

Morphologic evaluation of the gills as a tool in the diagnostics of pathological conditions in fish and pollution in the aquatic environment: a review

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ABSTRACT: Fish gills, owing to their status as a multifunctional organ, have always fascinated researchers. In spite of the intense work done on the morphologic examination of gills, the organ is relatively under-used in health evaluation in fish. The existing literature on this topic is reviewed here. Our review summarises important diagnostic guidelines for the examination of gill structure and describes the morphological lesions that develop under the influence of different biological and physicochemical factors. The picture that should emerge is that of an organ that is extremely sensitive to all types of handling and unfavourable changes in the external and internal environments. We conclude that studying the morphology of the fish gills provides an opportunity to assess fish health status as well as information on possible health hazards coming from their environment.

Keywords: gills; respiratory tract; morphological examination; pathomorphological changes

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1. Introduction

The gills in fish are a particularly sensitive organ responsible for respiration and maintaining the optimal osmotic pressure and acid-base balance of bodily fluids. They play an important role in excreting unnecessary and harmful metabolic products and in supporting food drainage (Genten et al. 2009). Because their large surface area contacts the external environment, the gills are sensitive to even minor chemical or physical changes in the surroundings and are the target or-

gan for many contaminants carried in water (Mallat 1985; Mazon et al. 1999; Wong and Wong 2000; Cerqueira and Fernandes 2002; Mir and Channa 2009; Movahedinia et al. 2009). Water flowing through the gills exposes the organ to constant contact with potentially harmful chemical and biological substances, which is reflected in morphological changes in the organ and impairment of its functions. Moreover, even minor mechanical damage to the structure of gills may be dangerous to fish. Lesions resulting from any of these influences may be a direct cause of increased mortality

in reared fish (Ferguson et al. 1994; Antychowicz and Matras 2008). Studies carried out under laboratory conditions (Karlsson 1983; Karlsson-Norrgren et al. 1985; Wester and Canton 1991; Schwaiger et al. 1992; Oliveira-Ribeiro et al. 1996; Schwaiger et al. 1996; Mazon et al. 2002; Thophon et al. 2003) and in ponds or other natural environments (Hinton and Lauren 1990; Jagoe and Haines 1997; Schwaiger et al. 1997; Teh et al. 1997; Stentiford et al. 2003; Flores-Lopes and Thomaz 2011) have demonstrated that the morphological evaluation of the gills may serve as an indicator for assessing fish health status and environmental pollution levels. This knowledge is particularly useful in intensive culture systems, especially in disorders caused by various environmental factors (Daoust et al. 1984; Capkin et al. 2009; Bradford et al. 2010; Poleksic et al. 2010; Mitchell and Rodger 2011).

In addition, many researchers, such as Wilson et al. (1983), Rucker et al. (1952), and Karges and Woodward (1984), have observed that a deficit in pantothenic acid may result in hypoplasia of the gill epithelium whereas a deficit in vitamin C causes gill deformation and functional impairment, manifested mainly as dysplastic lesions at the ends of distal gill filaments (Soliman et al. 1986). Thus, it seems justifiable to infer that nutrient availability and feeding affect gill appearance.

2. Structure of the gills and their functions and dysfunctions

The gills in teleostei consist of five pairs of gill arches, which are curved bone structures located on both sides of the head (Kilarski 2007). Two rows of gill filaments are distributed on the external side of the first four arches; the free ends of the filaments extend towards the gill chamber (Wilson and Laurent 2002). Filaments do not develop on the last, or fifth, gill arch. In teleosts and sturgeons, a skin fold covers the gill arches from the external side, and the bony plates that develop on it form a movable operculum that limits the gill opening with its distal end (Kilarski 2007).

Each gill arch bears two rows of gill filaments that form the so-called “hemibranchia” (Kilarski 2007). A thorough examination has demonstrated that they are composed of a row of long, thin filaments, i.e. primary lamellae protruding from the gill arch like teeth in a comb. Additionally, the sur-

face of each primary lamella is expanded by regular crescent folds, i.e. secondary lamellae, found on the ventral and dorsal surfaces.

The gill arch is a curved bony structure from which gill radii of the primary lamellae extend radially (Roberts 2001). The inclination angle of these lamellae and the corresponding degree of ventilation may vary because of the abductor and adductor muscles. The vascular system of the gills consists of two components: arterial–arterial and arterial–venous. The respiratory lamellae are abundantly vascularised with capillaries and are the site of respiration and oxygenation of the blood.

The gill filament consists of a cartilaginous core and vessels and is covered with stratified epithelium. Specialised mucous and chloride (salt) cells are located between the epithelial cells at the base of the respiratory lamellae (Movahedinia et al. 2012). Chloride cells contain numerous mitochondria, which are essential for intensive energy processes associated with osmotic regulation. They also remove excess sodium chloride from the body (Hibiya 1982). These cells are common in marine and anadromous fish but are rarely found in freshwater species. They are located at the margins of gill lamellae, because their number increases in pathological conditions. The cells of the gill filament also include double-concave supporting cells (pillar cells) that are contractile. These cells are combined in pairs and constitute a casing for the capillaries of the gill lamellae.

Given the relatively limited number of structural components in the gills (epithelium, endothelium, fibrinous-cartilaginous matrix, basal cells, and specialised cells such as mucous, chloride, and eosinophilic granular cells), the extent of the pathological response is somewhat limited (Roberts 2001). A general examination may reveal oedema, excessive production of mucus, anaemia, or necrosis. These lesions are typical for acute pathological processes caused by different biological and physicochemical factors found in water as solutions or suspensions (Ferguson 1989). Anomalies associated with the permeability of the membrane at the cellular or tissue level are most common. They result in oedema of the epithelium or sub-epithelial spaces. In the early stages, oedema affects only single cells; subsequently, the thickness and length of the whole secondary lamellae increase. In addition, these lesions are often accompanied by excessive mucus production.

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3. Gross examination

Gill appearance is best evaluated on live fish, and gross post-mortem sampling of this organ may bias results because of the rapid development of lesions. Samples for histopathological examination should be taken no later than one minute after sacrifice and placed in a vessel with 5% buffered formalin or Bouin's solution (Speare and Ferguson 1989) to protect against post-mortem artefacts that mimic lesions (Walsh and Ribelin 1975; Ferguson 1989; Speare and Ferguson 1989).

A colour change is one of the first signs associated with gill dysfunction. Healthy gills are bright red, but with anaemia, they become pale pink, and paleness with haemorrhages most often indicates viral disease. Paleness of this organ detected in fish immediately after capture is a sign of ischaemia of the whole body and is an excellent indicator of health status in fish. When the gills are brown and pale brownish, methaemoglobinaemia associated with nitrite poisoning is the cause (Antychowicz and Matras 2008), while a light red colour of gills is caused by cyanide poisoning (Svobodova et al. 1993). Mosaic colouring may develop with branchiomycosis, branchionecrosis, and mass infestation with *Sanguinicola inermis* eggs (Antychowicz and Matras 2008). Necrotic lesions represent a more advanced symptom, and these macroscopically detected pathological changes may result in fragments becoming separated from the respiratory lamellae and gill filaments.

In the case of low oxygen content in water (Scott and Rogers 1980; Fernandes and Mazon 2003), pH changes, accumulation of nitrogenous metabolic products in the aquatic environment (Capkin et al. 2009), or irritation of the gills by toxins or parasites, the first reaction is an increase in mucus production by this organ (Speare et al. 1991; Thiyagarayah et al. 1996; Lease et al. 2003; Svobodova et al. 2005) (Figure 1C). Mucus pushes pathogenic factors away from the surface of the gills and thus reduces their harmful impact and hinders tissue penetration by parasites. In addition, its proteinaceous components neutralise the physicochemical effects of many parasites. However, excessive accumulation of mucus impairs ion and gas exchange, and even minor temperature or pH oscillations may result in death. If hyperaemia of the gills, clubbed gill filaments, and, later, frayed margins and necrotic foci are seen in addition to excessive production

of mucus, bacterial gill disease may be suspected. Abbas (2006) demonstrated that non-ionised ammonia strongly induces mucus production. Roberts (2001) and Turnbull (1993) confirmed that ammonia which forms in ponds with excessive stock density and enzymes found in fish excrement affect the properties of mucus in the gills. Different suspensions found in water very often accumulate between the gill filaments and constitute an excellent medium for bacterial and fungal growth.

4. Microscopic evaluation

Microscopically, acute lesions include hypertrophy of the respiratory epithelium, elevation of the epithelial cells and their separation from the basal membrane, swelling of the epithelial cells, excessive production of mucus, infiltration of mononuclear inflammatory cells and necrosis and adhesions of gill lamellae (Takashima and Hibiya 1995; Myers and Fournie 2002). Hyperplasia of the epithelium and chloride cells (Bais and Lokhande 2012), hyperplasia of mucous cells, microaneurysms, telangiectasia, infiltration with eosinophilic cells, hypertrophy and fusion of the epithelium on the respiratory lamellae are lesions of a chronic nature.

5. Oedema of the lamellae and separation of the epithelium of the respiratory lamellae

Oedema of the respiratory lamellae is often found as a result of exposure to substances such as heavy metals, pesticides, and therapeutics (e.g. hydrogen peroxide, formalin), as well as the effect of water acidification after acid rainfall or irritation by various suspensions (Walsh and Ribelin 1975; Roberts 2001). The impact of the latter and their accumulation in the gills causes oedema of the gill lamellae with accompanying dilatation of the vessels. Oedema is often associated with cell infiltrates and is a symptom of disorders of osmoregulation; it represents a kind of defence mechanism (Movahedinia et al. 2012).

At the terminal stage of oedema, the respiratory epithelium separates entirely from both the primary and secondary lamellae (Figure 1A,B), and this process is accompanied by necrosis of the epithelial cells, which consequently leads to res-

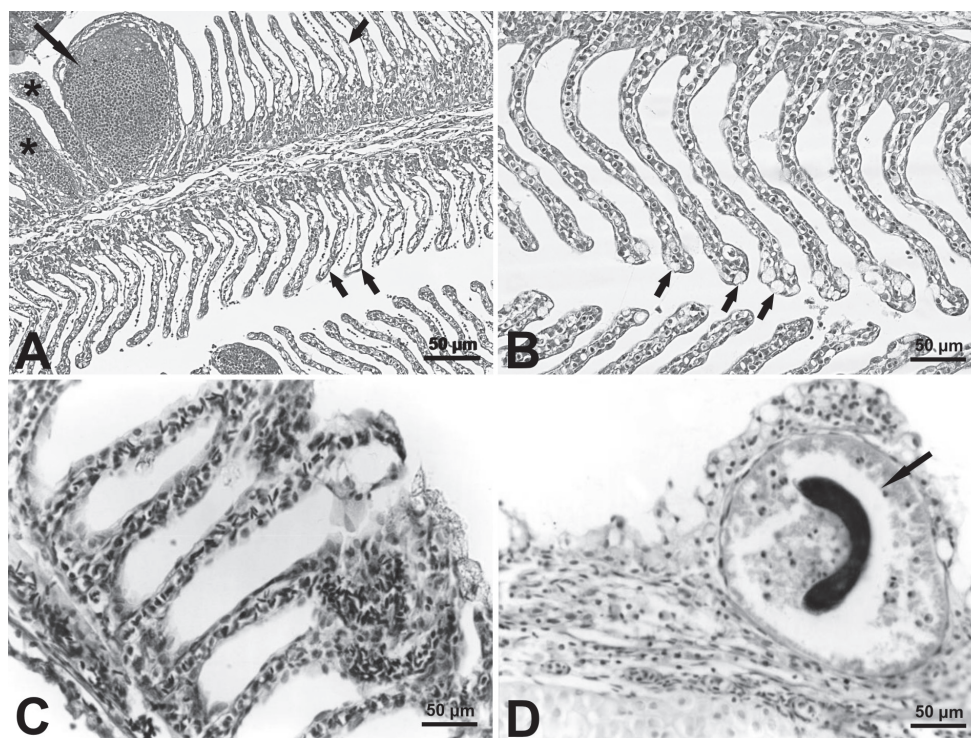


Figure 1. The morphological changes in gills of rainbow trout (*Oncorhynchus mykiss*) – A,B and carp (*Cyprinus carpio*) – C,D. **A:** Congestion (asterisks), formation of aneurysms (long arrow), elevation of epithelial cells (short arrows), lamellar fusion (upper right side). **B:** The oedema of epithelial cells at the top of lamellae (arrows). **C:** Hyperplasia of mucous cells with fusion at the top of lamellae. **D:** *Ichthyophthirius multifiliis* with infiltration of lymphoid cells and blood congestion. HE staining

piratory and osmotic regulation disturbances and even death (Monteiro et al. 2008). Singhadach et al. (2009) suggested that elevation of the epithelium of gill lamellae is an immediate effect of different toxic substances and reflects an acute inflammatory process in the gills. Many authors, such as Arellano et al. (1999), Pane et al. (2004), Schwaiger et al. (2004), Authman and Abbas (2007), Monteiro et al. (2008), Singhadach et al. (2009), have described the process of separation of the respiratory epithelium from the basal membrane. This phenomenon is often observed as a result of intoxication with, for instance, chloride, ammonia, or phenol (Satchell 1984) and initially affects a limited area before spreading over the entire respiratory lamella. It may act as a protective mechanism by increasing pollutant-diffusion distance (Mohavedinia et al. 2012). Bullock (1990) reported that separation of the epithelium from the supporting cells of the respiratory lamellae is a process that initiates necrosis of the gills. Oedema of the gill lamellae with concurrent dilatation of the blood vessels often results from an accumulation of suspensions in the gills.

6. Petechiae

Physical examinations of the gills often reveal petechiae, which are generally seen as small red dots or lines. Microscopically, damage and loss of double-concave cells (pillar cells) are observed at these sites and, as a result, the capillaries of the respiratory lamella are fused and form a uniform space filled with blood, which results in dilatation of the lamella, blood congestion, microaneurysms (Figure 1A) and sometimes destruction of lamellae. Petechiae may result from mechanical injuries (during catching, prolonged transport, or intensified culture) and poisoning with toxins and heavy metals such as cadmium (Noga 1996; Al-Attar 2007; Pantung et al. 2008; Bais and Lokhande 2012) or with pesticides (Van den Heuvel et al. 2000; Cengiz and Unlu 2002; Thophon et al. 2003; Schwaiger et al. 2004; Cengiz 2006). Over time, extravasated blood coagulates, and a fibrinous clot forms that is subsequently reorganised and absorbed. The presence of numerous petechiae may impair respiration, an effect commonly seen in response to high tempera-

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tures in the environment when oxygen demand in fish increases and oxygen content is low in water.

7. Hyperplasia/hypertrophy

Irritating factors often cause a substantial increase in the number of mucous, epithelial, and chloride (salt) cells present in the gills in fish. This phenomenon is particularly pronounced when exposure to irritants is chronic. The effects of heavy metals, ammonia intoxication, excessively high or low water pH, and parasitic infestations that destroy the gill cells are thought to be the cause of excessive proliferation. Kantham and Richards (1995) and Singhadach et al. (2009) reported that chronic exposure to even diluted toxic substances causes proliferation of the cells between the respiratory lamellae. Initially, such a state is believed to represent an adaptation to new conditions and is aimed at protecting against excessive penetration of toxins from water to the blood vessels in the gills and thus to the blood (Mallat 1985; Hinton and Lauren 1990; Fernandes and Mazon 2003); subsequently, however, it leads to a decrease in the respiratory-excretory surface of the gills and impairs their function.

Adhesions of the respiratory lamellae are common and result from a change in the electrical charge of glycoproteins in the gill cells, which consequently attracts neighbouring lamellae (Daoust et al. 1984) and causes hyperplasia and hypertrophy. The predisposing factors include heavy metals (Authman et al. 2013), pesticides (Eller 1969), ammonia, and parasites (Szarek et al. 2006). Adhesions of the gill lamellae over a large area (as a result of hyperplasia and hypertrophy) and accompanying destruction of the supporting cells are a sign of poor health status in fish (Ghorashi et al. 2013). Disturbances of the acid-base and ion balance then develop, which may cause acidosis and even suffocation (Satchell 1984; Svobodova et al. 1993).

In differential diagnostics, it is important that in comparison with other factors, the adhesions of the lamellae caused by external parasites, such as freshwater ick, are limited only to the locations of parasites on the gill surface (Szarek et al. 2006; Movahedinia et al. 2012).

8. Exudate and cellular infiltration

Oedema of the gills and concurrent exudate and cellular infiltration reflect disturbances in the

regulation of osmotic pressure. Infiltration with mononuclear granular cells may be caused by various toxic substances (Kantham and Richards 1995; Authman and Abbas 2007).

Intoxication with gaseous ammonia, even at sub-threshold doses, results in eosinophilic infiltration at the base of gill filaments and in an increase in the number of mucous cells and their hypertrophy, even though no lymphocytic infiltration is seen in such locations (Scott and Rogers 1980). Such a phenomenon is often accompanied by petechiae near large blood vessels. Infiltration with lymphocytes and macrophages frequently occurs together with infestations of unicellular parasites. Intoxication with Cd, Cu, and Zn ions is reflected in the histopathologic picture as an increase in the number of chloride cells (Crespo et al. 1981). These cells then migrate from the epithelium of respiratory filaments and accumulate on the surface of respiratory lamellae.

9. Necrotic foci in the gills

Necrosis of the gills develops as a result of prolonged exposure to irritants that most often include suspensions found in water. It is also caused by microorganisms, for instance, koi herpes virus and *Branchiomyces sanguinis*. Rodger (2007) reported that necrosis of the epithelial cells in the gills results from toxins, the irritating effects of phytoplankton, or excretions of nematocysts of hydra or jellyfish. At the initial stage of necrosis, pyknosis or karyorrhexis are observed microscopically; these phenomena are typical of degenerative processes. Necrosis is the final stage. In the most advanced cases, necrosis may result in complete atrophy of the soft tissue covering the gill filaments and, consequently, in uncovering of the cartilaginous elements (Noga 1996).

10. Evaluation of morphological lesions in the gills as a tool for monitoring the environment

According to Mallat (1985), Mazon et al. (1999) and Cerqueira and Fernandes (2002), increasing levels of pollution in ecosystems and inflows of toxic substances that consume substantial amounts of oxygen cause numerous changes in the morphological picture of the gills and their functions.

The gills are sensitive not only to high doses that induce acute lesions but also to sub-threshold doses that exert their effects over a longer period of time (Johnson et al. 1993; Bernet et al. 1999). Morphological evaluation, particularly microscopic, allows detection of these disturbances at an early stage and constitutes an excellent “early warning” tool for changes in the environment. Early diagnosis may thus prevent mass deaths. Knowledge of gill pathology, both general and microscopic, is a valuable tool for veterinarians who supervise fish cultures. Its pathognomonic nature and relatively low cost are its main advantages. Many authors emphasise the importance of utilising histopathology as the method of choice for monitoring the impact of the environment on fish breeding (Haaparanta et al. 1997; Poleksic et al. 1999; Poleksic et al. 2002). According to Schwaiger et al. (1997) histopathological examination allows differentiation of the pathological changes caused by the disease and those caused by pollution. In marine ecosystems, there are many national and regional programs investigating the impact of environmental pollution on the arrangement of morphological lesions (Johnson et al. 1993; Myers et al. 1993; ICES 1997).

Evaluation of morphological lesions is also a useful tool for assessing the impact of xenobiotics, e.g. pesticides or pharmaceuticals on the health status of fish (Eller 1969; Schwaiger et al. 1997; Capkin et al. 2009; Velmurugan et al. 2009). Pharmaceuticals and pesticides very often react with water leading to changes in pH; this, in turn, elicits a stress response that starts in the gills and results in reduction of gas exchange and the emergence of respiratory distress (Treves-Brown 2000) as reflected in the morphology of the gills. Substances as dichlorvos (Velmurugan et al. 2009) cypermethrin (Raksheskar 2012), deltamethrin (Cengiz 2006), endosulfan (Cengiz and Unlu 2002), malathion (Cengiz and Unlu 2003) cause gill lesions of varying severity and nature, from the raising up of the epithelium, through petechiae, and finally necrosis plaques and deformation of gill structure.

11. Morphological lesions in the gills in the course of selected disease conditions

Generalised bacterial infections may also be reflected in the gills. Furunculosis caused by *Aeromonas salmonicida* bacterium may cause fo-

cal thrombi in the primary or secondary lamellae or their oedema with accompanying infiltration of inflammatory cells (Roberts 2001). Infection with *F. columnaris* results in microscopically thread-like bacteria located at the ends of gill filaments. In addition, a massive infection combined with hypertrophy of the cells covering the gill filaments may cause the interlamellar spaces to disappear.

Fungal infections such as branchionecrosis are characterised by haemorrhagic foci in gills that become pale (as a result of ischaemia) and necrotic over time. Numerous petechiae and adhesions of gill lamellae as well as dark, round spores of *Branchiomyces sanguinis* in the capillaries are visible in such locations. Hyphae grow from the spores and invade the gill tissues and blood vessels, and the mycelia that develop cause emboli and circulatory disturbances in the gills. Intensive hyperaemia with dilatation of the blood vessels is seen with concurrent ischaemia and paleness of the gill filaments, which gives them a characteristic marbled appearance. In ischaemic locations, necrotic lesions develop from the margins towards the gill arches. In such cases, the ends of gill filaments often die, tissue defects develop, and necrotic gill lamellae tend to adhere to each other, forming a uniform necrotic wall.

Parasites such as *Sanguinicola*, *Salminicola* spp., and *Ergasilus* spp. cause hyperplasia of the epithelial tissue of blood vessels in the gills and infiltration with leukocytes and fibrinous tissue. *Sanguinicola* eggs enter small blood vessels and cause their obstruction while miracidia elicit mechanical injuries and inadequate blood supply combined with local hyperaemia. *Dactylogyrus vastator* infestation damages the supporting cells of the gill filaments and thus causes deformation of the gills; the gill filaments become club-shaped because of overfilling with blood. In a massive infestation, defects in the gills develop (Antychowicz and Matras 2008). Moreover, intensive invasions with flukes of the *Dactylogyrus* genus microscopically appear as cells at different stages of disintegration and infiltrating bi-nucleated giant cells and erythrocytes on a background of hypertrophic connective tissue. Deeper damage results in extravasations of blood that stay between the respiratory lamellae. In fish reared in ponds, the parasite also damages the cartilaginous tissue in addition to the epithelium of the gills (Jara and Chodynieski 1999).

Ichthyophthirius multifiliis is visible in gills as white spots, not more than 0.5 mm in diameter; microscopically, trophozoite growth causes the el-

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evation and displacement of the epithelial cells and subsequently vacuolisation and necrosis (Bruno et al. 2013) (Figure 1D).

Amoeboid gill disease is generally seen as multifocal, disseminated spots and dots on a background of grey and swollen gill tissue with an accompanying accumulation of excessive amounts of mucus surrounding the gill arches. Histopathological examinations then reveal hyperplasia or complete adhesions of the respiratory lamellae. Small interlamellar vesicles are also formed. Nowak and Munday (1994) observed lesions in the structure of gills in Atlantic salmon after transfer to seawater. Within three weeks following a transfer, the number of salt cells substantially increased, the integrity of epithelium changed, and hyperaemic foci developed. Nodules and clusters of mononuclear cells and macrophages were seen within one week. After approximately four weeks, colonisation with amoebas was found near the lesions.

12. Summary

Pathologic lesions in the gills are a significant problem in fish cultures, but reviews of histopathologic lesions in the gills of fish are scarce. As this review of the literature reveals, an unnatural appearance of this organ and microscopic lesions are proof of the poor health status of fish or are a reflection of changes in the aquatic environment. Morphological examination of the gills is an excellent tool for evaluating disturbances in fish cultures and does not require a large financial outlay. Therefore, its application should become widely adopted by both breeders and veterinarians who supervise the aquaculture industry.

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