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The Histopathological Effects of Water Soluble Fractions (WSF) of Crude Oil on the Gills of *Xiphophorus helleri* (Poeciliidae, Teleostei)

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Abstract: In this investigation, it was aimed to evaluate the toxicity potential of water soluble fractions of crude oil histopathologically in order to identify the problems originating from water soluble fractions of crude oil in *Xiphophorus helleri*. An acute toxicity experiment was performed by exposure to three concentrations (10%, 20%, 40%) of water soluble fractions of crude oil on the specimens for 24, 48, 72 and 96 hours. The tissues of control and experimental groups prepared by the Hematoxylin-Eosin staining method, examined under light microscope, by passing through routine histological process. In parallel with the increase in concentration it was seen to be increase in histopathological findings in the gills. It was observed that disorganization of secondary lamellae, hyperplasia and fusion in the form of ballooning depending on the exposure time as deteriorating in the test group of 10% concentration. It was observed that adhesion in addition to obvious irregularity in secondary lamellae, hyperplasia which is more pronounced especially in the distal ends of the lamellae, lamellar edema and epithelial separation in parallel with increasing exposure time in the test group of 20% concentration. It is in question that the findings increasingly worsening depending on the increase of time in the test group of 40% concentration. It was observed that hypertrophy in mucous cells and increase in the amount of mucous secretion, hyperplasia in the maximum level in balloon appearance at distal ends of secondary lamellae in addition to proliferation of mitochondria-rich cells and mucous cells as more pronounced than other test groups. As a result, it was concluded that water soluble fractions of crude oil exposure will primarily effects primitive vertebrates in particular *Xiphophorus helleri*, and also will effects all vertebrates by means of food chain and interactions of living groups in generally, all living things will suffer due to crude oil intoxication to aquatic environments.

Keywords: Crude oil, Histopathology, Gills, *Xiphophorus helleri*.

The Effects of Water Soluble Fraction of Crude Oil on The Testis Histology of *Xiphophorus helleri* Heckel, 1848 (Poeciliidae, Teleostei)

Özet: Bu çalışmada; *Xiphophorus helleri*'de ham petrolün suda çözünebilen kısımlarından kaynaklanan sorunların belirlenebilmesi için, histopatolojik olarak ham petrolün suda çözünebilen kısımlarının toksisite potansiyelinin değerlendirilmesi amaçlandı. Örneklerin üç farklı konsantrasyonda (%10, %20, %40), 24, 48, 72 ve 96 saat süreli maruziyetle bir akut toksisite testi gerçekleştirildi. Kontrol ve deneme grubu dokuları rutin histolojik süreçlerden geçirilerek, Hematoksilen-Eosin boyama metoduna göre boyanıp ışık mikroskobunda incelendi. Konsantrasyon artışına paralel olarak solungaçlardaki histopatolojik bulgularda artış olduğu görüldü. %10 test grubunda maruziyet süresi arttıkça kötüleşen şekilde sekonder lamellerde düzensizleşme, hiperplazi ve sekonder lamellerin uç kısımlarında balonlaşma şeklinde füzyon gözlendi. %20 test grubunda artan maruziyet süresi paralelinde, sekonder lamellerde belirgin düzensizleşmeye ilave olarak adhezyon, lamellerin distal uçlarında daha belirgin olan hiperplazi, lamellar ödem ve epiteliyal separasyon görüldü. %40 test grubunda maruziyet süresi artışına bağlı olarak ağırlaşan bulgular söz konusu olup; diğer test gruplarından çok daha belirgin olarak, mukus hücrelerinde hipertrofi ve mukus salgısı miktarında artış, mitokondri zengini hücrelerde ve mukus hücrelerinde proliferasyona ek olarak sekonder lamellerin uç kısımlarında balon görünümünde maksimum düzeyde hiperplazi görüldü. Sonuç olarak, ham petrolün suda çözünebilen kısımlarına maruziyetin öncelikle *Xiphophorus helleri* özelinde primitif omurgalıları, genel anlamda ise besin zinciri ve canlı grupları arası etkileşimlerle bütün omurgalıları etkileyeceği ve sucul ortamlara ham petrol intoksikasyonuyla tüm canlı gruplarının zarar göreceği kanaatine varıldı.

Anahtar Kelimeler: Ham petrol, Histopatoloji, Solungaçlar, *Xiphophorus helleri*.

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Introduction

Due to human urban and industrial activities, rivers are dumped with different kinds of chemicals such as polynuclear aromatic hydrocarbons (PAHs), affecting the inhabiting aquatic organisms individually and thereby potentially harming the ecosystem. Environmental accidents, such as oil spills, aggravate this problem, and biomonitoring studies associated with new methods to evaluate the detrimental effects on biota have been increasing in number over the past few decades. The pollution resulting from the release of oil into the aquatic environment due to recent accidents has become a global concern because of its effects on aquatic ecosystems.

Production facilities in offshore platforms, and especially in the transport process the crude oil pollution caused from invasions, in some cases, reach to estuaries which are important spawning and brooding areas and coastal areas closed to seaside, besides the maritime (Ramachandran et al., 2006). Hydrocarbons are the most important organic contaminant in the crude oil content and Whatever the spread of results is the rare accident, Exxon Valdez, Amoco Cadiz tanker accidents will be remembered as the very striking example for the coastal population is extraordinarily damaging (Marty et al., 2003).

The water-soluble fraction (WSF) of crude oil is consist of toxic mixtures of hydrocarbons and heterocyclic compounds; and affects all of the aquatic organisms included fish, which are widely used for biomonitoring about water pollution (Ruddock et al., 2003; Harmon & Wiley, 2010). These compounds are absorbed across the body surface (Varanasi et al., 1989; Hesni, et al., 2011), and alimentary canal; and can reach to humans via food chain.

Teleost gills are primarily areas of interest in ecotoxicological researches as the first contact surface to environmental pollutants (Brand et al., 2001; Pereira et al., 2013). Also gills are considered as the extended surfaces to be respiratory gas exchange is provided, ion balance is regulated, and also ion balance is regulated (İşisağ&Karakişi, 1997; Machado&Fanta, 2003). By this point, the observable effects of water soluble fractions of crude oil on gill histology of *Xiphophorus helleri* are intended to reveal.

Materials and Methods

Animals:

The swordtail is grouped within the family Poeciliidae which interestingly contains some of the most popular ornamental species in the aquarium trade. The genus *Xiphophorus*

includes species of the swordtail and the platy fish. *Xiphophorus helleri* samples which is in the same development level is obtained from commercial fish supplier.

Petroleum Water Soluble Fraction (WSF):

We have provided crude oil of petroleum from Tüpraş Petroleum Refinery. Then we processed crude oil into water soluble fractions which includes 10 percent of crude oil and 90 percent tap water by solving it during 24 hours with magnetic mixer. After that, the mixture kept in the separation funnel during 12 hours. The upper layer was heavy oil layer and the bottom layer was crude oil that we used in our research. Then we prepared three different concentrations as 10%, 20% and 40%. Water soluble fractions of crude oil's pH was 7.2 and tap water's pH was 6.2.

Experimental Design for Acute Exposure:

This study was carried out in accordance with the Animal Ethics Commitee Report (No. 2008-49) based on Decisions of Ethical Commitee for Experimental Animals prepared by Faculty of Pharmacy, Ege University.

Forty animals were divided into four groups including a control group. One of them is a control group without any chemical contribution, and three other groups that have involved different concentrations of WSF as 10%, 20% and 40%. Also the experiment was designed for 24, 48, 72, 96 hours. Test organisms are fed daily during the experiments by commercial feed (Sera-San). After 24, 48, 72, 96 hours exposure, individuals were sacrificed in by Trikain metansülfonat (MS 222). Gill samples were collected for histopathological studies for light microscopy.

Light Microscopy Procedure:

Gill tissue was fixed in Bouin fixative for 24h at room temperature; dehydrated in alcohol series, and embedded in paraffin. Sagittal sections in 5µm thickness were stained by routine Hematoxylin-Eosin (HE) procedure; examined and photographed light microscopically (Olympus BX-51; Altra20 Soft Imaging System).

Findings

Control Group

Gill filaments consist of primary lamellae which contain cartilage axis in the centers and secondary lamellae which are connected to primary lamellae by definite angle (Figure 1A, B). Lamellae is covered by the gill epithelium. Mucous cells monitored at the epithelium in patches. Pillar cells provide support functions. The massive and small number of mitochondria rich cells which is light coloured is took place among epithelial

cells. Moreover, erythrocytes can be seen in large quantities (Figure 1B).

10% Test Group

10% solution applied in the group 24 hours after any significant change was observed (Figure 2A). After 48 hours, secondary lamellae monitored as irregular forms in shape and curled up with each other to combine their almost as (Figure 2B). After 72 hours exposure, epithelial hyperplasia in the terminals of secondary lamellae starts, to increase the amount of mucous secretion draws attention (Figure 2C). After 96 hours there is fusion at the distal parts of the secondary lamellae, hyperplasia are defined as those of the balloon increased (Figure 2D).

20% Test Group

20% solution applied in the group, after 24 hours, some of the secondary lamellae hyperplasia is typical (Figure 3A). After 48 hours a significant irregularity in the secondary lamellae and adhesion is added to hyperplasia (Figure 3B). At the end of 72 hours, being more distinctive at the distal parts of secondary lamellae, in addition to widespread hyperplasia observed as balloons, lamellar edema initial is characterized that separation of respiratory epithelial cells from pillar cells is foregoing (Figure 3C). At the end of the 96 hours, being observed edema occasionally, the most striking change is to be fused that lamellae at the distal parts is distinguished from each other of rising hyperplasia (Figure 3D).

40% Test Group

24 hours after, in the 40% application group, edema formation is significantly better than the previous group; an intense hyperplasia and irregularity are obviously observed at secondary lamellae (Figure 4A). After 48 hours, in parallel with an increase in mucous secretion, disorganization and fusion stems from increasing of hyperplasia, also occasionally edema are observed in the secondary lamellae (Figure 4B). At the end of the 72 hours application, fusion at the secondary lamellae, proliferation at the mucous cells and mitochondria-rich cells are foresaid; mucous cells are seen markedly (Figure 4C). After 96 hours, widespread hyperplasia in the form of balloons was noted at the distal parts of the secondary lamellae and also mucous cells were more evident (Figure 4D).

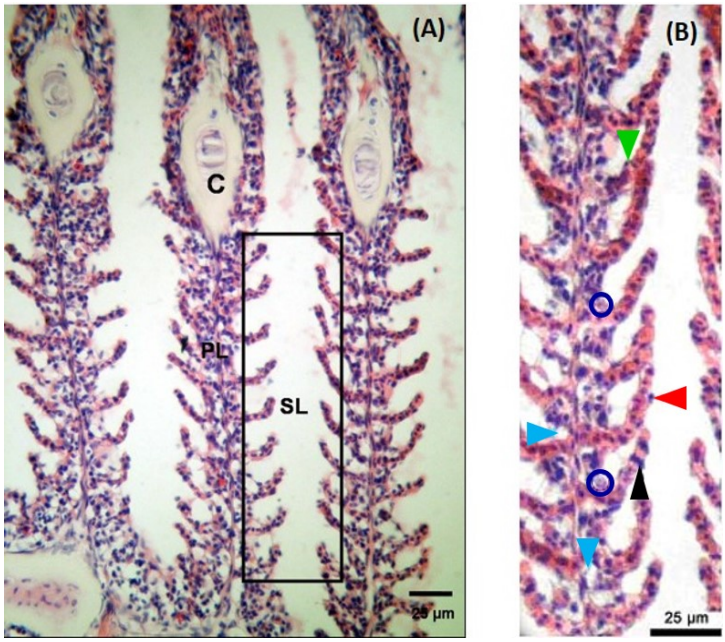

Figure 1. Control group, cross-section of gill; **(A)** Cartilage axis (C); primary lamellae (PL) and secondary lamellae in rectangle (SL), **(B)** The histological structure of gill filament; red arrow: mucous cells, black arrow: pillar cells, green arrow: epithelial cells, blue arrow: capillary blood vessels, in circle: mitochondria rich cells (HE).

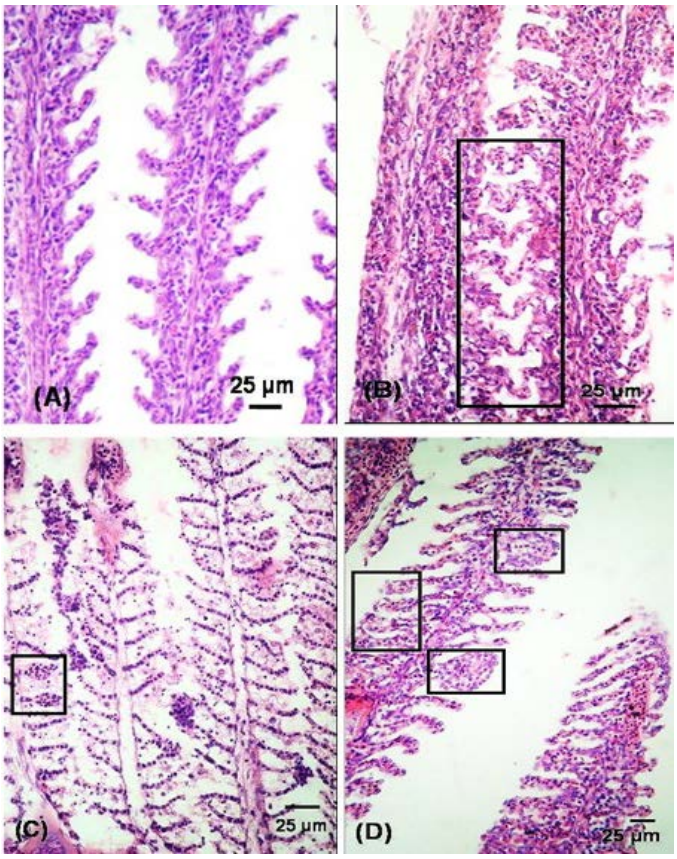

Figure 2. Test group, 10% , cross-section of gill; **(A)** 24 h, **(B)** 48 h, in rectangle: irregularity of secondary lamellae, **(C)** 72 h, in rectangle: epithelial hyperplasia, **(D)** 96 h, in rectangle: fusion and hyperplasia (HE).

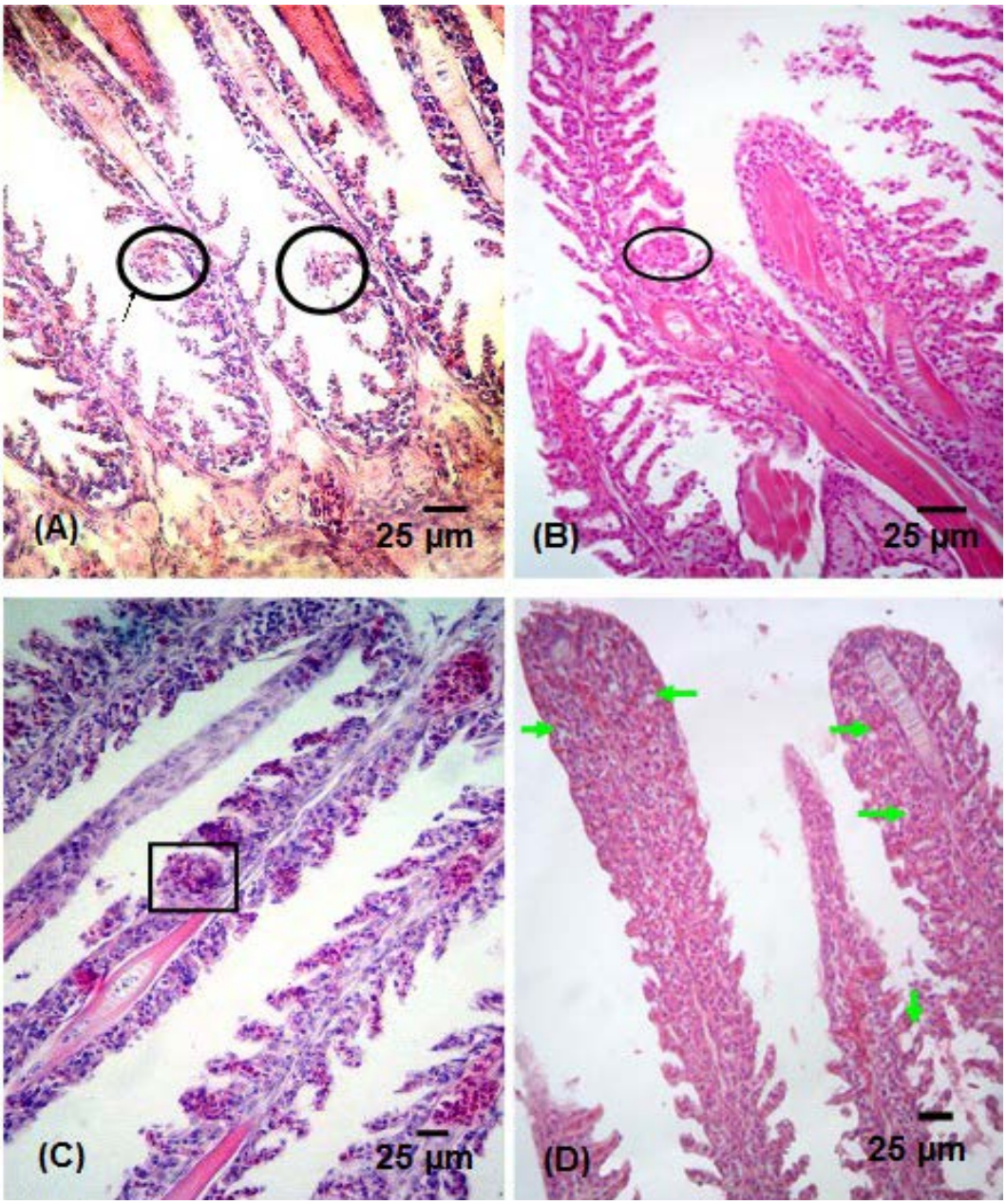

Figure 3. Test group, 20%, **(A)** 24 h, in circle: hyperplasia, **(B)** 48 h, in circle: hyperplasia, **(C)** 72 h, in rectangle: hyperplasia, **(D)** 96 h, green arrows: edema (HE).

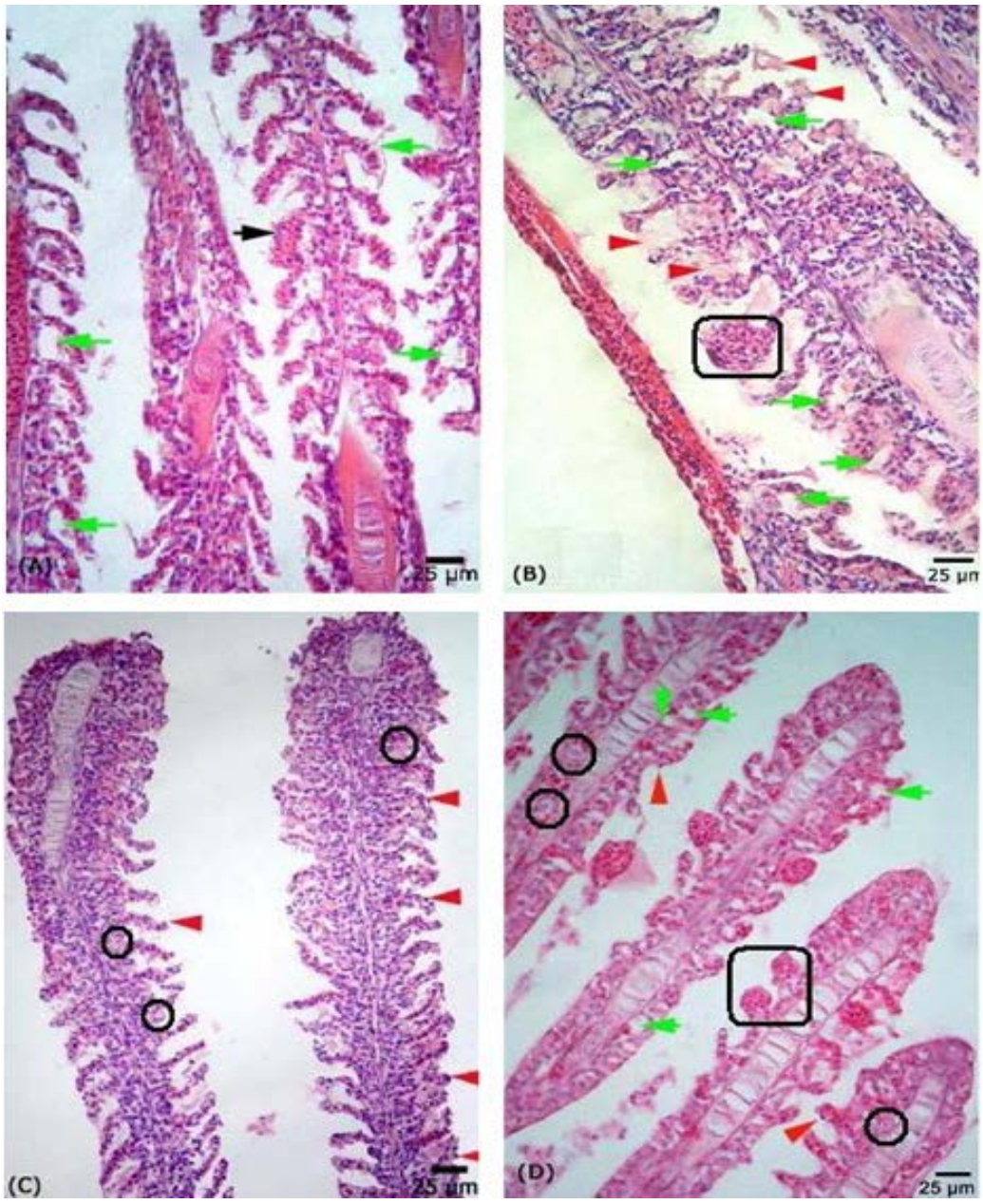

Figure 4. Experiment group, 40%, **(A)** 24 h, green arrow: edema, black arrow: hyperplasia, **(B)** 48 h, green arrow: edema, red arrow: an increase of mucous secretion, in rounded rectangle: disorganization and fusion stems from increasing of hyperplasia, **(C)** 72 h, red arrow: proliferation at the mucous cells, in circle: proliferation at the mitochondria-rich cells, **(D)** 96 h, red arrow: marked mucous cells, in rounded rectangle: hyperplasia at the distal ends of secondary lamellae (HE).

Discussion and Results

Water soluble fractions of crude oil which is among the pollutants as a result of human activity spread in aquatic environments is of great importance in terms of acute and chronic toxicity; the accidents which can

occur especially in the transport process may be suicidal for some fish communities. Primarily in the extension of gas exchange parameters, shown response to aquatic pollutants at the respiratory surfaces will affect osmoregulation mechanisms and other physiological parameters (Brand et al., 2001). Although it is not specific against the chemicals can be found in the water (van den Heuvel et al., 2000; Furia, 2004), it is displayed that histological structure of gills shows high sensitivity at many species as *Astyanax sp.* and *Onchorhyncus mykiss* (Akaishi et al., 2004). The findings which were monitored in the context of histological changes and were assessed as morphological lesions generally indicate compliance to this reports in general means.

Lamellar epithelium, which constitutes the first contact surface with pollutants at the cover-protection/substance exchange processes, is considered as the first barrier of the defense functions. In this context hyperplasia is a typical defense function and it is aimed to increase the distance which is bounded to pass that pollutants are able to enter into the body inside. Thus the organism thickens the respiratory surfaces which is good vascular structurings and can prevent to pass the chemicals to blood tissue to a certain extent. Also the structurings followed the irregulation at secondary lamellae are similarly changes increased diffusion distance. These basic histopathological defense processes seen at *X. helleri* were also reported at *Oncorhynchus gorbuscha* (Brand et al., 2001), *Prochilodus scrofa* (Mazon et al., 2002), *Mugil sp.*, *Cyprinus carpio* and *Barbus sp.* (Ortiz et al., 2003), *Metynnis roosevelti* (Machado and Fanta, 2003); *Trachinotus sp.* (Furia 2004) and *Astyanax sp.* (Akaishi et al., 2004), too.

Increase and hypertrophy of mucous cells assessed as markers of another protection/defense method (Brand et al. 2001). The largely glycosaminoglycan content of mucous creates a biochemical barrier in addition to physical barriers. However, it shouldn't be neglected that such a barrier can also block the oxygen transmission to a certain extent, in all respiratory surfaces including the skin (Akaishi et al., 2004). After the Exxon Valdez disaster, the changes about mucous cells were also noted in similar format by Marty et al. (2003) in demmersal *Sebastes spp.* examples which was obtained from Gulf of Alaska. Although an increase in number of mucous cells and hypertrophy in mucous cells were tracked according to long time/ increasing concentration parameters in our study, any biometric evaluation has not done.

It is reported to work through acetyl cholinesterase enzyme of the effect mechanisms of constituent parts

hydrocarbons which were content of WSF and to change motor reactions by reason of obstruction of enzyme activity in *Astyanax sp.* examples which were exposed to these chemicals acutely (Akaishi et al., 2004). Acetyl cholinesterase inhibition leads to accumulation of acetylcholine in the synaptic connections of cholinergic nerves. If it is evaluated in the context of exposure time and dose, it is obvious that such an accumulation may cause even death by blocking the transfer of neural message in respiratory system and respiratory centers.

When enzyme efficiencies are thought together with changes in gill histology however, reduction in the quantity of oxygen which is necessary for all physiological functions will leads to negativities in the attitudes of swimming-action, nutrition and escape-defense, the energy metabolism through. Because the presented study planned as a pilot experiment relating to determine only acute effects in gill histology, biochemical methods concerning enzyme efficiency were not used. Detailed studies that these methods will also be used, still continue in our department.

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