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Conventional Methods of Diagnosis of Toxicosis in Fishes

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SUMMARY

This article provides an overview of conventional methods used in fish toxicology to assess the impacts of environmental contaminants on fish health. Those techniques include clinical observation, histopathology, biochemical assays, chemical analyses, and toxicity tests. Clinical observation focuses on detecting physical abnormalities and behavioural changes, while histopathology and microscopic analyses offer detailed insights into tissue damage and cellular alterations in vital organs such as the liver, gills, and kidneys. Biochemical assays are employed to measure physiological responses to contaminants, including oxidative stress and enzyme activity. Chemical analyses detect and quantify the presence of contaminants in the environment and fish tissues, aiding in the correlation between contaminant levels and toxic effects. Toxicity tests, including LC50 studies, provide critical data on the lethality of specific pollutants. As such, advanced techniques, such as molecular biomarkers, metabolomics, proteomics and advanced imaging, are increasingly integrated into toxicological research to enhance diagnostic capabilities and improve the assessment of aquatic ecosystem health.

INTRODUCTION

The pollution of the aquatic environment by point and non-point sources, such as agricultural runoff, industrial effluents, oil spills, mine waste, and other chemical contaminants, has been recognized for many years. In the field of fish toxicology, conventional approaches to evaluating the health and well-being of fish exposed to environmental insults have primarily focused on short-term studies using whole-animal responses, such as gross abnormalities, behavioural changes, altered length and weight, and mortality, often resulting from toxic effects like heavy metals and pesticides. In contrast, mammalian and veterinary toxicology often incorporate clinical techniques alongside whole-animal responses to assess the health of animals exposed to toxic chemicals (Mehrle & Mayer, 1980). Fish, however, exhibit a range of clinical signs during disease progression, some of which are pathognomonic, but the majority are non-specific. As a result, a systematic approach is needed to evaluate disease cases (Noga, 2010). Common clinical techniques used in mammalian toxicology, such as blood chemistry profiles, blood cell analyses, and histopathological examinations, allow for a deeper understanding of physiological and biochemical responses to toxins. Unfortunately, these diagnostic techniques are less commonly applied in aquatic toxicology due to the relatively limited biochemical and physiological research in this field. Currently, lethality tests are the most widely used method for assessing environmental hazards to aquatic life, followed by chemical analysis, biochemical assays, toxicity tests measuring reproductive effects, and residue accumulation studies. In contrast, histological tests and physiological/biochemical assessments rank lower in utility, as they are not yet sufficiently linked to adverse environmental impacts. This highlights the challenges of developing effective diagnostic tests for fish and underscores the need for further research and application of clinical techniques in aquatic toxicology (Mehrle & Mayer 1980).

Building on the need for effective diagnostic methods in fish toxicology, the evaluation of organ damage in fish plays a pivotal role in understanding the broader impacts of environmental stressors. Given the complex relationship between fish and their habitats, these organisms have become key subjects in studying the effects of environmental contaminants, diseases, and other stressors on aquatic ecosystems. A critical aspect of this research is assessing organ damage, which provides valuable insights into the health, resilience, and overall status of fish populations and their ecosystems. This review explores a range of diagnostic approaches, from conventional histopathological assessments to advanced molecular and biochemical assays, each offering unique strengths and challenges. We examine the application of histopathology, biomarker analysis, oxidative stress indicators, and non-invasive imaging techniques. Through these methods, we seek to unravel the intricate dynamics of organ damage in fish, focusing on how stressors affect critical organs such as the liver, gills, kidneys, and reproductive systems (Jerald *et al*., 2024).

Conventional Methods:

Clinical Observations

This process involves monitoring behavioural and physical changes in fish that may signal toxic exposure, such as abnormal swimming patterns, reduced feeding, or physical deformities. It also includes visual examination of the fish organs and tissues. The condition of the fish is crucial for determining the success of the tests; sending fish that are already decaying is ineffective. Ideally, live fish showing clinical signs of distress should be analysed. Ensuring that the fish are delivered to the laboratory under fresh and undamaged conditions is crucial, although this may not always be achievable. Fish specimens should not be transported in the water in which they die, and the use of preservatives, such as formalin or alcohol, should be avoided, as these substances may interfere with diagnostic procedures. For general examinations, frozen samples are recommended when fish are submitted for chemo-toxicological analysis to check for metals, pesticide residues, and other contaminants. Chemo-toxicological assessments are relevant when metal poisoning is suspected or when evaluating the metal content of fish intended for human consumption. Such tests are also conducted in cases in which phenols and pesticides may be involved. Under certain circumstances, these analyses can aid in diagnosing ammonia toxicity in fish. However, this method is not suitable for diagnosing fish toxicity caused by specific compounds like cyanides, sulphides, or chlorine. Atomic Absorption Spectrophotometry (AAS) has been used to detect metals in fish organs and tissues. Elevated metal concentrations are typically found in parenchymatous organs and gills. For instance, acute copper poisoning can be diagnosed through chemical analysis of the gills, where metal concentrations can increase significantly. In non-contaminated water, the copper content in fish gills can reach up to 10 mg per kg of dry matter. (https://www.fao.org/fishery).

Histopathology:

The method essentially involves acquiring extremely thin sections of animal organs, allowing for the detection of cellular and tissue abnormalities once they are stained. The degree of these anomalies, which are frequently seen in organs like the intestines, liver, spleen, heart, and gills, could be directly related to the conditions under which the animals were kept in captivity or to their exposure to the outside world (Martins *et al*., 2018). This involves the microscopic examination of afflicted tissue, representing a crucial investigative method within the medical domain. This practice is rooted in the scrutiny of histology, which pertains to the microscopic anatomy of humans or animals. The procedure entails the inspection of slender tissue slices under light microscopes. Histotechnique encompasses a series of methods that facilitate the visualization of microscopic characteristics of tissues and cells, aiding in the identification of distinct structural alterations associated with various diseases (Slaoui & Fiette 2011). The histological examination of the liver of fish can serve as a useful model when examining the impact of stressors, such as contaminants, infectious agents, parasites, biological contaminants, and physical-chemical variables. Exposure to these stressors can induce pathological alterations in fish, including kidney tubular damage, abnormalities in gill lamellae, and liver necrosis. Consequently, it is crucial to conduct histopathological studies to characterize and evaluate potential abnormalities in aquatic animals exposed to diverse infestation and noxious materials in aquaculture environments (Gobinath & Ramanibai 2014).

Tissue Preparation → Fixation → Paraffin Infiltration → Dehydration and Embedding

Biochemical Assays:

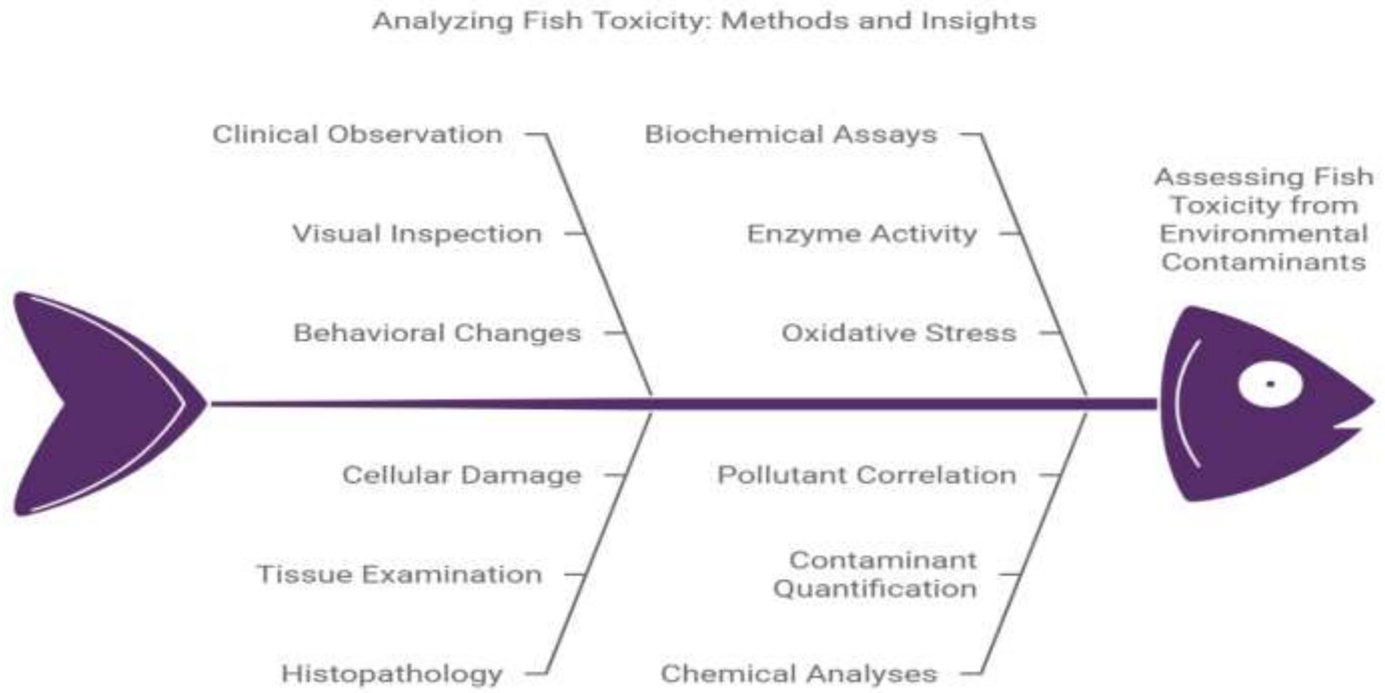
Evaluation of enzymatic activity, protein concentration, and additional biochemical indicators. Biochemical assays are a common method for gauging the level of organ damage in fish, especially when assessing harm to the liver and muscle due to chemical contaminants or illnesses. In fish, a diverse array of serum proteins found in complex combinations plays a pivotal role in a broad spectrum of physiological functions, relevant to both their well-being and when they are afflicted by diseases. These proteins hold significant value in comprehending various physiological aspects of fish (Ahmed & Sheikh 2019). The serum biochemical profile serves as a valuable tool for gaining insights into the internal health of fish, often detecting issues before visible disease symptoms arise due to their close association with aquatic environments (Wang *et al*., 2022).

Toxicology Test:

The toxicology test evaluates the concentration of test substances causing mortality to 50% (LC_{50}) of juvenile fish after 96hr exposure period. Toxicology tests are essential for diagnosing fish health issues, particularly when chemical exposure or environmental toxins are suspected causes of the disease. These tests involve the analysis of various biological samples, such as blood, tissues, or water, to detect and quantify harmful substances that may impact fish health. The process begins with sample collection from the affected fish or their environment, followed by sophisticated analytical techniques such as gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), and inductively coupled plasma mass spectrometry (ICP-MS). These methods allow the detection of a wide range of toxic substances, including heavy metals, pesticides, and industrial pollutants. By comparing the levels of these toxins in affected fish to known toxic thresholds, researchers can assess whether exposure to specific chemicals contributes to the observed health problems. Additionally, toxicology tests help to identify the mechanism of toxicity, such as oxidative stress or disruption of cellular functions, which can provide insights into the underlying pathology. This diagnostic approach is crucial for managing and mitigating the impact of environmental pollutants on aquatic ecosystems and for ensuring the health of fish populations in both wild and aquaculture settings. (OECD, 2000**).**

Chemical Analysis

Chemical analysis in fish toxicology involves the systematic examination of toxic substances in fish and their aquatic environments to assess the health impacts on fish populations and ecosystems. This analysis is essential for identifying and quantifying pollutants, such as heavy metals, pesticides, and other organic contaminants, that can accumulate in fish tissues and affect their physiological functions. The process typically begins with sample collection, where fish and water samples are taken from various habitats. These samples undergo various analytical techniques, including Gas Chromatography (GC), High-Performance Liquid Chromatography(HPLC), Mass Spectrometry(MS), etc. Each method is chosen based on the type of contaminant being studied and the required sensitivity and specificity. By conducting chemical analyses, researchers can determine the presence and concentration of toxicants, assess the potential risks to fish health, and evaluate the effects on reproduction, growth, and survival. This information is crucial for environmental monitoring, regulatory assessments, and the development of strategies to mitigate pollution and protect aquatic ecosystems. Overall, chemical analysis plays a vital role in understanding and addressing the impacts of anthropogenic activities on fish and their habitats. (Khan *et al*., 2016).



CONCLUSIONS

Techniques like H&E staining, metabolomics, and advanced imaging offer in-depth structural and functional assessments, helping to bridge the gap between cellular changes and broader physiological outcomes. Integrating traditional histological methods with modern analytical and molecular techniques enables comprehensive evaluations of organ health. This approach facilitates the development of targeted strategies for disease prevention and environmental monitoring. Ultimately, this multidisciplinary strategy is vital for advancing biological research, enhancing aquaculture practices, and protecting biodiversity.

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