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Investigating and Identifying Fish Illness in Aquaculture of Raised Fish: An Overview from the Proteomics Angle

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Abstract

The growing demand for fish protein throughout the world is mostly met by aquaculture. However, the sector confronts issues including disease outbreaks that can have a major effect on fish health and productivity. Recognizing anomalous behavior, physical symptoms, and changes in appearance among grown fish are all part of diagnosing fish sickness in aquaculture. To preserve the health of the aquaculture system and to perform appropriate treatments, effective diagnosis is essential. The susceptibility of farmed fish to illnesses by improper husbandry techniques, outside variables like pollution and climatic change, and even modifications to the way products are traded within this industry, be one of the primary obstacles to aquaculture production. This study underscores the significance of using proteome analysis to uncover biomarkers, since this facilitates the creation of diagnostic instruments for the early identification of illness. Aquaculturists can lessen the effects of diseases on fish populations by taking targeted actions based on the identification of certain protein signatures linked to different fish illnesses.Proteomics is an example of a high-throughput technology that may be a valuable characterization implement, concentrated on the identification of pathogens and the virulence mechanisms connected to host pathogen interactions in the study and diagnosis of diseases that enforce the control, avoidance, in addition to treating illnesses in farmed fish. One important and promising method for studying fish illness is proteomics, which plays a significant role in knowing fish responses to environmental cues such stress and temperature, as well as pathogenic processes.

Keywords: Fish Pathology, Aquaculture, Fish Illnesses, Proteomics, And Fish Welfare

INTRODUCTION

Firming encompasses the breeding, growing, and harvesting of fish, aquatic plants, crabs, mollusks, and aquatic animals. This is linked to the term aquaculture. Food and industrial possessions are produced by means of the regulated cultivation of freshwater and saltwater organisms (1). Aquaculture is mostly divided into two categories. Mariculture is the first; it involves raising marine life for food and other products, including jewelry, cosmetics, nutraceuticals, and medications. Marine farms can be found in enclosures on land and on the sea, such as raceways, ponds, and cages, and also discovered in their natural marine habitat (2). Among many different species being farmed throughout the coastlines of the world are seaweeds, mollusks, shrimp, marine fish, and several lesser species like sea cucumbers and sea horses. Fish farming is the second, which involves the commercial growing of fish in tanks and other enclosures constructed by humans. Common fish kept in these cages include catfish, tilapia, salmon, carp, cod, and trout. To fulfill the demand for fish products, the fish farming sector has expanded in the modern era. This type of aquaculture has long been popular because it is to provide an inexpensive source of protein (3). Fish raised in aquaculture are more vulnerable than fish in the wild to a variety of viral, bacterial, parasitic, and fungal diseases as a result of husbandry practices. Live production is always risky when it comes to infectious disease loss. Modifications in that microbe, infectious pathogens, and people interact were also caused by the trend toward higher density production systems, pollution and climate change related disruptions of the ecological systems' equilibrium, along with the projected increase in global commerce in products from aquaculture and their byproducts (4). This affects the rates of reproduction and proliferation of pathogens, which results in a wider geographic dispersion of pathogenic agents and a rise in the number of species that are impacted by disease outbreaks. Disease outbreaks are a major barrier to this business, having a big influence on the quantity, quality, and safety of fish produced globally. These also cause the producer to lose access to some markets and experience significant costs (5), as shown in Figure (1).

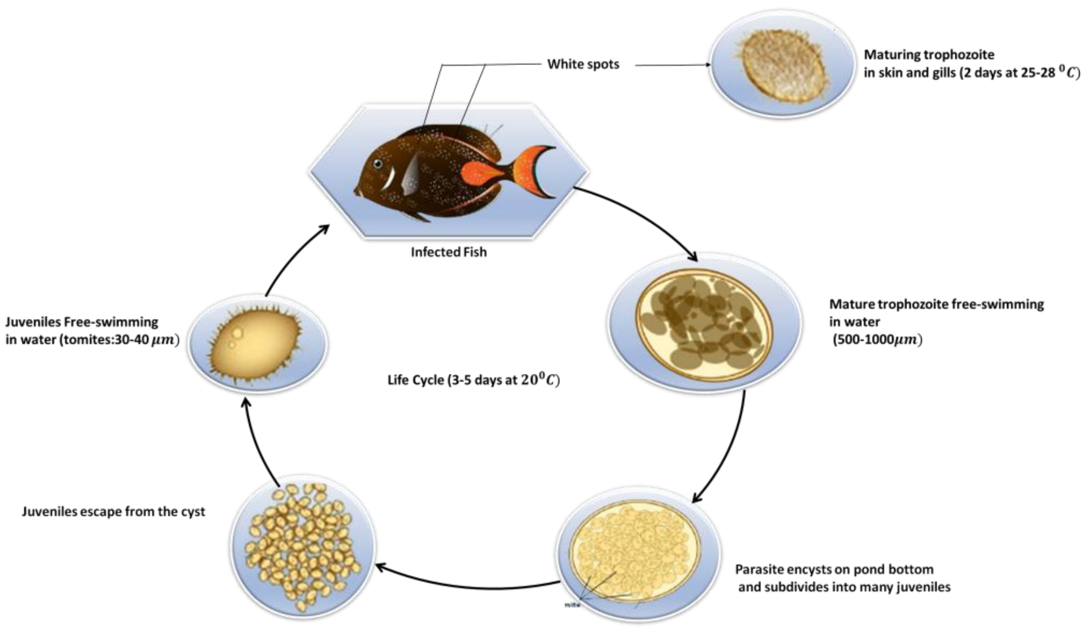

Figure (1). The white-spot disease-causing lchthyophthiriusmultifilis life cycle

(Source: https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.fao.org%2Ffishery%2Fstatic%2FFAO_Training%2FFAO_Training%2FGeneral%2Fx6709e%2Fx6709e15.htm&psig=AOvVaw1YoBq0uVDf7K9JCcWG4PYA&ust=1702194924939000&source=images&cd=vfe&opi=89978449&ved=0CBIQjRxqFwoTCOjy7K_wgYMDFQAAAAAdAAAAABAD)

The paper (6) employed metabolomics in farmed salmon research, the goal of this review is to highlight discoveries and pinpoint shortcomings. The majority of the salmonids and sample types that have been researched were identified, analytical platform utilization was emphasized, and methodological information was collected for comparison. The paper (7) developed farmed fish eggs would need to be sent to the moon. The effects on fish embryos and young larvae of lunar mission circumstances, including microgravity during travel and hyper gravity during rocket launch, to assess the viability of this idea. The paper (8) used examples from farmed fish, namely salmonids and lumpfish, the conference aimed to increase understanding of fish ethics, severity categorization, and humane outcomes in fish research. The workshop's main objectives were to improve the definition of humane endpoints in fish studies and to have a discussion about ideas for creating and applying score sheets that are used to evaluate endpoint-related clinical indications. The paper (9) used bacterial, viral, and parasite illnesses found in salmonids and other cold-water fish kept in captivity across the world. Furthermore, precautionary and controlling

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strategies that might aid in the fight against these microbial diseases in aquaculture are discussed. The paper (10) prevented pathogens from establishing a host, and it's crucial to comprehend the interactions that occur within the fish gut microbiota. As a result, the close-knit links and interactions between pathogens and gut microbiota that may cause dysbiosis and illness are discussed. The paper (11) investigated that fish farming in Bangladesh is affected by climate change and makes recommendations for how geospatial technology might be used to address these problems. To address climate-related issues in Bangladeshi aquaculture for long-term sustainable output, the study was anticipated to be beneficial to stakeholders such as researchers, scientists, policymakers, and aquafarmers. The paper (12) described the idea of a protein-protein interaction (PPI) network, along with its general applications and methods of discovery.The paper (13) examined the use of edge computing, 5G, Internet of Things (IoT), artificial intelligence (AI) algorithms, and fisheries intelligent equipment in contemporary aquaculture. They also examines the challenges that exist and the opportunities for future advancement. Concurrently, the design frameworks for the major functional modules in the building of the intelligent fish farm are suggested, depending on various business needs. The paper (14)described the obstacles, achievements, and opportunities Tanzania faces in building a sustainable aquaculture sector. These include inadequate funding for research on fish diseases, a lack of expertise in diagnosing and treating fish diseases, limited management options, and limited extension services that reflect farmer's limited knowledge of pond management practices. The paper (15)examined the body of research on the fundamental and practical characteristics of oocyte loss due to atresia, as well as the morpho-physiological elements and the variables that lead to a supraphysiological rise in follicular atresia. The utilization of early follicular atresia detection in aquaculture's induced spawning selection process and the consequences of follicular atresia for fisheries management are finally covered in the review, as shown in Figure (2).

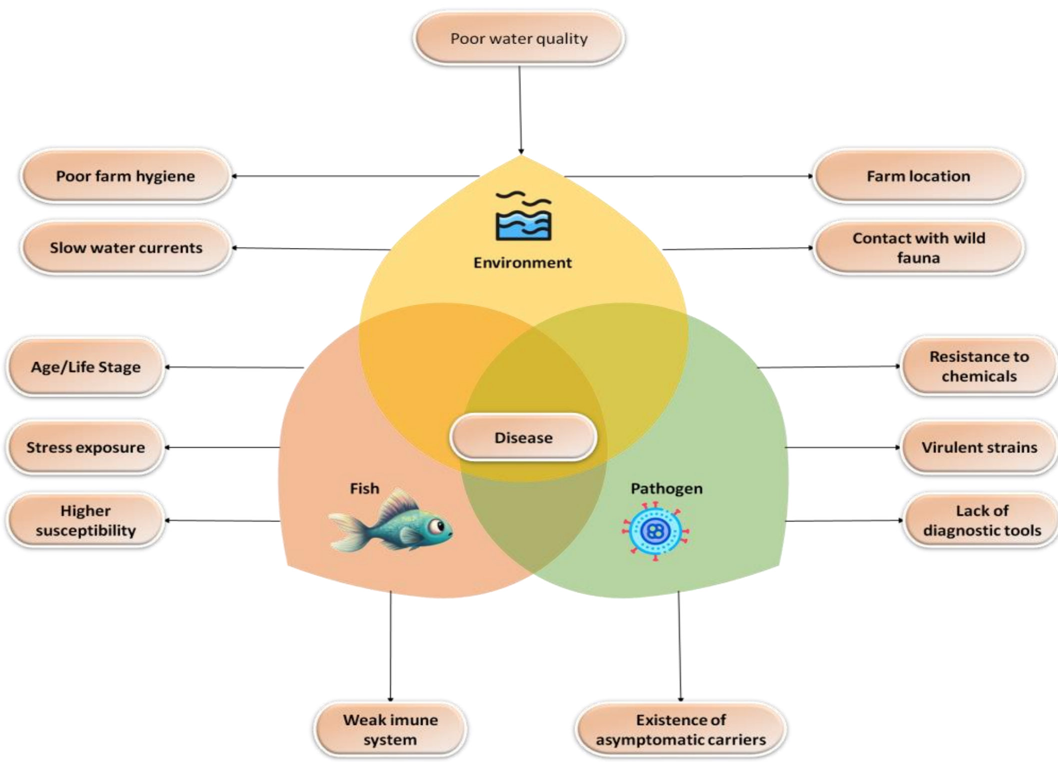

Figure (2). Diagram of aquaculture disease, showing the key elements for pathogen assessment and host-pathogen interactions influencing fish disease outbreaks

(Source: Author)

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FISH WELFARE, STRESS, AND HEALTH

The wellbeing of fish is rarely given the same consideration as that of other vertebrates, even though they are the most eaten animal. The body of knowledge on fish welfare is new when compared to other terrestrial creatures reared for human consumption. Theyrequire a common definition of intelligence, and the discrepancy between the public's opinion of intelligence and the available scientific data is partly to blame for this disregard. The majority of definitions focus on a function-based and feelings-based technique. While excellent welfare is characterized as a lack of bad feelings and the existence of happy ones, the first takes into the emotional condition of the animal. A fish is considered to be in excellent welfare if it can adapt to its surroundings and maintain homeostasis, according to the second definition, it gives the animal's physiology, biology, and overall health more weight. When assessing a fish's wellbeing, its health status can give objective standards, but it is not a comprehensive assessment. It is not always the case that a fish in excellent health is in an effective welfare condition, but good health is necessary to ensure a healthy fish. However, poor health, or the animal's diminished capacity to perform normally, handle stress, and fend off illness, typically indicates or results in a low level of welfare in several circumstances. For example, dead unhealthy fish can spread infections and lower the quality of the water. The use of chemical remedies for specific outbreaks may damage fish populations to some extent. Notably, an acute illness can strike a healthy animal in an ideal habitat at any time, severely impairing its wellbeing. For example, diseases are ingrained in the environment when fish are raised in cages (16).

Generally speaking, bad husbandry circumstances lead to poor welfare status, which in turn results in poor health. In conclusion, there is a close relationship between health and welfare, and it is possible to view poor wellbeing as both a cause and an effect of bad health. Examining the most current methods used to investigate the connection between specific diseases/pathologies and welfare, this part centers on health as the basis for evaluating fish wellbeing and the impact of stressors on disease resistance. In aquaculture, common stresses in culture systems include improper husbandry conditions or even regular farming methods. Fish welfare gets threatened by infections as a result of the allostatic load placed on the animals, and it may also compromise the effectiveness of the immune system. For example, in Mozambique tilapia (Oreochromismossambicus), significant temperature variations resulted resulting in a decrease in immunity and disease tolerance (17). More recently, it was demonstrated that using a transcriptomics method, it’s possible to determine how much the rearing density of Nile tilapia affects its receptivity to oomyceteSaprolegniaparasitica, as shown in Figure (3).

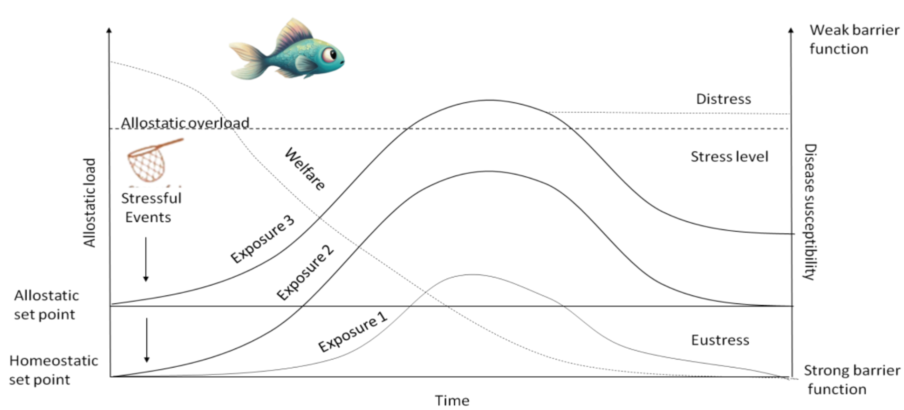

Figure (3). Interaction between the fish's assessment of repeated or ongoing stressful events, wellbeing, allostatic load, and susceptibility to illness

(Source: Author)

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Stress is viewed as a situation in which equilibrium is in danger of being compromised. Allostasis and homeostasis are restored by a complex web of adjustments to the physiological systems. Fish, like all other vertebrates, have a broad physiological stress response that they use in response to perceived stressors. This response enables the ability of the person that adjust to manage both expected and unexpected changes in their surroundings, also known as eustress. In the circulation, cortisol and catecholamines are produced as a first response, which sets off a chain of subsequent events. As it happens, tension does not always mean that one's wellbeing has been jeopardized. These include the release of hormones, a rise in inflammatory markers, and acute phase protein synthesis. These energy reserves force ultimately run out if the stressor continues, even if the fish has been able to adjust over time. The fish may become unable to adjust as a result of the allostatic overload caused by the complete depletion of energy reserves, which can result in immunosuppression, illness, and in more extreme cases, even death. Additionally, a number of studies have shown how the capacity of epithelial barriers to function is impacted by stressful husbandry settings.Rainbow trout (Oncorhynchusmykiss), Atlantic cod (Gadusmorhua), and Atlantic salmon (Salmosalar) have been shown to have changes in these barriers when exposed to various acute stressors. It was also shown that intestinal barrier function was compromised in Atlantic salmon raised in environments with low dissolved oxygen concentrations. Endogenous opioids, pituitary hormones, and catecholamines are examples of hormones, and serotonin all play a role in these disruptions, although elevated cortisol levels have been linked (18).

It has been demonstrated that cortisol has an immunomodulatory effect, which means that it may enhance certain immune system components while suppressing others, such as apoptosis induction, differentiation pattern changes, cytokine release inhibition, and immunocyte migration inhibition. However, there is a chance that the correlation between immunological state and stress determination can be masked by the fact that the cortisol response varies between species and even in individuals, depending on a number of other factors. The authors of this review have cited recent articles for a full explanation of the processes behind these immunoregulatory alterations and the mounting endocrine-immune response.For the aquaculture sector to be sustainable, it is critical that we expand our scientific understanding of the systems governing stress, fish welfare, and health. Recent years have the successful application of sophisticated high-throughput technologies, such as proteomics, to research on aquaculture, encompassing the examination of fish health and wellbeing. These technologies have given researchers a comprehensive comprehension of the chemical processes that underlie the physiological stress reaction, along with important new insights into the distinct proteins implicated in inflammatory and immunological reactions.Fish proteome studies mostly focus on the hepatic, but mucus and blood plasma are becoming important, primarily from an immunological perspective, since fish skin mucus is one of their main defense mechanisms, along withplasma is a recorder and mirror of both normal and pathological circumstances. Proteomics has significant implications in this field, including the examination of host-pathogen interactions and the impact of certain illnesses and parasites on the quantity and changes of proteins. For instance, there is collaborative research assessing the proteome alterations in fish exposed to a particular disease following exposure to a rearing stressor (19).

DIAGNOSTICS OF DISEASES

Disease diagnosis in aquaculture requires a multi-level approach, taking into account factors such as environmental etiology, cellular morphology, genome, and proteome complexity, as shown in Figure (4).

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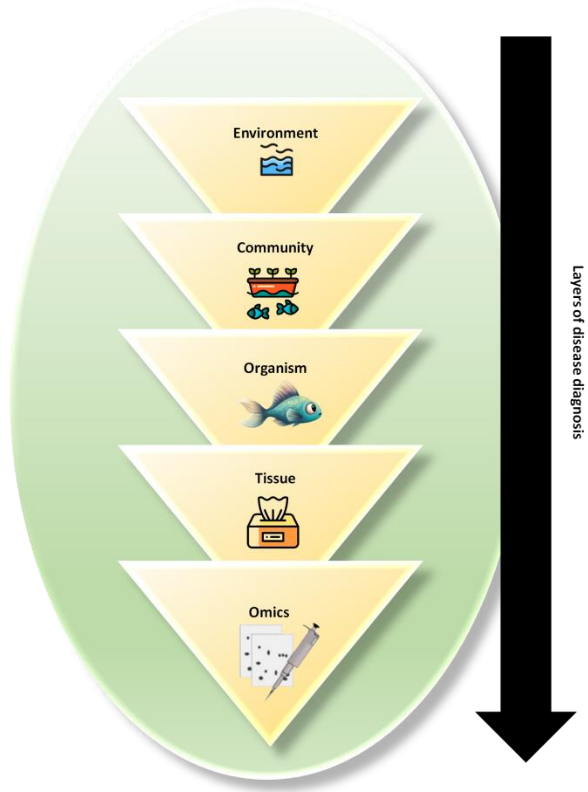

Figure (4). Layers of disease diagnosis represented as a concentric ring (Source: Author)

To get a successful illness diagnosis in aquaculture, new fields such as proteomics might be a valuable addition to more traditional procedures such as pathogen identification, disease symptomatology, and histological examination. In the field of proteomics, the primary objective is to identify as many proteins as possible inside complicated protein mixtures, which may have thousands and even hundreds of proteins.Following one dimensional (1 DE) and two dimensional (2 DE) gel electrophoresis, proteins are identified in mass spectrometry in gel based techniques; conversely, mixtures of proteins are left in the liquid before identifying the protein in gel free techniques.Peptides are produced by digesting protein samples using an enzyme unique to the sequence, commonly trypsin, to differentiate it from the top-down proteomics examination of whole proteins in each instance. This process is known as peptide-based bottom up proteomics. Liquid chromatography linked to tandem mass spectrometry (LC-MS/MS) is a technique that may be used to separate and analyze peptide samples. As a general practice, for MS analysis, the peptides are converted into gas state ions using ESI. Peptide samples can be examined using Time-of-flight (TOF) mass spectrometries with Matrix-assisted laser desorption/ionization (MALDI). The selection of a method is contingent upon several factors, including the primary research aim, expenses, and expertise. For the purpose of microbiological identification and diagnosis, MALDI-TOF MS-based techniques are most suitable because they are quick, sensitive, and cost-effective (20).

IDENTIFICATION OF PATHOGENS

A crucial component of illness diagnosis and treatment is pathogen identification. Numerous and comprehensive applications have been made to explore this issue using conventional, immunological, and molecular methods. Nonetheless, proteomics has developed over the last ten years into a powerful tool for epidemiological research,

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damage characterization, and pathogen detection. The process of identifying a pathogen, an organism that causes disease in a particular sample, involves figuring out whether and not it exists. Numerous methods, including those from molecular biology, microbiology, and immunology, are used in this important diagnostic process. These techniques are used by scientists and medical experts to pinpoint the bacteria, viruses, fungus and parasites that cause infections or illnesses. The study's objective is to determine which bacteria causes the illness, allowing for focused with efficient treatment plans. Technological breakthroughs like DNA sequencing and sophisticated imaging have greatly increased the speed and accuracy of pathogen detection, facilitating quicker and more precise diagnoses in the fields of public health and medicine (21).

SYMPTOMATOLOGY

The fact that the degree of infection varies based on several factors, such as the kind of host, the age, and the physiological condition of the fish, environmental circumstances, as well as illness stage, various pathogens affect fish in different ways. Diseases can manifest in a variety of ways and are exported, turning a disease from acute to chronic and vice versa. In this case, there is an epidemic of infectious salmon anemia (ISA) in Atlantic salmon, when the fish have mild changes in appearance but initially have insignificant mortality. Inaction on diagnostic measures may result in the chronic stage being unreported. The severity of the disease may increase if acute disease phases with significant mortality occur occasionally. In addition, the acute stage of an ISA infection is more common in the spring, whereas the chronic stage appears in autumn.

Fish are susceptible to secondary infections in addition to diseases caused by bacteria, viruses, parasites, and fungi, which can worsen their condition and raise the death rate. Fish infection can be identified by looking for clinical symptoms and changes in behavior. Symptoms that are displayed in reaction to a sickness may not be exclusive to that illness and may be quite similar across infections with various pathogens. Furthermore, the fish may exhibit few or none of these symptoms. Following these findings, certain infections can be confirmed by gross and microscopic pathology; however, more precise methods of diagnostics are frequently required for identification (22).

INSTRUMENTS FOR INVESTIGATING HOST PATHOGEN INTERACTIONS

Holobiome method: metaproteomics and metagenomics

The instruments available for examining the interactions between host pathogens are quite complex and may be utilized to comprehend interactions between people and ecosystems as well as those at the molecular, cellular, and physiological levels. When a pathogenic agent, such as a virus, bacterium, prion, fungus, viroid, or parasite, challenges the host organism, a biological reaction is triggered; in turn, the pathogen responds by going on the offensive. This interaction suggests that both sides induce gene expression and protein synthesis. If the host's defensive mechanisms or reaction to the pathogenic challenge are ineffective, the host may develop an infectious process that eventually leads to death.

It is possible to approach host-pathogen interactions from a broader angle, taking into the related microbial communities. In fact, evidence suggests that the microbiota may be crucial for an organism's immunological response. A crucial component for the continued fish host pathogen interactions is studied using the holobiome method, which focuses on the creation of rational solutions for disease prevention and resistancethat interactions between the pathogen, the host, and other environmental microorganisms. Additionally, by lowering the usage of antibiotics, which has a detrimental effect on the environment, this comprehensive understanding of interactions between pathogens and fish hosts might improve aquacultures sustainability. The most potent and recently developed high-throughput techniques for revealing the relevant microbial populations' genomes and proteomes in marine and oceanic settings are metagenomics and metaproteomics. Although they may be very helpful while

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examining the microbial groups that are native to the fish farms' surroundings, these approaches are currently rare in aquaculture research. Furthermore, by describing the bacteria associated with the mucus on fish skin and the fish stomach, metagenomics and metaproteomics approaches help to understand important immune related genes and proteins, which might operate as a coherent biosystem throughout interactions between fish hosts and pathogens through intricate networks (23).

Networks of Protein-Protein Interaction (PPI) and Omics-Based Strategies

The utilization of high-throughput technologies like mass spectrometry-based proteomics, RNA sequencing (RNA Seq) and metabolomics has helped to make significant advancements in the last few years in our understanding of the proteins, metabolites, and genes that interact with the host during infectious events. However, utilizing zebrafish (Daniorerio) larvae as models of infection and merging in vivo techniques with omics based methodologies to examine connections at all scales, including single cells and entire animals, this approach advanced our knowledge between fish pathogens and their cellular processes.

Extensive proteome analysis of the fish host and pathogen in healthy and diseased settings made it possible to discover proteins that are important for disease defense mechanisms. These proteins' regulatory complexity is represented by networks of protein protein interaction (PPIs). To develop novel treatment methods for fish farming, proteomics may be integrated with other omics-based techniques to create model networks that may anticipate the dynamics of interactions among cellular bio-components associated with fish-pathogen immune responses. Given that they catalyze and regulate every cellular process and play a crucial role in host-pathogen interactions, proteins can be considered the fundamental actors and building blocks of every living organism.

The PPI networks, which may be predicted by computational methods and identified experimentally, for example, using interactome proteomic approaches, are becoming more and more well-liked and are quickly emerging as the best resources for learning about pathogenesis. The PPI networks have the potential to provide novel insights into the interactions between pathogens and hosts, along with speeding up the development of vaccinations, treatments for fish illnesses, and preventive measures by discovering relevant health and disease biomarkers. Without a doubt, PPI network analysis is one of the most potent and economical methods for supporting the control of fish diseases in the aquaculture industry. In summary, a multitude of novel instruments are being developed to tackle host-pathogen interactions in fish farming, which can aid in the management, avoidance, and cure of illnesses in fish. It is becoming apparent that these interactions are highly intricate, necessitating comprehensive, complementary, and integrated learning experiences (24).

CONCLUSION

Proteomics presents a highly promising avenue for fish pathology investigation and assessment, offering thorough comprehension of pathogenesis mechanisms, important details on identifying pathogens and virulence mechanism description, previously unidentified physiological congregation responses, and novel stress response pathways.Fish farming is relatively new to the study of proteins, and its use is limited to a small number of sequenced species. The application of proteomic methods in aquaculture will increase with further advancements in the definition of extensive datasets from diseased fish and fish diseases, as well as aquaculture proteomes, which result in novel and interesting findings in this sector. However, there is significant potential for a novel, comprehensive, and integrated understanding of fish pathogenesis through the study of the interplay between holobiome host pathogens, and predict that may determine the direction of future research to better understand how infections affect fish. The future to improve disease control and overall sustainability in farmed fish farming by enabling real-time monitoring and early fish sickness identification.

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