

DETECTION OF EPIZOOTIC ULCERATIVE SYNDROME IN FRESHWATER EDIBLE FISH USING AI TECHNIQUES



Podeti Koteswar Rao

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PREFACE

Aquaculture is emerging as one of the most vital sectors in global food production, contributing significantly to nutrition, employment, and economic development. Among freshwater resources, edible fish species serve as a crucial protein source for millions of people, particularly in developing countries. However, the sustainability of aquaculture is severely threatened by infectious diseases, of which Epizootic Ulcerative Syndrome (EUS) is among the most destructive. EUS is a trans boundary aquatic disease, characterized by deep ulcerative lesions, extensive tissue necrosis, and high mortality rates in susceptible fish species. Its outbreaks not only cause severe economic losses to farmers but also affect food security and ecosystem health.

Traditionally, the diagnosis of EUS has relied on clinical observation, histopathological examination, and microbiological or molecular techniques. While effective, these methods are often limited by delays, the need for specialized expertise, and high operational costs. In an industry where timely detection is the difference between containment and catastrophic loss, there is an urgent need for innovative, rapid, and reliable diagnostic solutions.

This research introduces Artificial Intelligence (AI) as a transformative tool for the detection of EUS in freshwater edible fish. AI techniques, particularly machine learning, deep learning, and image recognition, offer the ability to analyze complex datasets, identify subtle pathological features, and provide real-time diagnostic support. By applying AI to fish health monitoring, this work seeks to bridge the gap between traditional diagnostic practices and the need for scalable, automated systems that can be deployed even in resource-limited aquaculture settings. The preface to this study emphasizes the interdisciplinary nature of the work. It brings together insights from aquaculture, veterinary pathology, computational biology, and artificial intelligence. Such convergence is vital for developing next-generation solutions that ensure both the economic viability of aquaculture and the safety of food supplies. Moreover, the approach discussed herein demonstrates how AI can be harnessed not only for accurate disease detection but also for predictive surveillance, enabling farmers and policymakers to anticipate and mitigate outbreaks before they escalate.

This endeavor is motivated by the vision of fostering sustainable aquaculture through technological innovation. By combining biological understanding with computational intelligence, the study aspires to contribute toward improved fish health management, reduced economic vulnerability, and strengthened food security. The application of AI in detecting EUS is more than a technological advancement; it represents a paradigm shift towards precision aquaculture, where science and technology work hand in hand to secure the future of freshwater resources.

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ABSTRACT

Epizootic Ulcerative Syndrome (EUS) is a highly destructive fish disease that affects a wide range of freshwater edible species, causing ulcerative lesions, high mortality, and substantial economic losses in aquaculture. Early and accurate detection of EUS is crucial for disease management, food security, and the protection of fish farmers' livelihoods. Conventional diagnostic methods, including histopathology and molecular assays, though reliable, are often time-consuming, costly, and dependent on specialized expertise. To address these challenges, Artificial Intelligence (AI) offers a promising alternative by enabling rapid, automated, and precise disease detection. This study explores the application of AI techniques, including machine learning, deep learning, and image-based recognition, in diagnosing EUS in freshwater edible fish. By analyzing fish images and associated datasets, AI models can identify characteristic disease patterns with high sensitivity and specificity. The integration of AI tools with aquaculture health monitoring not only reduces diagnostic delays but also supports the development of predictive disease surveillance systems. The findings highlight the potential of AI-driven platforms to revolutionize fish health management, minimize economic risks, and promote sustainable aquaculture. This research underscores the role of interdisciplinary approaches in advancing precision aquaculture and safeguarding aquatic resources.

KEYWORDS

Epizootic ulcerative syndrome, Freshwater edible fish, Artificial intelligence, Machine learning, Deep learning, Image recognition, Aquaculture, Fish disease detection, Precision aquaculture, Predictive surveillance.

CHAPTER 1**Epizootic Ulcerative Syndrome (EUS)****INTRODUCTION**

Epizootic Ulcerative Syndrome (EUS) was delineated at a DFID Regional Seminar in Bangkok in 1994 as “a seasonal epizootic condition affecting freshwater and estuarine warm water fish, characterized by a complex infectious etiology, the presence of invasive *Aphanomyces* infection, and necrotizing ulcerative lesions that typically elicit a granulomatous response” (Roberts *et al.*, 1994a). Nevertheless, research conducted since then, as outlined in the aetiology and epidemiology portions of this book, indicates that a complicated aetiology is not invariably present in all cases. The case definition for EUS may now have a reference to the specific fungal infection *Aphanomyces invadans*. Given these advancements, EUS may be seen as transcending the classification of a syndrome; yet, the term “epizootic ulcerative syndrome” is widely recognized among fish health professionals and will persist in usage for this ebook.

A prior review, released by AAHRI and NACA in 1992, consolidated a significant amount of literature on the topic from national and international articles, reports, and conference proceedings. This article aims to provide practical applications to aid fish health professionals in diagnosing and managing EUS. The annex section contains extensive information on fungal and viral isolation and identification, a framework for outbreak investigations, and EUS reporting datasheets. It is important to note that other separate ulcerative fish diseases exist, and a valid EUS diagnosis can only be established through histological confirmation of the specific characteristics outlined on page 31. Consequently, it is anticipated that this manual will motivate fish health professionals to explore alternative causes of ulcerative disease. For more than 25 years, outbreaks of an ulcerative disease, histologically characterized by mycotic granulomas, have impacted freshwater and estuarine fish across much of Asia and Australia. The condition is referred to by many names: Mycotic Granulomatosis (MG) in Japan, Red Spot Disease (RSD) in Australia, and Epizootic Ulcerative Syndrome (EUS) in Southeast and South Asia. MG, RSD, and EUS were previously characterized as separate entities; however, recent research indicates that the same pathogenic *Aphanomyces* fungus is implicated in all instances (refer to the Aetiology section on “Fungi”). Consequently, a comprehensive history of EUS necessitates the inclusion of outbreaks in Japan and Australia.

Mycotic Granulomatosis (MG)

The initial documentation of an EUS-like condition occurred in the summer of 1971, involving farmed ayu (*Plecoglossus altivelis*) in Oita Prefecture, Japan (Egusa and Masuda, 1971). The defining lesion, a granulomatous reaction to invasive hyphae, was identified, leading to the designation of the condition as mycotic granulomatosis (Miyazaki and Egusa, 1972). It swiftly disseminated to multiple Prefectures and impacted various fish species, primarily cultured ayu and goldfish (*Carassius carassius auratus*), as well as wild Formosan snakehead (*Channa maculata*), crucian carp (*Carassius auratus*), bluegill (*Lepomis macrochirus*), and grey mullet (*Mugil cephalus*) (Miyazaki and Egusa, 1972; 1973a; b; c). Notably, common carp (*Cyprinus carpio*) remained unaffected. Hatai *et al.* (1977) identified the invasive Oomycete fungus from afflicted fish and later designated it *Aphanomyces piscicida* (Hatai, 1980). *A. piscicida* is now recognized as conspecific with the EUS pathogen, *Aphanomyces invadans* (Lilley *et al.*, 1997a; b). Despite the absence of significant MG epizootics in Japan since 1973, outbreaks have persisted intermittently. Hatai *et al.* (1994) recently documented a comparable disease in imported ornamental dwarf gourami (*Colisa lalia*) from Singapore, which was also shown to be associated with the same *Aphanomyces* pathogen (Lilley *et al.*, 1997a).

In 1972, outbreaks of a cutaneous ulcerative illness known as Red Spot Disease (RSD) impacted estuarine fish, especially grey mullet (*Mugil cephalus*), in Queensland, Australia (McKenzie and Hall, 1976). The illness subsequently advanced to impact freshwater and estuarine fish in coastal rivers of New South Wales (Callinan *et al.*, 1989), Northern Territory (Pearce, 1990), and Western Australia. Fraser *et al.* (1992) isolated an *Aphanomyces* fungus from afflicted fish, demonstrating its ability to induce the disease in fish by bath challenges, contingent upon the artificial abrasion of the experimental fish's skin (Callinan, 1994b). Consequently, an additional component was deemed to be implicated in the illness process. Virgona (1992) demonstrated that RSD outbreaks in estuarine fish in the Clarence River, NSW, were correlated with reduced catchment rainfall, as noted by Callinan *et al.* (1995a), who connected this to runoff originating from acid sulfate soils. Ultrastructural analysis of fish gills and skin revealed that low pH and increased levels of monomeric aluminium, indicative of estuary acidification, cause substantial lesions in fish (Sammur *et al.*, 1996). In aquarium experiments, RSD was subsequently triggered in fish subjected to sublethal levels of artificially acidified water (at pH 3 and pH 5) and pathogenic *Aphanomyces* spores, even with modest quantities of monomeric aluminium (Callinan *et al.*, 1996; Callinan, 1997). Similar to the pathogenic RSD-*Aphanomyces* has been identified as the same species as the EUS pathogen, *A. invadans* (Callinan *et al.*, 1995a; Lilley *et al.*). Subsequent to the outbreaks of MG and RSD, a progressive

westward dissemination across Asia occurred of a syndrome linked to cutaneous ulceration, resulting in significant mortalities among several freshwater and estuarine fish species. The condition was designated as Epizootic Ulcerative Syndrome (EUS) in 1986 during the Consultation of Experts on Ulcerative Fish Diseases in Bangkok (FAO, 1986). EUS outbreaks have been documented in 18 countries within the Asia-Pacific area (Fig. 1), but not all have been definitively verified as EUS following the outbreak of an ulcerative disease, presumed to be EUS, that transpired in the rivers of southern Papua New Guinea between 1975 and 1976 (Haines, 1983). In 1982-1983, there were elevated mortality rates in gudgeon (*Ophieleotris aporos* and *Oxyeleotris heterodon*) from upland regions and mullet from estuaries in northern Papua New Guinea (Coates *et al.*, 1989). Introduced tilapia (*Oreochromis mossambicus*) is prevalent in certain regions and has demonstrated resistance. Roberts *et al.* (1986) subsequently studied the preserved afflicted fish and confirmed the findings.



Fig. (1). A map illustrating the distribution of EUS in the asia-pacific area. there is uncertainty about the dates that signify the onset of the initial significant outbreak.

In 1980, outbreaks of an epizootic hemorrhagic syndrome transpired in Java, Indonesia, especially impacting cultured cyprinid and clariid fish, although the identification of this as EUS remains disputed (Roberts *et al.*, 1986). Ulcerated snakeheads and catfish have been later documented in the Indonesian regions of Sumatra, Sulawesi, and Kalimantan (Widagdo, 1990). Invasive hyphae have been detected in sand gobies (*Oxyeleotris marmoratus*) from eastern Kalimantan (Rukyani, 1994), and D. Bastiawan (personal communication) separated.

CHAPTER 2**Hematological Analysis****INTRODUCTION**

Blood serves as an effective signal for assessing the health of an organism (Joshi *et al.*, 2002a). There is a growing recognition among fish pathologists regarding the utilization of hematological and biochemical parameters as diagnostic instruments for assessing the physiological health status of fish. Joshi *et al.* (2002b) and Chagas and Val (2003) indicated that hematological parameters serve as a pathological indicator for the entire organism and are crucial for evaluating the physiological condition of fish affected by pathogens and for diagnosing their functional status. The initial study on the hematology of Indian fish was likely published by Dhar in 1948. This was the first study on the shape of erythrocytes and leukocytes, clotting time, *etc.*, in the air-breathing fish *Ophiocephalus punctatus*. Das (1958) reported the haematological data from three Indian main carps: *Labeo rohita*, *Catla catla*, and *Cirrhinus mrigala*. Houston and DeWilde (1968) proposed in their study on *Salmogardnerii*, *Cyprinus carpio*, and *Salvelinus fontinalis* (brook trout) that hematocrit values should serve as a general indicator of hematological state in regular examinations.

Hematological and biochemical assays are not extensively utilized for diagnosing fish diseases; nonetheless, these tests could serve as effective diagnostic tools for monitoring physiological and pathological alterations in fish. Various factors, including season (Mahajan *et al.*, 1979b), water contamination (Witeska, 1998), physicochemical parameters of water stress (Joshi, 1982; Nikoo *et al.*, 2010), age and sex (Svetina *et al.*, 2002), and fish species, influence haematological parameters (Nikoo *et al.*, 2012).

Blood is a vital fluid connective tissue of the body. It constitutes a vascular tissue. Blood forms the organism's internal milieu and performs numerous functions. It executes the essential processes of metabolism and respiration. Blood necessitates all conditions requisite for the provision of oxygen (O₂), vital for tissue oxidation. It functions as a conduit for the elimination of metabolic wastes and metabolites from a system through excretion. The quality and quantity of blood components will aid in assessing the physiological condition of the animal. Hematological studies in fish are typically conducted for toxicological research, environmental monitoring, and as potential indicators of physiological or pathological changes in

fishery management and disease investigations. The data also aid in assessing physiological responses to specific stressors and in establishing hematological guidelines for different animals (Kawamoto 1929; Khan and Siddiqui, 1969).

The escalation of urbanization, industrialization, population growth, and humanity's insatiable greed to exploit natural resources has engendered a significant threat to all forms of life, culminating in a global crisis. Substantial quantities of industrial waste, pesticides, and other organic chemicals and fertilizers utilized in agriculture readily infiltrate water bodies *via* runoff, leading to water pollution. Aquatic pollution is particularly concerning, as all forms of life rely on water. Natural ecosystems are contaminated by industrial waste and other anthropogenic activities among all forms of water contaminants (Velez and Montoro 1998; Conacher *et al.*, 1993). Industrial wastes represent the primary cause of metal pollution in natural water (Livingstone, 2001). Hematological indicators are critical criteria for assessing the physiological health of fish. Their alterations are contingent upon fish species, age, the reproductive cycle of spawners, and diseases (Luskova 1997; Golovina Trombicky 1989; Golovina, 1996). Freshwater fish exhibit a close relationship with their environment, rendering them highly vulnerable to physical and chemical fluctuations, which may manifest in their blood constituents.

Blood is acknowledged as a potential indicator of fish responses to water quality and can be utilized to evaluate the impact of environmental toxins, as outlined by Hesser (1960) in procedures for routine fish hematology. Larson and Snieszko (1961) evaluated different techniques for measuring haemoglobin in trout blood. Bouck and Ball (1966) asserted that haematology might serve as a valuable instrument for assessing the stress levels in fish due to water pollution. Gelineo (1969) examined the concentration of hemoglobin in 53 species of freshwater and marine fish. Blaxhall and Daisely (1973) delineated standard haematological techniques for analyzing fish blood, encompassing haemoglobin quantification, Packed Cell Volume (PCV), erythrocyte enumeration, Erythrocyte Sedimentation Rate (ESR), Total Leukocyte Count (TLC), Differential Leukocyte Counts (DLC), and cytochemical staining. This is a description of stained blood cells, along with the range and mean values for these tests on brown trout (*Salmo trutta*) as reported by Hussein *et al.* (1974), who conducted a hematological investigation on *Anguilla vulgaris* and *Mugil cephalus*. Seasonal fluctuations in erythrocyte count, haematocrit values, and haemoglobin content have been noted for both species, with elevated levels in summer and diminished levels in winter. Denten and Yousuf (1975) documented seasonal alterations in the haematology of *Salmo gairdneri*. Clark *et al.* (1979) investigated physiological stress induced by environmental factors in largemouth bass, *Micropterus salmonids*, and found that Haematocrit (Ht), Haemoglobin (Hb), and total plasma protein exhibited a

positive correlation with fish length; Hb and Ht were positively correlated with fish age, whereas mean corpuscular haemoglobin showed a negative correlation with fish age. Haemoglobin and packed cell volume were correlated with erythrocyte counts, as noted by Siddiqui and Nasim (1979), who observed that *Cirrhina mrigala* had higher haemoglobin and erythrocyte concentrations in males compared to females.

Wedemeyer *et al.* (1983) examined the physiological stress response in *Oncorhynchus kisutch* and determined that hematocrit levels were a sensitive predictor of physiological stress caused by population density and temperature fluctuations. Kumar *et al.* (1984) investigated the haematological alterations in the cold water fish *Schizothorax plagiostomus* (Heckel), which were naturally infected with metacercariae of *Diplostomium tertare*. The study revealed a decrease in total erythrocyte count, Packed Cell Volume (PCV), Hemoglobin (Hb), and Total Leukocyte Count (TLC) compared to controls, indicating pollution. Murray (1984) correlated haematological traits with the sex of fish and the seasonal period. Ralio *et al.* (1985) indicated that fish blood parameters, including erythrocyte and leucocyte counts, hemoglobin, hematocrit, and leucocyte differential counts, are significantly influenced by incidental factors such as physical and environmental stress from water contaminants. Rao *et al.* (1989) noted that active, fast-moving fish (*Scomberomorus guttatus* and *Rastrelliger kanagurata*) exhibited elevated erythrocyte parameters to accommodate their high metabolic rates compared to sluggish predatory fish (*Arius maculatus*) and bottom detritus feeders (*Liza parsia*).

The leukocyte characteristics were unrelated to the fish's activity and habitat. Thakur and Pandey (1990) investigated the impact of BHC on *Clarias batrachus* and determined that lymphocytosis, neutropenia, and eosinopenia were associated with BHC toxicity. Høglund *et al.* (1992) investigated the haematological changes in a population of *Anguilla anguilla* naturally infected with *Anguillicola crassus* throughout the Swedish Baltic coast, specifically in an area receiving heated cooling water from a nuclear power plant. Infection led to decreased lymphocyte counts and elevated granulocyte counts, suggesting a humoral and cellular immune response that influenced hematological parameters in *Oreochromis aureus* (Allen, 1993).

The findings indicated that parameters of *Aureus* seem to resemble those of *O. niloticus* and *M. mossambicus*. Hemoglobin concentration was elevated in *O. aureus* and subsequently in other species. Rauthan and Grover (1994) noted that blood parameters are influenced by both intrinsic and external variables. Research on the hematological parameters of *Barilius bendelisis* across various seasons indicated an increase in total erythrocyte count, hemoglobin, packed cell volume,

Histopathology

INTRODUCTION

This chapter provides a comprehensive overview of the common histological changes observed in fish afflicted by EUS, referencing specific species observations. The initial skin lesions in certain samples have been identified as mostly regions of epithelial necrosis accompanied by adjacent edema, hemorrhaging of the underlying dermis, and some infiltration of inflammatory cells. Confirmation of fungal involvement in the majority of these early samples has not been feasible. Nevertheless, a limited number have included a minor quantity of hyphae. Fungal hyphae were identified in the epidermis of certain early -stage infected fish from India (Viswanath *et al.*, 1997). Roberts *et al.* (1989) similarly investigated early lesions during an EUS outbreak in a captive population of Indian large carp. They noted an acute necrotizing myopathy, more severe than often found in wild fish, disseminated over a broad region beneath the active skin lesion. The epidermis near the ulcer borders was degenerated and thickened, including a minimal quantity of fungal hyphae encased within an epithelioid capsule. The dermal blood vessels exhibited significant hyperaemia, with some surrounded by a sheath of lymphoid or myeloid cells, potentially linked to viral infection, despite the absence of detectable viral inclusion bodies, and subsequent clinical changes.

All infected fish species exhibit significant degenerative changes in skin and muscle tissue, with minimal disruption of internal organs. Advanced lesions have widespread necrotizing granulomatous mycosis of the underlying muscle fibers, marked by distinctive branching aseptate, invasive fungal mycelium. A considerable amount of germs may be present on the surface of specific advanced lesions. As the lesion progresses, fungal cells are progressively enveloped by dense layers of host epithelioid cells, and specific areas may show signs of myophagia and healing. In specific advanced lesions, fungal hyphae may infiltrate the abdominal viscera, possibly acting as the primary cause of death. A substantial number of mycotic granulomas have been detected in the kidneys, livers, and digestive tracts of many fish species, including spiny eels, *Cirrhinus mrigala*, *Colisa lalia*, *Channa* sp., *Puntius* sp., *Esomus* sp., *Mugil* sp., *Valamugil* sp., *Therapon* sp., and *Glossogobius* sp. Sillago species. (Chinabut, 1990; Ahmed &

Hoque, 1998; Wada *et al.*, 1994; Viswanath *et al.*, 1998). Wada *et al.* (1994) identified mycotic granulomas in the abdominal adipose tissue, pancreas, gonads, spleen, central nervous system, and heart of dwarf gourami. Vishwanath *et al.* (1998) subsequently demonstrated fungal invasion of the esophagus and spinal cord of mullet, as well as intermuscular bones of *Puntius*. The internal organs of ill fish, except those impacted by fungal hyphae, display minimal histological changes, which Roberts *et al.* (1986) observed may occasionally arise from background pathology. Palisoc (1990) observed minimal tissue damage in the kidneys of striped snakeheads, marked by an increased presence of melanomacrophage centers, haemosiderin pigments, and a restricted quantity of mitotic figures. Regions of the spleen demonstrated a considerable enhancement in white pulp formation, but the heart, liver, and gills showed minimal histological modifications. Still, no abnormalities were observed in the stomach and intestine. Supplementary kidney samples have revealed tubular vacuolar degeneration, along with granular obstruction and degeneration or localized proliferation of hematopoietic tissue.

Chinabut (1990) determined that these traits were consistently more apparent in armed spiny eels. Pancreatic specimens sometimes display acinar necrosis (Callinan *et al.*, 1989) along with eosinophil and inflammatory cell infiltration. Prior to bacterial or fungal involvement, mild localized hepatic cellular degeneration may manifest in the liver. The only consistent haematological change in affected fish is a significantly diminished hemoglobin concentration resulting from extra- and intra-vascular erythrocyte deterioration (Tangtrongpiros *et al.*, 1985).

THE MATERIALS INCLUDING TECHNIQUES SAMPLE SELECTION

Channa striatus and *Channa punctatus* exhibiting visible indications of microbial and mycotic infection were obtained from Hasanparthy, Dharmasagar, and Bandham lakes in Warangal district, Telangana, India. The specimens (fish) were gathered alive using a fishing net and promptly transported to the laboratory for additional clinical assessment in plastic containers filled with oxygenated water.

The specimens were profoundly anesthetized by immersion in a 5 ml/L water solution of ethylene glycol monophenyl ether. Following the dissection of the fish, tissue samples from the skin, gills, liver, and pancreas were meticulously extracted and preserved in fixatives. Skin, gill, liver, and pancreatic tissues were preserved in Bouin's, Zenker's, Susa, Carnoy, and Formal Calcium standard fixatives according to established techniques. The diseased fish exhibited red blotches on their bodies, excessive mucus production, damage, and lethargy. The microbial examination was conducted on various infected tissues obtained from

Channa striatus and *Channa punctatus*, specifically the skin, gills, liver, and pancreas. The bacterial species include *Aeromonas hydrophila*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella salmonicida*. The fungal germs identified in the aforementioned organs include *Aspergillus flavus*, *Fusarium solani*, *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Penicillium chrysogenum*, *Aspergillus niger*, and *Trichoderma viride*.

PROCESSING FOR HISTOPATHOLOGICAL INVESTIGATIONS

Samples of skin, gills, liver, and pancreatic tissues were extracted from the fixative.

Hydration

The tissues were washed with saline solutions according to established protocols.

Dehydration

The process involved placing the tissue in sufficient 70% alcohol, then transferring it to 90% alcohol for 1 change and 2 changes of 100% alcohol.

Clearing

Tissues were cleared using a clearing agent, specifically xylene.

Embedding and Impregnating

The impregnation agent employed was paraffin wax, which enabled effortless entry into the tissue without inducing structural damage or significant shrinkage or crystal formation.

Trimming

The embedding block containing tissue was meticulously clipped to ensure that just a single tissue specimen was prepared for sectioning.

Mounting of the Block

An iron block measuring 30 mm was utilized to secure the block at the appropriate angle and position for cutting.

Section Cutting

The tissue block was sectioned to a thickness of 5µm using a microtome.

Histochemical Analysis

INTRODUCTION

Histochemistry has significantly advanced the comprehension of biological phenomena and clinical medicine. Histochemical techniques facilitate the analysis of the localization of proteins, lipids, glycogen, and other substances, as well as molecular alterations at the cellular level. The primary benefit of histochemistry is its ability to analyze biological events within specific cells. The modified condition of cellular components can be examined by histochemical methods. The histochemical assays indicate the location of chemical products resulting from cellular activity. This work examines the histochemical characteristics of the skin, gills, liver, and pancreas of *Channa striatus* and *Channa punctatus* to analyze the differences between control samples and those affected by bacterial and fungal infections.

The skin serves as the principal barrier against environmental factors, facilitating proper internal physiological functions (Sarasquetel *et al.*, 1998). This condition is significant in numerous disease processes. The skin mucus of fish may play a crucial role in natural defense against parasites and harmful microorganisms (Fletcher, 1978), in addition to potentially serving osmoregulatory and lubricating functions (Van Oosten, 1957; Rosen; Conford, 1971). The defense may be mechanical, resulting from the constant generation of mucus (Pickering, 1974). The presence of lysozymes and IgM complement components indicates that mucus plays an active role in the immune system (Hjelmeland *et al.*, 1983).

This encompasses a range of specific interests, from architecture to histochemistry and necrotic diseases of the skin, particularly its zoonotic significance. The structural integrity and histochemical intricacies are essential for the skin's reliance in this regard. Despite considerable uniformity in the fundamental organization of vertebrates, particularly regarding the dermis, epidermis, and basement membrane, light and electron microscopy have elucidated the structural architecture of the skin and its protective functions for the organism.

Most prior research in histochemical investigations of skin, such as Askawa (1970), examined the histochemistry of mucus on the epidermis of the eel

Anguilla japonica. Mittal and Munshi (1971) conducted a comparative research on the skin structure of specific air-breathing freshwater teleosts. Harris *et al.* (1973) conducted a histochemical examination of mucous cells in the epidermis of the brown trout, *Salmo trutta*. Banerjee and Mittal (1975) conducted research on the histochemistry and functional organization of the skin of the “livefish” *Clarias batrachus*. Mittal and Banerjee (1975) conducted investigations on histochemistry and the structural composition of the skin of the murrel, *Channa striata*. Mittal *et al.* (1976) investigated the protein and carbohydrate histochemistry concerning keratinization in the epidermis of *Barbus sophor* (Cyprinidae, Pisces). Mittal and Whitear (1979) documented the keratinization of fish skin, specifically focusing on the catfish *Bagarius bagarius*. Mittal *et al.* (1980) delineated the fine structure and histochemistry of the epidermis of the fish *Monopterus albus*, whereas Hjelmeland *et al.* (1983) examined skin mucus protease from the rainbow trout, *Salmo gairdneri* Richardson, and its biological importance.

Sultana and Rao (1990) reported observations regarding the structure and histochemistry of the skin of *Mystus gulio*. Mittal *et al.* (1994) conducted a histochemical investigation of glycoproteins in the unicellular glands of the epidermis of the Indian freshwater fish *Mastacembelus panculus*. The histochemical examination of glycoproteins in the unicellular glands of the epidermis of the Indian freshwater fish *Mastacembelus*. Mittal *et al.* (1995) described the surface architecture of the skin of the Indian catfish, *Bagarius bagarius*. Gonzalez *et al.* (1996) examined the histological and histochemical properties of lymphocytosis in seabream, *Sparus aurata*. Sarasquetel *et al.* (1998) examined the histochemical aspects of lymphocystis illness in the skin of gilthead seabream, *Sparus aurata*. Park and Kim (1999) documented the structure and histochemistry of the skin of the mud loach, *Misgurnus anguillicaudatus*.

Park *et al.* (2000) conducted a histological examination of the skin of the amphibious fish, *Periophthalmus modestus*. Park *et al.* (2001) investigated the morphology and histochemistry of the skin of the mud loach, *Misgurnus mizolepis*, concerning cutaneous respiration. Zacccone *et al.* (2001) elucidated the structural, histochemical, and functional characteristics of fish epidermis. Saikia and Rai (2002) conducted histochemical research on the functional organization, amounts, and distribution of various carbohydrate elements in the skin club cells of the catfish *Heteropneustes fossilis* (Bloch) (Heteropneustidae, Pisces) under acid stress. Park (2002) examined the integumentary anatomy of the air-breathing mudskipper fish, *Periophthalmus magnuspinnatus*. Jong-Young Park (2002) investigated the morphology and histochemistry of the skin of the Korean spined loach, *Iksookimia koreensis* (Cobitidae), concerning breathing. Park (2002a) examined the morphology and histochemistry of the skin of the spined cobitid fish, *Iksookimia koreensis*, concerning respiration. Park (2002b) studied the

structure of the skin of the air-breathing mudskipper fish, *Periophthalmus magnuspinnatus*. Park *et al.* (2003) contributed to the understanding of the structure and histochemistry of the skin of the torrent catfish, *Liobagrus medtadiposalis*. Swati Mittal *et al.* (2004) conducted a scanning electron microscopy and histochemical study on the operculum of the peppered loach, *Lepidocephalichthys guntea*.

The epidermis is separated into three layers: an uppermost layer, a middle layer, and a basal layer. The outermost layer consists of multiple strata of epithelial cells. Chloride cells, outermost cells, mucous cells, and sacciform granular cells are located at the surface. The intermediate layer consists of unicellular glands, mucous cells, and sacciform granular cells. Epithelial cells occupy the interstitial areas between the glandular cells (Sunita *et al.*, 2004).

Dutta and Rai (2005) conducted studies on lipids in the skin of the catfish *Clarias batrachus* and described the presence of proteins and nucleic acids in its skin. Guerra *et al.* (2006) characterized the stratum adiposum, a distinctive structure of the African catfish skin (*Clarias gariepinus*). Rai and Saikia (2007) examined the impact of acid stress on the histochemical response of alkaline phosphatase activity in the skin cells of the catfish *Heteropneustes fossilis*. Rai and Saikia (2008) observed the effects of acid stress on the functional organization and distribution of carbohydrate constituents in the skin of *Heteropneustes fossilis*. Bonilla *et al.* (2008) analyzed the skin histology and morphometry of the fish *Eremophilus mutisii*. Tarun *et al.* (2010) detailed the histochemistry and functional organization of the dorsal skin of *Andstrus ddlichopterus*. Mohamed *et al.* (2010) examined the effects of experimental infection with *Gyrodactylus* species on the density of skin mucus in catfish fry (*Clarias gariepinus*), focusing on the pathological alterations observed in the skin histochemistry of freshwater catfish *Clarias batrachus* (Laxma Reddy Benarjee, 2011).

The fish skin is the protective structure that envelops the body, safeguarding it from the infiltration of bacterial and fungal infections or allergens, as well as preventing the loss of water, solutes, or minerals. The outside-in and inside-out barrier functions rely on the epidermis, a stratified cellular layer. Mucus envelops the epidermis in fish and amphibian tadpoles, whereas distinct cornified cellular layers (stratum corneum) form the outermost epidermal barrier in adult amphibians, reptiles, birds, and mammals. Kubo (2011) states that the skin of teleosts is particularly distinctive and histologically diversified (Fast *et al.*, 2002). It markedly differs from that of mammals, as it secretes mucus that plays a role in immunological processes (Salinas *et al.*, 2011). The shape and function of the organism demonstrate its adaptability to the physical, chemical, and biological characteristics of the aquatic environment, as well as its evolutionary history. The

CHAPTER 5**Diagnostic Techniques Based on AI and Machine Learning****INTRODUCTION**

Epizootic Ulcerative Syndrome (EUS) is a catastrophic disease impacting aquaculture, primarily caused by the fungal pathogen *Aphanomyces invadans*, in conjunction with bacterial and viral diseases. It presents a considerable risk to global aquaculture, affecting the livelihoods of millions and the accessibility of sustainable seafood resources. The disease presents as ulcerative lesions, resulting in elevated mortality rates, diminished market value, and economic detriment for farmers. Conventional methods for controlling EUS depend on clinical observation, laboratory tests, and preventive strategies, which tend to be reactive, labor-intensive, and constrained by delays in identification and reaction.

In recent years, Artificial Intelligence (AI) and Machine Learning (ML) have become crucial instruments in tackling the complex difficulties presented by EUS. Utilizing extensive data from environmental monitoring systems, fish health evaluations, and genetics, AI and ML provide swift diagnosis, predictive modeling, and tailored intervention tactics. These technologies have proven capable of early disease outbreak detection, optimizing water quality and agricultural practices, and recommending exact treatments, thereby considerably improving the efficiency and sustainability of aquaculture operations.

This chapter examines the utilization of AI and ML in the diagnosis, prevention, and management of EUS. It examines AI-driven diagnostic methodologies, encompassing image-based lesion identification and predictive analytics, which improve precision and diminish reaction durations. Additionally, it analyzes how machine learning algorithms predict epidemics by incorporating environmental and historical data, thereby informing preventive strategies. The significance of AI in enhancing treatment procedures and creating novel monitoring systems is emphasized.

The chapter finishes by discussing the problems associated with the implementation of AI and ML technologies, including data restrictions, adoption

barriers, and technological constraints, while offering future perspectives and ideas to promote collaboration and creativity. This investigation highlights the revolutionary capacity of AI and ML in addressing EUS concerns and promoting aquaculture sustainability.

What is Endoscopic Ultrasound (EUS)?

Epizootic Ulcerative Syndrome (EUS) is a severe infectious disease impacting freshwater and brackish-water fish, primarily caused by the fungal pathogen *Aphanomyces invadans*. It is a complex illness, frequently aggravated by secondary bacterial and viral illnesses. EUS is marked by the formation of necrotic ulcers on the skin, resulting in considerable tissue damage, diminished physiological function, and elevated death rates. Initially recognized in the 1970s, the illness has proliferated throughout Asia, Africa, and certain regions of the Americas, emerging as one of the most commercially and ecologically detrimental diseases in aquaculture.

Symptoms of EUS manifest as tiny hemorrhage lesions on the skin that advance to extensive ulcerative wounds. These sores frequently reveal the underlying musculature and may lead to secondary infections, thereby exacerbating the health of the affected fish. The illness affects numerous species, including commercially significant fish such as catfish, tilapia, and carp, rendering it a global issue for aquaculture enterprises.

Economic and Ecological Importance

The economic influence of EUS on aquaculture is significant. The disease causes substantial production losses due to elevated mortality rates and diminished market value of fish exhibiting obvious lesions. EUS outbreaks can result in severe financial repercussions for small-scale fish farmers, who are essential to aquaculture in numerous developing areas, hence intensifying poverty and food insecurity.

EUS outbreaks ecologically damage aquatic habitats by impacting both cultivated and wild fish populations. The illness can be transmitted from aquaculture systems to natural water bodies, jeopardizing biodiversity and disrupting ecological balances. Initiatives to treat the disease, including the application of pesticides and antibiotics, may adversely affect the environment, underscoring the necessity for sustainable management techniques.

CHALLENGES IN ENDOSCOPIC ULTRASOUND MANAGEMENT

Constraints of Conventional Diagnostic Methods

Traditional diagnostic techniques for EUS, including histopathology, microbiological cultures, and PCR assays, are frequently laborious, resource-demanding, and reliant on laboratory infrastructure. Although these approaches are dependable, they are not consistently available to small-scale farmers, particularly in isolated or resource-constrained environments. The interval between sample collection, processing, and result acquisition frequently facilitates further disease dissemination, hence diminishing the efficacy of control efforts.

Control Techniques and Their Constraints

Preventive strategies, including water quality control, immunization, and antibiotic administration, have had little efficacy owing to the intricate nature of the disease and the absence of early detection methods. The overuse of antibiotics has heightened worries regarding antimicrobial resistance, hence hindering disease control initiatives.

The Necessity for Expeditious, Precise, and Scalable Solutions

To tackle these difficulties, there is an urgent requirement for technologies that can deliver rapid, precise, and economical illness detection, together with predictive capabilities to facilitate prompt therapies. Advanced technologies must be scalable to accommodate the varied requirements of global aquaculture systems.

THE FUNCTION OF ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING IN DISEASE MANAGEMENT

Fundamentals of Artificial Intelligence and Machine Learning Concepts

Artificial Intelligence (AI) denotes the emulation of human cognitive functions in machines, allowing them to execute activities including learning, thinking, and decision-making. Machine Learning (ML), a branch of AI, encompasses algorithms that acquire knowledge from data to generate predictions or judgments autonomously, without explicit programming. Machine learning methodologies, including supervised learning, unsupervised learning, and deep learning, are very effective for the analysis of intricate datasets.

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