

Unveiling Aquatic Organism Health Through eProteins: A Contemporary Perspective

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Abstract

Environmental DNA (eDNA) analysis has transformed our understanding of aquatic ecosystems, but traditional methods often lack insights into organismal health. The emerging field of environmental protein (eProtein) analysis offers a novel approach to monitoring the physiological status of aquatic organisms. Recent advancements in proteomic technologies have enabled the detection and characterization of stress-responsive proteins in water samples, providing valuable insights into organismal health and environmental stressors. This perspective paper explores the potential of eProtein analysis for monitoring aquatic organism health, disease dynamics and reproductive cycles. Methodological advancements in protein extraction and mass spectrometry have enhanced the sensitivity and specificity of eProtein analysis, facilitating comprehensive molecular profiling and biomarker identification. By integrating eProtein analysis into aquaculture management and environmental monitoring, researchers can proactively manage fish health, mitigate disease outbreaks and safeguard aquatic ecosystems. Future research directions include refining extraction methods, establishing standardized protocols and leveraging interdisciplinary collaborations to maximize the potential of eProtein analysis for aquatic research and conservation.

Keywords

protein, proteomics, environmental DNA, aquatic ecosystem

Overview and background

In recent years, advancements in environmental DNA (eDNA) analysis have revolutionised our ability to understand the distribution, abundance and genetic diversity of aquatic organisms (Thomsen and Willerslev 2015, Tsuji et al. 2019, Doi et al. 2021, Bonenfant et al. 2002, Banerjee et al. 2022). However, eDNA methods often fall short of providing insights into the physiological health and status of these organisms. In addition

to eDNA, environmental RNA (eRNA) acts as an intermediate between DNA and proteins, providing functional insights into gene expression and metabolic activity in nature (Tsuru et al. 2020, Hechler et al. 2023). Moreover, the detection and quantification of RNA can leverage quantitative PCR and sequencing technologies like eDNA analysis. While eRNA only provides the abundance of the genome, it does not directly estimate the protein in nature.

Addressing this gap, the emerging field of environmental protein (eProtein) analysis holds promise in elucidating the health, stress and reproductive status of aquatic organisms. This ideas paper aims to explore the potential of eProtein analysis as a novel approach for monitoring the physiological well-being of aquatic species, with implications for aquaculture, disease detection and ecosystem management.

Recent studies have underscored the potential of eProtein analysis in deciphering the physiological state of aquatic organisms. Wang et al. (2019) demonstrated the presence of stress-responsive proteins in aquatic environments, suggesting a link between environmental stressors and protein expression profiles. Brandão-Dias et al. (2021), Tank et al. (2010) and Brandão-Dias et al. 2021 extended these findings by successfully detecting genetically engineered crops in stream water samples through eProtein analysis, showcasing the versatility of this approach in environmental monitoring. Moreover, advancements in proteomic technologies have facilitated the identification and quantification of eProteins with unprecedented sensitivity and specificity, enabling researchers to unravel intricate molecular signatures indicative of organismal health and environmental stressors.

Methodological Advancements

To harness the potential of eProtein analysis, innovative extraction and analytical techniques have been developed. Building upon established methods, such as ammonium sulphate precipitation (Page and Thorpe 1996), researchers have refined extraction protocols to enhance protein recovery from water samples. The advancements in mass spectrometry have revolutionised the analytical landscape of eProtein analysis, enabling comprehensive molecular profiling and identification of stress-responsive biomarkers (Bonenfant et al. 2002). High-resolution mass spectrometry, coupled with advanced bioinformatics tools, allows for the identification of protein species and their post-translational modifications, providing valuable insights into organismal physiology and environmental health. With rapid development of proteomics techniques, the high-throughput proteomics allowing for the identification of molecular pathways and molecular interactions has been recognised as a perspective approach that enables analysis of the differential expression levels of proteins. High-resolution mass spectrometry and high-throughput proteomics can result in new paradigms in eProtein analysis.

Future direction

The integration of eProtein analysis into aquatic research holds immense promise for advancing our understanding of organismal health and ecosystem dynamics. By leveraging eProteins, researchers can monitor stress responses, track reproductive cycles and detect early signs of disease in aquatic populations. The non-invasive nature of eProtein analysis makes it particularly well-suited for large-scale environmental monitoring and aquaculture management. Moreover, the ability to detect subtle changes in protein expression patterns offers a window into the physiological adaptations of aquatic organisms to environmental stressors, providing crucial information for conservation efforts and ecosystem management strategies.

Furthermore, eProtein analysis has the potential to revolutionise aquaculture practices by enabling real-time monitoring of fish health and welfare. By analysing protein biomarkers associated with stress, disease and reproductive status, aquaculture operators can proactively manage their stock, mitigating the spread of diseases and optimising production efficiency. Additionally, eProtein analysis can aid in the development of targeted interventions, such as dietary supplements or environmental modifications, to enhance the resilience of farmed fish to environmental changes.

Combining the eProteins approach with environmental metabolomics can further broaden the scope and impact of aquatic research. For example, cyanotoxin-encoding genes can predict cyanotoxin production during harmful cyanobacterial blooms in a lakes (e.g. Duan et al. (2022)). Environmental metabolomics focuses on the comprehensive analysis of small molecules (metabolites) within an ecosystem, providing a snapshot of metabolic processes and environmental interactions. By integrating eProtein analysis with environmental metabolomics, we can achieve a more holistic understanding of aquatic health and ecosystem dynamics. This combined approach can reveal intricate relationships between protein expression and metabolic pathways, shedding light on how organisms respond to environmental stressors at both the molecular and systemic levels.

Conclusion

In conclusion, eProtein analysis represents a promising avenue for elucidating the physiological health and status of aquatic organisms. By harnessing the power of proteomics, researchers can unlock valuable insights into stress responses, disease dynamics and reproductive cycles in aquatic ecosystems. As we continue to refine extraction techniques and expand our analytical capabilities, eProtein analysis holds the potential to revolutionise aquatic research and management practices in the years to come. Through collaborative efforts and interdisciplinary approaches, we can harness the full potential of eProtein analysis to safeguard the health and resilience of aquatic ecosystems for future generations.

Author contributions

HD and TS contributed equally to this idea and HD wrote the ideas paper with reviewing by TS.

Conflicts of interest

The authors have declared that no competing interests exist.

References

- Banerjee P, Stewart KA, Dey G, Antognazza CM, Sharma RK, Maity JP, Chen CY (2022) Environmental DNA analysis as an emerging non-destructive method for plant biodiversity monitoring: a review. *AoB Plants* 14 (4): 031.
- Bonenfant D, Mini T, Jenö P (2002) Mass spectrometric analysis of protein phosphorylation *The Protein Protocols Handbook*. In: JM W (Ed.) *The protein protocols handbook*. Humana Press
- Brandão-Dias PFP, Rosi EJ, Shogren AJ, Tank JL, Fischer DT, Egan SP (2021) Fate of Environmental Proteins (eProteins) from Genetically Engineered Crops in Streams is Controlled by Water pH and Ecosystem Metabolism. *Environmental Science & Technology* 55, 8: 4688-4697. <https://doi.org/10.1021/acs.est.0c05731>
- Doi H, Minamoto T, Takahara T, Tsuji S, Uchii K, Yamamoto S, Katano I, Yamanaka H (2021) Compilation of real-time PCR conditions toward the standardization of environmental DNA methods. *Ecological Research* 36 (3): 379-388. <https://doi.org/10.1111/1440-1703.12217>
- Duan X, Zhang C, Struewing I, Li X, Allen J, Lu J (2022) Cyanotoxin-encoding genes as powerful predictors of cyanotoxin production during harmful cyanobacterial blooms in an inland freshwater lake: Evaluating a novel early-warning system. *Science of The Total Environment* 830 <https://doi.org/10.1016/j.scitotenv.2022.154568>
- Hechler R, Yates M, Chain FJ, Cristescu M (2023) Environmental transcriptomics under heat stress: Can environmental <scp>RNA</scp> reveal changes in gene expression of aquatic organisms? *Molecular Ecology* <https://doi.org/10.1111/mec.17152>
- Page M, Thorpe R (1996) Purification of IgG by precipitation with sodium sulfate or ammonium sulfate *The protein protocols handbook*. In: Walker J (Ed.) *The protein protocols handbook*. Humana Press https://doi.org/10.1007/978-1-60327-259-9_125
- Tank J, Rosi-Marshall E, Royer T, Whiles M, Griffiths N, Frauendorf T, Treering D (2010) Occurrence of maize detritus and a transgenic insecticidal protein (Cry1Ab) within the stream network of an agricultural landscape. *Proceedings of the National Academy of Sciences* 107 (41): 17645-17650. <https://doi.org/10.1073/pnas.1006925107>
- Thomsen PF, Willerslev E (2015) Environmental DNA-An emerging tool in conservation for monitoring past and present biodiversity. *Biological conservation* 183: 4-18. <https://doi.org/10.1016/j.biocon.2014.11.019>

- Tsuji S, Takahara T, Doi H, Shibata N, Yamanaka H (2019) The detection of aquatic macroorganisms using environmental DNA analysis-A review of methods for collection, extraction, and detection. *Environmental DNA* 1 (2): 99-108. <https://doi.org/10.1002/edn3.21>
- Tsuru K, Ikeda S, Hirohara T, Shimada Y, Minamoto T, Yamanaka H (2020) Messenger RNA typing of environmental RNA (eRNA): A case study on zebrafish tank water with perspectives for the future development of eRNA analysis on aquatic vertebrates. *Environmental DNA* 3 (1): 14-21. <https://doi.org/10.1002/edn3.169>
- Wang P, Yan Z, Yang S, Wang S, Zheng X, Fan J, Zhang T (2019) Environmental DNA: an emerging tool in ecological assessment. *Bulletin of environmental contamination and toxicology* 103, 5: 651-656. <https://doi.org/10.1007/s00128-019-02720-z>