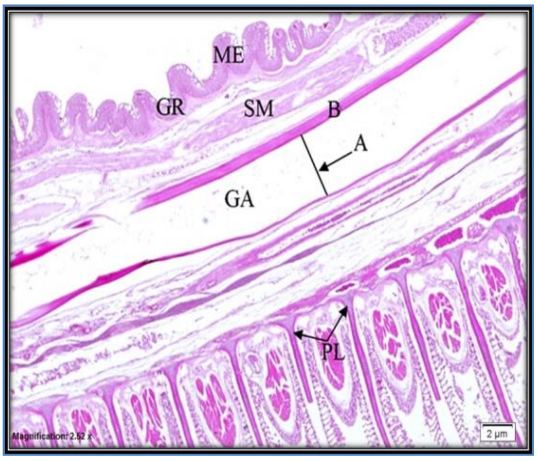

Fig-2 T.S of the gill arch (GA) of non-polluted *L. equulus*: adipose tissue (A); bone (B); gill raker (GR); mucosal epithelium (ME); primary lamella (PL) and sub mucosa (SM) (H&E, Bar = 2 µm).

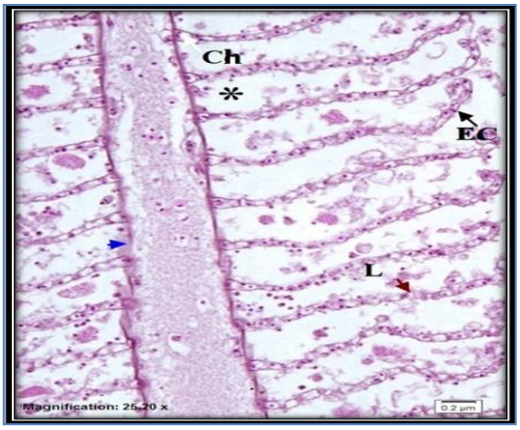

Fig-3 T.S of the gill of non-poluted *L. equulus* normal aspect showing the filament: Chloride cells (Ch); epithelial cell (EC); capillary lumen (blue arrow); lacuna (red arrow); lamellae (L) and the water channel (*) (H&E, Bar = 0.2µm).

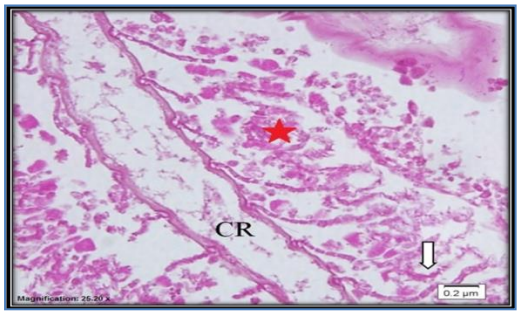

Fig-4 T.S of the gill tissue of polluted *L. equulus* showing lamellar disorganization (down head white arrow); Cartilage rupture (CR) and Oedema (red star) (H&E, Bar = 0.2µm).

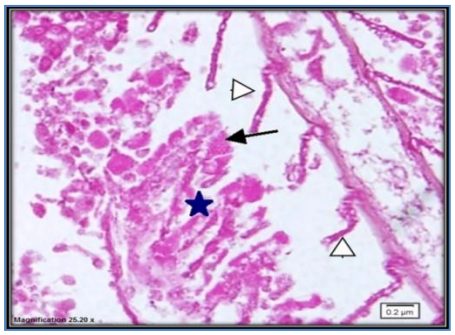

Fig-5 T.S of the gill tissue of polluted *L. equulus* showing ansumiria (black arrow); epithelial rupture with hemorrhage (blue star); shortage of length of secondary lamellae (white arrow heads) (H&E, Bar = 0.2µm).

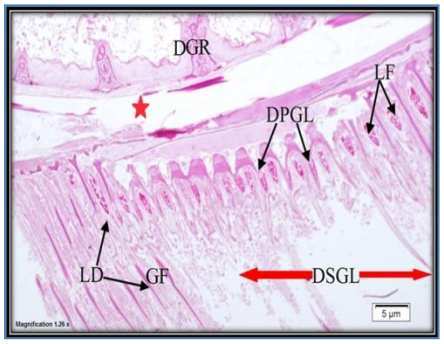

Fig-6 T.S of the gill tissue of polluted *L. equulus* showing degenerated gill rakers (DGR); Degenerated primary gill lamellae (DPGL); Degenerated secondary gill lamellae (DSGL); Gill filament (GF); Lamellae degenerated (LD); Lamellae lifting (LF) and Sub mucosa (red star) (H&E, Bar = 5 µm).

[36] observed the fusion of adjacent lamellae after exposure to heavy metals and chemicals, such as cadmium and copper which is in line with the present findings. The histological changes such as fusion of secondary lamellae and narrowed water channels in the gills of *Leiognathus equulus,* in this study may be an indication of their either reaction to high a mount of toxicant intake or physiological altering processing, lead to adaptation and preventing the pollutant entry through the gill surface. Gill are permanent contact with water, are primary and very sensitive receptor surface for aquatic pollution [37] and have been widely used to investigate the toxicity effects from heavy metals [38].

The polluted gills swollen in comparison to non-polluted fish because of the hypertrophy and hyperplasia of the gill epithelial cells. There was separation of the respiratory epithelia and proliferation of chloride cells in basilamellar regions. This lead to lamellar swelling fusion with swollen and degeneration of respiratory epithelial cells (stage I), shown in [Table-1] which describes the stages of severity histopathological alternation in gill. Toxic substances can injure gills, thus reducing the oxygen exchanges and consumption, also the disrupting the osmo-regulatory function of aquatic organisms [39]. The histopathology of fish gills collected from polluted region showed an abnormal and pathological effect. [40] observed hypertrophy, destruction of gill lamellae, these histopathological change due to the toxicity of cadmium chloride in experimental gills of fish as seen in this work also. Similar findings have been reported for other fish species that are exposed to different pollutants [41-43] observed hyperplasia of the epithelial cells, fusion of secondary lamellae, lifting of the lamellar epithelium and blood congestion in the gills of *P. lineatus* being caged in Brazilian Cambé stream being polluted by the industrial, domestic and agricultural wastes. [44] observed epithelial lifting, proliferation and necrosis and hyperplasia of mucous cells, shortening of the secondary lamella, abnormal raising of the epithelium and a swell as fusion of the secondary lamellae and excessive mucus secretion in fish exposed to diazin. The changes in appearance of the secondary lamellae result from the collapse of the pillar cell system and breakdown of vascular integrity with release of large quantities of blood that push the lamellar epithelium outward. The gill epithelium injured as is a common response of fish exposed to a variety of contaminants [45]. Hyperplasia of epithelial cells between the spaces of two adjacent filaments due to partially full filled with blood (Hyperplasia of epithelial cells.). Hyperemic changes were also found in the secondary lamellae [Fig-8].Morphological anomalies of gill observed in the present study indicates that structural damages such as hyperplasia with lamellar fusion, telangectasia, oedema, epithelial lifting, necrosis may be causes of respiratory and osmo-regulatory disorder, these similar finding in other research [46]. The alternations also may play a protective role against contamination, rather than have an irreversible toxic effect and may control the effect for long time. Histopathology provides a rapid method to detect effects of irritants in various organs [47]. The pathological changes in the chloride cells may indicate osmo-regulatory dysfunction, which is the main function of the chloride cells. Chloride cells proliferation due to an added function of oxygen transport due to injury to gill tissue proper. In certain abnormal conditions chloride cells may to be an oxygen transport functions.

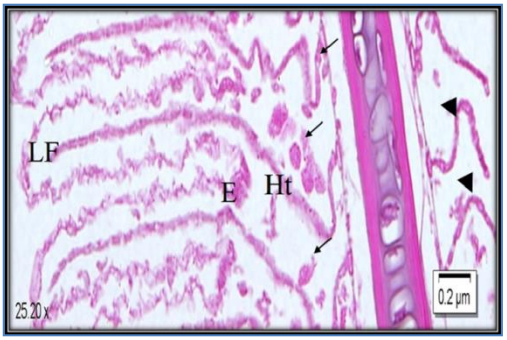

Fig-7 T.S of the gill tissue of polluted *L. equulus* showing edema (E) and decreasing of length, break and abnormal and curling of secondary lamella (arrow heads), increasing in lifting (LF); proliferation and hypertrophy (Ht) of mucous (goblet) cells and chloride cell (arrows) (H&E, Bar = 0.2 µm).

Fig-8 T.S of the gill tissue of polluted *L. equulus* showing disruption of cartilaginous core (whit arrow); hemorrhage (black stars) and hyperplasia in lamellar epithelium leading to lamellae fusion (black arrow) (H&E, Bar = 0.2 µm).

Blood vessels in some of the plates were filled with blood. Hyperemic changes were also found in the secondary lamellae [Fig-4]. Epithelial rupture with hemorrhage, ansumiria and shortage of length of secondary lamellae [Fig-5]. These changed in the gill structure were described as stage II [Table-1].

Sever alternations of the gills (stage III) were observed in [Fig-6 & 7]. Degenerated gill rakers, degenerated primary gill lamellae, degenerated secondary gill lamellae, gill filament and lamellae. Fusion, two or more lamellae (plates) were merged due to the hyperplastic processes in epithelial tissue [Table-1]. Similar observation have been reported in several histopathological researches in the gills of fish in response to agricultural, sewage and industrial pollutants [49]

The gill is a suitable organ for histological examination in order to determine the effect of pollution as gill covers more than 60% surface area of the fish and its external location renders it the most vulnerable target organs for the pollutants, for those histological biomarkers of toxicity in fish organs are a useful indicator of environmental pollution [48]. Many pollutants have to undergo metabolic activation in order to be able to provoke cellular and tissues change in the affected organism. It is generally, assumed that histopathological biomarkers became as a mirror to reflect the effects of exposure to a variety of pollutants and indicators of the general health of fish. Gill histopathological alterations, such as those observed in this study and findings from previous studies, could result in severe physiological problems, ultimately leading to the death of fish. The histological changes observed in the gills of the *L. equulus* in the present study indicate that the fish were responding to the direct effects of the contaminants as much as to the secondary effects caused by stress.

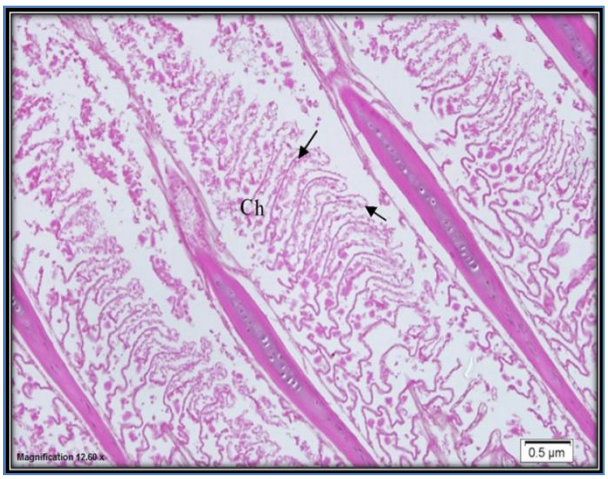

Fig-9 T.S of the gill tissue of polluted *L. equulus* showing complete architectural loss (Arrows) and Chloride cell (Ch) (H&E, Bar = 0.5 µm).

Table-1 *List of the histopathological alternations observed in the gills of L. equulusfrom Al-Shabab Lagoon. I, II and III - Stage of severity of alternation (Modified from [50]).*

Stage	Histopathological alternations in the gills
I	Hypertrophy and hyperplasia of gill epithelium. Dilation of marginal vascular channels. Lifting of respiratory epithelium. Fusion and disorganization of secondary gill lamellae. Shortening of secondary gill lamellae.
II	Hemorrhage and rupture of lamellar epithelium. Empty mucous cells or their disappearance.
III	Lamellar aneurysm. Cell degeneration. Lamellar telangiectasis.

Conclusion

The results from this study showed the different polluted material sources caused morphological and histological changes in the gill of *L. equulus* fish species, indicating the rapid responding to the direct effects of the pollution and contamination as much as to the effects caused by stress and these may lead directly or indirectly to the death of fish.

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Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest: None declared

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