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Review of fish histopathology as a technique for monitoring the aquatic environment.

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Abstract

In programmes monitoring the influences of environmental pollutants, pathological alterations are easily employed as powerful "biomarkers" to identify and explain the effects of contaminating chemicals. Researchers can examine specific target organs and cells during in vivo exposure to potentially dangerous, environment-polluting substances using histopathological examinations. In order to provide prognostic evidence of probable for both humans and animals, histopathology may also be employed to identify the precise patterns of acute and chronic detrimental effects on tissues and organs. The main pathogenic changes described in fish exposed to environmental pollutants are given a basic overview and appraisal in this article.

Keywords - Environmental Pollutants, Biomarker, Histopathology, Pathophysiological Effects

Introduction

The study of alterations in any tissue linked to a disease or ailment is known as histopathology. The name is derived from the words histos (tissue), pathos (sickness), and logia (research) in ancient Greek. To find the changes to the normal structure of tissues (and possibly their etiology), it entails the inspection of tissues and cells under a microscope. There are many approaches to do research on the health of aquatic creatures, whether they are wild or domesticated. Histological changes can be utilised as potential markers of environmental pollution, and it is a tool that aids in the understanding and prevention of diseases as well as enhancing production yields. The procedure essentially entails taking very thin slices of the animal organs so that, when stained, changes in cellular and tissue levels can be seen. The manner in which these animals are housed in captivity or the environment to which they are

exposed may have a direct impact on the degree of these abnormalities, which are typically observed in the gills, liver, spleen, heart, and gut.

When determining the general health of aquatic animals, information gleaned from the study of histopathological/histomorphological lesions in aquatic animals can be a useful contribution, especially if persistent stressors and/or pathogens are present. The maintenance of water quality parameters within the appropriate range for the cultivated species, the care with feeding management, prophylactic actions regarding everyday utensils, and the adoption of practices that minimize vector input are necessary to protect and/or improve the health of these animals. By doing this, there is a higher chance of discovering healthy fish with entire organs and their original roles. With the help of the most popular histological examination methods and illustrations of the key changes connected to various fish organs, the authors of this book hope to aid researchers and fish farmers.

Because of this, histological examinations are quite difficult and demanding, and not just from a histotechnical standpoint. Basic understanding of the physiological functions and histological structure of the researched species is a must for a successful study. The purpose of this Special Issue was to present a compilation of original articles for biologists, veterinarians, animal scientists, and other experts while also advancing our understanding of the histopathology of aquatic species. The topic is very broad, thus the published publications likewise have a wide range of research fields, methodologies, and species under investigation. This Special Issue includes eight original studies and two review papers. Their brief summaries are provided by the authors in the following three fields:

[a] Fish Nutrition histopathology -

Histopathology is commonly utilised in fish nutrition trials to characterise minor changes in the tissues and cells of the digestive system as a result of the addition of novel components or the substitution of fish meal in fish feed [Rašković et al. 2011 and Randazzo et al. 2021]. The histology technique could offer researchers potential explanations for this phenomena if the growth parameters of fish are unsatisfactory or are trailing in comparison to the control group of fish or accessible literature data. The appearance of cells involved in the digestion or metabolism of molecules derived from fish feed, such as entrecotes in the intestine or hepatocytes in the liver, will often be affected by new ingredients in fish feeds rather than significant histopathological changes [Ostaszewska et al. 2005].

A decreased cell size (or the size of their nucleus) is a marker for metabolic and physiological variations of organs in fish fed alternative feeds and can be used to calculate their surface area or volume [Rašković et al. 2019]. However, several ant nutritional components in plant-based fish feed cause enteritis in fish by inducing inflammation in the distal intestine. Both carnivorous and omnivorous fish experience this [Baeverfjord et al. 1996 and Urán et al. 2008], while enteritis in carnivorous fish species is more severe and wide spread. Rombenso et al. [2021] investigated the cause of enteritis in Atlantic salmon (*Salmo salar*) given soybean meal made from particular soybean genotypes that had changed glycinin profiles and low levels of oligosaccharides and lipoxygenases.

As neither of these compounds affected the severity of enteritis in Atlantic salmon, the researchers came to the conclusion that neither is a cause of distal intestine inflammation. For 56 days, Psofakis et al. [2021] fed gilthead seabream (*Sparus aurata*) meals containing varied concentrations of hydrolyzed feather meal and chicken by-product meal in place of fishmeal. According to the nutritional experiment, larger replacement levels (>50%) led to severe liver diseases such cirrhosis and necrosis of pancreatic acini. However, despite the absence of intestine inflammation, the intestine's histological structure continued to be devoid of pathological characteristics, which is a good illustration of why two (or even more) organs should be included in histological research. A semi-quantitative scoring system was applied in both research included in this Special Issue to assess histopathological alterations in the intestines and livers of experimental fish.

[b] Exposure assays histopathology -

One approach of preference in exposure testing is histopathology because it can provide mechanistic insight into toxicological pathology [Wester et al. 2002]. The US Environmental Protection Agency (EPA) and the Organisation for Economic Co-operation and Development (OECD) both issue guidelines for carrying out standardized exposure assays on aquatic species. The criteria allow for inter-laboratory comparison because they encompass a variety of experimental species, life stages, and exposure scenarios. Researchers will focus on various organs and evaluate changes in their micro-architecture depending on the stressor they choose to use in exposure experiments.

A single experiment in which an amphibian, *Xenopus laevis*, was subjected to atrazine, a commonly used herbicide (at least in the United States, since the European Union outlawed it), was the basis for two publications published by one set of writers in this Special Issue. The histology of the heart and cerebellum [Asouzu et al. 2021], as well as the liver and kidney [Sena et al. 2021], are used to assess the consequences of exposure. The authors used caspase-3 in the liver and kidney and inositol 1,4,5-trisphosphate in the heart and cerebellum as their immunohistochemical markers on the same types of tissue. The liver and kidney of this frog regularly exhibited a variety of distinct histological abnormalities, which both investigations demonstrated to be major unfavorable effects of the herbicide on the tissues.

Further histo morphometric techniques were used to demonstrate alterations in a variety of different cells and tissue architectures. Rainbow trout (*Oncorhynchus mykiss*) fingerlings were treated to a bath containing various species of *Aeromonas spp.* by Gómez de Anda et al. [2021]. They discovered many histological changes in the neural tissues (oblongata medulla, telencephalon, and nasal epithelium), which were unique to the fish with the more severe *A. lusitana* and *A. salmonicida* infections. The intensity and location of histopathological changes were moderate to less severe in fish infected with other bacterial species.

[c] Environmental factor histopathology -

Globally, freshwater or marine environments are often observed to gauge the general wellbeing of the aquatic species that live there. Histopathology is typically one of a number of indicators used during monitoring in order to get as much data as possible from the sampled fish

[Van der Oost et al. 2003]. Studies included in this Special Issue take a similar tack. Histopathological techniques were employed in three published publications to examine tissues of interest on different cyprinid fish from Balkan Peninsula environments. Rebok et al. [2021] provided a case study of a lipoma detected in the liver of a black barbell (*Barbus balcanicus*) in the Bregalnica River (North Macedonia). This collection of adipocytes was serially sectioned and examined under a microscope, while the authors also reconstructed a 3D image after applying software.

Over the course of a year, Tsakoumis et al. [2021] examined the histology of the gills of the endemic fish *Alburnus vistonicus* in Vistonis Lake (Greece). The authors describe the environment of this lake as "peculiar" in that it contains both freshwater (from three rivers) and seawater (from the Aegean Sea). In order to establish a link between salinity and changes in fish gills, the scientists tracked the aforementioned fish species using a battery of indicators, including histopathology. Salinity levels fluctuate as a result of the seasonal activity of rivers. The Tami River (Serbia) conducts research on fish in a natural habitat using histopathology as a biomarker [Marinović et al. 2021]. Metals from anthropogenic activities are present in this river, which is a burden. The common bream (*Abramis brama*) gills were evaluated using a semi-quantitative scoring system by the authors in order to explore the impacts of water quality during two seasons (autumn and spring perspective).

Progression of histopathology -

Techniques used in histopathology can offer a more analytical perspective on illness and how it affects tissues and cells. so that there are no restrictions on the histopathology approach in terms of laboratory setup, environment, climate, or aquatic species investigated. This technique is used on extremely different species in terms of phylogeny, age, sex, and physiology, and it is applied in both labs and the field. A variety of different tissues are also explored. The various section evaluation methods were selected based on the objectives of the given study. We noticed that this collection of articles will show the development of histopathology in the following ways, which we believe are many important methods for working in the field of histology/histopathology of aquatic organisms --

[A] Organs collection

1) In live animal treatments, abide by the Ethics Committee on Animal Use's guidelines.

2)To avoid damaging the tissues while extracting the organs for study, choose the proper tweezers, scissors, and scalpels.

3)The skin, kidney, liver, gastro-intestinal tract, and gills are the primary organs used for histological investigation.

Figure -1, Standard organs for removal from fish for histological analysis

Complete fish sample

[1] fish skin

[2] fish kidney

[3] fish liver

[4] fish stomach

[5] fish intestine

[6] fish gills

[B] Fixation of organs

1) Fix the organs in 10% buffered formalin, which is present in the vials that were previously identified, after removing them. The fixation in Bouin's solution is advised for gonads.

2) After being collected, the organs should stay in the fixative for 24 to 48 hours before being moved to 70% alcohol until processing time.

[C] Organ processing

1) Create two marks of identification for cassettes: a pencil inscription on the outside and a butter paper stamp inside, both written in pencil.

2) Remove the organ from the alcohol 70%-filled vial and set it on a petridish that had been previously prepared using dental wax that had been dipped in alcohol 70%.

3) Using a scalpel blade and a stereomicroscope, cut the organs as shown in Table 1 by following the instructions.
4) Insert the organs into the cassette and snap the lid shut.
5) Up to the subsequent stage (dehydration of the organs), keep the cassette within a beaker containing 70% alcohol.

[D] Organ impregnation, diaphanization, and dehydration

1) Place the cassettes in the beaker of 70% alcohol in the automatic tissue processor's cassette holder (Figure 2).

Figure-2- Automatic tissue Processor

2) Dehydration of the organs enables the exchange of the tissue's water for alcohol; diaphanization encourages the substitution of the sample's alcohol for Xylol; and impregnation entails the substitution of Xylol for paraffin (Table 1).

Table-1- Organ dehydration scheme for histology

S.N.	REAGENTS	TIME
1.	Alcohol 70%	1 hour
2.	Alcohol 70%	1 hour
3.	Alcohol 95%	1 hour
4.	Alcohol 95%	1 hour
5.	Alcohol 100%	1 hour
6.	Alcohol 100%	1 hour
7.	Xylol	1 hour
8.	Xylol	1 hour
9.	Paraffin	65°C (149°F) for 1 hour
10.	Paraffin	65°C (149°F) for 1 hour
Observation	After step 10, immediately start embedding	

D] Embedding of organs

1) Start the automatic embedding apparatus and heat the filtered paraffin to 65°C (149°F).
2) Set up the stainless-steel base moulds for the organs.
3) Set the paraffin base mould beneath the dispenser and fill it with stainless steel.
4) Take the organs out of the cassette and position them onto the steel base bold using heated tweezers.
5) After the paraffin solidifies, remove the blocks from the steel base mould by allowing it to cool to ambient temperature for 24 hours.

[E] Histological section cutting

1) Place the blocks in the freezer for at least 24 hours before cutting in the histological section.
2) Set the block on the block holder for the microtome, change the cutting scale (3 to 4 mm), and then thin the paraffin until all the organs are at the same level.
3) Start by cutting the block to the desired thickness, continuing until the paraffin ribbon containing the organs forms.
4) Insert the ribbon into a warm water bath at 45 °C (113 °F) to stretch it without touching or rupturing any organ points.
5) The ribbon should be swept up and placed onto a slide that has already been well cleaned; Histopathology instruction for freshwater fish.
6) Let the slide sit in a drying oven set to 30°C (85°F) after manufacturing it (in duplicate).

[F] Staining and permanent slides

1) The slide containing the paraffin ribbon and organs should be transferred in accordance with steps 1 through 19 of the Hematoxylin-Eosin (H-E) staining procedure (Table 2)

Table -2- Slide staining scheme for histology

S.N.	STEP	TIME
1.	Xylol	4 minutes
2.	Xylol	4 minutes
3.	Alcohol 100%	4 minutes
4.	Alcohol 100%	4 minutes
5.	Alcohol 90%	4 minutes
6.	Alcohol 80%	4 minutes
7.	Alcohol 70%	4 minutes
8.	Running tap water	5 minutes
.	Hematoxylin	2 minutes
10.	Running tap water	10 minutes
11.	Distilled water	3 immersions and quick suspensions
12.	Alcohol 70%	3 immersions and quick suspensions
13.	Eosin Y solution	11 minutes
14.	Alcohol 95%	3 immersions and quick suspensions

15.	Alcohol 100%	4 minutes
16.	Alcohol 100%	4 minutes
17.	Alcohol + Xylol	4 minutes
18.	Xylol	4 minutes
19.	Xylol	Until the moment of the slide assembly

2) It is advised that you perform tests on a few duplicate slides before processing all of the other slides because, in some circumstances, the periods given in table 2 may vary depending on the tissue.
3) After the process is complete, the stained slide should be covered with a cover glass and adhered.
4) Additional staining may be required depending on the research's goal and what to look for; Several examples are as follows: The Giemsa stain (Romanowsky) enables the identification of microorganisms generally, such as parasites, bacteria, and even fungi. Prussian blue, by the Pearl method, is used to evaluate iron deposits of biological origin in organs such as the spleen and kidneys. Collagen fibres can be identified using Masson's trichrome.

[G] Analysis and interpretation of tissue alterations

You must be familiar with the structure of healthy tissue in order to analyses and interpret the modifications; only then will it be possible to compare findings and spot potential anomalies at the cellular and tissue levels. Therefore, it is advised to consult bibliographical materials in advance such as articles and books to understand not only the anatomy but also the physical structure of the organs, including the type, shape, and arrangement of the cells, the presence of vessels, and the placement of the muscle fibres, to name a few.
It is advised to create a table before you begin reading histological slides. This table should include a list of potential alterations for each individual organ and allow you to check the changes both qualitatively and quantitatively. F (focal) refers to an alteration in a single point of the organ, whereas M (multifocal) refers to changes in several points of the organ. For modifications that impact the entire organ, use C (coalescent), 0 indicates no lesion, 1 indicates a lesion that compromises up to 25% of the organ, and 2 indicates a lesion that compromises up to 50% of the organ.

Review of tissue alteration manuscripts

The review articles are concentrated on histopathology investigations, which give researchers the opportunity to examine certain target organs and cells when they are exposed in vivo to potentially dangerous, environment-polluting substances. Histopathology can also be used to identify unique patterns of acute and long-term damage to tissues and organs, which can be used to predict probable pathophysiological effects in animals. The main pathogenic changes described in aquatic animals exposed to environmental pollutants are given a basic overview and appraisal in this article.

[1]Skin histopathological alterations

Because it serves as a partition between the fish's internal and exterior environments, the skin is regarded as a crucial organ in fish. When an animal comes into close touch with diseases, parasites, or poisonous substances in the water, this is their first line of defence. The skin of fish is moist, unkeratinized, and covered in a thick layer of mucus. Fish skin is extremely sensitive to physical stimuli and chemicals found in the water by nature. In water quality testing or environmental risk assessment programmes, using fish skin as a target organ or biomarker is not common practice. The acute effects of toxicants (such as metals, detergents, chlorine, acid, etc.) on the skin of fish are typically difficult to ascertain (Hylland et al. 2003).

The skin has, however, drawn a lot of attention in vitro and in vivo investigations because of direct skin contact with the environment and its crucial activities. H. fossil is treated to sub lethal concentrations of copper sulphate ($CuSO_4$) for seven days showed alterations in the surface epidermal cells, an increase in mucous production, and loss of epidermal and goblet cell form, size, and structure (Khangarot and Tripathi 1991). In *H. fossil* is subjected to the sub lethal concentration of malachite green, Rajan and Banerjee (1994) reported the modifications including a considerable decrease in the mucous cells of the dorsal and opercula epidermis and the decrease of mucus secretion.

The same alterations in the skin of perch Perca fluviatilis and goldfish Carassius auratus exposed to pulp mill waste were also noted by Lindesjoo and Thulin (1994).The integrity of the internal environment of fish may be harmed by changes to the mucous coat or underlying layers (epidermis or dermis). These modifications then result in deviant behaviour, illness, or even death. Iger et al. (1994) observed changes in the skin of common carp *Cyprinus carpio* exposed to sub lethal concentrations of cadmium, including an increase in mucous secretion and necrotic pavement cells, the migration of mature club cells, the appearance of chloride cells, leukocyte infiltration, mast cell appearance in the epidermis, the formation of new capillaries, and haemorrhage. The freshwater, air-breathing catfish *H. fossil* is that was exposed to ammonium sulphate underwent the same changes (Paul and Banerjee 1996).

[2] Kidney histopathological alterations

The kidney is the main organ for the elimination of water. For freshwater animals, this organ is particularly crucial. Ion loss in these fish is minimized by ion reabsorption systems in the kidney. In contrast, marine species have modest urine flow rates to reduce water loss. Therefore, removing divalent ions from the body is one of the kidney's main roles (Nishimura and Imai 1982). Fish exposed to hydrocarbons have been found to have hydropic vacuolation, proteinaceous droplets, and necrosis of tubular epithelial cells in their kidneys (Spies et al. 1996). In addition, glomerular abnormalities such as Bowman's space dilatation, glomerular rate hyperplasia, fibrosis, and thickening of the glomerular basement membrane were found (Adams et al. 2010; Spies et al. 1996 and The et al. 2005).

The kidney of trout (*Salmo trutta*) and tilapia (*Oreochromis mossambicus*) treated to mercuric chloride showed enlargement of the parietal cells of the Bowman's capsule and an increase in

melanomacrophage centres (Handy et al. 1993). Fish exposed to a variety of environmental pollutants also showed similar modifications (Pacheco et al. 2002). As a result, stressor-specific kidney histopathological changes could not be assumed. Although kidney changes can show signs of toxic damage, they can also show signs of xenobiotic influence when combined with pathological changes in other organs (Rhodes et al. 1987).

[3] Liver histopathological alterations

The regulation of metabolism, the creation of plasma proteins, the storage of energy and some vitamins and trace metals, as well as the transformation and excretion of steroids and xenobiotics, are all crucial bodily processes that the liver plays a significant part in. The liver is responsible for xenobiotic detoxification. The liver is typically a target organ because of the vast blood supply that results in significant exposure to toxicants and their buildup in the liver. Additionally, according to Schlenk and Benson (2005), it has a high metabolic rate. In toxicological studies, a number of pathological changes to the liver have served as trustworthy biomarkers (Moore et al. 1994 and Stentiford et al. 2003). Numerous national marine biological monitoring programmes in Europe and the USA have made use of liver histopathology (Feist et al. 2004).The liver changes in flatfish were divided into four classes by Myers et al. (1987) --

1. Degenerative lesions, such as those that affect the ciliary epithelial cells and hepatocytes' nuclei and are polymorphic.
2. Foci of cellular alteration (FCA), comprising vacuolated, clear cell, and eosinophilia foci.
3. Benign neoplasm's, such as hepatocytes adenoma, bile ducts cholangioma, and blood vessels and capillaries hemangioma.
4. Malignant neoplasm's, such as hepatocytes carcinoma, cholangiocarcinoma, and hemangiosarcoma.

A first class of liver damages are the variations in hepatocarcinogenesis (Hylland et al. 2003). The second category of hepatic lesions consists of specific or nonneoplastic proliferative lesions like hepatocytes regeneration, bile duct hyperplasia, and hepatic fibrosis in conjunction with general or non-specific degenerative alterations like cellular necrosis and hyaline inclusion bodies. Although this group provides more information on the general health state of fish, inflammatory changes are the third type of liver modifications and are regarded as the least relevant indicators of pollution exposure (Hylland et al. 2003).

In general, contaminants do not always cause liver histological changes. English sole (Pleuronectes vetulus) exposed to PAHs, polychlorinated biphenyls (PCBs), DDTs, chlorines', and dieldrin, for instance, developed liver changes such as neoplasm's, foci of cellular alteration (FCA), megalocytic heptosis (MH), hepatocellular nuclear pleomorphism (NP), and hydropic vacuolation (HV), The siluriform Corydoras paleatus subjected to organophosphate pesticides showed abnormalities such as hepatocytes polymorphism, cytoplasmic vacuolation, and nucleus in a lateral position, according to Fanta et al. (2003).

Hepatocytes vacuolation, according to Pacheco and Santos (2002), is a symptom of metabolic harm and may be related to exposure to tainted water. Fish exposed to the Camber stream's

cytoplasm and nuclear degeneration, nuclear vacuolation, and localized necrosis in the liver were documented by Camargo et al. 2007. Fish exposed to metals (Oliveira Ribeiro et al. 1996 and Paris-Palacios et al. 2000) and PCBs (Chang et al. 1998) commonly exhibit these more severe modifications. The liver of the catfish Clarias gariepinus treated to fenvalerate showed pathological changes such as hepatocytes vacuolation, leukocyte infiltration, blood congestion, necrosis, and fatty infiltration (Chang et al. 1998).

The liver of 7-day-old Sacramento split tail larvae subjected to sub lethal amounts of esfenvalerate for a week showed the same alterations, according to Teh et al. (2005). Although it takes a lot of time and effort to prepare liver samples and skilled pathologists to identify hepatological abnormalities, utilizing liver histopathology as a biomarker of pollution exposure may not be a useful strategy for pollution screening (Au 2004).

[4] Histopathological alterations of gastro-intestinal tract

Toxicants can cause minor changes in the motility, secretion, and absorptive activities of fish's gastro-intestinal tract or more serious abnormalities in the mucosal integrity, blood flow, or neuromuscular control of the digestive tract. The organism's capacity to thrive is eventually impacted by these changes (Schlenk and Benson 2005). The hydropic degeneration of the digestive glands (Haensly et al. 1982), the proliferation of mucous cells, hyperemia, atrophy, and metaplasia were the principal abnormalities noted in the gastrointestinal tract. According to several research, eating a diet heavy in certain metals may cause an increase in intestinal cell death (Berntssen et al. 1999).

Few studies have documented changes in the intestine's histomorphological in fish that have been exposed to heavy metals (Bernet et al. 1999). Both Bano et al. and 1990 and Bernet et al. 1999 showed some changes in the guts of mercury-exposed *Channa punctatus* and *Heteropneustes fossilis*. *Salvelinus alpinus* treated to methyl and inorganic mercury did not exhibit any histological changes, however (Oliveira Ribeiro et al. 2002). According to Giari et al. (2007), several xenobiotics (petroleum, chlorinated biphenyl, benzopyrene, terbuthylazine, and cadmium) have been shown to change the epithelium in the intestine of fish. According to Hylland et al. (2003), the gastro-intestinal tract does not appear to have pathological alterations that are beneficial for understanding the biological consequences of toxicants.

[5] Gill histopathological alterations

The fish gill is a multipurpose organ that regulates ions, excretes nitrogenous waste, and regulates acid-base levels. It makes up more than half of the animal's entire surface area, making it vulnerable to water pollution. Numerous data on gill modifications in fish exposed to various toxicants were supplied by Mallatt (1985), Wood (2001), and Au (2004). The most frequent lesion is the lifting of the lamellar epithelial cells brought on by the fluid penetration. It could result in a decrease in respiratory gas exchange and an increase in diffusion distance. The more severe gill damages caused by pavement cell raising include lamellar fusion and epithelial

rupture (Au 2004). Another pathogenic response of the gills is the necrosis of several lamellar and filament cells.

However, because metals directly interact with ion transport proteins and decrease their activity, it is frequently observed in the gill of fish that have been exposed to metals. Diffusion of ions and water should increase during necrosis. According to research using transmission electron microscopy, necrotic cells have enlarged cytoplasm and organelles that are more electron dense. The cell membranes would eventually burst, releasing the content. According to Bury et al. (1998), leukocyte infiltration should also be viewed as an adaptive reaction. The expansion of the necrotic cell typically occurs in conjunction with the pavement cell's hypertrophy. Fish exposed to metals have also been observed to develop this lesion (Goss et al. 1995).

Fish exposed to metal contaminants show higher mucous cell growth and increased mucous production than fish exposed to organic pollutants. Pavement cells, mucous cells, and chloride cells are constantly growing, and while this might be seen as a protective mechanism that prevents pollutants from reaching the branchial surface, it also has the potential to impair respiratory gas exchange, which could result in animal mortality (McDonald et al. 1993). Chloride cell multiplication was first identified by Haaparanta et al. (1997) in roaches (Rutilus rutilus) taken from Finland's contaminated lakes as a substantial pathogenic change.

The separation between the blood and environment is increased by the epithelial raising, hyperplasia, and hypertrophy of the epithelial cells as well as the partial fusion of lamellae, which prevents pollutants from entering the organism (Poleksic et al. 1994). More reports of these changes come from long-term exposures. They extend the blood's and water's diffusion distances. According to Perry et al. (1996), they also reduce the inter lamellar distance and the diffusion conductance of breathing gases into the gills. Fish exposed to a severe kind of stress may exhibit inflammatory reactions such as lamellar aneurysm, blood clotting, dilatation of marginal channels, and leukocyte infiltration (Rosety- Rodrguez et al. 2002).

Aneurysms are categorized as significant changes that are challenging to treat. It happens when the pillar cells burst because of an increase in blood flow or a direct chemical reaction (Poleksic et al. 1994). Organ chlorines, petroleum chemicals, organophosphates, carbonates, herbicides, and heavy metals are only a few of the pollutants that might cause these changes (Au 2004; Hemalatha et al. 1997 and Van der Oost et al. 2003). Although the majority of gill modifications are concentration-dependent, they are not species-specific and are independent of the kind of toxin, exposure intensity (acute vs. chronic), exposure medium (freshwater vs. seawater), or fish species. When rainbow trout were exposed to petroleum wastes, epithelial lifting and lamellar fusion were seen (Engelhardt et al. 1981).

The same alterations have also been reported in fish exposed to metals (Camargo et al. 2007) and organic toxicants (Rosety-Rodrguez et al. 2002) in their gills. Giari et al. (2008) observed desquamation, edema with epithelial lifting, telangiectasia, hyperplasia and lamellar fusion in the gills of European sea bass (Dicentrarchus labrax L., 1758) exposed to sub lethal mercury concentrations. Shorthorn sculpin (Myoxocephalus scorpius) gills exposed to pulp and paper mill wastewater also showed signs of hyperplasia (Barker et al. 1994). Indeed, the host's ability to

breathe is impacted by alterations in the respiratory epithelium. Even if slight changes don't kill fish, they have a negative impact on their ability to do their daily tasks. On the other side, significant or severe injury may result in death.

Final comments

Histology is a crucial method for determining the health state of fish, together with macro-, microscopic-, parasitological, hematological, and immunological analyses. It is crucial to stress that this instrument should be used carefully and deliberately because a mistake at one stage can affect how the test results are read and interpreted. Therefore, it is advised to do some planning before beginning the histopathological analysis, taking into account the purpose of the analysis, the appropriate number of animals (sufficient to avoid compromising the result and to use fewer than necessary), the procedures advised by the Ethics Committee on the Use of Animals, the quality of the utensils used to process the samples, technical training, the appropriateness of Personal Protective Equipment and containment, and so on. We anticipate that this collection of publications will benefit readers and researchers interested in the histology and histopathology of aquatic creatures, as well as spark new interest in this field of study.

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