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HISTOPATHOLOGICAL EFFECTS OF ETIOLOGICAL AND NON-ETIOLOGICAL AGENTS IN SOME FISH GILLS

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ABSTRACT

The gill histology in fish is used as an identification of infectious and non-infectious diseases. This approach has also been used in recent years as a biomarker for the determination of pollution and as a tool in environmental monitoring. The gill structure is extremely influenced by external factors, and might serve as an indicator of many problems. In the present study, pathology associated with *Ichthyobodo necator*, *Ichthyophthirius multifiliis*, *Apiosoma* sp., *Dactylogyrus* sp., *Gyrodactylus* sp. (parasites), *Flavobacterium* sp. (bacteria) and concrete contamination were investigated on different fish gills (*Oncorhynchus mykiss*, *Salmo coruhensis*, *Salvelinus fontinalis*, *Acipenser baeri* and *Symphysodon discus*). The gill tissues of all examined fish were fixed in 10% neutral buffered formalin embedded in paraffin blocks and stained with hematoxylin and eosin. Hyperplasia and lamellar apposition were observed as the most common pathologies on the gills analyzed. However, abundant necrotic cells, as an indication of a more serious complication, were also observed on the gills of *Symphysodon discus* infested by *Gyrodactylus* sp. The vacuolisation of the gills in brook trout, infected with *Flavobacterium* sp. was recorded as another notable finding.

KEYWORDS:
Fish, histology, parasites, bacteria, concrete contamination

INTRODUCTION

Infectious and non-infectious factors in aquatic ecosystems are extremely damaging for aquatic organisms, especially fish. The gills of fish are in direct contact with the external environment and are sensitive to external fluctuations in the aquatic environment. The gills are used for exchange of gases, ammonia and ions [1, 2].

Damage to the gill structure might result in several morphological and physiological complications to fish. Among them, respiratory problems and the accumulation of ammonia are the most apparent results of gill damage. Whereas most common gill pathologies include hyperplasia, hypertrophy, lamellar fusion, thrombosis, epithelial necrosis, epithelial lifting, edema and multiple structural disorders [3, 4]. In regard to the gill pathology, earlier studies have focussed on the histologic effects of different chemicals, including pesticides [5, 6], disinfectants [7], and fertilizers [8] on reared and wild-type fish gills and damage due to of infectious fish pathogens, especially protozoan [9] and metazoan [10] fish parasites on the gills.

The gill histopathology of fish has also been used recently as a biomarker for determination of pollution and a tool for environmental monitoring [11, 12]. The interaction between gill structure and external environment can be employed while seeking the cause of a problem. Therefore, gill histology should be investigated for different fish species considering the variables of aquatic systems. The aim of the present study was to investigate the effects of external variables such as water quality, chemicals and infectious agents on the gills of different fish species.

MATERIAL AND METHODS

Different fish species affected by infectious and non-infectious agents were used in this study. The fish species and their pathologic agents are presented in Table 1. All fish were obtained from the aquaria research unit or aquaculture research unit. Sturgeons (*Acipenser baeri*) were obtained from accidentally concrete contaminated water, anesthetized with MS-222, and then immediately fixed with neutral buffered formalin (10%). To determine the pathologic agents, some of the fish species, such as coruh trout (*Salmo coruhensis*), brook trout (*Salvelinus fontinalis*), discus (*Symphysodon discus*), and rainbow trout (*Oncorhynchus mykiss*) showing disease symptoms, were sampled for bacteriological [13] and parasitological [14, 15] examinations.

Anacker-Ordal Agar (AOA) medium was used for bacteriological examination. The gill samples of infested brook trout were aseptically inoculated on AO agar. Then, the medium was incubated at 20 ±2°C. Pure bacterial colonies were selected and examined according to Sanders and Fryer [16] and

TABLE 1
Fish species, infectious and noninfectious agents in the study. N; number of fish, RTEU; Recep Tayyip Erdoğan University, KTU; Karadeniz Technical University.

Fish Species	N	Pathologic effects	Source
Rainbow trout (*Oncorhynchus mykiss*)	5	*Ichthyobodo necator*	Research Unit RTEU Fisheries Sciences
	6	*Apiosoma* sp.	
Çoruh trout (*Salmo coruhensis*)	5	*Ichthyophthirius multifiliis*	
Brook trout (*Salvelinus fontinalis*)	2	*Flavobacterium* sp.	Research Unit KTU Fisheries Sciences
Sturgeon (*Acipencer baeri*)	3	Concrete contamination	Research Unit RTEU Fisheries Sciences
Discus (*Symphsodon discus*)	1	*Dactylogyrus* sp.	Aquarium Unit RTEU Fisheries Sciences
	2	*Gyrodactylus* sp.	

Pickett [17]. The infected live fish were anesthetized with MS-222 and immediately fixed with 10% neutral buffered formalin; in case of large fish, only gills were removed and fixed.

The second gill arch of all fish samples was removed and processed for paraffin embedding. The tissue sections of 5 µm were cut, placed on microscope slides and stained with hematoxylin and eosin [18]. All slides were examined and imaged using a microscope (Leica DFC365 FX model).

RESULTS AND DISCUSSION

A bacteriological and parasitological examination of the fish was carried out; and as an infectious agent following pathogens were isolated: *Ichthyobodo necator*, *Ichthyophthirius multifiliis*, *Apiosoma* sp., *Dactylogyrus* sp., *Gyrodactylus* sp. and *Flavobacterium* sp. (Table 1). High mortality rates and cataracts in the eyes of the concrete exposed fish were observed. The histopathologic changes observed on the gills are presented in Table 2 and Figure 1–7.

According to the results, edema was a common histological finding on the gills of rainbow trout infested by protozoan parasites except for *Ichthyophthirius multifiliis* infestation. In addition, an epithelial lifting was also observed on the gills infested with protozoans (Figure 1,2,3).

TABLE 2
Histologic changes on different fish gills and their pathologic agents.

Fish	Pathologic agents	Histologic changes	Figures
Rainbow trout (*Oncorhynchus mykiss*)	*Apiosoma* sp.	Edema Epithelial lifting Necrosis Hyperplasia	Figure 1
Rainbow trout (*Oncorhynchus mykiss*)	*Ichthyobodo necator*	Edema Epithelial lifting Multiple deformation	Figure 2
Çoruh trout (*Salmo coruhensis*)	*Ichthyophthirius multifiliis*	Epithelial lifting Deformation	Figure 3
Discus (*Symphsodon discus*)	*Gyrodactylus* sp.	Lamellar apposition Hyperplasia Necrosis	Figure 4
Discus (*Symphsodon discus*)	*Dactylogyrus* sp.	Hyperplasia Loss of erythrocytes Lamellar apposition	Figure 5
Brook trout (*Salvelinus fontinalis*)	*Flavobacterium* sp.	Vacuolization Hyperplasia Deformation Lamellar thrombosis	Figure 6
Sturgeon (*Acipencer baeri*)	Concrete contamination	Hyperplasia Lamellar apposition Deformation	Figure 7

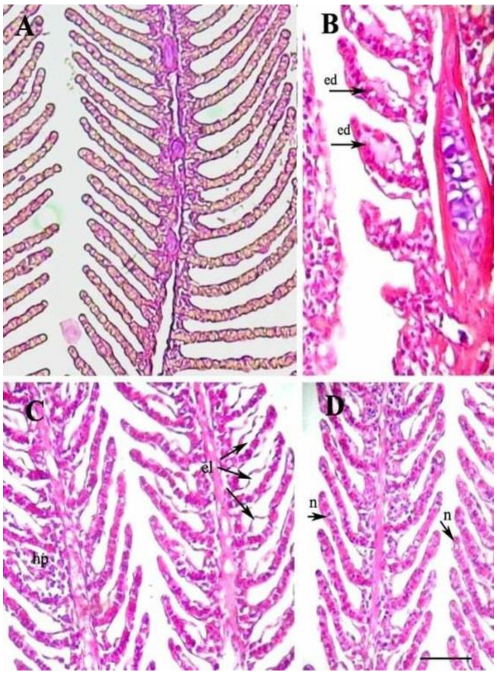

FIGURE 1
Effects of *Apiosoma* sp. infestation on gills of rainbow trout (*Oncorhynchus mykiss*). A: control, B: edema (ed), C:, epithelial lifting (el), and hyperplasia (hp), D: necrosis (n) (scale bar: 50µm).

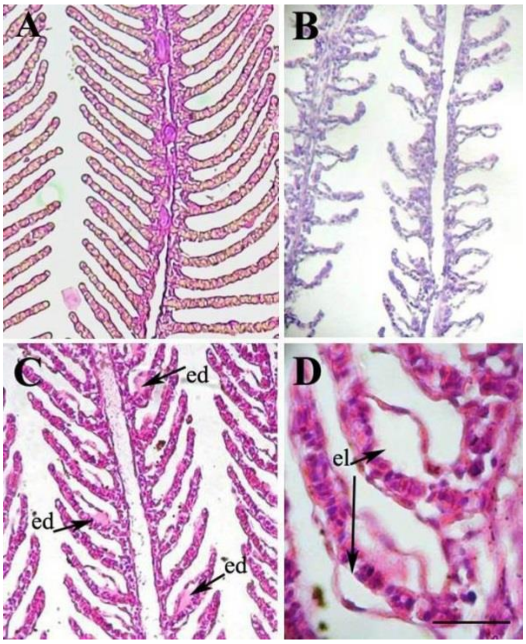

FIGURE 2
Effects of *Ichthyobodo necator* infestation on gills of rainbow trout (*Oncorhynchus mykiss*). A: control, B: multiple deformation, C:, edema (ed), D: ephitel lifting (el), (scale bar: 25µm).

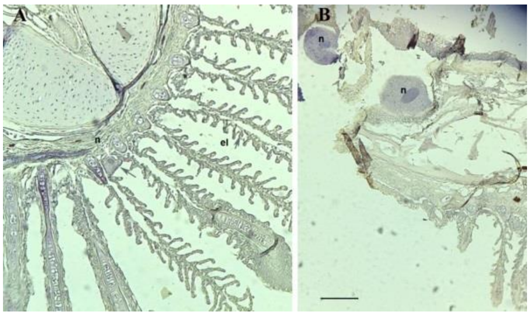

FIGURE 3
Effects of *Ichthyophthirius multifiliis* infestation on gills of çoruh trout (*Salmo coruhensis*). A: *I. multifiliis* on base of lamellae (black arrow) and its nucleus (white arrow), ephitel lifting (el), B: *I. multifiliis* and their nucleus (n), on gill rakers of *S. coruhensis* (scale bar: 300µm).

Lamellar opposition defined as the initial phase of hyperplasia was observed in the gills of discus infested with trematodes (*Dactylogyrus* sp. and *Gyrodactylus* sp.) (Figure 4,5). The most prominent histologic changes observed on the gills of brook trout were multiple vacuolisation (Figure 6).

There was a development of pale yellow bacterial colonies on the Anacker-Ordal agar. The isolated bacteria were gram-negative, thin, long bacilli, oxidase positive and catalase weak positive. The gliding movement of the bacteria was also observable under a microscope. Based on these characteristics, the bacteria could be identified as *Flavobacterium* sp.

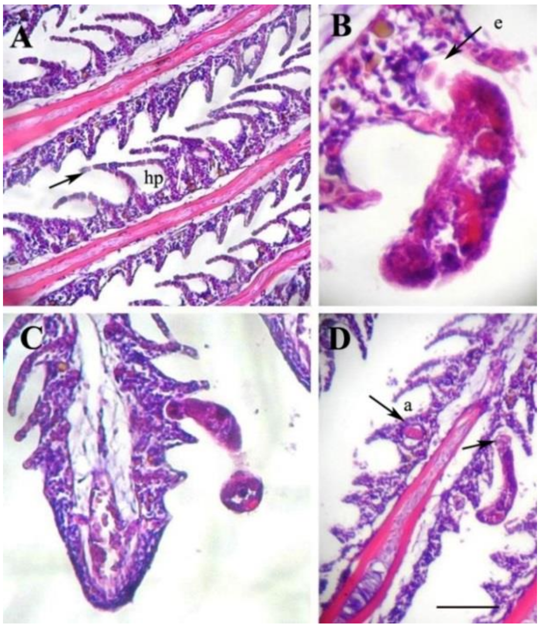

FIGURE 4
Effects of *Dactylogyrus* sp. infestation on gills of discus (*Symphsodon discus*). A: lamellar apposition (black arrow) and hyperplasia (white arrow), B: *Dactylogyrus* sp. and loss of blood from lamellea, erythrocytes (e). C: *Dactylogyrus* sp. longitudinal section, D: *Dactylogyrus* sp. longitudinal and cross-section (a), (scale bar: 400µm).

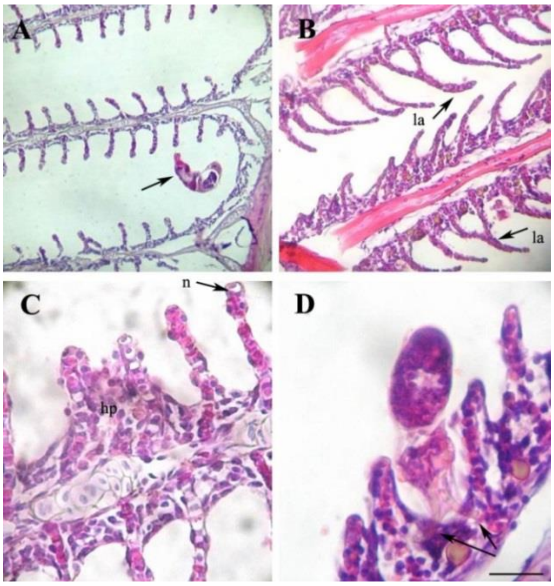

FIGURE 5
Effects of *Gyrodactylus* sp. infestation on gills of discus (*Symphsodon discus*). A: individual of the parasites (black arrow), B: lamellar apposition (la), C: necrosis (n) and hyperplasia (hp), D: *Gyrodactylus* sp. and its hooks in the base of lamella (black arrows), (scale bar: 200µm).

The parasitic infestations on the external surface of the wild and reared fish species are very common problems.

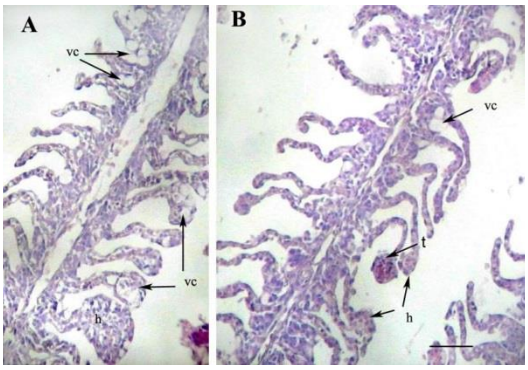

FIGURE 6
Effects of *Flavobacterium* sp. infection on gills of brook trout (*Salvelinus fontinalis*). A, B: multiple deformation, hyperplasia (hp), vacuolization (vc) and lamellar thrombosis (t), (scale bar: 30µm).

Such infestations result in a high fish mortality and economic lose in intensive culture conditions. The main disorder that occurs in the gill structure of the fish, infested with ciliate and flagellate parasites, is hyperplasia. A hyperplasia on gills can cause hypoxia [19]. In the present study, hyperplasia was observed on all fish gills infested with protozoan and metazoan parasites except for *Ichthyophthirius multifiliis*, the causative agent of white spot disease.

Epithelial lifting and edema were described by Fernandes and Mazon [20] as the early defense mechanism of the gills against an external abuse. However, Nascimento et al. [21] stated that necrosis caused by different abuses is an irreversible damage occurring on the fish gill. Necrotic cells (although a small amount) were observed on the secondary lamellae of the rainbow trout infested with *Apiosoma* sp. The other necrotic cells were observed on the gills of discus infested with *Gyrodactylus* sp. We also observed that the hooks of *Gyrodactylus* sp. were embedded in the base of secondary lamellae (Figure 4). These situations possibly explain the difficulty in the treatment of *Gyrodactylus* sp. infestation. Haidar and Ansari [22] reported a lower amount of red blood cell in *Cyprinus carpio* infected with monogenean parasites (*Dactylogyrus* and *Gyrodactylus*) compared with the healthy fish. The present study shows how erythrocytes are lost from the gill lamellae in discus (Figure 5).

The fusion of gill lamellae causes reduction in the surface area of the gills, which unavoidably results in hypoxia. Lamellar apposition and hyperplasia are defined as the beginning of lamellar fusion [3]. Typical lamellar appositions were observed on the discus infested with monogenean parasites and sturgeon exposed to concrete contamination (Figure 4, 7).

Concrete is the most preferred building material having binding properties for different materials. Bridges, retaining walls, hydroelectric power plants, and dams are commonly built in the aquatic systems throughout the world. However,

FIGURE 7
Effects of concrete contamination on gills of sturgeon (*Acipencer baeri*). A: control, B: hyperplasia (hp), C: multiple deformation, hyperplasia (hp) and lamellar apposition (la), (scale bar: 35µm).

the contents of concrete can cause a significant damage to aquatic organisms. Cement and other chemicals in concrete having high pH levels are the main contributors of the adverse effects of the concrete contamination in aquatic ecosystems [23, 24]. In the present study, multiple deformations, lamellar apposition and hyperplasia were observed on the gills of sturgeon in the water contaminated by concrete. Our results show that concrete contamination can cause massive fish death due to multiple gill pathologies in both intensive aquatic systems and their natural habitats. Therefore, the construction of concrete structure in the aquatic systems must be strongly controlled by authorized government agencies.

The genus *Flavobacterium* causes several diseases such as: *Flavobacterium columnare* causes columnaris diseases, *Flavobacterium psychrophilum* causes bacterial coldwater disease and *Flavobacterium branchiophilum* causes bacterial gill diseases [25]. Wakabayashi et al. [26] found swelling of gill lamellae in juvenile rainbow trout while infested with *Flavobacterium branchiophilum*. Declercq et al. [27] observed that *F. columnare* spreads rapidly throughout the gill lamellae and causes respiratory distress. They also reported lamellar fusion and gill epithelium destructions on the coi carp in the same study. Many bacteria produce some proteolytic enzymes, which disrupt host protein structure and also affect carbohydrates and lipid metabolism. These enzymes can severely damage host enzymes. In this case, vacuolisation occurs in the cells due to defective enzyme system dealing with glycogen metabolism.

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