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Using of Fish Pathological Alterations to Assess Aquatic Pollution: A Review

Negin Salamat and Mehdi Zarie

Department of Marine Biology, Khorramshahr University of Marine Science and Technology, Faculty of Marine Science, P.O. Box: 669, Khorramshahr, Iran

Abstract: Using of fish pathology is well established as an essential section of the set of 'biomarkers' applied in biological effects of variety pollutants monitoring programs in Europe such as International Council for the Exploration of the Sea (ICES). Use of histopathological techniques allows investigators to examine specific target organs and cells as they are affected by exposure to environmental chemicals. Moreover, it offers a means of detecting acute and chronic harmful effects of exposure in the tissues and organs of individual animals. This review provides a general evaluation of the main pathological alterations reported in fish exposed to contaminants.

Key words: Histopathology % Biomarker % Fish % Contamination

INTRODUCTION

Xenobiotics derived from urban communities, agricultural operations and industrial effluents, finally released into the environment. In the context of pollution, aquatic systems are highly at risk because of their trend for accumulation relatively high concentrations of chemicals entering from surrounding terrestrial systems, thus, regardless of their source of entry to the environment, water bodies are frequently stores for a large variety of stressor chemicals [1].

During past several decades, aquatic toxicology changed its way from a explanatory approach, that was needed to investigate those concentrations of particular pollutants in the water which were not well-matched with the life of individual fishes, to deliberations of nonlethal concentrations that do not lead to death over the short duration but do hurt the individual, so making it use resources to stay alive in a changed condition. Just the existence of particular xenobiotic in the part of an aquatic ecosystem does not signify harmful effects. Establishment of links between external levels of exposure, internal levels of tissue contamination and early detrimental effects is needed [2].

Biomarkers were derived from investigation in this field, with the emphasis on host responses. They demonstrate the multiple organ, tissue and cellular sites of action and the range of responses that were possible [3].

The term 'biomarker' has been defined in several ways. It is generally included approximately any measurement indicating an interaction between a biological system and a possible risk, which may be chemical, physical or biological [4]. It is exactly described as an alteration in a biological response (ranging from molecular, cellular and physiological alterations to behavioral changes) due to exposure to environmental chemicals [5]. Van Gastel and Van Brummelen [6] redefined the term 'biomarker' as any biological response to an environmental toxicants at the subindividual level, which evaluated inside an organism or in its products (urine, feces, hair, feathers, etc.) and reflecting a departure from the normal situation that cannot be detected in the intact organism [6]. During the past two decades, attempts have been made to identify and characterize biomarkers in a range of organisms from bacteria to humans to predict disease or detrimental ecological effects [7- 10].

The NRC [11] described biomarkers as "indicators signaling events in biological systems or samples following chemical exposure" and recommended using of biological indicators to determine: (1) biologically effective concentration, (2) harmful effects and (3) sensitive populations or individuals, in order to forecast and maybe avert clinical disease. Actually, NCR had placed the emphasis on human health in its original

Corresponding Author: Negin Salamat, Department of Marine Biology, Khorramshahr University of Marine Science and Technology, Faculty of Marine Science, P.O. Box: 669, Khorramshahr, Iran.
Tel: +989166165146, Fax: +98 63242 333 22.

classification [11]. Adams [7] modified the original NRC classification to comprise characteristics of organisms, populations, or communities that respond in assessable ways to the environmental changes [7]. With the advance of measurements for including other organisms such as fish, their utility as a "marker" or as an "indicator" in ecological settings was really in doubt [12].

The NRC suggested three kinds of biomarkers in order to categorize responses as markers of exposure, effect and susceptibility, each of which has been discussed in terms of its potential use in ecological risk assessment patterns [13]. Stressor exposure may resulted in effects that may be defined as an early acclimatize nonpathogenic result or as a more critical functionally change, depending on the toxicant concentration and mechanism of its action [3, 9].

Exposure to sublethal concentrations of environmental chemicals may lead to the histological structure alterations which can significantly alter the function of tissues and organs. Histological and ultrastructural changes in cells, tissues or organs can afford good biomarkers of pollutant stress. Using of histopathological changes as a biomarker has the benefit of permitting researchers to examine specific target organs and cells as they are affected by exposure to environmental chemicals. Moreover, histopathology makes it possible to detect both acute and chronic changes of in the tissues and organs of individual organisms. Hinton [14] proposed it is the common characteristics of cellular organization that make histopathological examination a precious tool in the biomarker approach [14]. The histopathology biomarker is a higher level response following chemical and cellular interaction [3]. Histo-cytological responses are relatively easy to determine and it can be possible to relate them to individual's health and fitness which, sequentially, permits more extrapolation to population/community effects. A wide range of histo-cytological alterations in fish have been developed and recommended as biomarkers to screen the effects of toxicants [15]. Cells and tissues changes in fish are frequently used biomarkers in many studies, but such alterations have been observed in all vertebrates and invertebrates livening in aquatic ecosystems. Histopathological biomarkers represent tissue injuries occur in response to previous or current contact of the organism to one or more pollutants [12].

The opinion that disturbances in structure or function of individual cells form the basis of a disease or toxicity was first proposed in the 19th century with the perception of "cellular pathology" suggested by Rudolf Virchow, known as the father of pathology [16]. Pathology has been a crucial and vigorous method in established routine toxicology studies, carried out for the purpose of risk assessment. In aquatic toxicology, application of the histopathology techniques in assessing toxicologic pathology of organisms has many advantages [16].

The present study reviews the most important histo-cytopathological alterations in fish, which have been used as biomarkers in different international pollution monitoring programs. These responses provide information concerning the exposure, effects and susceptibility of fish in the context of environmental monitoring and the biomarker approach.

Histopathological Changes of Gill: The gills of a fish comprise a multifunctional organ (respiration, ion regulation, acid-base balance and nitrogenous waste excretion) constitute over 50 percent of the total surface area of the animal that make it sensitive to chemicals in water. The reviews of Mallatt [15], Wood [18] and Au [17] have provided extensive information on gill structural alterations in fish as a result of toxicants exposure. Mallatt [17] has provided a quantitative synthesis of more than 100 toxicological studies in which structural changes in the gills were examined by light or electron microscopy.
Lifting of the lamellar epithelial cells away from the basement membrane due to a penetration of fluid is the most common lesion, which could be give rise to reduce respiratory gas exchange by increasing diffusion distance and decreasing interlamellar distance. Fusion of neighboring lamellae and epithelial rupture are perhaps the direct results of pavement cell lifting and represent more severe gill damage [15].

Necrosis of different lamellar and filament cells like chloride and pavement cells is another most commonly reported responses, but is more common for metals than for organics or other pollutants, possibly since metals directly interact with ion transport proteins and inhibit their activity. Necrosis would be expected to increase diffusion of ions and water. In true necrosis, Transmission Electron Microscopy shows that organelles and cytoplasmic volume swell and become more electrons dense in necrotic cells. Ultimately cell membranes would be ruptured and the contents possibly would be lost by swamping to the external water. Leukocyte infiltration should be also considered an adaptive response [19]. Hypertrophy of the pavement cells is possibly an event associated with necrosis cell swelling. This lesion is also

more commonly associated with metals. However, cell hypertrophy sometimes indicates the origin of pavement cells which occurs when they shrink back to expose increased chloride cell-surface area in return to acid-base and ionic interruptions [20, 21]. Proliferation of mucous cell, associated with excess mucus secretion, seems to occur more frequently in result of exposure to metals than to organic pollutants. Proliferation of pavement cells, mucous cells and chloride cells seem to be protective which limit the accesses of chemicals with the branchial surface, on other hand they may also block respiratory gas exchange and then lead to animal smothering [22]. Haaparanta *et al*. [23] reported chloride cell proliferation as a significant pathological change in roach *Rutilus rutilus* collected from polluted lakes in Finland [23].

Epithelial lifting, hyperplasia and hypertrophy of the epithelial cells with partial fusion of lamellae are defense mechanisms which result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants [24, 25]. These alterations, more commonly associated with chronic exposures than acutely lethal exposures, are greatly increase the blood-to-water diffusion distance, decrease interlamellar distance and lead to an total reduction in the diffusive conductance of the gills to respiratory gases [26, 27]. Lamellar aneurysms and blood congestion with dilation of marginal channels together with leukocyte infiltration could be considered part of an inflammatory response and occur when fishes suffer a more severe type of stress [28]. Aneurysm is related to the pillar cells rupturing [29] as a result of a bigger flow of blood or direct effects of chemicals on these cells. This alteration is a severe change, which its improvement is possible, but more difficult than the epithelial changes [24].

These are the gill lesions in response to a wide range of contaminants, including organochlorines, petroleum compounds, organophospates, carbonates, herbicides and heavy metals with a greater reported occurrence [15, 30, 32]. The most of gill alterations reported in the literature, even though concentration dependent (i.e. more severe in acute lethal exposures than in chronic sublethal exposures), are actually non-specific and are not correlated with the kind of toxicant, exposure level (acute or chronic), exposure medium (freshwater or seawater), or fish species. Epithelial lifting and lamellar fusion were observed in rainbow trout (*Oncorhynchus mykiss*) exposed to petroleum residues [33]. The same changes have also been reported in the gills of the fishes exposed to organic toxicants [28] and metals [29, 34, 35]. Giari *et al*. [36] documented hyperplasia with lamellar fusion, telangiectasia, edema with epithelial lifting and desquamation, in the gills of farmed European sea bass (*Dicentrarchus labrax* L.) acutely treated with mercury [36]. Field exposure to pulp and paper mill sewage resulted in hyperplasia in the gills of shorthorn sculpin (*Myoxocephalus Scorpius*) [37].

Definitely, the respiratory epithelium changes cooperates the host respiratory ability. Moderate changes don't lead to mortality directly, but can harmfully affect the functioning of the fish. On the other hand, severe or extensive damage may directly cause death. Overall, though tissue preparation for histopathological study is time consuming, gill histopathology seems to be a promising biomarker for general environmental pollution monitoring.

Histopathological Changes of Liver: The liver carries out essential body functions including regulation of metabolism, synthesis of plasma proteins, energy storage, storage of certain vitamins and trace metals and transformation and excretion of steroids and detoxification of xenobiotics. In general, liver is a target organ due to its large blood supply that causes noticeable toxicant exposure and accumulation and also its clearance function and its pronounced metabolic capacity [38].

Numerous categories of liver pathology are present as reliable biomarkers of toxic damage [39, 43], therefore studies on liver histopathology in fish have increasingly been incorporated in national marine biological effects monitoring programs in both Europe and the USA [44, 45].

Myers *et al*. [46] generally classified flatfish hepatic alterations into several distinct groups and it could be possible to rank them according to their relative importance as indicators of toxicant exposure: 1. Degenerative lesions such as biliary epithelial cells degeneration and polymorphism of hepatocytes and their nucleus; 2. Foci of cellular alteration (FCA), including basophilic, eosinophilic, clear cell and vacuolated foci; 3. Benign neoplasm, including hepatocyte adenoma, bile ducts cholangioma and blood vessels and capillaries hemangioma; 4. Malignant neoplasms, including hepatocyte carcinoma, cholangiocarcinoma and hemangiosarcoma.

Specific non-neoplastic proliferate lesions including hepatocytes regeneration, hyperplasia of bile duct and hepatic fibrosis in addition to general or non-specific degenerative alterations such as cellular necrosis, hyaline inclusion bodies comprise a second group of hepatic lesions. Ultimately, inflammatory changes consists a third group of liver alterations, which is considered as minimal significant indicator of pollutant exposure, although this

group can offer more information on the general health status and condition of the fish [47].

Overall, liver histopathological lesions are not specific to pollutants. For example, exposure to PAHs, PCBs, DDTs, chloranes and dieldrin increases the prevalence of liver lesions including neoplasm's, foci of cellular alteration (FCA), megalocytic heptoses (MH), hepatocellular nuclear polymorphism (NP), hydropic vacuolation (HV) in English sole (*Pleuronectes vetulus*); while in winter flounder (*Pleuronectes americanus*), exposed to PAHs, DDTs or chlordanes, non-neoplastic proliferative lesions and non-specific necrotic lesions significantly increased [48, 49, 50].

Fanta *et al*. [51] reported abnormalities such as irregular shaped hepatocytes, cytoplasmic vacuolation and nucleus in a lateral position in the siluriform *Corydoras paleatus* exposed to organophosphate pesticides. Pacheco and Santos [52] observed increased vacuolation of the hepatocytes as a sign of metabolic damage, perhaps correlated to exposure to polluted water. Camargo and Martinez [35] observed signs of degeneration (cytoplasmic and nuclear degeneration and nuclear vacuolation) and the focal necrosis in the liver parenchyma of fishes exposed to the water of the Cambe River. These alterations have been reported as more severe changes, which are more commonly associated with the exposure of the fishes to contamination by metals, such as copper [53] and mercury [54] and by polychlorinated biphenyls (PCBs) [55].

Lesions including hepatocellular cytoplasmic vacuolization, leucocytes infiltrations, blood congestion, necrosis and fatty infiltrations were found in the liver of catfish *Clarias gariepinus* treated with fenvalerate [56]. The same changes were reported by Teh *et al*. [57] in the liver of 7-day-old larvae of the fish *Sarcamento splittail* exposed to sublethal concentrations of esfenvalerate for one week. However, using of liver histopathology as a biomarker of contamination exposure may not be a highly cost-effective method for pollution screening because it needs much time and effort to prepare liver samples and expected pathologists are also required to distinguish hepatological alterations [15].

Histopathological Changes of Kidney: Kidney is the primary organ for water elimination and is especially vital for freshwater species in which efficient ion reabsorption mechanisms in the kidney minimize the loss of ions. Vice versa, the urine flow rate is low in seawater to minimize water loss and therefore one of the primary functions of kidney is to eliminate of divalent ions [58].

Hydropic vacuolation, presence of proteinaceous droplets and necrosis of the tubule epithelial cells have been documented in the kidney of fishes exposed to hydrocarbons [59]. In addition, glomerulus lesions including dilation of Bowman's space, hyperplesia and fibrosis of the glomerular rate and thickening of basement membrane were also reported by many researchers [60, 62]. Swollen Bowman´s capsule cells and melanomacrophages were found in the kidney of trout (*Salmo trutta*) and tilapia (*Oreochromis mossambicus*) exposed to mercuric chloride [63]. Similar alterations were found in fishes exposed mixed environmental contaminants [52, 64]. According to these reports the kidney histopathological alterations, as same as gill, could not be considered specific to the stressors.

Although the renal alterations can provide evidence of toxic affront by themselves but in combination with the other organs pathological change they could provide stronger indications of xenobiotic effect [65].

Histopathological Changes of Gastro-intestinal Tract: The gastro-intestinal tract is one of the main routes for the uptake of xenobiotics present in the diet or in the water that the fish inhabit [66, 67]. The effects of toxicant on the gastro-intestinal tract of fish may range from slight changes in motility, secretion and absorptive functions to more severe effects associated with mucosal integrity, blood flow or neuromuscular control. These effects could ultimately influence the ability of the organism to thrive [68]. The main changes reported in gastro-intestinal tract included hydropic degeneration of the digestive gland [69], proliferation of mucous cells, hyperaemia, atrophy and metaplasia. Some studies have indicated that high levels of some metals in diet may cause increased apoptosis of intestinal cells [70]. Only a few studies have documented the histomorphological alterations of intestine in the fish exposed to heavy metals [67, 71].

Bano and Hasan[72] and Banerjee and Bhattacharya [67] demonstrated some alterations in the gut of *Channa punctatus* and *Heteropneustes fossilis* following mercury intoxication [67, 72]. However, amazingly no histopathological alterations were observed in *Salvelinus alpinus* following exposure to inorganic mercury and methyl mercury in their feed [73]. Changes of intestinal epithelium have been reported by different investigators in the fishes affected by different xenobiotics (petroleum, chlorinated biphenyl, benzopyrene, terbuthylazine, cadmium) [36, 66, 74].

However, based on current information, tissues of the

gastro-intestinal tract do not seem to exhibit lesions which may be of value for biological effects monitoring [47].

Histopathological Changes of Skin: The fish skin is considered as an important organ because it is the interface between the external and internal environment of fish. It forms the first line of defense in animal which is in direct contact with waterborne toxic chemicals, parasites and disease organisms.

Fish skin is hydrated, unkeratinized and totally covered by a layer of slimy mucus. Due to the nature of fish skin, it may be very susceptible to waterborne chemicals and physical stressors.

Using of fish skin as a target organ or biomarker in the water quality tests or environmental risk assessments is not routine yet. In most cases it is difficult to determine exactly the acute, local dermal toxicity of waterborne stimulators or scathing chemicals (e.g. metals, detergents, chlorine, acid, etc.), because other organs (gills in particular) with more susceptibility are also exposed to chemicals [47].

Nonetheless, as a result of direct contact of skin with the environment and many of its important functions, the skin has received extensive attention in in-vitro and in vivo studies. However, up to the present time, application of the skin toxicity data is limited.

Architectural abnormalities in the surface epidermal cells; aboundant mucus secretion; loss of shape, size and structure of epidermal cells and goblet cells were observed in *Heteropneustes fossilis* during 7 days' sublethal exposure to copper (CuSO4) [75]. Rajan and Banerjee [76] reported the alterations like significant decrease in the number of mucous cells of dorsal and opercular epidermis and rapid breakdown in the mucous barrier in *Heteropneustes fossilis* treated with sublethal concentrations of malachite green [76]. The similar results were also reported by Lindesjoo and Thulin [77] in perch *Perca fluviatilis* and goldfish *Carassius auratus* treated with pulp mill waste.

Destruction of the mucous coat or the underlying layers (epidermis or dermis) could disturb the integrity of the fish internal environment, lead to abnormal behavior, disease or death. Abundant mucus on surface and many mucous cells near surface; necrotic pavement cells; mature club cells migrated out, many were newly differentiated; chloride cells appeared; massive extravasations of leukocytes; mast cells appeared in the epidermis and formation of new capillaries; melanosomes became common, extended into epidermis; erythrocytes in matrix of dermis were reported by Iger *et al*. [78] in the skin of common carp *Cyprinus carpio* exposed to sulethal concentration of cadmium [78]. The same results were observed in freshwater, air-breathing catfish *Heteropneustes fossilis* exposed to ammonium sulfate [79].

CONCLUSION

On the basis of the information presented in different studies, there is no doubt that the application of histopathological changes as a biomarker of organism exposure to contaminated sites, offers important information that can contribute to environmental monitoring programs designed for surveillance, hazard assessment or regulatory compliance.

One of the most important benefits of the use of histopathological biomarkers in environmental screening is possibility of examining specific target organs, including gills, kidney and liver.

However, the fish are responding to the direct effects of the pollutants as well as to the secondary effects caused by stress. This information verifies that histopathological changes are valuable biomarkers for field evaluation, especially in tropical regions that are naturally affected by variety of environmental variations. It should be highlighted that histopathology is able to assess the initial effects and reactions to acute exposure to chemical stressors.

REFFERENCES

1. Bernet, D., H. Schmidt, W. Meier, P. Burkhardt-Hol and T. Wahli, 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. J. Fish Disease, 22: 25-34.
2. Hogarh, J.N., J.N. Fobil, G.K. Ofosu-Budu, D. Carboo, N.A. Ankrah and A. Nyarko, 2008. Assessment of heavy metal contamination and macro-nutrient content of composts for environmental pollution control in Ghana. Global J. Environmental Research , 2(3): 133-139.
3. Schlenk, D., R. Handy, S. Steinert, M.H. Depledge and W. Benson, 2008. Biomarkers. In The toxicology of fishes, Eds., Di Giulio, R.T. and D.E. Hinton. CRC Press, Taylor & Francis Group, Boca Raton, New York, pp: 683-733.
4. WHO International Programme on Chemical Safety (IPCS), 1993. Biomarkers and risk assessment: concepts and principles. Environmental Health Criteria 155, World Health Organization, Geneva.
5. Peakall, D.W., 1994. Biomarkers: the way forward in environmental assessment. Toxicology Ecotoxicology News, 1: 55-60.

6. Van Gestel, C.A.M. and T.C. Van Brummelen, 1996. Incorporation of the biomarker concept in ecotoxicology calls for a redefinition of terms. Ecotoxicol., 5: 217-225.
7. Adams, S.M., 1990. Status and use of biological indicators for evaluating the effects of stress on fish. In American Fisheries Society Symposium, Ed., Adams, S.M. American Fisheries Society, Bethesda, MD, pp: 1-8.
8. Depledge, M.H., J.J. Amaral-Mendes, B. Danial, R.S. Halbrook, P. Kloepper-Sams, M.N. Moore and D.B. Peakall, 1993. The conceptual basis of the biomarker approach. In Biomarkers: Research and Application in the Assessment of Environmental Health, Eds., Peakall, D.B. and R.L. Shugart. Springer-Verlag, Berlin, pp: 15-29.
9. Decaprio, A.P., 1997. Biomarkers: coming of age for environmental health and risk assessment. Environmental Science Technol., 31: 1837-1848.
10. Adams, S.M., 2002. Biological indicators of aquatic ecosystem stress: introduction and overview. In Biological Indicators of Aquatic Ecosystem Stress, Ed., Adams, S.M. American Fisheries Society, Bethesda, MD, pp: 1-12.
11. National Research Council (NRC), 1987. Biological markers in environmental health research. Environmental Health Perspectives, 74: 3-9.
12. McCarty, L.S. and K.R. Munkittrick, 1996. Environmental biomarkers in aquatic toxicology: fiction, fantasy or functional. Hum. Ecological Risk Assessment, 2: 268-274.
13. Schlenk, D., 1999. Necessity of defining biomarkers for use in ecological risk assessments. Marine Pollution Bulletin, 39:48-53.
14. Hinton, D.E., 1994. Cells, cellular responses and their markers on chronic toxicity of fishes. In Aquatic Toxicology: Molecular, Biochemical and Cellular Perspectives, Eds., Malins, D.C. and G.K. Ostrander. Lewis Publishers, Boca Raton, pp: 207-239.
15. Au, D.W.T., 2004. The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. Marine Pollution Bulletin, 48: 817-834.
16. Cotran, R.S., V. Kumar and S.L. Robbins, 1994. Robbins Pathologic Basis of Disease. W.B. Saunders, Philadelphia, PA.
17. Mallatt, J., 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. Canadian J. Fisheries and Aquatic Sciences, 42: 630-648.
18. Wood, C.M., 2001. Toxic responses of the gill. In: Target Organ Toxicity in Marine and Freshwater Teleosts Organs, Eds., Schlenk, D. and W.H. Benson. vol. 1. Taylor & Francis, London, pp: 1- 89.
19. Bury, N.R., J. Li, G. Flik, R.A.C. Lock and S.E. Wendelaar Bonga, 1998. Cortisol protects against copper induced necrosis and promotes apoptosis in fish gill chloride cells in vitro. Aquatic Toxicol., 40: 193-202.
20. Perry, S.F. and P. Laurent, 1993. Environmental effects on fish gill structure and function. In Fish Ecophysiology, Eds., Rankin, J.C. and F.B. Jensen, Chapman and Hall, London, pp: 231-264.
21. Goss, G.G., S.F. Perry and P. Laurent, 1995. Ultrastructural and morphometric studies on ion and acid—base transport processes in freshwater fish. In Cellular and Molecular Approaches to Fish Ionic Regulation, Fish Physiology, Eds., Wood, C.M. and T.J. Shuttleworth. 14: 257-284.
22. McDonald, D.G. and C.M. Wood, 1993. Branchial acclimation to metals. In Fish Ecophysiology, Eds., Rankin, J.C. and F.B. Jensen. Chapman and Hall, London, pp: 297-321.
23. Haaparanta, A., E.T. Valtonen and R.W. Hoffmann, 1997. Gill anomalies of perch and roach from four lakes differing in water quality. J. Fish Biology, 50: 575-591.
24. Poleksic, V. and V. Mitrovic-Tutundzic, 1994. Fish gills as a monitor of sublethal and chronic effects of pollution, pp: 339-352.
25. Fernandes, M.N. and A.F. Mazon, 2003. Environmental pollution and fish gill morphology. In Fish adaptations, Eds., Val, A.L. and B.G. Kapoor. Enfield, Science Publishers, pp: 203-231.
26. Greco, A.M., K.M. Gilmour, J.C. Fenwick and S.F. Perry, 1995. The effects of softwater acclimation on respiratory gas transfer in the rainbow trout, Oncorhynchus mykiss. J. Experimental Biol., 198: 2557-2567.
27. Perry, S.F., S.G. Reid, E. Wankiewicz, V. Iyer and K.M. Gilmour, 1996. Physiological responses of rainbow trout (Oncorhynchus mykiss) to prolonged exposure to soft water. Physiological Zool., 69: 1419-1441.
28. Rosety-Rodríguez, M., F.J. Ordoñe, Rosety, J.M. Rosety, A. Ribelles and C. Carrasco, 2002. Morpho-histochemical changes in the gills of turbot, Scophthalmus maximus L., induced by sodium dodecyl sulfate. Ecotoxicology and Environmental Safety, 51: 223-228.

29. Martinez, C.B.R., M.Y. Nagae, C.T.B.V. Zaia and D.A.M. Zaia, 2004. Morphological and physiological acute effects of lead in the neotropical fish *Prochilodus lineatus*. Brazilian J. Biol., 64(4): 797-807.
30. Nahed, S.G. and A.S. Saad, 2008. Effect of Environmental Pollution by Phenol on Some Physiological Parameters of *Oreochromis niloticus.* Global Veterinaria, 2(6): 312-319.
31. Hemalatha, S. and T.K. Banerjee, 1997. Histopathological analysis of acute toxicity of zinc chloride on the respiratory organs of airbreathing catfish heteropneustes (Saccobranchus) fossilis (Bloch). Veterinarski Arhiv, 67: 11-24.
32. GlobalTox, 1997. Technical evaluation of histopathology as an environmental monitoring tool for the mining industry in Canada. Report prepared for Aquatic Effects Technology Evaluation (AETE) Program, Ottawa. Natural Resources Canada by Global Tox International Consultants Inc., Ottawa, pp: 153.
33. Engelhardt, F.R., M.P. Wong and M.E. Duey, 1981. Hydromineral balance and gill morphology in rainbow trout Salmo gairdneri, acclimated to fresh and sea water as affected by petroleum exposure. Aquatic Toxicol., 1: 175-186.
34. Metwally, M.A.A. and I.M. Fouad, 2008. Biochemical Changes Induced by Heavy Metal Pollution. Global Veterinaria, 2(6): 308-311.
35. Camargo, M.M.P. and C.B.R. Martinez, 2007. Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. Neotropical Ichthyol., 5(3): 327-336.
36. Giari, L., E. Simoni, M. Manera and B.S. Dezfuli, 2008. Histo-cytological responses of *Dicentrarchus labrax* (L.) following mercury exposure. Ecotoxicology and Environmental Safety, 70: 400-410.
37. Barker, D.E., R.A. Khan, E.M. Lee, R.G. Hooper and K. Ryan, 1994. Anomalies in sculpin (Myoxocephalus spp.) sampled near a pulp and paper mill. Archives of Environmental Contamination and Toxicol., 26: 491-496.
38. Mohamed, F.A.S., 2009. Histopathological Studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarun, Egypt. World J. Fish and Marine Sciences, 1(1): 29-3.
39. Kohler, A., 1990. Identification of contaminant-induced cellular and subcellular lesions in the liver of flounder (Platichthys flesus L.) caught at differently polluted estuaries. Aquatic Toxicol., 16: 271-294.
40. Kohler, A., H. Deisemann and B. Lauritzen, 1992. Histological and cytological indices of toxic injury in the liver of the dab Limanda limanda. Diseases of Aquatic Organisms, 91: 141-153.
41. Moore, M.J. and M.S. Myers, 1994. Pathobiology of chemical-associated neoplasia in fish. Aquatic Toxicol., 24: 327-386.
42. Stehr, C.M., M.S. Johnson and M.S. Myers, 1998. Hydropic vacuolation in the liver of three species of fish from the US West Coast: lesion description and risk assessment associated with contaminant exposure. Diseases of Aquatic Organisms, 32: 119-135.
43. Stentiford, G.D., M. Longshaw, B.P. Lyons, G. Jones, M. Green and S.W. Feist, 2003. Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Marine Environmental Res., 55: 137-159.
44. Lang, T., 2002. Fish disease surveys in environmental monitoring: the role of ICES. ICES Marine Science Symposia, 215: 202-212.
45. Feist, S.W., T. Lang, G.D. Stentiford and A. Kohler, 2004. Biological effects of contaminants: use of liver pathology of the European flatfish dab (Limanda limanda L.) and flounder (Platichthys flesus L.) for monitoring. ICES Techniques in Marine Environmental Sciences No. 38, ICES, Copenhagen.
46. Myers, M.S., L.D. Rhodes and B.B. McCain, 1987. Pathologic anatomy and patterns of occurrence of hepatic neoplasms, putative preneoplastic lesions and other idiopathic hepatic conditions in English sole (*Parophrys vetulus*) from Puget Sound, Washington, USA. J. Natl. Cancer Inst, 78: 333-363.
47. Hylland, K., S. Feist, J. Thain and L. Forlin, 2003. Molecular/Cellular Processes and the Healthof the Individual. In Effects of Pollution on Fish: Molecular Effects and Population Responses, Eds., Lawrence, A. and K. Hemingway. Blackwell Science Ltd, Garsington Road, Oxford, UK, pp: 145-151.
48. Schiewe, M.H., D.D. Weber, M.S. Myers, F.J. Jacques, W.L. Reichert, C.A. Krone, D.C. Malins, B.B. McCain and U. Varanasi, 1991. Induction of foci of cellular alteration and other hepatic lesions in English sole (Parophrys vetulus) exposed to an extract of an urban marine sediment. Canadian J. Fisheries and Aquatic Sciences, 48: 1750-1760.
49. Johnson, L.L., C.M. Stehr, O.P. Olson, M.S. Myers, S.M. Plerce, C.A. Wigren, B.B. McCain and U. Varanasi, 1993. Chemical contaminants and hepatic lesions in winter flounder (*Pleuronectes americanus*) from the northeast coast of the United States. Environ. Science Technol., 27: 2759-2771.
50. Myers, M.S., C.M. Stehr, O.P. Olson, L.L. Johnson, B.B. McCain, S.L. Chan and U. Varanasi, 1993. National benthic surveillance project: Pacific

Coast--Fish histopathology and relationships between toxicopathic lesions and exposure to chemical contaminants for cycles I to V (1984-88). National technical information service, US, pp: 1-147.

51. Fanta, E., F.S. Rios, S. Romao, A.C.C. Vianna and S. Freiberger, 2003. Histopathology of the fish Corydoras paleatus contaminated with sublethal levels of organophosphorus in water and food. Ecotoxicology and Environmental Safety, 54: 119-130.
52. Pacheco, M. and M.A. Santos, 2002. Biotransformation, genotoxic and histopathological effects of environmental contaminants in European eel (*Anguilla anguilla* L.). Ecotoxicology and Environmental Safety, 53: 331-347.
53. Paris-Palacios, S., S. Biagianti-Risbourg and G. Vernet, 2000. Biochemical and (ultra)structural hepatic perturbations of *Brachyodanio rerio* (Teleostei, Cyprinidae) exposed to two sublethal concentrations of copper sulfate. Aquatic Toxicol., 50: 109-124.
54. Oliveira Ribeiro, C.A., E. Fanta, N.M. Turcatti, R.J. Cardoso and C.S. Carvalho, 1996. Lethal effects of inorganic mercury on cells and tissues of Trichomycterus brasiliensis (Pisces; Siluroidei). Biocell, 20: 171-178.
55. Chang, S., V.S. Zdanowicz and A. Murchelano, 1998. Associations between liver lesions in winter flounder (*Pleuronectes americanus*) and sediment chemical contaminants from north-east United States estuaries. J. Marine Sciences, 55: 954-969.
56. Sakr, S.A., S.M. Hanafy and N.E. El-Desouky, 2001. Histopathological, histochemical and physiological studies on the effect of the insecticide, hostathion, on the liver of the catfish *Clarias gariepinus.* Egyptian J. Aquatic Biology Fish, 6(2): 103-124.
57. The, S., S. Hung, F. Teh, D. Deng and I. Werner, 2005. Sublethal toxicity of orchard stormwater runoff in Sacramento splittail (*Pogonichthys macrolepidotus*) larvae. Marine Environmental Res., 59(3): 203-216.
58. Nishimura, H. and M. Imai, 1982. Control of renal function in freshwater and marine teleost. Federation Proceeding, 41: 2355-2360.
59. Spies, R.B., J.J. Stegeman, D.E. Hinton, B. Woodin, R. Smolowitz, M. Okihiro and D. Shea, 1996. Biomarkers of hydrocarbon exposure and sublethal effects in embiotocid fishes from a natural petroleum seep in the Santa Barbara channel. Aquatic Toxicol., 34: 195-219.
60. Veiga, M.L., E.L. Rodrigues, F.J. Pacheco, M.J.T. Ranzani-Paiva, 2002. Histopathologic changes in the kidney tissue of Prochilodus lineatus, 1836 (Characiformes, Prochilodontidae) induced by sublethal concentration of Trichlorfon exposure. Brazilian Archives of Biology and Technol., 45: 171-175.
61. Thophon, S., M. Kruatrachue, E.S. Upathan, P. Pokethitiyook, S. Sahaphong and S. Jarikhuan, 2003. Histopathological alterations of white seabass, Lates calcarifer in acute and subchronic cadmium exposure. Environmental Pollution, 121: 307-320.
62. Adams, D.H., C.H. Sonne, N. Basu, R. Dietz, D. Nam, P.S. Leifsson and A.L. Jensen, 2010. Mercury contamination in spotted seatrout, Cynoscion nebulosus: An assessment of liver, kidney, blood and nervous system health. Science of the Total Environment, 408: 5808-5816.
63. Handy, R.D. and W.S. Penrice, 1993. The influence of high oral doses of mercuric chloride on organ toxicant concentrations and histopathology in rainbow trout, Oncorhynchus mykiss. Comparative Biochemistry and Physiol. (C), 106: 717-724.
64. Schwaiger, J., R. Wanke, S. Adam, M. Pawert, W. Honnen and R. Triebskorn, 1997. The use of histopatological indicators to evaluate contaminant-related stress in fish. J. Aquatic Ecosystem, Stress and Recovery, 6: 75-86.
65. Rhodes, L.D., M.S. Myers, W.D. Gronlund and B.B. McCain, 1987. Epizootic characteristics of hepatic and renal lesions in English sole, Parophrys vetulus, from Puget Sound. J. Fish Biol., 31: 395-407.
66. Lemaire, P., J. Berhaut, S. Lemaire-Gony and M. Lafaurie, 1992. Ultrastructural changes induced by benzo[a]pyrene in sea bass (*Dicentrarchus labrax*) liver and intestine: importance of the intoxication route. Environmental Researches, 57(1): 59-72.
67. Banerjee, S. and S. Bhattacharya, 1995. Histopathological changes induced by chronic nonlethal levels of elsan, mercury and ammonia in the small intestine of Channa punctatus (Bloch). Ecotoxicology and Environmental Safety, 31: 62-68.
68. Schlenk, D. and W.H. Benson, 2005.Target organ toxicity in marine and freshwater teleosts, Volume 1: Organs, Taylor & Francis Group, Boca Raton, New York.
69. Haensly, W.E., J.M. Neff, J.R. Sharp, A.C. Morris, M.F. Bedgood and P.D. Boem, 1982. Histopathology of *Pleuronectes platessa* L. from Aber Wrac'h and Aber Benoit, Brittany, France: long-term effects of the Amoco Cadiz crude oil spill. J. Fish Diseases, 5: 365-391.

70. Berntssen M.H.G., K. Hylland, B.S.E. Wendelaar and A. Maage, 1999. Toxic levels of dietary copper in Atlantic salmon (Salmo salar L.) parr. Aquatic Toxicol., 46: 87-99.
71. Ferrando, S., M. Malsano, V. Parrino, T. Ferrando, L. Girosi and G. Tagliaferro, 2006. Gut morphology and metallothionein immunoreactivity in *Liza aurata* from different heavy metal polluted environments. Italian J. Zool., 73: 7-14.
72. Bano, Y. and M. Hasan, 1990. Histopathological lesions in the body organs of cat-fish (*Heteropneustes fossilis*) following mercury intoxication. J. Environmental Science Health B, 25(1): 67-85.
73. Oliveira Ribeiro C.A., L. Belger, E. Pelletier and C. Rouleau, 2002. Histopathological evidence of inorganic mercury and methyl mercury toxicity in the arctic charr (*Salvelinus alpinus*). Environmental Res., 90: 217-225.
74. Giari L., M. Manera, E. Simoni and B.S. Dezfuli, 2007. Cellular alterations in different organs of European sea bass *Dicentrarchus labrax* (L.) exposed to cadmium. Chemosphere, 67: 1171-1181.
75. Khangarot B.S. and D.M. Tripathi, 1991. Changes in humoral and cell-mediated immune responses and in skin and respiratory surfaces of catfish, *Saccobranchus fossilis*, following copper exposure. Ecotoxicology and Environmental Safety, 22: 291-308.
76. Rajan, M.T. and T.K. Banerjee, 1994. Toxicity of a fish disinfectant, malachite green to Heteropneustes fossilis and induced changes in the mucocyte density of its skin. J. Freshwater Biol., 6: 177-182.
77. Lindesjoeoe, E. and J. Thulin, 1994. Histopathology of skin and gills offish in pulp mill effluents. Diseases of Aquatic Organisms, 18: 81-93.
78. Iger, Y. and R.A.C. Lock, J.C.A. Van derMeij and S.E. Wendelaar Bonga, 1994. Effects of water-borne cadmium on the skin of the common carp (*Cyprinus carpio*). Archives of Environmental Contamination and Toxicol., 26: 342-350.
79. Paul, V.I. and T.K. Banerjee, 1996. Ammonium sulphate induced stress related alterations in the opercular epidermis of the live fish Heteropneustes (Saccobranchus) fossilis (Bloch). Current Science (Bangalore), 70: 1025-1029.