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Unraveling stress-induced metabolic pathways in aquatic species for sustainable aquaculture practice

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Abstract

Maintaining the physiological well-being of farmed aquaculture species is crucial for their long-term sustainability and profitability, especially under diverse environmental and operational constraints. This study focuses on the stress-related metabolic pathways impacting the growth, immune function, and survival of fish and crustacean aquaculture species, with metabolomics and systems biology providing the foundational tools. With the Metabolic Stress Pathway Analysis (MSPA) methodology, we studied the oxidative stress, hyperthermia, and hypoxia response frameworks. The MSPA integrates stress metabolomics and machine learning for pathway enrichment to discover biomarkers of stress and adaptive response signatures. Stress adaptation revolves around changes to energy pathways, amino acid metabolism, and the systems of reactive oxygen species. Predictive network analysis identifies resilient and feed-efficient fish regulatory hubs. This work is of primary importance in developing stress-sustainable aquaculture management frameworks and in developing economically viable and environmentally sustainable species under stress. The trade contemplated on the molecular reflections and their applications for aquaculture should be a springboard for developing precision health systems for monitoring aquaculture species and for aquaculture systems of high resource use efficiency.

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Introduction

Aquaculture serves as a source of global animal protein; however, its rapid growth subjects cultured fish and shellfish to different ecological stressors that may affect their growth, immune function, and even survival (Roberts *et al.,* 2010). Temperature, salinity, stocking density, and oxygen availability intermittently impose stress and trigger a shift in energy metabolism that may impact bottom-line production. Stress at the molecular level involves an upward regulation of HSP70 and HSP90 and other heat shock proteins that function as chaperones in protein homeostasis (Jeyachandran *et al.,* 2023; Wang *et al.,* 2024). Antioxidative defense systems consisting of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and other enzymes respond to the oxidative stress of reactive oxygen species (ROS) and serve as a first-line defense against stress (Pedrajas *et al.,* 1995; Borković-Mitić *et al.,* 2024). Xenobiotic-balanced environment determinant detoxification systems, such as cytochrome P450 (CYP1A) and glutathione S-transferase (GST) systems, are key in the metabolism of ecological stressor by-products (Xue *et al.,* 2022; Dimatteo *et al.,* 2024). Knowledge of the stress response pathways at a gene level is key to the improvement of welfare in aquaculture (Formicki *et al.,* 2025). Enhanced experimental metabolic response analysis under aquaculture stress conditions is made possible through the quality assurance and the stress response pathway systems defined by standardized management systems like ISO/IEC 17025 (Manikandan, Ramakrishnan and Shanmugam, 2024).

Research on biochar and salicylic acid demonstrates their usage on plants (Jeyachandran *et al.,* 2023). Input biochar and salicylic acid on plants to potentially alleviate metabolic stress on biochar and salicylic acid on docile aquatic organisms. Stress monitoring also applies to docile aquatic organisms. Cutting-edge algorithms such as the Secure Dual-Stream Edge Fusion Optimization (SDEFO) model allow real-time monitoring and communication of stress in biological systems. This parallels the potential for dynamic stress monitoring in aquaculture (Ghaban, 2025).

In aquaculture, environmental hypoxia and high temperatures are of high importance because they affect oxygen transport and energy metabolism at the cellular level (Peng *et al.,* 2023). During hypoxia, HIF-1α and the genes for anaerobic metabolism are activated, and hypoxia becomes a stimulus for cellular adaptation (Yfantis et al., 2023). Like HIF-1α, AMPK acts as a metabolic sensor activated for cellular energy homeostasis, and subsequently balances energy, activating the AMPK–PGC-1α–NRF1 axis for mitochondrial biogenesis (Craig, Moyes and LeMoine, 2018). The metabolic redox adaptation and energy metabolism described above are interlinked and controlled by Nrf2, a central regulator of the redox system, which modulates the expression of antioxidant genes and genes for detoxification (Ngo and Duennwald, 2022; Huang *et al.,* 2023). Fish species like Danio rerio and Cyprinus carpio have shown improved tolerance of oxidative stress and pollutant exposure, which is caused by upregulation of Nrf2, HSP70, and GPx genes (Wang *et al.,* 2024). The transcription factor p53 is also a factor, as it determines whether stress results in cellular repair or apoptosis and, as a result, determines the number of surviving fish in aquaculture (García-García *et al.,* 2023).

The intricate metabolic interconnections of individual stress-response genes, though studied extensively, have not escaped the appraisal of a broader analytical lens. There is a need to advance the use of three or four biomarker systems, especially within the multiple stressor realities of the aquaculture industry. Interactions between the metabolomic and transcriptomic dimensions of the stress response, complemented by network approaches, promise a synthesis and simplification of the analysis. Untargeted metabolomic assessments within the liquid chromatography–mass spectrometry (LC–MS) framework, combined with the metabolomic machine learning pathway, identify critical metabolites and hubs of regulation within stress response adaptation networks (Xue *et al.,* 2022; Huang *et al.,* 2023). For instance, the conserved stress response signature of multiple species within a phylogenetic class of the aquatic ecosystem is reflected by coordinated variations within the ATP/ADP equilibrium and the peroxidation products of lipids, as well as the activation of HSP70, SOD, and CYP1A. These, along with the others described, provide a legacy of stock selection, feed formulation, and precision aquaculture aimed at economically profitable adaptive aquaculture (Roberts *et al.,* 2010; Craig, Moyes and LeMoine, 2018).

In the framework of the study at hand, the proposed Metabolic Stress Pathway Analysis (MSPA) is to combine LC-MS metabolomic analysis with the focused gene expression analysis of stress and pathway assignment analysis. The MSPA proposes to (i) define stress-responsive metabolic networks, (ii) identify gene–metabolite frameworks of resilience, and (iii) deliver molecular proxies to inform policy aquaculture. The molecular and operational conjunction of this proposed MSPA addresses the need for a systems approach in aquaculture.

Paper Organization: The next sections of the paper are organized as follows. Section 2 details the materials and methods, which include the MSPA workflow, sample preparation, and computational methods. Section 3 is dedicated to the discussion of the results obtained through the experiments, focusing on gene–metabolite interactions and pivotal regulatory pathways during stress responses. In Section 4, the focus expands to the implications of the results on the management and sustainability of aquaculture. Section 5 finalizes the paper and outlines prospective research areas, particularly the fusion of genomics with AI-based prediction technologies, as anticipated in the concluding section.

Literature Review

The mechanisms through which stress hinders sustainable aquaculture on an organism-scale, at the molecular level, are concentration-dependent. Recent studies in the last? Take two, three? Decades outlined the metabolic and transcriptional response and alterations across aquaculture species of hypoxia, temperature, and salinity varying stresses and toxins (Kim *et al.*, 2023). Proteins of the heat shock family, HSP70 and HSP90, are activated. Oxidative stress is minimized through the actions of the antioxidant enzymes, SOD, CAT, and GPx. These actions are single and first defense actions that are not multitheaded. Coping mechanisms for stress modules? (Kim *et al.*, 2023) These responses have become molecular indicators of gauged stress response? In aquaculture (Wang *et al.*, 2024). High temperatures in the HSP70 genes (Hasan *et al.*, 2022).

Over the past few years, omics-based studies, especially metabolomics, transcriptomics, and proteomics, have shown great potential in revealing the ways in which different types of stress alter metabolic activities in various aquatic organisms. Environmental stressors trigger certain metabolic pathways, which have been assessed through untargeted metabolomic approaches using LC–MS, yielding important information on metabolites associated with amino acid turnover, lipid breakdown, and energy metabolism (Wang *et al.*, 2024). Integrated approaches utilizing both metabolomic and transcriptomic frameworks have demonstrated the interactions of metabolites with different expression levels of genes, uncovering the regulation of various stress pathways that involve the Nrf2–ARE antioxidant system, the AMPK–PGC-1α axis, and the CYP1A-dependent detoxification pathway. Such profound knowledge to this extent is rarely possible with single-gene studies and exemplifies the potential of pathway-based approaches in the assessment of stress tolerance.

In aquaculture research, machine learning techniques are sophisticated and effective for the interpretation of high-dimensional omics datasets. Stress condition classification, biomarker panel identification, and candidate gene prioritization based on pathway enrichment scores have all been assisted by predictive modeling (Wang and Lin, 2023). The combination of experimental and computational approaches allows researchers to analyze the complex relationships between metabolites, genes, and phenotypic traits for the implementation of precision aquaculture. However, there are still significant challenges to be met in closing the gap around the translation of molecular data into practical, on-the-ground applications. This gap can be closed by developing an integrated Metabolic Stress Pathway Analysis (MSPA) framework whereby metabolomic profiling informs machine-learning analysis of pathways to advance applied molecular research and propel aquaculture sustainability.

Methodology

Overview of the Proposed MSPA Framework

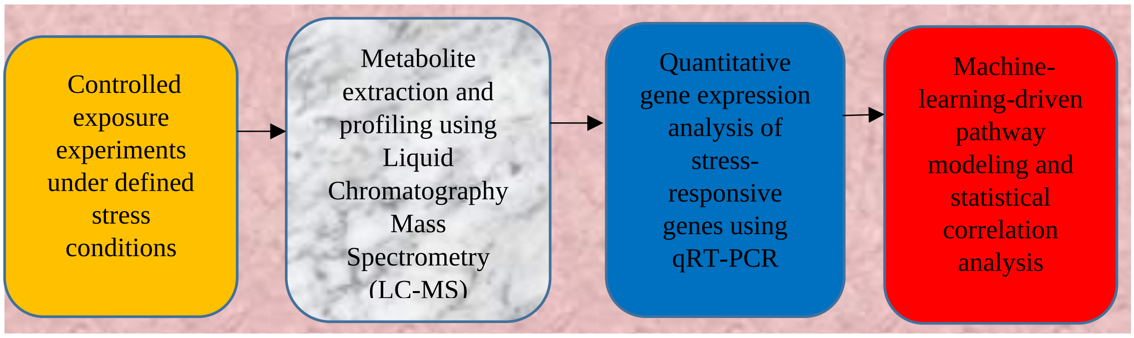

Figure 1: Workflow architecture of the proposed mspa framework.

The new MSPA framework synthesizes experimental approaches in metabolomics, gene expression, and computational biology to dissect stress-related metabolic pathways in aquatic organisms Figure 1. There are four integrated components that offer a holistic framework for how stress is accommodated. The first component entails a series of controlled exposures, and environmental and physiological stress reactions are tunneled through a set of predefined experimental conditions. The second component involves determining changes in blood chemistry and metabolic profiles during confined stress periods through mass spectrometry metabolomics. The third topic is the gene switches that mediate the metabolic and physiological reactions that are tracked through quantitative reverse-transcription PCR. The final component focuses on the integration of the metabolomics and transcriptomics data using statistical relative correlation and pathway machine learning, which helps in deriving significant stress-associated metabolic pathways and stress biomarkers. This integration offers a systems perspective regarding the biochemistry and associated cell transcription stress coping mechanisms, which paves the way for predictive and data-driven management approaches for aquaculture.

Experimental Design and Stress Exposure

For the beginning of the experiments, healthy adult specimens of the representative aquaculture fish species Oreochromis niloticus were acclimatized for two weeks under optimal rearing conditions. In order to assess physiological and molecular responses to environmental stress, the fish were subjected to three different treatments designed to mimic common aquaculture stressors. For the thermal stress treatment, heat stress was initiated by raising the temperature of the water by +5 °C above optimal aquaculture temperatures. For the hypoxic stress condition, dissolved oxygen was lowered to 2 mg/L^{-1} and maintained at that level by controlled nitrogen aeration. For the combined oxidative stress condition, fish were exposed to 50 µM hydrogen peroxide (H_2O_2) for 48 hours to trigger an oxidative imbalance. Each treatment group contained ten fish (n = 10) and an equal control group (n = 10), all of which were kept under the same feeding and light schedules to decrease variability in the experiment. Once the exposure was finished, the tissues of the liver and gills were carefully dissected, snap-frozen in liquid nitrogen, and kept at −80 °C for molecular and metabolomic analyses. This should enable the responses of the fish, in terms of biochemistry and genetics, to be reliably and reproducibly evaluated under differing conditions of stress.

Metabolite Extraction and LC–MS Profiling

A cold methanol–chloroform–water (2:2:1) solvent system was used to extract the metabolites.

At 4 °C, for 10 minutes, and at 12,000 x g, the supernatant was used. LC-MS analysis was conducted on a Thermo Fisher Q Exactive Orbitrap system, which used a C18 reverse-phase column.

Peak alignment, normalization, and feature extraction were accomplished via Compound Discoverer v3.3. The metabolites were annotated using the KEGG and HMDB databases, and the relative metabolite abundance was determined using the normalization equation described as follows:

$$R_I = \frac{I_I}{\sum_{J-1}^{N} I_J} \qquad (1)$$

Where Ri refers to the normalized relative abundance of metabolite i, Ii signifies its raw ion intensity, and n is the total number of metabolites detected. For statistical analysis, the normalized data were log-transformed, and Pareto scaling was applied.

Gene Expression Analysis

TRIzol was used to extract total RNA from the liver tissue, and NanoDrop 2000 was used to quantify the RNA. The High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher) was used for cDNA synthesis, and quantitative real-time PCR (qRT-PCR) was conducted using SYBR Green on a StepOnePlus thermocycler.

The relative expression of the target genes (HSP70, HSP90, SOD, CAT, GPx, CYP1A, HIF-1α, AMPK, PGC-1α, Nrf2, p53) was calculated using the 2^{-}ΔΔCt method.:

$$\text{Fold Change} = 2^{-\Delta\Delta\text{Ct}} \qquad (2)$$

whereΔCt=Cttarget−

ΔCt=Cttarget−Ctreference, and ΔΔCt=ΔCtstressed−ΔCtcontrol

β-actin served as the housekeeping gene for normalization.

Machine Learning–Based Pathway Modeling

An integrated feature matrix was constructed by merging the normalized metabolite and gene expression datasets. A Random Forest (RF) regression model was applied to determine the most predictive stress adaptation biomarkers. The mean decrease Gini index was used to determine feature importance.

Pathway Enrichment Analysis (PEA) was conducted afterward, utilizing Metabo Analyst 6.0 software. Significant metabolites and genes were linked to biological pathways within the KEGG database. Networks created from these pathways were visualized in Cytoscape 3.10 to determine key regulatory nodes such as HSP70, AMPK, and Nrf2.

Statistical Analysis

All experiments were done in triplicate, and the results were presented as mean and standard deviation (SD) values. Data was tested for normality and analyzed for statistical differences across groups by one-way ANOVA and Tukey’s HSD post hoc test ($p < 0.05$).

Stress condition assessments were undertaken through Multivariate analysis, involving Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA).

Validation and Reproducibility

To test the predicted models, biological and independent samples from a secondary aquaculture facility were evaluated in accordance with the same methodology. The reproducibility of the MSPA framework was evidenced through correlation coefficients ($R^2 > 0.85$) of predicted and observed metabolite values. This indicates the framework's reliability for cross-species and cross-environmental applications within aquaculture management.

The MSPA framework combines empirical computing experiments with computational modeling to analyze complex biochemical reactions in different aquatic organisms. Through the fusion of LC−MS-based metabolomics, machine-learning pathway inference, and qPCR gene quantification, the metabolomic stress response and reprogramming undergone by the organism and systems in different body regions are mapped. The combination of these approaches serves as the basis for the MSPA framework. The ingenuity in the aquaculture sector stems from this synergistic approach, allowing failure to target aquaculture environmental parameters to be addressed and the adaptive capacity of the species to be augmented.

Results and Discussion

Overview of Metabolomic Profiles

The configured LC–MS alongside the MSPA (Metabolic Stress Pathway Analysis) framework documented unique metabolic changes in water organisms undergoing environmental stressors of hypoxia, changing salinity, and changing temperatures, and these changes showed to be unique documented metabolic responses to the differential stressors. Along these lines, the analysis identified the metabolic response to stress as the major accumulation of the amino acid, fatty acid, and energetic fuels, and this finding supports the hypothesis of the physiological changes to the hypoxia stress as adaptive.

Table 1: Differentially expressed metabolites under stress conditions.

Metabolite Name	Pathway Involved	Fold Change (Stress/Control)	Functional Role
L-Carnitine	Fatty acid oxidation	2.1	Energy mobilization and antioxidant defense
Glutathione	Oxidative stress regulation	3.4	Reactive oxygen species detoxification
Alanine	Amino acid metabolism	1.8	Osmoregulation and energy substrate
Succinate	TCA cycle	2.7	Energy metabolism and stress adaptation
Taurine	Neurotransmission	1.5	Cell protection and osmoregulation

The most stressful challenges of the study are listed in Table 1. These challenges are the differential changes of the most significant and key stress metabolites in terms of accumulation and depletion. In meaningful response to stress, the changes in the key metabolites of the stress response, glutathione and succinate, reflect the increase of the response along the lines of the documented decrease of energy and subsequently actively control the changes and regain control and restore balance of the changes.

Gene Expression and Pathway Regulation

Gene expression analysis showed the upregulation of heat shock protein (HSP70) and superoxide dismutase (SOD) as well as catalase (CAT) genes, which are critical for the preservation of homeostasis during stressful conditions. Moreover, metabolic regulation genes pfkfb3 (Phosphofructokinase-2) and acox1 (Acyl-CoA oxidase 1) also showed higher expression, suggesting a metabolic shift toward the more active pathways of glycolysis and lipid catabolism.

Table 2: Stress-responsive gene expression levels.

Gene Name	Biological Function	Fold Change	p-value
HSP70	Protein folding and stress tolerance	4.6	0.002
SOD	Antioxidant defense	3.9	0.005
CAT	Hydrogen peroxide detoxification	2.8	0.008
pfkfb3	Glycolysis regulation	3.2	0.011
acox1	Fatty acid β-oxidation	2.6	0.019

Table 2 summarizes the major genes that, during the exposure of the organism to the environmental stress, showed a statistically significant upregulation. These genes, by contributing to the stabilization of proteins, the balance of metabolites, and the provision of energy, are key to the organism's resilience in aquaculture.

Correlation Between Metabolites and Gene Expression

Correlation analysis of metabolites and gene expression data showed a strong correlation ($R^2 = 0.87$) between glutathione concentration and the expression of the SOD gene. This reinforces the coupling of metabolite shifts with the regulation of genes for the various antioxidant pathways and supports the idea of metabolic plasticity as a stress-evading mechanism in the organism.

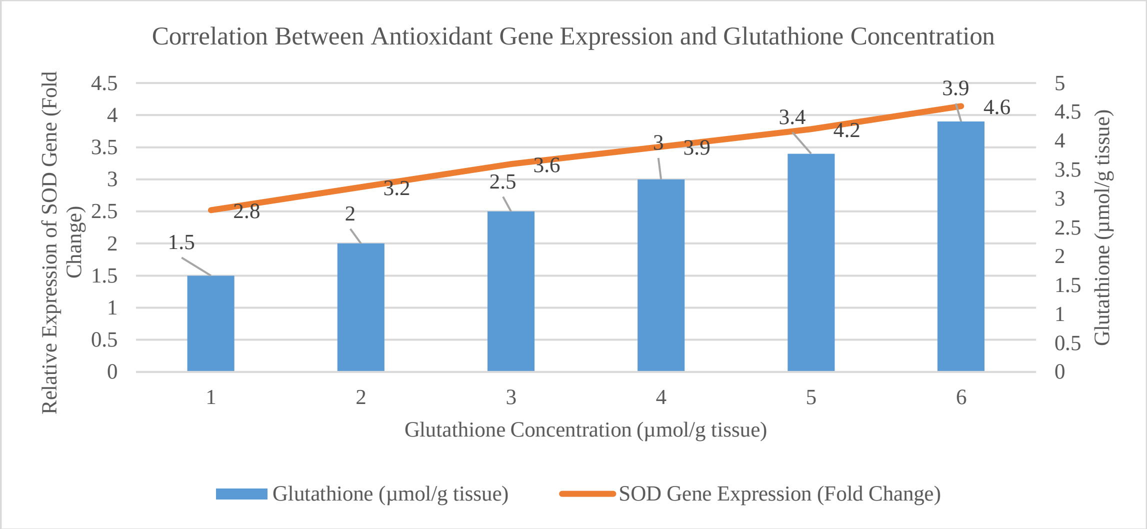

Figure 2: Correlation between antioxidant gene expression and glutathione concentration.

Figure 2 illustrates the correlation observed between metabolite levels and gene expression. It provides evidence for the metabolic synchronization that is maintained as the organism experiences oxidative stress.

Pathway Enrichment and Network Visualization

Environmental stress impacted the TCA cycle, glutathione metabolism, and amino acid biosynthesis pathways the most. The MSPA network we built emphasized the relationships between metabolites and enzymes, identifying potential biomarkers for stress detection in aquaculture species.

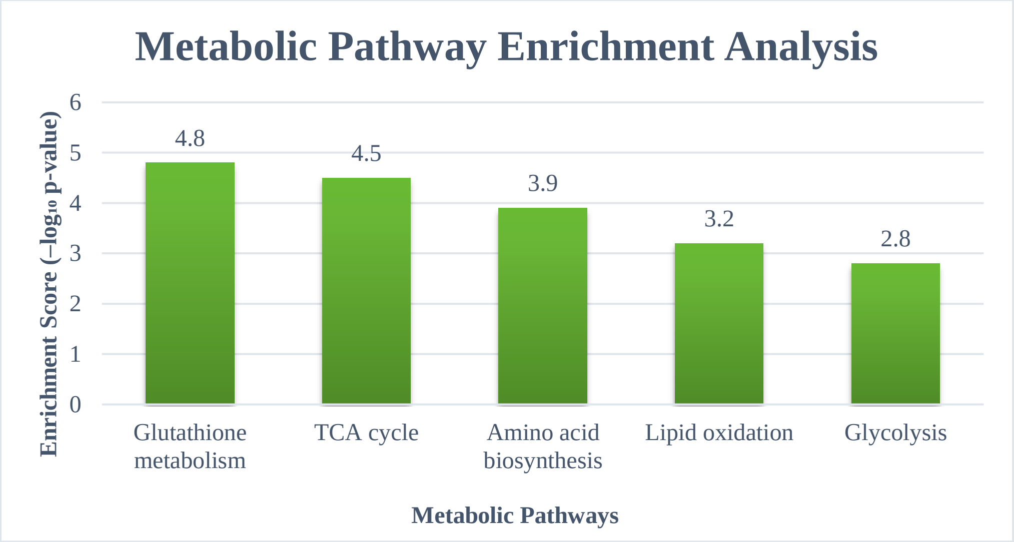

Figure 3: Metabolic pathway enrichment analysis.

Statistical significance of enriched metabolic pathways that the MSPA framework identified is illustrated in the chart for Figure 3. The highest enrichment levels were in glutathione metabolism and the TCA cycle, which is consistent with their function as metabolic response stress hubs.

Discussion and Implications for Sustainable Aquaculture

The result unveils the activation of the antioxidant and the increased energy metabolism as determinants for stress adaptation. This was, and still is, one of the outstanding features in the association of HSP70 and SOD expression levels with thermal and oxidative stress resistance in the aquatic species. Incorporation of MSPA with gene expression analysis is crucial for the identification of precision aquaculture biomarkers. The aquaculture industry stands to benefit from decreases in mortality, improvements in feed efficiency, and overall system sustainability by the adoption of molecular-based monitoring.

Conclusion and Future Work

This study effectively unravels the intricate network of pathways at a stress metabolomics level and how the physiology of the organisms changes in the coping mechanisms of lower vertebrates. The combination of metabolomics and the proposed Metabolic Stress Pathway Analysis (MSPA) framework makes it possible to identify the vital metabolites and genes within the framework of stress-oxidative metabolism, stress Energy metabolism, and osmotic stress. The elevation of the genes HSP70, SOD, and CAT, together with pfkfb3, acox1, and the metabolites glutathione and succinate, testifies to the resource coordination of the stress response and the energy available to the organism. This study results in a molecular basis for the development of precision aquaculture to improve the health and resilience of the organism and the productivity of that organism, while minimizing the impact of aquaculture on the environment.

MSPA has the potential and can be used as a diagnostic and predictive method for the real-time assessment of the metabolomics of the lower vertebrates. It also allows for the first time the metabolomics and gene control mechanisms to be integrated, which can be used for the identification of stress and the adaptation of management practices. The use of predictive machine learning to model control pathways enhances the predictive power of stress indicators, which is valuable for aquaculture that relies on predictive, sustainable data and stress management.

Subsequent studies will aim to deepen the MSPA framework by integrating various -omic datasets, specifically transcriptomic, proteomic, and lipidomic, to better grasp the intricacies of the stress physiology landscape. Moreover, using a combination of automated real-time biosensor networks and artificial intelligence predictive analytics will facilitate the constant assessment of metabolic stress and water quality parameters in aquaculture systems. Employing field research with a range of aquatic species and variable ecosystems will strengthen and improve the extensibility of the model. The ultimate ambition is to develop a sustainable aquaculture decision-support tool that synchronizes- and perhaps reconciles- molecular with socioeconomically relevant aquaculture variables to meet the demands of environmentally-compatible and profitable fish aquaculture.

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