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Review

Mass Spectrometry-Based Proteomics of Algal Toxins: A Scoping Review of Methodological Trends, Reporting Standards, and Analysis of Comparative Protein Responses

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Abstract

Algal toxins pose critical threats to aquatic ecosystems and public health. Mass spectrometry-based proteomics has become an increasingly important tool in ecotoxicology, enabling researchers to characterize molecular and cellular responses to a variety of environmental stressors, including chemical pollutants and naturally occurring toxins. Among these, algal toxins have emerged as a key area of application, with proteomics offering valuable insights into organismal responses, toxicity mechanisms and biomarker discovery. A systematic literature search of SciFinder, Scopus, and Web of Science identified 76 eligible mass spectrometry-based proteomic studies published from 2015 through May 2026. This review critically examines the current state of mass spectrometry-based proteomics, focused on key algal toxins including microcystins, anatoxins, BMAA, brevetoxins, saxitoxins, domoic acid, cylindrospermopsin, and okadaic acid/*Dinophysis* toxins, highlighting methodological trends, data transparency, adherence to reporting standards, and emerging themes in protein and pathway-level responses. We assess reporting quality and transparency using the Minimum Information About a Proteomics Experiment (MIAPE) guidelines and the Criteria for Reporting and Evaluating Ecotoxicity Data (CRED) framework. Additionally, a comparative protein and pathway analysis was conducted, identifying recurring functional responses across toxin classes while highlighting toxin-specific signatures at both the protein and biological process levels. Because the proteomics of microcystins has been comprehensively reviewed elsewhere, microcystin studies were included only in the methodological assessment and were excluded from the protein and pathway analysis. This review concludes with recommendations to improve reproducibility, promote data sharing, and guide the next phase of proteomic applications in algal toxin research.

Keywords: algal toxins; proteomics; mass spectrometry; ecotoxicology; biomarkers

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Academic Editor: Marco Pelin

Received: 28 August 2026
Revised: 9 November 2026
Accepted: 13 November 2026
Published: 17 November 2026

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1. Introduction

The significance of microalgae as primary producers in aquatic ecosystems cannot be overstated. However, some marine algae and cyanobacteria produce toxic secondary metabolites that can pose serious risks to human health, wildlife, domestic animals, and local economies [1,2]. These toxins may contaminate water, forage items, prey, or food such as fish and shellfish. High toxin levels are often linked to harmful algal blooms (HABs): a rapid proliferation of one or a few species of microalgae, cyanobacteria, or even macroalgae

that causes negative impacts on aquatic ecosystems [3], public health, or economic activity (e.g., fisheries and tourism). However, a visible bloom is not required for toxin-related hazards. Algal toxins can persist in the environment after a bloom subsides, be released through cell lysis, accumulate in biota, and transfer through food webs, resulting in toxic exposure even when bloom conditions are not present [4].

1.1. Geographic, Chemical and Biological Diversity of Key Algal Toxins

Algal toxins are produced by a wide array of marine and freshwater microalgae and cyanobacteria, and they have been reported from every continent, including Antarctica [5,6], reflecting their broad geographic distribution. These toxins occur in both temperate and tropical waters, inland lakes and rivers, coastal zones, and open ocean ecosystems. The production of algal toxins is influenced by complex environmental factors such as nutrient availability, temperature, salinity, and light, making their occurrence widespread and variable across ecosystems [7].

Structurally, algal toxins are highly diverse, spanning multiple chemical classes. These include cyclic peptides such as microcystins (MC); alkaloids such as saxitoxin (STX) and the related gonyatoxins (GTX), collectively known as paralytic shellfish poisons (PSPs), and cylindrospermopsin (CYN); amino acid derived toxins such as β-*N*-methylamino-L-alanine (BMAA), and domoic acid (DA); and polyethers including brevetoxins (PbTX), ciguatoxins (CTX), okadaic acid (OA), and the related *Dinophysis* toxins (DTXs) collectively referred to as the diarrhetic shellfish toxins (DSTs) [8]. Cyclic peptides and alkaloids tend to be water soluble while the polyethers are highly lipophilic. This diversity of structure and physical properties contributes to their broad range of biological targets and toxic effects. Algal toxins affect numerous molecular pathways and organ systems. They act as neurotoxins, hepatotoxins, nephrotoxins, and gastrointestinal irritants, among others. Mechanisms of action include inhibition of protein phosphatases, modulation of ion channels, disruption of redox homeostasis, and protein synthesis inhibition. These varied chemical structures and modes of action not only pose challenges for detection and management [9] but also offer insights into fundamental biological processes and potential biomedical applications [10].

1.2. Proteomics in Environmental Toxicology and Ecotoxicology

Environmental toxicology is the study of the effects of environmental contaminants on human health, whereas ecotoxicology examines the effects of environmental contaminants on individual organisms, populations, communities, and ecosystems. Elucidating the molecular pathways affected by toxicant exposure is a major objective in both fields which increasingly rely on omics technologies [11,12]. Transcriptomics was among the first high-throughput approaches to be widely adopted because it enabled rapid and sensitive characterization of changes in gene expression [11]. However, transcript abundance is often a poor predictor of protein abundance, activity, or post-translational regulation. Proteomics provides a complementary perspective by directly measuring the molecules responsible for cellular function and phenotype [13]. Consequently, proteomic studies can improve mechanistic understanding of toxicant-induced responses, identify affected biological pathways, elucidate changes in cellular signaling and regulatory networks, and support the development of biomarkers of exposure and effect [14].

1.3. Methodological Approaches in Proteomics

Approaches to proteomics can be broadly divided into bottom-up or top-down strategies. Top-down proteomics involves the mass spectrometric (MS) analysis of full-length proteins, whereas the inputs for bottom-up proteomics are enzymatically digested peptides, which are analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS)

and mapped onto full-length proteins from a user-specified database [15]. Shotgun proteomics is a discovery-based approach and a particular case of bottom-up proteomics that aims to identify and quantify as many proteins as possible in a sample in an unbiased manner. It is used to get a broad overview of protein composition and to identify differentially abundant proteins (DAPs) between two conditions. On the other hand, targeted proteomics is a complementary, hypothesis-driven approach that focuses on quantifying specific proteins of interest by incorporating selected reaction monitoring (SRM), multiple reaction monitoring (MRM), or parallel reaction monitoring (PRM) in the MS analysis [16]. These methods involve monitoring pre-selected precursor and fragment ion pairs for targeted quantification. Targeted proteomics strategies limit the number of features monitored to optimize sensitivity and precision for validation and high throughput analysis.

MS-based proteomics are typically combined with separation techniques prior to MS analysis: either 2D gel electrophoresis or liquid chromatography (LC). Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) was instrumental in the early development of proteomics in the 1980s [17]. Orthogonal separation methods are applied wherein proteins are separated in the first dimension by isoelectric point (pI) followed by a second dimension of separation by molecular weight. After visualization, spots of interest are excised from the gel, then analyzed directly (top-down) or enzymatically digested (bottom-up) followed by MS/MS analysis for identification. While 2D gel electrophoresis was foundational in the early development of proteomics and has become a standard approach, it tends to be labor intensive and not well suited for high-throughput analysis. Reverse phase LC-MS/MS has largely supplanted traditional gel-based methods for protein/peptide separation and identification, marking a clear shift in preferred platforms.

Proteomic investigations rely on various MS data acquisition strategies, the choice of which depends on the experimental goals, sample complexity, and desired depth of analysis. The most used strategies include Data-Dependent Acquisition (DDA), Data-Independent Acquisition (DIA), and targeted approaches such as SRM, MRM and PRM. In DDA, precursor ions are first detected in a full MS scan, after which a user specified number of the most intense ions are selected for fragmentation (MS/MS). This widely adopted strategy is most suitable for discovery-based proteomics. However, DDA suffers from stochastic sampling which can limit reproducibility and introduce missing data across replicates. Perhaps most importantly, significantly altered low-abundance proteins can be overlooked.

In the DIA approach, precursor ions within defined mass-to-charge (m/z) isolation windows are fragmented systematically, regardless of their intensity, rather than selecting individual precursors for fragmentation as in DDA. Although precursor ions can be detected in MS1 scans, the resulting complex MS2 spectra contain fragment ions from multiple co-isolated precursors, and quantification commonly relies on fragment-ion intensities. DIA offers greater reproducibility, quantitative accuracy, and data completeness across replicates. However, it is also computationally intensive, and data analysis has historically relied on high-quality spectral libraries, often generated through DDA and specific to instruments and sample types [18]. More recently, computational approaches have enabled library-free and hybrid analysis strategies, reducing dependence on previously generated spectral libraries [18,19].

Quantification of proteomic data may be performed through label free (LFQ) or labeled approaches. When proteins are first separated by 2D-gel, quantification is typically performed by densitometric analysis of stained protein spots. Alternatively, proteins from different experimental conditions may be labeled with spectrally distinct fluorescent cyanine (Cy2, Cy3, and Cy5) dyes and co-resolved on the same two-dimensional gel using Difference In-Gel Electrophoresis (2D-DIGE), allowing accurate relative quantification by fluorescence

imaging while minimizing gel-to-gel variation. In DDA, the peptide precursor signal is used for peptide quantification. In DIA, the intensities of specific fragment ions are used to quantify a peptide. Stable Isotope Labeling by Amino acids in Cell culture (SILAC) is a metabolic labeling technique used in quantitative proteomics. In SILAC, cells are cultured in media containing either "light" (normal) or "heavy" (isotope-labeled) amino acids (usually lysine or arginine) [20]. These labeled amino acids are incorporated into all newly synthesized proteins as cells grow. As peptides from different samples will have unique labels, samples may be combined and analyzed in a single LC-MS/MS run. Alternatively, peptides may be labeled with isobaric tags that are unique to a sample or treatment [21]. The tags are cleaved during collision induced dissociation (CID) and reporter ion intensities detected in the low mass range, serve as a proxy for peptide intensity. This approach allows for multiplexing of samples (up to sixteen for TMT-pro) as each isobaric tag produces a unique reporter. However, this approach is subject to error due to ratio compression: true fold change is artificially compressed due to co-fragmentation of non-target precursors. Isobaric labeling approaches are generally not compatible with DIA as all precursor ions are fragmented simultaneously making it impossible to assign a reporter to a specific ion.

Following data acquisition, MS/MS spectra are processed through bioinformatic workflows that typically include database searching for peptide and protein identification, quantitative analysis, statistical evaluation of differential abundance, and biological interpretation through visualization and pathway analysis. Because analytical choices made during these stages can substantially influence protein identification and quantification, transparent reporting of data processing parameters is essential for reproducibility and interpretation of proteomic datasets. Consequently, reporting guidelines have been proposed to improve transparency and facilitate cross-study comparisons.

1.4. Reporting Standards in Proteomics and Ecotoxicology MIAPE and CRED

The Human Proteome Organization created the Proteomics Standards Initiative (HUPO-PSI) in 2005 to establish common guidelines for reporting proteomics data. The guidelines, entitled Minimum Information about a Proteomics Experiment (MIAPE), were first described in 2007 [22]. These guidelines provide a framework for assessing reporting quality, reliability, results and significance of proteomic studies, and facilitate replication and validation of findings. Similarly, the Criteria for Reporting and Evaluating Ecotoxicity Data (CRED) project, developed by a collaboration of international researchers and regulatory bodies, is an evaluation framework designed to improve transparency, reproducibility, and consistency of ecotoxicity studies used in environmental risk assessments [23]. The CRED project has established 50 data reporting recommendations and shares significant overlap with MIAPE regarding experimental design.

Given the heterogeneity of toxin classes, test organisms, experimental designs, and proteomic methodologies represented in this literature, a scoping review approach was used to map the available evidence and characterize the current state of algal toxin proteomics. Published studies were evaluated using selected MIAPE and CRED reporting criteria to assess transparency and reproducibility, while reported DAPs were compiled and compared across toxin classes. The objectives of this scoping review were to (1) characterize methodological trends and reporting practices in algal toxin proteomics; (2) identify recurring protein responses across studies and toxin classes; and (3) distinguish conserved biological processes from toxin-specific proteomic responses.

2. Results

2.1. Overview of Subjects, Objectives and Methods

During the approximate 11-year period from 2015 through the first half of 2026, 76 studies were published fitting criteria described for our literature search [24–99]. Details of these studies are available in Supplementary File S1 and are summarized in Figure 1. Microcystin topped the list by far with more than half (39) of the studies focused on this cyclic peptide either alone (34) or in combination with other algal toxins (5). This preponderance of studies is likely owing to its widespread occurrence and significant health risk as a hepatotoxic protein phosphatase inhibitor. DSTs and BMAA tied for second place, each with nine studies. A wide variety of test species were used for these studies: sixteen using fish or fish larvae (including 8 on zebrafish), twenty-two studies using mammals (mice, rats, manatees and sea lions), twenty using mammalian cell lines, and 8 studies on bivalves. Other test organisms included cyanobacteria, fungi (yeast), invertebrates, sea turtles, earthworms and lettuce. Seven studies were opportunistic involving test subjects that were caught in the wild after exposure to algal toxins under natural conditions. These included Florida manatees and sea turtles exposed to brevetoxins and California sea lions exposed to domoic acid.

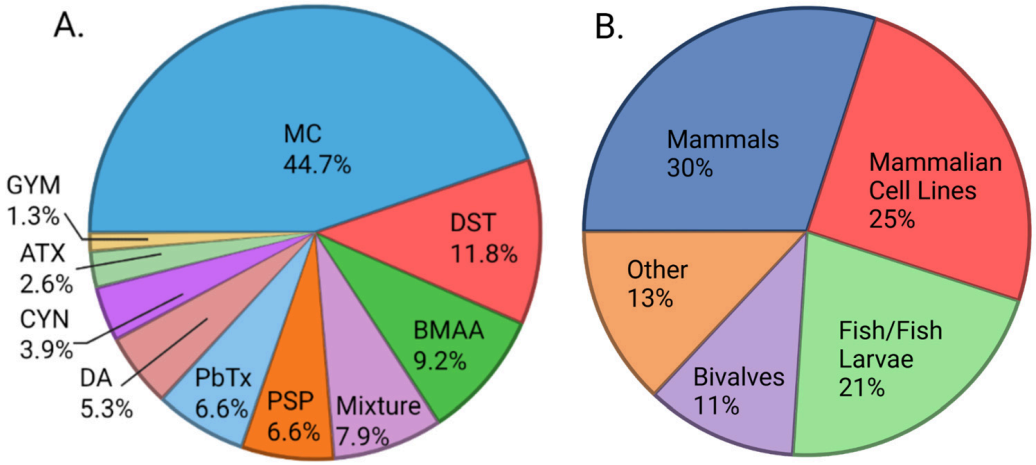


Figure 1. Distribution of (**A**) algal toxin classes and (**B**) test organisms among the proteomic datasets included in this review.

The most frequently stated rationale for conducting these studies was to clarify the mechanism of toxicity (67 studies, 88%). However, the specific aims within this category varied considerably, ranging from investigating sex-based differences, gestational and developmental toxicity, species-specific responses, and the effects of acute versus chronic sub-lethal exposures, to exploring adaptive mechanisms, environmental influences such as temperature, immune and defense responses, and organ-specific effects. The second most common rationale was biomarker discovery (10 studies, 13%), aiming to identify protein signatures indicating exposure or effect. Six studies addressed both mechanism of toxicity and biomarker discovery. Five studies (7%) focused on other research questions, including ecotoxicology, chemical ecology, physiology, and xenobiotic metabolism. Lastly, one study evaluated a potential therapeutic intervention using a cell culture model. Because individual studies could address multiple research objectives, these categories were not mutually exclusive. The research rationale assigned to each study is provided in Supplementary File S1.

During the decade covered by this review, not a single top-down approach was reported: every study included in this review applied a bottom-up approach, and only two targeted studies were described. Nearly one third (28%) of studies (21) used a 1D or 2D gel approach for protein separation and visualization. One study applied a 1D SDS-PAGE separation followed by LC-MS, and 20 studies applied a 2D SDS-PAGE separation followed by analysis of excised proteins. While the shift from gel-based to LC-based separations was observed in our dataset, it was certainly not linear with fluctuating adoption rates observed over the decade covered in this review. Nonetheless, during the first year covered by this period (2015) seven out of ten studies applied gel-based separations. However, no gel-based separations were reported after 2023.

Gel-based studies quantified proteins by densitometry or DIGE. For LC-based separations, LFQ and labeled quantifications were roughly equal (28 vs. 26 respectively). Data-independent acquisition (DIA) has been applied only in a single study to date [24], yet its potential for improving reproducibility and depth of coverage is noteworthy and warrants broader uptake. Finally, the targeted studies applied SRM or PRM to monitor specific proteins [58].

2.2. Adherence to Reporting Criteria MIAPE and CRED

To evaluate the transparency and reproducibility of proteomics studies involving algal toxins, we systematically assessed 76 publications using key parameters drawn from MIAPE [22] and CRED [23] guidelines. The MIAPE experimental design checklist shows significant overlap with the CRED guidelines to include: test species, age, number of pooled organisms, and number of biological replicates. Our assessment focused on experimental design, sample preparation, mass spectrometry settings, and bioinformatics reporting. The results of this assessment are summarized in Table 1 with details described in Supplementary File S2.

Table 1. Select reporting parameters for all 76 studies analyzed in this review with the percentage of studies identified that reported these parameters ($n = 76$ unless otherwise indicated). Minimum Information about a Proteomics Experiment (MIAPE) represents relevant reporting guidelines for proteomics studies and Criteria for Reporting and Evaluating Ecotoxicity Data (CRED) represents relevant reporting guidelines for toxicology studies.

	Select Reporting Parameters	**Reported in Literature (%)**	**Parameters Included in Reporting Guidelines**	
			MIAPE	**CRED**
Experimental Design	Test compound	100	✗	✓
	Concentration verification ($n = 69$)	20	✗	✓
	Use of testing guidelines	4	✗	✓
	Test organism species	100	✓	✓
	Test organism age ($n = 53$)	74	✓	✓
	Test organism sex ($n = 48$)	73	✓	✓
	Test organism tissue type	100	✗	✓
	Number of pooled organisms	21	✓	✓
	Biological replicates	97	✓	✓
	Technical replicates	46	✓	✗
	Exposure method	100	✗	✓

Table 1. *Cont.*

	Select Reporting Parameters	Reported in Literature (%)	Parameters Included in Reporting Guidelines	
			MIAPE	CRED
Sample Preparation	Lysis buffer	91	✓	✗
	Lysis methods	91	✓	✗
	Reducing agent	84	✓	✗
	Alkylating agent	78	✓	✗
	Digestion agent	92	✓	✗
	QC protein quantification	80	✓	✗
Mass Spectrometry	MS instrument type	91	✓	✗
	Chromatography gradient ($n = 58$)	79	✓	✗
	Gel conditions ($n = 21$)	95	✓	✗
	Mass of sample analyzed	13	✓	✗
	MS quality control samples	21	✓	✗
	Image analysis ($n = 20$)	100	✓	✗
Bioinformatics	Software (version and algorithm)	62	✓	✗
	Database	86	✓	✗
	Peptide mass tolerance	54	✓	✗
	Fragment mass tolerance	54	✓	✗
	Fixed modifications	55	✓	✗
	Variable modifications	58	✓	✗
	Missed cleavages	53	✓	✗
	Statistical testing	80	✓	✓
	False discovery rate (FDR)	51	✓	✗
	Raw data publicly available	25	✓	✓

2.2.1. Experimental Design

All studies reported the test compound, test organism species and tissue type. Some studies lacked complete information on important variables such as age and sex of the organism. Of the 53 studies in which age was considered applicable, 39 (74%) reported this information, while 35 of 48 studies (73%) reported sex. These parameters were not applicable in some studies, particularly those involving cell lines, microorganisms, or organisms for which age or sex could not be reliably determined. No studies involving bivalves reported sex, which may reflect biological complexity, as some bivalve species are hermaphroditic. Reporting the number of pooled organisms was relatively infrequent (21%). However, many studies analyzed tissues or samples from individual organisms rather than pooled specimens, making this parameter inapplicable in those cases.

Although only 4% of studies explicitly reported the use of standardized testing guidelines (e.g., OECD, ISO, or USEPA), this finding should be interpreted cautiously. CRED recognizes that adherence to guideline methods is not, by itself, an indicator of study reliability, and well-designed non-guideline studies may provide equally robust data when experimental procedures are adequately described. Because many proteomic investigations

are hypothesis-driven mechanistic studies rather than regulatory toxicity assessments, the limited use of standardized testing guidelines is not unexpected.

While biological replicates were frequently included (97%), the use of technical replicates was absent or unreported in 54% of studies. While replicate injections can provide information on analytical reproducibility, advances in LC-MS instrumentation have substantially improved analytical precision [100]. Consequently, many contemporary proteomic studies prioritize increasing biological replication over performing technical replicates. Concentration verification, a core element of the CRED guidelines, was reported in only 20% of applicable studies. This relatively low percentage may reflect the widespread use of commercially sourced toxins at nominal concentrations, for which investigators may consider additional analytical verification unnecessary, rather than a lack of attention to experimental rigor. Seven studies were opportunistic, collecting samples from wild organisms with confirmed or suspected algal toxin exposure. For these studies, precise exposure concentrations and durations could not be verified. Therefore, concentration verification was not applicable. However, some of these studies confirmed toxin exposure analytically [35,36,38]. In several experimental studies, organisms were exposed to toxin-producing algal cultures rather than purified toxins [39,42,50–52,59,73,78,83,88,94,96]. Although these exposures included additional algal components, the concentration of the target algal toxin was analytically verified, and these studies were therefore considered to have met the concentration verification criterion. Exposure methods varied widely, including waterborne, injection, and dietary exposure routes, and were consistently reported, yet justification for the chosen model or exposure strategy was rarely provided.

2.2.2. Mass Spectrometry

In terms of instrumentation, studies employed a broad range of MS platforms, from MALDI-TOF, MALDI-TOF/TOF to Orbitrap-based systems. Nearly all studies (91%) reported the type of instrument used. Reporting of separation methods was also strong. All studies described the separation approach used. Among the 58 LC-based studies, chromatographic gradient conditions were reported in 46 (79%). Twenty of the 21 studies that used gel-based separation reported gel conditions, and all 20 studies that quantified proteins from gels reported their image analysis procedures. Three studies combined gel-based and LC-based separation and are counted in both groups. Details on MS quality control samples, which are important for documenting analytical reproducibility, were reported in only 21% of studies. This likely reflects underreporting rather than an absence of quality control, as routine instrument performance monitoring and system suitability testing are standard practice in many proteomics core facilities but are often omitted from published methods. Nevertheless, documenting these procedures would improve transparency and allow readers to better assess analytical reliability. Similarly, the mass of protein or peptide analyzed was reported in only 13% of studies. Although the amount analyzed is necessarily known during experimental setup and sample loading, it is frequently omitted from published methods, limiting reproducibility and comparisons among studies.

2.2.3. Sample Preparation

Sample preparation protocols demonstrated considerable variability across studies. Lysis buffer composition and methods were among the consistently reported parameters, both documented in 91% of studies. The digestion enzyme was reported in 92% of studies, with trypsin being the predominant choice. Protein quantification methods such as Bradford or BCA assays were noted in 80% of studies but occasionally marked as unknown or omitted entirely. Reducing and alkylating agents were reported less consistently at 84% and 78% respectively. The lack of standardization in this domain poses a barrier to reproducibility.

2.2.4. Bioinformatics

Bioinformatics information was variable, with some parameters consistently documented and others frequently omitted. The software used for protein identification was reported in 92% of studies (Figure 2). However, MIAPE requires both the version number and the database search algorithm used, and only 62% of studies met this requirement. The most frequently used software were Mascot (25), Proteome Discoverer (22), MaxQuant (10), and ProteinPilot (5). The protein sequence database searched was reported in 86% of studies. Statistical testing methods were reported in 80% of studies. Six studies used custom or organism-specific databases to improve identification rates in non-model organisms [42,51,81,96]. Peptide mass tolerance was reported in 54% of studies, fragment mass tolerance in 54%, fixed modifications in 55%, variable modifications in 58%, and allowed missed cleavages in 53%. False discovery rate (FDR) thresholds, which are critical for controlling the rate of false protein identifications, were reported in only 51% of studies. Together, these represented some of the least consistently reported analytical parameters. Perhaps most concerning, raw mass spectrometry data were deposited in *public* repositories in only 25% of studies, substantially limiting data transparency, independent validation, and opportunities for reanalysis as bioinformatic tools continue to evolve.

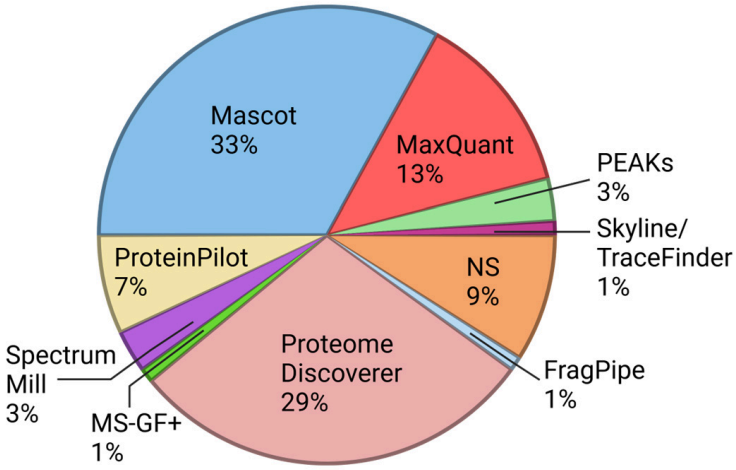


Figure 2. Distribution of protein identification software used across the 76 proteomic studies included in this review.

2.3. Recurring Proteins and Pathways in Algal Toxin Proteomic Studies

To place algal toxin proteomic responses within the broader context of cellular stress biology, the algal toxin dataset was compared with proteins previously identified as recurrently altered in proteomic studies of stress across diverse taxa, including *Homo sapiens*, *Mus musculus*, *Drosophila melanogaster*, *Caenorhabditis elegans* [101,102], and *Danio rerio* [103]. Following standardization of protein names and consolidation of homologs and isoforms, a reference dataset containing 58 commonly recurring proteins and protein families was assembled from previous reviews.

Substantial overlap was observed between the algal toxin dataset and the previously reported stress-responsive proteins (Figure 3). Among the most frequently recurring protein families identified across the 38 algal toxin proteomic datasets were ribosomal proteins (62 occurrences), protein folding chaperones, including heat shock proteins (24), protein disulfide isomerases (12) and peptidyl-prolyl cis-trans isomerases (9), actin/actin related proteins (24), myosin (14), annexins (12), apolipoproteins (11), cathepsins (11), glutathione S-transferases (9), tubulin (9), Fibrinogen (8), ATP synthase complex proteins

(7), fructose-bisphosphate aldolase (8), glyceraldehyde-3-phosphate dehydrogenase (7), and eukaryotic translation initiation factors (9).

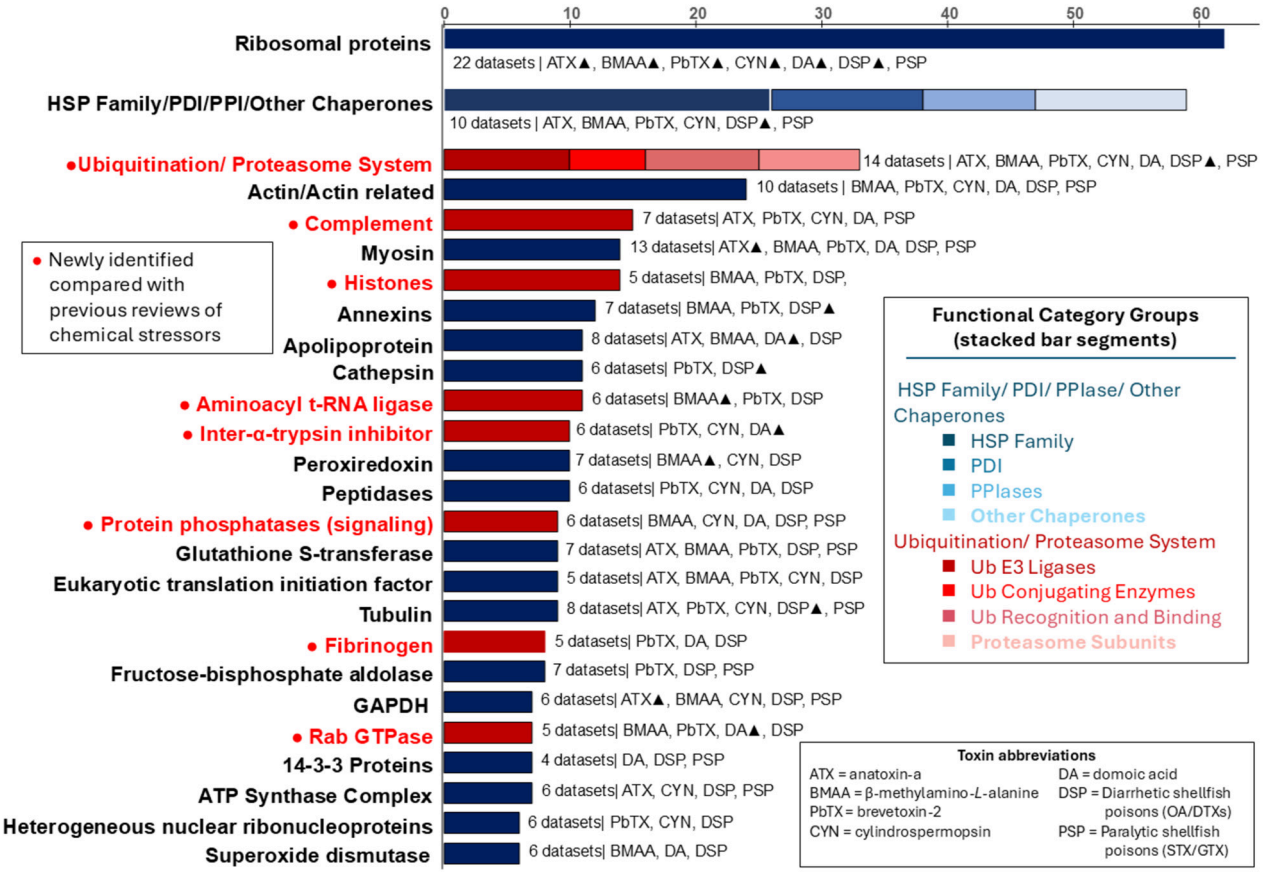


Figure 3. Most frequently recurring standardized protein families represented among the DAPs identified across 38 algal toxin proteomic datasets. Bar length represents the total number of occurrences. The number to the right or below the vertical bar indicates the number of independent studies reporting the protein family. Toxin abbreviations indicate the toxin classes in which the protein family was identified, and ▲ denotes occurrence in ≥50% of studies for that toxin class. Blue bars represent protein families previously identified in general stress proteomic reviews [101–103], whereas red bars represent additional recurrent protein families identified in the present analysis. Data were compiled from the 38 studies included in the protein and pathway analysis (Supplementary File S3).

Several additional recurrent protein families were identified that were not included in the reference dataset compiled from previous stress proteomic reviews. This included ubiquitination machinery and proteasome subunits (33 occurrences across 14 studies), complement proteins (15 occurrences across 7 studies), protein phosphatases (10 occurrences across 6 studies), aminoacyl-tRNA ligases (17 occurrences across 7 studies), histones (15 occurrences across 5 studies), inter-α-trypsin inhibitor heavy chain proteins (10 occurrences across 6 studies), and Rab GTPases (7 occurrences across 5 studies). The distribution of these protein families across toxin classes is summarized in Figure 3 with blue bars representing proteins previously reported as recurring in stress response and red bars representing new proteins or protein families identified in the algal toxin studies.

It is noteworthy that the recurrence of several protein families differed according to proteomic separation method. Ribosomal proteins, aminoacyl-tRNA ligases, eukaryotic translation initiation factors, RNA-processing proteins/hnRNPs, and histones were absent from all gel-based studies yet occurred with sufficient frequency in LC-based studies to be included among the most frequently recurring protein families. In contrast, protein-

folding chaperones and ubiquitin/proteasome-associated proteins were reported at similar frequencies in gel- and LC-based studies, while actin and myosin were more frequently reported in gel-based studies.

While individual proteins provide useful points of comparison among studies, nomenclature varies substantially among species, and many proteins participate in multiple biological processes. Therefore, proteins were consolidated into 19 primary functional categories for subsequent analyses. The functional categories and the number of DAPs assigned to each category are summarized in Table 2.

Table 2. Primary functional categories used for classification of standardized DAPs and the number of DAP observations assigned to each category [1].

Functional Class	Count
Metabolism/Energy	245
Protein Quality Control	177
RNA Processing/Transcription	174
Signaling/Stress Adaptation	167
Cytoskeleton/Structural Remodeling	124
Immune/Inflammatory	124
Lipid Metabolism/Transport	113
Vesicle Trafficking/Localization	108
Translation/Gene Expression	99
ECM/Adhesion/Remodeling	81
Ion Channels/Transport	77
Oxidative Stress/Redox	49
Cytoskeletal Transport	33
DNA Replication/Repair	31
Photosynthesis/symbiont proteins	22
Nervous System	12
Programmed Cell Death	6
Storage Protein	4
Uncharacterized	267

[1] Data were compiled from the 38 independent datasets in the protein and pathway analysis (Supplementary File S3).

The distribution of functional categories among toxin classes is shown in Figure 4A,B. Figure 4A presents a heat map of the percentage of studies within each toxin class reporting at least one DAP assigned to a given category, whereas Figure 4B shows a heat map of the percentage of all proteins assigned to each category within a toxin class. Across all toxin classes, Metabolism/Energy, Protein Quality Control, Lipid Metabolism/Transport, and Signaling/Stress Adaptation were the most frequently represented categories identified in 85, 77, 70 and 68% of studies respectively over all toxin classes. Metabolism/Energy was reported in 100% of studies for BMAA, DA, DSTs, and in at least 60% of studies for all remaining toxin classes. Protein Quality Control was reported in 100% of ATX and DSTs studies and prominent in other toxin classes except for PSP toxins at 43% of studies reporting. Signaling/Stress Adaptation and Lipid Metabolism/Transport were both identified in 50–100% of studies depending on toxin class.

A. Percent of Studies Reporting							B. Percent of Proteins							Functional Class
ATX	BMAA	PbTX	CYN	DA	DSP	PSP	ATX	BMAA	PbTX	CYN	DA	DSP	PSP	
75	100	60	80	100	100	86	18.3	9.1	10.7	11.1	9.8	22.9	25.0	Metabolism / Energy
100	83	60	80	75	100	43	11.0	7.9	14.0	17.8	8.2	13.9	7.4	Protein Quality Control
75	100	60	40	75	86	57	7.3	6.2	2.7	7.4	8.2	7.7	9.0	Lipid Metabolism / Transport
50	100	60	60	75	57	71	12.2	11.4	8.7	13.3	12.3	5.3	12.2	Signaling / Stress Adaptation
50	67	40	60	50	100	57	4.9	6.6	4.0	9.6	5.7	12.3	4.8	Cytoskeleton / Structural Remodeling
75	33	80	40	100	71	43	6.1	1.7	22.0	7.4	23.8	8.0	3.7	Immune / Inflammatory
50	50	80	60	100	57	14	7.3	4.0	6.0	5.9	15.6	2.9	2.1	ECM / Adhesion / Remodeling
50	100	40	40	25	71	43	11.0	19.5	12.0	3.0	0.8	4.3	5.3	RNA Processing / Transcription
50	83	60	20	50	57	57	3.7	3.9	2.7	3.0	4.1	3.5	13.3	Ion Channels / Transport
75	67	40	60	25	86	29	6.1	8.2	3.3	7.4	4.9	5.1	7.4	Vesicle Trafficking / Localization
25	67	60	40	50	71	29	1.2	2.9	2.7	1.5	3.3	4.0	3.2	Oxidative Stress / Redox
100	50	40	0	25	57	43	7.3	1.5	1.3	0.0	0.8	2.9	2.1	Cytoskeletal Transport
50	83	20	40	0	29	43	2.4	10.1	4.7	3.0	0.0	5.9	2.1	Translation / Gene Expression
25	67	40	20	0	14	14	1.2	3.5	2.7	0.7	0.0	0.5	1.1	DNA Replication / Repair
0	17	20	0	25	0	14	0.0	1.0	0.7	0.0	2.5	0.0	1.1	Nervous System
0	17	20	20	0	0	0	0.0	1.5	2.0	7.4	0.0	0.0	0.0	Photosynthesis / Symbiont Protein
0	17	0	20	0	14	0	0.0	0.5	0.0	1.5	0.0	0.3	0.0	Programmed Cell Death
0	17	0	0	0	14	0	0.0	0.3	0.0	0.0	0.0	0.5	0.0	Storage Protein

Figure 4. Heat map showing the distribution of primary functional categories among algal toxin proteomic studies. (**A**) The percentage of studies within each toxin class reporting at least one DAP assigned to a given functional category. (**B**) The percentage of all standardized DAPs within each toxin class assigned to that category. Functional categories were assigned based on UniProtKB annotations, supplemented by the Human Protein Atlas and published literature where necessary. Color intensity reflects increasing percentages from red (low) to green (high). Toxin abbreviations are ATX, anatoxin; BMAA, β-methylamino-L-alanine; PBTX, brevetoxin; CYN, cylindrospermopsin; DA, domoic acid; DSTs, okadaic acid/*Dinophysis* toxins; PSP, paralytic shellfish poisoning toxins (saxitoxins). Data were compiled from the 38 independent datasets included in the protein and pathway analysis (Supplementary File S3). Created in https://BioRender.com.

Several functional categories showed marked variation among toxin classes. Immune/Inflammatory proteins were reported in all DA studies and 80% of PbTX studies but were less frequently reported in BMAA, CYN and PSP studies. ECM/Adhesion/Remodeling proteins were reported in 100 and 80% of DA and PbTX studies respectively, but in only 14% of PSP studies. BMAA stood out from the other algal toxins by consistently altering proteins involved in gene expression. Proteins associated with RNA processing and transcription were identified in every BMAA proteomic study (100%), while proteins involved in translation and gene expression were reported in 83% of studies. In contrast, these functional categories were much less frequently represented in studies of the other algal toxins and were low or even absent from domoic acid studies. Oxidative Stress/Redox proteins were reported in 71% of DST studies but only 25% of ATX studies. Programmed Cell Death, Storage Proteins, and Nervous System categories were among the least frequently represented categories across all toxin classes.

When evaluated based on protein abundance rather than study frequency (Figure 4B), Metabolism/Energy, Protein Quality Control, Signaling/Stress Adaptation, Immune/Inflammatory, Cytoskeleton/Structural Remodeling accounted for the largest proportions of proteins in most toxin classes. However, the relative contribution of individual categories varied again among toxins, indicating differences in the functional composition of the reported proteomic responses.

Hierarchical clustering based on the relative distribution of proteins among the 19 functional categories revealed two major groups of toxins (Figure 5). DSTs, ATX, BMAA, CYN, and PSP exhibited broadly similar functional response profiles, whereas DA and PbTX formed a separate cluster. Minor differences in branching order among the former group likely reflect the relatively small differences in their functional category distributions and should not be overinterpreted. This analysis is descriptive and exploratory, and because

cluster stability was not statistically evaluated, the observed groupings should not be interpreted as evidence of statistically distinct toxin groups.

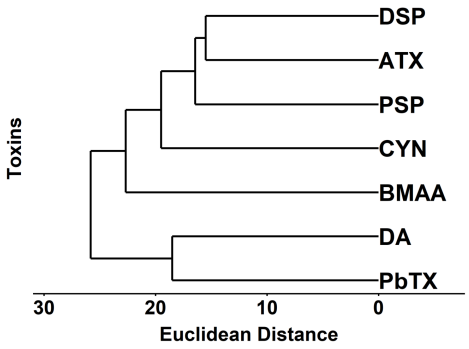


Figure 5. Hierarchical clustering of algal toxin classes based on functional proteomic responses. Dendrogram showing the similarity among algal toxin classes based on the relative distribution of DAPs across the 19 primary functional categories (Figure 4B). Toxin abbreviations are ATX, anatoxin; BMAA, β-methylamino-L-alanine; PbTX, brevetoxin; CYN, cylindrospermopsin; DA, domoic acid; DSP (diarrhetic shellfish poisons), okadaic acid/*Dinophysis* toxins; and PSP, paralytic shellfish poisoning toxins (saxitoxins).

3. Discussion

3.1. Methodological Trends and Reporting Quality (MIAPE/CRED Analysis)

The rapid expansion of proteomic studies investigating algal toxin exposure has generated a substantial and growing body of data. However, meaningful cross-study comparisons depend on both methodological transparency and consistent reporting practices. Therefore, before considering the biological insights emerging from these studies, it is important to evaluate the strengths and limitations of the available literature. Our assessment of 76 studies against selected MIAPE and CRED criteria revealed a pattern common to emerging applications in proteomics: reporting was strong for prominent parameters but weak for technical details that are important for reproducibility.

Reporting of the fundamental experimental design parameters was well-documented in nearly all studies. However, no systematic improvement in technical reporting was observed over the decade that this review covered. The greatest deficiencies in reporting were associated with database search parameters, protein identification criteria, and data accessibility. Unlike several experimental design parameters that may not apply to every study or may simply be underreported, peptide and fragment mass tolerances, search modifications, missed cleavages, FDR thresholds, and raw data deposition are fundamental components of nearly all discovery proteomics workflows. Without this information, readers cannot fully evaluate identification confidence, reproduce database searches, or independently reanalyze the data. Improving reporting of these parameters should therefore be a priority for future proteomics studies of algal toxins. Although these parameters are required by MIAPE, they remain absent from a substantial portion of the literature, limiting the ability to assess identification confidence across studies.

Although most studies reported the software platform used for protein identification, fewer than two-thirds reported both the software version and database search algorithm, as recommended by MIAPE. In some cases, the search algorithm can be inferred from the software platform (e.g., MaxQuant employs the Andromeda search engine). However, this is not universally true, as platforms such as Proteome Discoverer support multiple search engines (e.g., Sequest HT, Mascot, MS Amanda, and Byonic). Because different software versions and search algorithms can yield different peptide and protein identifications from the same raw mass spectrometry data, explicit reporting of both the software version

and search algorithm remains essential for reproducibility and meaningful comparison among studies.

Another challenge across the dataset was the prevalence of non-model organisms for which proteomes are incomplete or absent. This includes bivalves, sea turtles, manatees, corals, and cyanobacteria. These incomplete proteomes may affect data quality and comparability yet are not covered by MIAPE reporting criteria.

Future studies would benefit from greater adherence to MIAPE reporting guidelines to ensure that essential experimental and database search parameters are consistently documented. Protein or peptide quantification prior to LC-MS analysis should be routinely reported, raw mass spectrometry data should be deposited in publicly accessible repositories at the time of publication, and complete lists of identified proteins and DAPs should be provided as Supplementary Material. Adoption of these practices would substantially improve transparency, reproducibility, data reuse, and cross-study comparisons, thereby accelerating progress in understanding the molecular mechanisms of algal toxin toxicity.

3.2. Biological Insights from Comparative Proteomics

Comparison with previously published compilations of recurrent stress-responsive proteins demonstrated substantial overlap between algal toxin studies and the broader stress proteomics literature. Frequently recurring protein families included ribosomal proteins, heat shock proteins, actin, myosin, protein disulfide isomerases, annexins, cathepsins, glutathione S-transferases, tubulins, and translation initiation factors. Many of these proteins are associated with maintenance of protein homeostasis, cellular structure, energy metabolism, and antioxidant defense and have been repeatedly reported across diverse stressors and taxa. The recurrence of these protein families across multiple toxin classes suggests that many algal toxin responses reflect recurring cellular stress response rather than toxin-specific biomarkers.

Although considerable overlap existed among toxin classes, several recurring protein families were identified that were not represented in earlier algal toxin stress-proteomics compilations. These included ubiquitination machinery, aminoacyl-tRNA ligases, protein phosphatases, histones, Rab GTPases, complement proteins, fibrinogens, and inter-alpha-trypsin inhibitor heavy chains. Some of these proteins are likely to reflect advances in proteomic depth and the improved detection of lower-abundance regulatory proteins in more recent studies, whereas others may reflect differences in sample type.

One of the most consistent themes emerging from the protein and pathway analysis was the prominence of proteins associated with proteostasis. Heat shock proteins, protein disulfide isomerases, ribosomal proteins, translation initiation factors, aminoacyl-tRNA ligases, ubiquitination machinery, and proteasome components were recurrently identified across multiple toxin classes. Collectively, these proteins span the major components of the proteostasis network, including protein synthesis, folding, quality control, trafficking, and degradation. Ubiquitination machinery was particularly prominent, occurring in 14 independent studies and representing one of the most recurrent protein families identified in the dataset. Although ubiquitination-related proteins were not highlighted in earlier reviews of common stress-responsive proteins, more recent studies increasingly recognize ubiquitin-mediated protein turnover as a central component of cellular stress adaptation [104–106]. Furthermore, a recent review of environmental pollutant-induced proteotoxicity identified disruption of protein quality control networks as a common consequence of toxicant exposure, highlighting coordinated effects on molecular chaperones, ubiquitin-proteasome pathways, endoplasmic reticulum-associated degradation, and autophagy [107]. These observations are consistent with the present analysis, in which proteins associated with multiple components of the proteostasis network were recurrently identified across toxin

classes, supporting the broader relevance of protein homeostasis in cellular responses to algal toxins.

A second notable feature was the frequent occurrence of proteins involved in translation and translational regulation. Ribosomal proteins represented the most frequently recurring protein family, while translation initiation factors, RNA-processing proteins, and aminoacyl-tRNA ligases were also repeatedly identified. Aminoacyl-tRNA ligases were particularly interesting because they were not included among previously compiled common stress proteins yet occurred across multiple toxin classes. This observation is particularly relevant for BMAA, where aminoacyl-tRNA ligases were identified in more than half of the studies. Recent works provide mechanistic links between BMAA exposure and translational fidelity. BMAA has been shown to interact with alanyl-tRNA synthetase, forming BMAA-tRNA^{Ala}, escaping intrinsic proofreading, and inhibiting both amino acid activation and editing functions [108]. In cyanobacteria, BMAA sensitivity is also linked to tRNA modification pathways that influence translational fidelity and ribosome assembly [109]. However, aminoacyl-tRNA ligases were identified across multiple toxin classes, suggesting that their recurrence in algal toxin proteomics is not solely BMAA-specific but may reflect broader translational regulation and proteostasis responses. Regulation of translation allows cells to rapidly reallocate resources, selectively synthesize stress-response proteins, and limit production of damaged proteins. The repeated identification of translation-associated proteins therefore suggests that reorganization of protein synthesis is a common response to algal toxin exposure.

Although three studies focused on BMAA reported only enriched biological pathways [26,31,32] and not the underlying DAPs, the pathway-level results were remarkably consistent with the findings of the present analysis. Frequently enriched pathways included unfolded protein response, protein ubiquitination, oxidative stress responses, actin cytoskeleton remodeling, oxidative phosphorylation and mitochondrial dysfunction, EIF2 signaling, PI3K/AKT and mTOR signaling, calcium signaling, and apoptosis-related pathways. This concordance provides independent support for the functional categories identified in the present review while also highlighting an important limitation of pathway-only reporting. Without access to the underlying protein lists, recurring proteins cannot be identified, independently validated, or incorporated into cross-study comparative analyses. Future proteomic studies should therefore report both enriched pathways and complete lists of DAPs to maximize the value of these datasets for reanalyses.

Hierarchical clustering revealed substantial similarities among toxin classes but also identified several distinctive patterns. Brevetoxin and domoic acid studies clustered separately from the remaining toxins and shared relatively large proportions of immune/inflammatory and extracellular matrix-associated proteins. Interpretation of these patterns should be approached cautiously because several brevetoxin and domoic acid studies analyzed plasma or cerebrospinal fluid rather than whole tissues or cells. The frequent occurrence of complement proteins, fibrinogens, and inter-alpha-trypsin inhibitor heavy chains likely reflects, at least in part, the predominance of plasma-derived proteins in these studies. Nevertheless, these proteins are well-established components of acute-phase and inflammatory responses and therefore represent important indicators of organismal stress.

While all toxin classes demonstrated alterations in the Protein Quality Control category, cathepsins were observed only in PbTX and DST studies. Because cathepsins function primarily in lysosomal protein degradation, their restricted distribution may suggest a greater contribution of lysosomal degradation pathways in response to these toxin classes. Additional studies will be required to determine whether this pattern reflects a toxin-specific mechanism or differences in experimental design and sample selection.

Although Signaling/Stress Adaptation was among the most frequently represented functional categories, canonical protein kinases were not among the most recurrent protein families identified. Instead, the category was dominated by kinase-associated proteins, receptors, small GTPases, phosphatases, scaffold proteins, and other signaling regulators. This observation likely reflects a limitation of abundance-based proteomics, which detects changes in protein abundance but often fails to capture signaling events regulated primarily through post-translational modification. The frequent occurrence of kinase-associated proteins suggests that phosphoproteomic approaches may provide important additional insights into algal toxin signaling mechanisms that are not apparent from conventional proteomic analyses.

Interestingly, proteins associated with autophagy and programmed cell death were comparatively rare despite the well-established role of these pathways in cellular stress responses [110,111]. Most studies included in the present review examined acute or sub-lethal exposures or analyzed field-collected organisms with unknown exposure histories. Consequently, the algal toxin proteomic literature appears to be dominated by early-to-intermediate stress responses characterized by translational regulation, enhanced protein quality control, and ubiquitin-mediated degradation rather than terminal stress responses such as autophagy or cell death.

Comparison with the recent microcystin-focused proteomic review by Welten et al. [112] reveals both similarities and differences in the recurring protein responses to algal toxins. Consistent with our findings, metabolism and cytoskeletal remodeling were among the most frequently affected biological processes. Notably, cytoskeletal/structural remodeling was particularly prominent in the DST class, where it was identified in 100% of studies, mirroring the extensive cytoskeletal disruption reported for microcystin-LR. The prominence of cytoskeletal remodeling in both MC and DST classes is consistent with their established inhibition of the serine/threonine protein phosphatases PP1 and PP2A, which regulate cytoskeletal organization. In contrast, oxidative stress and oxidoreductase activity were considerably more prominent in the microcystin literature, likely reflecting the central role of reactive oxygen species generation in microcystin toxicity. Our broader analysis encompassing multiple algal toxin classes instead identified protein quality control, signaling/stress adaptation, and immune-related processes as more consistently represented across diverse algal toxins. These findings suggest that while many algal toxins converge on common stress-response pathways, individual toxin classes also exhibit distinct functional response profiles.

3.3. Challenges in Cross-Study Comparison

A major challenge in synthesizing proteomic studies of algal toxin exposure is that the reported protein-level results are not directly comparable across studies. The studies included in this review span diverse toxin classes, experimental designs, exposure routes, tissues, cell types, and subject organisms, ranging from mammals or mammalian cell lines to fish, bivalves, corals, and algae. Several studies examined animals captured or rescued from the wild after exposure [35,36,38,46–49]. Although toxin exposure was confirmed analytically in some cases [36,37,39], the dose and duration of exposure prior to sampling were unknown. This biological diversity is a strength of the literature, but it complicates efforts to identify conserved proteomic responses across studies.

One of the most persistent barriers is inconsistent protein nomenclature. The same protein may be reported using different names depending on the organism, database, search strategy, or annotation pipeline. Conversely, similar names may refer to distinct paralogs or protein family members. These inconsistencies are especially problematic in non-model organisms, where genome annotations may be incomplete and protein identi-

fications may rely on homologous sequences from related taxa. Because multifunctional proteins were assigned to a single primary functional category, alternative classifications could influence the relative representation of functional categories and, consequently, the hierarchical clustering. The clustering is therefore intended to provide a descriptive comparison of reported functional response patterns rather than a definitive classification of toxin-specific responses.

Another issue is that orthology is not always straightforward. Many studies relied on proteomic databases derived from non-model organisms or predicted transcriptomes, resulting in incomplete or unstable protein annotations. In some cases, protein accessions corresponded to deprecated or removed database entries, particularly for zebrafish. When possible, proteins were normalized to standardized human homologs or broader functional classes to facilitate cross-study comparison. Mapping proteins from diverse organisms to human homologs can improve cross-study comparison, particularly for pathway analysis, but this approach has limitations. Some proteins have clear one-to-one orthologs, whereas others belong to expanded gene families with multiple paralogs. In these cases, assigning a single human homolog may overstate the precision of the comparison. For this study, a formal orthology analysis was not performed, and some protein family assignments required judgment based on available annotations, sequence descriptions, and UniProt information. As a result, alternative classification schemes are possible, particularly for proteins with limited annotation or multiple biological functions. Where proteins possessed multiple annotated functions, assignment to a primary functional category was guided by the biological context of toxicant-induced stress and the predominant role most relevant to the objectives of this review. Consequently, other investigators might reasonably assign some proteins to different functional categories. These uncertainties could influence estimates of protein recurrence across studies and the relative representation of functional categories, particularly when proteins from evolutionarily distant organisms are consolidated under common protein family names.

The problem is further compounded by differences in proteomic methodology. Gel-based studies often report proteins identified from excised spots, which may contain multiple proteins or protein isoforms, whereas LC-MS/MS-based studies typically report larger protein lists derived from database searches. A single study in this analysis contributed 426 (or 22.3%) of the DAPs whereas three studies reported only six proteins each. Differences in database completeness, false discovery rate reporting, protein inference rules, quantification strategy, and statistical filtering can all influence which proteins are reported as differentially abundant. Therefore, absence of a protein from one study should not be interpreted as biological absence; it may reflect differences in detection depth, annotation quality, or reporting thresholds.

The influence of analytical methodology was apparent when recurring protein families were compared between gel- and LC-based studies. Gel-based studies preferentially identified several abundant structural proteins, whereas multiple protein families associated with translation, RNA processing, and gene regulation were observed only in LC-based studies. This pattern likely reflects, at least in part, differences in proteome coverage and physicochemical biases associated with gel-based separations, including protein abundance, solubility, molecular weight, and isoelectric point.

Another important consideration is that frequently observed proteins are not necessarily toxin-specific biomarkers. Proteins involved in cytoskeletal structure, glycolysis, mitochondrial metabolism, heat shock response, proteasomal degradation, and antioxidant defense are repeatedly identified across many proteomic stress studies. Their recurrence across algal toxin studies may reflect genuine conserved cellular responses, but these proteins are often abundant, highly conserved, and broadly responsive to stress [111]. As a

result, they may be more informative as indicators of generalized cellular injury or adaptation than as toxin-specific biomarkers.

Given these challenges, the most defensible approach is to integrate protein-level recurrence with functional and pathway-level interpretation. In this review, recurring proteins are interpreted within broad categories such as cytoskeletal remodeling, oxidative stress, proteostasis, mitochondrial function, immune activation, apoptosis, and signal transduction. This framework preserves biologically meaningful patterns while avoiding overinterpretation of imperfectly matched protein names across species and platforms.

In addition to limitations inherent in the available literature, this scoping review has several limitations. The literature search was restricted to English-language publications from 2015 onward and to three bibliographic databases, and therefore some relevant studies may not have been identified. Protein-level synthesis was further limited to studies that provided sufficiently complete lists of DAPs. In addition, standardization of protein names and assignment to primary functional categories required manual curation and, in some cases, interpretive judgment, particularly for non-model organisms with incomplete annotations. Although these decisions were made using consistent criteria and established protein databases, alternative classifications may be possible.

4. Materials and Methods

4.1. Literature Search, Study Selection, and Reporting Quality Assessment

The literature search and study selection were conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) guidelines [113]. To evaluate recent applications of mass spectrometry-based proteomics to algal toxin research, the SciFinder, Scopus, and Web of Science databases were searched for publications from 2015 through May 2026. The search was restricted to studies published from 2015 onward to focus the review on contemporary proteomic methodologies and reporting practices. Searches were initially conducted in November 2024, updated on 15 June 2025, and finalized in May 2026 by two authors (J.W. and E.C.). Separate searches were performed in each database for each major algal toxin class: anatoxin, β-N-methylamino-L-alanine (BMAA), brevetoxin, ciguatoxin, cylindrospermopsin, domoic acid, gymnocin, microcystin, okadaic acid, and saxitoxin. Each search combined three concepts with AND: (1) toxin names and toxin synonyms (e.g., anatoxin OR anatoxin a); (2) exposure and toxicity terms (toxin OR exposure OR toxicology OR toxicosis OR toxicity); (3) proteomic terms (proteome OR proteomic OR proteomics). Review articles were excluded with "AND NOT review or literature review," and again during screening. No controlled vocabulary was used, and all searches relied on free text terms. Any variants were entered explicitly rather than through truncation. Scopus searches were run in the "Title/Abstract/Keywords" field, Web of Science in "All Fields" field, and SciFinder in "Abstract/Keywords" field. For domoic acid and okadaic acid in SciFinder, the toxin term was restricted to "Title" because it originally returned an unmanageable volume of papers unrelated to toxicological exposure. Because the initial search using the toxin name gymnocin yielded no results, an additional search was performed using the producing organism, *Karenia mikimotoi*, which identified a single relevant study. Proteome-related terms were used as the defining search vocabulary because the review is limited to studies presenting proteomic analysis as a primary endpoint. Mass spectrometry terms (e.g., "mass spectrometry," "LC-MS/MS," "LC-MS") were evaluated during pilot searching but not retained because this primarily returned studies on toxin detection and quantification rather than proteomic investigations. A review protocol was not registered prior to conducting this scoping review. Search parameters for Scopus, Web of Science and SciFinder along with detailed exclusion criteria are shown in Supplementary File S4.

Primary research articles published in English from 2015 through May 2026 were eligible if they applied mass spectrometry-based proteomics to characterize the response of an organism, tissue, or cell lines to one of the algal toxins included in the search. Mass spectrometry had to be included in the proteomic workflow. No restrictions were placed on test organisms or biological matrix. Exposures to purified toxins, toxin-producing algae, and natural exposures in wild-caught organisms were eligible. Studies were excluded if no organism, tissue, or cell line was exposed to the toxin or if the toxin examined was outside the scope of this review (e.g., tetrodotoxin). Studies of the proteome of a toxin-producing species were excluded because these address the toxin-producing organism rather than the response of an exposed organism.

The database searches identified 536 records (Figure 6). Following removal of 229 duplicates and 132 records published before 2015, 175 records remained for title and abstract screening. Of these, 55 were excluded based on publication type ($n = 3$), absence of toxin exposure ($n = 7$), toxins outside the scope of the review ($n = 2$), or studies examining the proteome of toxin-producing organisms rather than responses to toxin exposure ($n = 43$). Of the 120 reports sought for retrieval, one could not be obtained. The remaining 119 reports underwent full-text eligibility assessment, resulting in the exclusion of 43 additional reports that did not meet the proteomics criteria ($n = 39$) or did not employ mass spectrometry ($n = 4$). A total of 76 publications met the eligibility criteria and were included in the review [24–99]. An Excel database was compiled by all authors containing publication information (authors, title, publication year, organism, toxin, sample type, methodological approach, separation, quantification, identification methods), stated study objectives, funding source, and DOI for each study (Supplementary File S1). Extracted data were independently verified by three authors (K.S.R., C.C., and E.C.), and discrepancies were resolved by review of the original publications. Disagreements or uncertainties regarding study inclusion were resolved by corresponding author (K.S.R.).

To assess reporting quality, each manuscript was evaluated using the Minimum Information About a Proteomics Experiment (MIAPE) checklist [22] while broader study design and ecotoxicological reporting were assessed using the Criteria for Reporting and Evaluating Ecotoxicity Data (CRED) framework [23]. Reporting parameters were grouped into four MIAPE categories: (1) experimental design, including toxin characterization and biological metadata; (2) mass spectrometry methodology, including instrumentation and analytical conditions; (3) sample preparation and workflow, including protein extraction, digestion, and quality control procedures; and (4) bioinformatics and data analysis, including database searching, search parameters, false discovery rate, and protein identification criteria. Each MIAPE- and CRED-related reporting parameter was evaluated using a binary classification (Yes/No) based on whether the specified information was reported. Information provided in the publication, Supplementary Materials, or cited methodological references was considered. When necessary, citation trails were followed until the relevant information was located or no further references were available. Analytical verification of the toxin concentration in the administered exposure medium or dosing solution was required to satisfy this criterion; reporting only the concentration of a stock solution was insufficient. Studies that measured toxin concentrations in biological tissues or fluids following exposure, but did not verify the administered concentration, were designated as tissue measured (TM) and were not counted as having verified exposure concentrations. Identification of a commercial sample preparation kit was considered sufficient reporting of the associated procedure because the manufacturer's documentation provides the relevant methodological information. For most parameters, explicit identification was sufficient (e.g., naming the reducing agent or mass spectrometer), whereas software reporting re-

quired identification of the software version and search algorithm. Parameters for which the required information could not be located were classified as not reported.

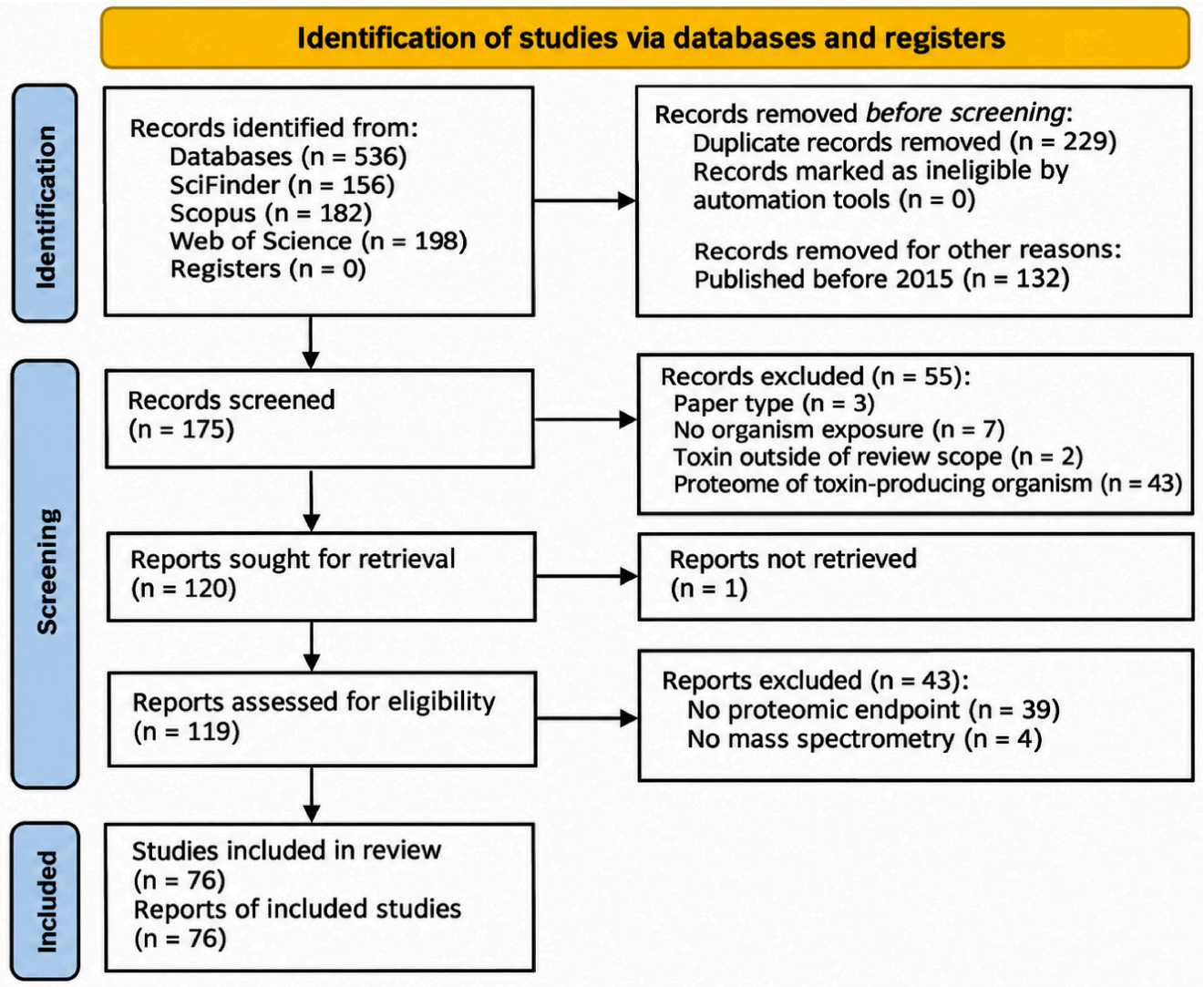


Figure 6. PRISMA-ScR flow diagram illustrating the literature search and study-selection process. Records identified through SciFinder, Scopus, and Web of Science were screened for eligibility, with reasons for exclusion documented at each stage. A total of 76 publications met the inclusion criteria and were included in the scoping review.

The MIAPE/CRED reporting assessments were independently performed by two authors (J.W. and E.C.). Discrepancies were resolved through joint reexamination of the original publications. Cases that could not be resolved by consensus were referred to a third author (K.S.R.) for final determination. The third author also provided guidance on the assessment criteria and reviewed approximately 15% of the assessments for accuracy and agreement. The complete manuscript-by-manuscript MIAPE and CRED assessments, including all individual reporting parameters and scoring criteria, are provided in the multi-sheet Supplementary Spreadsheet (Supplementary File S2).

4.2. Protein Functional Classification

Listings of DAPs were retrieved from 38 proteomic datasets when available [24,25,27–30,34–42,44–57,94–99]. Because microcystin proteomics was recently reviewed [112], studies of microcystins were included only for evaluation of reporting practices and method-

ological trends. Rather than duplicate the previous analysis, findings from the present comparative analysis were discussed in relation to those reported by Welten et al. [112], allowing similarities and differences in functional responses to be considered across toxin classes. For the purposes of this analysis, a dataset was defined as a distinct set of differentially abundant proteins (DAPs) associated with a particular toxin and experimental test subject. Targeted studies [43,58], studies that did not provide a complete list of DAPs, studies that reported only pathway-level results, reported only results for mixtures [26,31,32], or represented the sole study of a toxin class [59], were excluded from the analysis. Protein level data from our own study [37] which reported pathway level analysis was retrieved from our records and included in the analysis. Studies reporting results using multiple species as test subjects [25] or male and female subjects of the same species [25,99] were considered unique datasets. Two studies that examined the same toxin in the same test subject by the same author [56,57] were combined and considered a single dataset.

Because many proteins participate in multiple biological processes, each protein was assigned to a single primary functional category to facilitate quantitative comparisons among toxin classes. Classifications were based primarily on the annotated molecular function and biological process in UniProtKB [114] and, when necessary, supplemented by the Human Protein Atlas [115] and published literature. The assigned category reflected the protein's predominant biological role rather than all known functions. Protein isoforms and closely related homologs or alternative names were standardized to common protein or protein family names, where appropriate, to facilitate comparisons across studies. Proteins annotated as "-like," "putative," "predicted," or "low quality" were accepted according to their database annotation and classified based on the annotated homolog or predicted function; no independent functional reannotation was performed. Proteins annotated only as "hypothetical protein," without an associated homolog or functional descriptor, were retained as "uncharacterized". DAPs were included using the significance criteria (e.g., fold change and/or *p*-value) defined by the original authors.

The final protein and pathway analysis included 38 independent datasets comprising 1913 differentially abundant protein (DAP) observations. Of these, 1038 appeared only once and retained their original protein names, while 837 observations corresponded to proteins reported more than once and were assigned standardized names to reconcile differences in nomenclature across studies. An additional 38 observations appeared only once but were also assigned standardized names for consistency. This process resulted in 223 standardized protein or protein family identifiers. Of the 1913 DAP observations, 1646 were assigned to one of 18 characterized functional categories, while 267 were classified as uncharacterized, yielding 19 categories in total (Table 2). For multifunctional proteins, the primary functional category was selected based on the predominant annotated biological function. UniProtKB Gene Ontology (GO) annotations were examined using the GO ribbon visualization, with greater consideration given to categories containing larger numbers of annotations, as indicated by darker shading. On that basis, Rab GTPases were assigned to Vesicle Trafficking/Localization despite having fewer annotations associated with cellular organization and immune processes. When multiple functional assignments remained plausible, relevance to cellular stress responses was used to guide the final classification. For example, ISG15 is a multifunctional ubiquitin-like modifier involved in protein modification and immune signaling. Although its ubiquitin-like activity could support classification with Protein Quality Control, its prominent role in interferon-mediated stress responses supported its assignment to the Immune/Inflammatory category. The counts in Table 2 represent DAP observations rather than unique proteins. The complete compilation of DAPs, organized by toxin and study, including original protein names, standardized names,

up to four UniProt GO Slim categories (some with multiple categories in a single cell), and primary functional categories, is provided in Supplementary File S3.

Recurring proteins were evaluated using two measures: the total number of DAP occurrences and the number of datasets in which they were reported. Each protein was counted as a separate occurrence, even when multiple members of the same protein family were identified within a single study. However, each dataset was counted only once when determining the number of datasets reporting a particular protein or protein family. For example, ribosomal proteins accounted for 62 occurrences across 22 datasets (Figure 3). Because datasets varied substantially in the number of reported DAPs, those with larger protein lists contributed more heavily to the pooled functional distributions. This approach emphasizes the functional patterns represented across the available proteomics literature, while recognizing that studies with greater numbers of reported DAPs may exert a stronger influence on the observed distributions.

To compare algal toxin-responsive proteins with proteins commonly reported in proteomic studies of stress, protein and protein family lists were retrieved from the reviews reporting repeatedly detected proteins [101–103]. Protein names, synonyms, isoforms, and homologs were standardized using the same nomenclature and consolidation criteria applied to the algal toxin dataset to ensure direct comparability. Following consolidation and removal of duplicate entries, a curated reference dataset of 58 commonly recurring proteins and protein families was generated and used for comparison with the standardized algal toxin protein dataset.

4.3. Hierarchical Clustering

To compare overall proteomic response patterns among algal toxin classes, the percentage of DAPs assigned to each of the 19 primary functional categories was calculated for each toxin class. These percentages were treated as a 19-dimensional feature vector representing the functional response profile of each toxin. Pairwise Euclidean distances were calculated between toxin classes, and hierarchical agglomerative clustering was performed using the unweighted pair group method with arithmetic mean (UPGMA; average linkage) implemented in R (version 2026.06.0+242). The resulting dendrogram was used to visualize similarities in the functional distributions of protein responses among toxin classes.

5. Conclusions

5.1. Challenges and Reporting Gaps

A recurring limitation across the 76 studies included in the methodological assessment is inconsistent adherence to established reporting guidelines, despite the availability of frameworks such as MIAPE and CRED that were specifically designed to improve transparency and reproducibility. Parameters directly tied to analytical quality, such as mass tolerances, FDR thresholds, and MS quality control procedures, were the most frequently left out. Also, fewer than one third of studies deposited raw data in a public repository, which limits access to raw, primary data. Without these data, independent reanalysis, cross-study integration, and meta-analyses become constrained. Standardization of reporting would produce practical benefits. If future studies consistently reported the parameters assessed in this review, systematic meta-analyses could be conducted with greater confidence, and biomarkers could be validated across datasets. Additionally, the value of individual studies would be improved. Deposition of complete protein lists, search parameters, and raw data in recognized repositories should be considered a minimum standard for future publications.

5.2. Future Research Directions

The protein and pathway analysis of 38 independent datasets revealed Signaling/Stress Adaptation to be among the most frequently represented of the 19 functional categories. Several recent studies implicate toxin-associated signaling pathways, including NF-κB/MAPK activation by microcystin-LR [116], NF-κB-dependent JAK/STAT activation by okadaic acid [117], FXR/SHP antagonism by microcystin-LR [118], BMP signaling disruption during microcystin-LR/nanoplastic co-exposure [119], calcium signaling changes following DST exposure [120], and PSP toxins have been shown to increase protein ubiquitination [121]. Transcriptomic studies of saxitoxin, domoic acid and *Prymnesium parvum* exposure also point to altered inflammatory, apoptotic, neurotransmitter, calcium, and MAPK-associated pathways [122–124]. However, these signaling responses are often inferred from transcriptomic, pathway, or targeted biochemical analyses rather than directly measured at the protein activity level. Future studies should place greater emphasis on signaling pathways and post-translational regulation. Many of the signaling pathways implicated in algal toxin toxicity, including MAPK, NF-κB, JAK/STAT, BMP/TGF-β, proteostasis and ER-stress signaling, are regulated primarily through phosphorylation, ubiquitination, and other post-translational modifications rather than changes in protein abundance. The relevance of phosphoproteomics is particularly apparent for microcystins and DSTs, whose primary mechanism of action involves inhibition of serine/threonine phosphatases PP1 and PP2A. Such effects are expected to produce widespread alterations in protein phosphorylation that may not be accompanied by corresponding changes in protein abundance. Consequently, conventional abundance-based proteomics may underestimate the importance of signaling pathways and explain the relative scarcity of recurring protein kinases in the present analysis. Future studies employing phosphoproteomics and other PTM-focused approaches are likely to reveal important layers of algal toxin biology that are missed by conventional proteomics.

Another important future direction is the broader adoption of data-independent acquisition (DIA) proteomics. Compared with traditional data-dependent acquisition (DDA) workflows, DIA systematically fragments all detectable precursor ions within a specified mass range, reducing the stochastic sampling that can contribute to missing values and improving quantitative accuracy, precision, and reproducibility across large sample sets [125]. Recent advances in DIA instrumentation, spectral libraries, and computational analysis have also substantially increased proteome coverage and sensitivity, enabling more consistent detection of low-abundance proteins and regulatory pathways [126,127]. These advantages are particularly relevant to algal toxin research, where many studies involve non-model organisms, complex environmental samples, and subtle biological responses. Greater proteome depth may improve detection of signaling proteins, phosphatases, ubiquitination machinery, aminoacyl-tRNA ligases, and other low-abundance regulators that were infrequently reported in the current literature. In addition, DIA workflows are increasingly being applied to phosphoproteomics and other post-translational modification analyses, creating new opportunities to investigate toxin-induced signaling pathways that may not be apparent from abundance-based proteomics alone [128].

Finally, greater emphasis should be placed on independent validation of proteomic findings. Although proteomics provides a powerful discovery platform, relatively few algal toxin studies have validated DAPs using orthogonal approaches. Validation can strengthen confidence in biological interpretations, facilitate biomarker development, and help distinguish robust responses from study-specific observations. Common validation approaches include ELISA, immunoblotting, enzyme activity measurements, targeted mass spectrometry, and other biochemical assays. In studies involving non-model organisms, where species-specific antibodies are often unavailable, indirect functional measurements

or surrogate assays may provide practical alternatives. Increased integration of discovery proteomics with targeted validation approaches will improve confidence in candidate biomarkers and enhance the translational value of algal toxin proteomics research.

Despite the diversity of algal toxins, organisms, and experimental systems examined, this review demonstrates that common proteomic signatures emerge across toxin classes. Protein quality control, translational regulation, metabolism, signaling, and cytoskeletal remodeling constitute recurring functional patterns to algal toxin exposure, while individual toxin classes exhibit varying response profiles. At the same time, inconsistent reporting and limited data availability continue to constrain cross-study comparisons. Continued adoption of standardized reporting practices together with emerging technologies such as DIA, phosphoproteomics, and improved validation strategies will enable a more comprehensive understanding of algal toxin biology and accelerate the development of robust biomarkers and mechanistic insights.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/md24110414/s1: Supplementary File S1: Characteristics of Included Studies (authors, title, publication year, organism, toxin, sample type, methodological approach, separation, quantification, identification methods, stated study objectives, funding source, and DOI). Supplementary File S2: Reporting Parameters by Manuscript. Supplementary File S3: Compilation of All Available DAPs (standardized protein names and assigned functional categories, pivot tables for the construction of Figures 3 and 4). Supplementary File S4: Database Specific Search Strategies.

Author Contributions: Conceptualization, K.S.R. and C.C.; Methodology, K.S.R.; Validation, K.S.R., C.C. and E.N.C.; Formal Analysis, K.S.R., C.C., E.N.C. and J.M.W.; Investigation, K.S.R., C.C., E.N.C. and J.M.W.; Resources, K.S.R.; Data Curation, K.S.R., C.C., E.N.C. and J.M.W.; Writing—Original Draft Preparation, K.S.R., C.C. and E.N.C.; Writing—Review & Editing, K.S.R., C.C. and E.N.C.; Visualization, K.S.R., C.C. and E.N.C.; Supervision, K.S.R.; Project Administration, K.S.R.; Funding Acquisition, K.S.R. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported in part by NIH/NIEHS Grant R03ES037119 and by The Water School at Florida Gulf Coast University.

Institutional Review Board Statement: Not applicable

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Acknowledgments: During the preparation of this manuscript, the authors used ChatGPT (OpenAI, GPT-5.5) to assist with language editing, improving organization, and evaluating the clarity of scientific arguments. All AI-generated suggestions were critically reviewed, revised as appropriate, and verified against the primary literature. The authors take full responsibility for the content of this publication.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

2D-PAGE	Two-dimensional polyacrylamide gel electrophoresis
ATX	Anatoxin
BMAA	β-N-methylamino-*L*-alanine
BMP	Bone Morphogenetic Protein
CID	Collision induced dissociation
CRED	Criteria for reporting and evaluating ecotoxicity data
CTX	Ciguatoxins

CYN	Cylindrospermopsin
DA	Domoic acid
DDA	Data-dependent acquisition
DAPs	Differentially abundant proteins
DIA	Data-independent acquisition
DIGE	Difference gel electrophoresis
DST	Diarrhetic shellfish toxins
DSP	Diarrhetic shellfish poisoning
DTXs	*Dinophysis* toxins
FDR	False discovery rate
FXR	Farnesoid X Receptor
GYM	Gymnocin
GTX	Gonyatoxins
HABs	Harmful algal blooms
iTRAQ	Isobaric tags for relative and absolute quantitation
JAK	Janus kinase
LC	Liquid chromatography
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LFQ	Label free quantification
MAPK	Mitogen-activated protein kinase
MC	Microcystin
MIAPE	Minimum information about a proteomics experiment
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem Mass spectrometry
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
OA	Okadaic acid
PbTX	Brevetoxin
PRM	Parallel reaction monitoring
PSP	Paralytic shellfish poisons (STX, GTX)
SHP	Small heterodimer partner
SILAC	Stable isotope labeling by amino acids in cell culture
SRM	Selected reaction monitoring
STAT	Signal transducer and activator of transcription
STX	Saxitoxin
TMT	Tandem mass tags

References

1. Liu, X.; Bian, Z.; Hu, S.; Dickinson, C.F.; Benjamin, M.M.; Jia, J.; Tian, Y.; Place, A.; Hanna, G.S.; Luesch, H.; et al. The Chemistry of Phytoplankton. *Chem. Rev.* **2024**, *124*, 13099–13177. [CrossRef] [PubMed]
2. Pinto, A.; Botelho, M.J.; Churro, C.; Asselman, J.; Pereira, P.; Pereira, J.L. A Review on Aquatic Toxins—Do We Really Know It All Regarding the Environmental Risk Posed by Phytoplankton Neurotoxins? *J. Environ. Manag.* **2023**, *345*, 118769. [CrossRef] [PubMed]
3. Kazmi, S.S.U.H.; Yapa, N.; Karunarathna, S.C.; Suwannarach, N. Perceived Intensification in Harmful Algal Blooms Is a Wave of Cumulative Threat to the Aquatic Ecosystems. *Biology* **2022**, *11*, 852. [CrossRef] [PubMed]
4. Flewelling, L.J.; Naar, J.P.; Abbott, J.P.; Baden, D.G.; Barros, N.B.; Bossart, G.D.; Bottein, M.-Y.D.; Hammond, D.G.; Haubold, E.M.; Heil, C.A.; et al. Red Tides and Marine Mammal Mortalities. *Nature* **2005**, *435*, 755–756. [CrossRef] [PubMed]
5. Hallegraeff, G.; Enevoldsen, H.; Zingone, A. Global Harmful Algal Bloom Status Reporting. *Harmful Algae* **2021**, *102*, 101992. [CrossRef] [PubMed]
6. Kleinteich, J.; Hildebrand, F.; Wood, S.A.; Cifs, S.; Agha, R.; Quesada, A.; Pearce, D.A.; Convey, P.; Ḱpper, F.C.; Dietrich, D.R. Diversity of Toxin and Non-Toxin Containing Cyanobacterial Mats of Meltwater Ponds on the Antarctic Peninsula: A Pyrosequencing Approach. *Antart. Sci.* **2014**, *26*, 521–532. [CrossRef]
7. Murphy, J.C.; Gorney, R.M.; Lucas, L.V.; Zwart, J.A.; Graham, J.L. Fifty Years of Riverine Harmful Algal Bloom Modeling: A Global Synthesis of Approaches, Challenges, and Opportunities. *Water Res.* **2026**, *303*, 126240. [CrossRef] [PubMed]

8. Parsaeimehr, A. Detection to Treatment and Global Impacts of Algal Toxins. *Front. Biosci.* **2019**, *11*, 214–235. [CrossRef] [PubMed]
9. Howard, M.D.A.; Smith, J.; Caron, D.A.; Kudela, R.M.; Loftin, K.; Hayashi, K.; Fadness, R.; Fricke, S.; Kann, J.; Roethler, M.; et al. Integrative Monitoring Strategy for Marine and Freshwater Harmful Algal Blooms and Toxins across the Freshwater-to-Marine Continuum. *Integr. Environ. Assess. Manag.* **2023**, *19*, 586–604. [CrossRef] [PubMed]
10. Geada, P.; Gkelis, S.; Teixeira, J.; Vasconcelos, V.; Vicente, A.A.; Fernandes, B. Cyanobacterial Toxins as a High Value-Added Product. In *Microalgae-Based Biofuels and Bioproducts*; Elsevier: Amsterdam, The Netherlands, 2017; pp. 401–428. ISBN 978-0-08-101023-5.
11. Ebner, J.N. Trends in the Application of "Omics" to Ecotoxicology and Stress Ecology. *Genes* **2021**, *12*, 1481. [CrossRef] [PubMed]
12. Zhang, X.; Xia, P.; Wang, P.; Yang, J.; Baird, D.J. Omics Advances in Ecotoxicology. *Environ. Sci. Technol.* **2018**, *52*, 3842–3851. [CrossRef] [PubMed]
13. Gouveia, D.; Almunia, C.; Cogne, Y.; Pible, O.; Degli-Esposti, D.; Salvador, A.; Cristobal, S.; Sheehan, D.; Chaumot, A.; Geffard, O.; et al. Ecotoxicoproteomics: A Decade of Progress in Our Understanding of Anthropogenic Impact on the Environment. *J. Proteom.* **2019**, *198*, 66–77. [CrossRef] [PubMed]
14. Gajahin Gamage, N.T.; Miyashita, R.; Takahashi, K.; Asakawa, S.; Senevirathna, J.D.M. Proteomic Applications in Aquatic Environment Studies. *Proteomes* **2022**, *10*, 32. [CrossRef] [PubMed]
15. Jiang, Y.; Rex, D.A.B.; Schuster, D.; Neely, B.A.; Rosano, G.L.; Volkmar, N.; Momenzadeh, A.; Peters-Clarke, T.M.; Egbert, S.B.; Kreimer, S.; et al. Comprehensive Overview of Bottom-Up Proteomics Using Mass Spectrometry. *ACS Meas. Sci. Au* **2024**, *4*, 338–417. [CrossRef] [PubMed]
16. Lesur, A.; Domon, B. Advances in High-resolution Accurate Mass Spectrometry Application to Targeted Proteomics. *Proteomics* **2015**, *15*, 880–890. [CrossRef] [PubMed]
17. Marcus, K.; Lelong, C.; Rabilloud, T. What Room for Two-Dimensional Gel-Based Proteomics in a Shotgun Proteomics World? *Proteomes* **2020**, *8*, 17. [CrossRef] [PubMed]
18. Li, J.; Smith, L.S.; Zhu, H.-J. Data-Independent Acquisition (DIA): An Emerging Proteomics Technology for Analysis of Drug-Metabolizing Enzymes and Transporters. *Drug Discov. Today Technol.* **2021**, *39*, 49–56. [CrossRef] [PubMed]
19. Phetsanthad, A.; Carr, A.V.; Fields, L.; Li, L. Definitive Screening Designs to Optimize Library-Free DIA-MS Identification and Quantification of Neuropeptides. *J. Proteome Res.* **2023**, *22*, 1510–1519. [CrossRef] [PubMed]
20. Chahrour, O.; Cobice, D.; Malone, J. Stable Isotope Labelling Methods in Mass Spectrometry-Based Quantitative Proteomics. *J. Pharm. Biomed. Anal.* **2015**, *113*, 2–20. [CrossRef] [PubMed]
21. Sivanich, M.K.; Gu, T.; Tabang, D.N.; Li, L. Recent Advances in Isobaric Labeling and Applications in Quantitative Proteomics. *Proteomics* **2022**, *22*, 2100256. [CrossRef] [PubMed]
22. Taylor, C.F.; Paton, N.W.; Lilley, K.S.; Binz, P.-A.; Julian, R.K.; Jones, A.R.; Zhu, W.; Apweiler, R.; Aebersold, R.; Deutsch, E.W.; et al. The Minimum Information about a Proteomics Experiment (MIAPE). *Nat. Biotechnol.* **2007**, *25*, 887–893. [CrossRef] [PubMed]
23. Moermond, C.T.A.; Kase, R.; Korkaric, M.; Ågerstrand, M. CRED: Criteria for Reporting and Evaluating Ecotoxicity Data. *Environ. Toxicol. Chem.* **2015**, *35*, 1297–1309. [CrossRef] [PubMed]
24. Carneiro, M.; Gutiérrez-Praena, D.; Osório, H.; Vasconcelos, V.; Carvalho, A.P.; Campos, A. Proteomic Analysis of Anatoxin-a Acute Toxicity in Zebrafish Reveals Gender Specific Responses and Additional Mechanisms of Cell Stress. *Ecotoxicol. Environ. Saf.* **2015**, *120*, 93–101. [CrossRef] [PubMed]
25. Langan, L.M.; Lovin, L.M.; Taylor, R.B.; Scarlett, K.R.; Kevin Chambliss, C.; Chatterjee, S.; Scott, J.T.; Brooks, B.W. Proteome Changes in Larval Zebrafish (*Danio rerio*) and Fathead Minnow (*Pimephales promelas*) Exposed to (±) Anatoxin-a. *Environ. Int.* **2024**, *185*, 108514. [CrossRef] [PubMed]
26. Beri, J.; Nash, T.; Martin, R.M.; Bereman, M.S. Exposure to BMAA Mirrors Molecular Processes Linked to Neurodegenerative Disease. *Proteomics* **2017**, *17*, 1700161. [CrossRef] [PubMed]
27. Chiu, A.S.; Braidy, N.; Marçal, H.; Welch, J.H.; Gehringer, M.M.; Guillemin, G.J.; Neilan, B.A. Global Cellular Responses to β-Methyl-Amino-l-Alanine (BMAA) by Olfactory Ensheathing Glial Cells (OEC). *Toxicon* **2015**, *99*, 136–145. [CrossRef] [PubMed]
28. Frøyset, A.K.; Khan, E.A.; Fladmark, K.E. Quantitative Proteomics Analysis of Zebrafish Exposed to Sub-Lethal Dosages of β-Methyl-Amino-L-Alanine (BMAA). *Sci. Rep.* **2016**, *6*, 29631. [CrossRef] [PubMed]
29. Karlsson, O.; Berg, A.-L.; Hanrieder, J.; Arnerup, G.; Lindström, A.-K.; Brittebo, E.B. Intracellular Fibril Formation, Calcification, and Enrichment of Chaperones, Cytoskeletal, and Intermediate Filament Proteins in the Adult Hippocampus CA1 Following Neonatal Exposure to the Nonprotein Amino Acid BMAA. *Arch. Toxicol.* **2015**, *89*, 423–436. [CrossRef] [PubMed]
30. Koksharova, O.A.; Butenko, I.O.; Pobeguts, O.V.; Safronova, N.A.; Govorun, V.M. The First Proteomic Study of Nostoc Sp. PCC 7120 Exposed to Cyanotoxin BMAA under Nitrogen Starvation. *Toxins* **2020**, *12*, 310. [CrossRef] [PubMed]
31. Martin, R.M.; Stallrich, J.; Bereman, M.S. Mixture Designs to Investigate Adverse Effects upon Co-Exposure to Environmental Cyanotoxins. *Toxicology* **2019**, *421*, 74–83. [CrossRef] [PubMed]
32. Pu, L.; Steele, J.R.; Phillips, C.R.; Violi, J.P.; Rodgers, K.J. The Cyanobacterial Toxins BMAA and 2,4-DAB Perturb the l-Serine Biosynthesis Pathway and Induce Systemic Changes in Energy Metabolism in Human Neuroblastoma Cells: A Proteomic Study. *Toxicol. In Vitro* **2025**, *106*, 106058. [CrossRef] [PubMed]

33. Yan, T.; Zheng, X.; Jia, G.; Liang, Z.; Guo, L.; Ding, F.; Fang, Z.; Li, Y.; Zhao, Y. L-BMAA Induces Neurotoxicity through AMPK/Akt-TSC1/2-mTOR-Mediated Mitophagy Dysregulation and Apoptosis. *J. Hazard. Mater.* **2026**, *509*, 142074. [CrossRef] [PubMed]
34. Martin, R.M.; Bereman, M.S.; Marsden, K.C. BMAA and MCLR Interact to Modulate Behavior and Exacerbate Molecular Changes Related to Neurodegeneration in Larval Zebrafish. *Toxicol. Sci.* **2021**, *179*, 251–261. [CrossRef] [PubMed]
35. Ceballos, C.; Rein, K.S.; Walsh, C.J. Brevetoxicosis in Loggerhead (Caretta Caretta) and Kemp's Ridley (Lepidochelys Kempii) Sea Turtles: Plasma Proteomics for Florida Red Tide Biomarkers. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2026**, *306*, 110541. [CrossRef] [PubMed]
36. Ceballos, C.; Walsh, C.J.; Cabrera, J.; Rein, K.S. Brevetoxicosis in Florida Manatees (Trichechus Manatus Latirostris): Plasma Proteomics for Florida Red Tide Biomarkers. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2026**, *306*, 110525. [CrossRef] [PubMed]
37. Jobson, J.; Tsegay, P.S.; Beltran, M.T.; Taher, E.A.; Rein, S.R.; Liu, Y.; Rein, K.S. Brevetoxin Induces a Shift in the Redox State of the Proteome and Unfolded Protein Response in Human Lymphoblast Cells That Can Be Alleviated with the Acrolein Scavenger MESNA. *Environ. Toxicol. Pharmacol.* **2023**, *100*, 104137. [CrossRef] [PubMed]
38. Lazensky, R.; Silva-Sanchez, C.; Kroll, K.J.; Chow, M.; Chen, S.; Tripp, K.; Walsh, M.T.; Denslow, N.D. Investigating an Increase in Florida Manatee Mortalities Using a Proteomic Approach. *Sci. Rep.* **2021**, *11*, 4282. [CrossRef] [PubMed]
39. Reynolds, D.A.; Yoo, M.-J.; Dixson, D.L.; Ross, C. Exposure to the Florida Red Tide Dinoflagellate, Karenia Brevis, and Its Associated Brevetoxins Induces Ecophysiological and Proteomic Alterations in Porites Astreoides. *PLoS ONE* **2020**, *15*, e0228414. [CrossRef] [PubMed]
40. Diez-Quijada, L.; Puerto, M.; Gutiérrez-Praena, D.; Turkina, M.V.; Campos, A.; Vasconcelos, V.; Cameán, A.M.; Jos, Á. In Vitro Toxicity Evaluation of Cyanotoxins Cylindrospermopsin and Microcystin-LR on Human Kidney HEK293 Cells. *Toxins* **2022**, *14*, 429. [CrossRef] [PubMed]
41. Freitas, M.; Campos, A.; Azevedo, J.; Barreiro, A.; Planchon, S.; Renaut, J.; Vasconcelos, V. Lettuce (*Lactuca Sativa* L.) Leaf-Proteome Profiles after Exposure to Cylindrospermopsin and a Microcystin-LR/Cylindrospermopsin Mixture: A Concentration-Dependent Response. *Phytochemistry* **2015**, *110*, 91–103. [CrossRef] [PubMed]
42. Oliveira, F.; Diez-Quijada, L.; Turkina, M.V.; Morais, J.; Felpeto, A.B.; Azevedo, J.; Jos, A.; Camean, A.M.; Vasconcelos, V.; Martins, J.C.; et al. Physiological and Metabolic Responses of Marine Mussels Exposed to Toxic Cyanobacteria *Microcystis aeruginosa* and *Chrysosporum ovalisporum*. *Toxins* **2020**, *12*, 196. [CrossRef] [PubMed]
43. Chowdhury, R.R.; Grosso, M.F.; Gadara, D.C.; Spáčil, Z.; Vidová, V.; Sovadinová, I.; Babica, P. Cyanotoxin Cylindrospermopsin Disrupts Lipid Homeostasis and Metabolism in a 3D in Vitro Model of the Human Liver. *Chem.-Biol. Interact.* **2024**, *397*, 111046. [CrossRef] [PubMed]
44. Liebel, S.; Regina Grötzner, S.; Dietrich Moura Costa, D.; Antônio Ferreira Randi, M.; Alberto De Oliveira Ribeiro, C.; Filipak Neto, F. Cylindrospermopsin Effects on Protein Profile of HepG2 Cells. *Toxicol. Mech. Methods* **2016**, *26*, 554–563. [CrossRef] [PubMed]
45. Ziesemer, S.; Meyer, S.; Edelmann, J.; Vennmann, J.; Gudra, C.; Arndt, D.; Effenberg, M.; Hayas, O.; Hayas, A.; Thomassen, J.S.; et al. Target Mechanisms of the Cyanotoxin Cylindrospermopsin in Immortalized Human Airway Epithelial Cells. *Toxins* **2022**, *14*, 785. [CrossRef] [PubMed]
46. Ghosh, G.; Neely, B.A.; Bland, A.M.; Whitmer, E.R.; Field, C.L.; Duignan, P.J.; Janech, M.G. Identification of Candidate Protein Biomarkers Associated with Domoic Acid Toxicosis in Cerebrospinal Fluid of California Sea Lions (*Zalophus Californianus*). *J. Proteome Res.* **2024**, *23*, 2419–2430. [CrossRef] [PubMed]
47. Neely, B.A.; Ferrante, J.A.; Chaves, J.M.; Soper, J.L.; Almeida, J.S.; Arthur, J.M.; Gulland, F.M.D.; Janech, M.G. Proteomic Analysis of Plasma from California Sea Lions (Zalophus Californianus) Reveals Apolipoprotein E as a Candidate Biomarker of Chronic Domoic Acid Toxicosis. *PLoS ONE* **2015**, *10*, e0123295. [CrossRef] [PubMed]
48. Neely, B.A.; Soper, J.L.; Gulland, F.M.D.; Bell, P.D.; Kindy, M.; Arthur, J.M.; Janech, M.G. Proteomic Analysis of Cerebrospinal Fluid in California Sea Lions (*Zalophus Californianus*) with Domoic Acid Toxicosis Identifies Proteins Associated with Neurodegeneration. *Proteomics* **2015**, *15*, 4051–4063. [CrossRef] [PubMed]
49. Solosky, A.M.; Claudio, I.M.; Chappel, J.R.; Kirkwood-Donelson, K.I.; Janech, M.G.; Bland, A.M.; Gulland, F.M.D.; Neely, B.A.; Baker, E.S. Proteomic and Lipidomic Plasma Evaluations Reveal Biomarkers for Domoic Acid Toxicosis in California Sea Lions. *J. Proteome Res.* **2024**, *23*, 5577–5585. [CrossRef] [PubMed]
50. Huang, L.; Zou, Y.; Weng, H.; Li, H.-Y.; Liu, J.-S.; Yang, W.-D. Proteomic Profile in Perna Viridis after Exposed to Prorocentrum Lima, a Dinoflagellate Producing DSP Toxins. *Environ. Pollut.* **2015**, *196*, 350–357. [CrossRef] [PubMed]
51. Martins, J.C.; Domínguez-Pérez, D.; Azevedo, C.; Braga, A.C.; Costa, P.R.; Osório, H.; Vasconcelos, V.; Campos, A. Molecular Responses of Mussel Mytilus Galloprovincialis Associated to Accumulation and Depuration of Marine Biotoxins Okadaic Acid and Dinophysistoxin-1 Revealed by Shotgun Proteomics. *Front. Mar. Sci.* **2020**, *7*, 589822. [CrossRef]
52. Matus Hernández, M.A.; Hernández Saavedra, N.Y. Effect of Different Species of Prorocentrum Genus on the Japanese Oyster Crassostrea Gigas Proteomic Profile. *Toxins* **2021**, *13*, 504. [CrossRef]

53. Siddiqui, A.; Abidin, S.A.Z.; Shah, Z.A.; Othman, I.; Kumari, Y. Behavioural, Genomics and Proteomic Approach to Examine Alzheimer's Disease in Zebrafish. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2023**, *271*, 109636. [CrossRef] [PubMed]
54. Wang, J.; Lin, L.; Wang, D.-Z. Quantitative Proteomic Analysis Reveals Novel Insights into Hepatic Toxicity in Mice Exposed Chronically to Okadaic Acid. *Sci. Total Environ.* **2021**, *775*, 145772. [CrossRef]
55. Wang, X.; Wang, D.; Zhang, T.; Zhang, Q.; Zhao, J. iTRAQ-Based Quantitative Proteomic Analysis Reveals Toxicity Mechanisms in Chlamys Farreri Exposed to Okadaic Acid. *Front. Mar. Sci.* **2021**, *8*, 792050. [CrossRef]
56. Wuerger, L.T.D.; Birkholz, G.; Oberemm, A.; Sieg, H.; Braeuning, A. Proteomic Analysis of Hepatic Effects of Okadaic Acid in HepaRG Human Liver Cells. *EXCLI J.* **2023**, *22*, 1135–1145. [CrossRef] [PubMed]
57. Wuerger, L.T.D.; Sprenger, H.; Krasikova, K.; Templin, M.; Stahl, A.; Herfurth, U.M.; Sieg, H.; Braeuning, A. A Multi-Omics Approach to Elucidate Okadaic Acid-Induced Changes in Human HepaRG Hepatocarcinoma Cells. *Arch. Toxicol.* **2024**, *98*, 2919–2935. [CrossRef] [PubMed]
58. Wuerger, L.T.D.; Hammer, H.S.; Hofmann, U.; Kudiabor, F.; Sieg, H.; Braeuning, A. Okadaic Acid Influences Xenobiotic Metabolism in HepaRG Cells. *EXCLI J.* **2022**, *21*, 1053–1065. [CrossRef] [PubMed]
59. Kwok, C.S.-N.; Lai, K.K.-Y.; Lam, W.; Xu, S.J.-L.; Lam, S.-W.; Lee, F.W.-F. Proteome Analysis of Whole-Body Responses in Medaka Experimentally Exposed to Fish-Killing Dinoflagellate Karenia Mikimotoi. *Int. J. Mol. Sci.* **2021**, *22*, 11625. [CrossRef] [PubMed]
60. Carneiro, M.; Reis, B.; Azevedo, J.; Campos, A.; Osório, H.; Vasconcelos, V.; Martins, J. Glutathione Transferases Responses Induced by Microcystin-LR in the Gills and Hepatopancreas of the Clam Venerupis Philippinarum. *Toxins* **2015**, *7*, 2096–2120. [CrossRef] [PubMed]
61. Chen, L.; Hu, Y.; He, J.; Chen, J.; Giesy, J.P.; Xie, P. Responses of the Proteome and Metabolome in Livers of Zebrafish Exposed Chronically to Environmentally Relevant Concentrations of Microcystin-LR. *Environ. Sci. Technol.* **2017**, *51*, 596–607. [CrossRef] [PubMed]
62. Chen, X.-D.; Liu, Y.; Yang, L.-M.; Hu, X.-Y.; Jia, A.-Q. Hydrogen Sulfide Signaling Protects Chlamydomonas Reinhardtii Against Allelopathic Damage From Cyanobacterial Toxin Microcystin-LR. *Front. Plant Sci.* **2020**, *11*, 1105. [CrossRef] [PubMed]
63. Chen, Y.; Chen, G.; Xu, Y.; Wei, X.; Li, X.; Zhou, Y.; Chen, Z. Prenatal Microcystin-LR Exposure Alters Pancreatic Proteome and Impairs Insulin Secretion in Offspring Mice. *Toxicol. Lett.* **2025**, *410*, 159–168. [CrossRef] [PubMed]
64. Du, B.; Liu, G.; Ke, M.; Zhang, Z.; Zheng, M.; Lu, T.; Sun, L.; Qian, H. Proteomic Analysis of the Hepatotoxicity of *Microcystis aeruginosa* in Adult Zebrafish (*Danio rerio*) and Its Potential Mechanisms. *Environ. Pollut.* **2019**, *254*, 113019. [CrossRef] [PubMed]
65. Du, X.; Fu, Y.; Tian, Z.; Liu, H.; Xin, H.; Fu, X.; Wang, F.; Zhang, H.; Zeng, X. Microcystin-LR Accelerates Follicular Atresia in Mice via JNK-Mediated Adherent Junction Damage of Ovarian Granulosa Cells. *Ecotoxicol. Environ. Saf.* **2023**, *252*, 114592. [CrossRef] [PubMed]
66. He, J.; Li, G.; Chen, J.; Lin, J.; Zeng, C.; Chen, J.; Deng, J.; Xie, P. Prolonged Exposure to Low-Dose Microcystin Induces Nonalcoholic Steatohepatitis in Mice: A Systems Toxicology Study. *Arch. Toxicol.* **2017**, *91*, 465–480. [CrossRef] [PubMed]
67. He, Y.; Shen, A.; Salam, M.; Liu, M.; Wei, Y.; Yang, Y.; Li, H. Microcystins-Loaded Aged Nanoplastics Provoke a Metabolic Shift in Human Liver Cells. *Environ. Sci. Technol.* **2023**, *57*, 10521–10531. [CrossRef] [PubMed]
68. Ikumawoyi, V.O.; Lynch, K.D.; Iverson, D.T.; Call, M.R.; Yue, G.E.; Prasad, B.; Clarke, J.D. Microcystin-LR Activates Serine/Threonine Kinases and Alters the Phosphoproteome in Human HepaRG Cells. *Toxicon* **2024**, *249*, 108072. [CrossRef] [PubMed]
69. Jia, W.; Zhong, L.; Ren, Q.; Teng, D.; Gong, L.; Dong, H.; Li, J.; Wang, C.; He, Y.-X.; Yang, J. Microcystin-RR Promote Lipid Accumulation through CD36 Mediated Signal Pathway and Fatty Acid Uptake in HepG2 Cells. *Environ. Res.* **2024**, *249*, 118402. [CrossRef] [PubMed]
70. Kongsintaweesuk, S.; Intuyod, K.; Roytrakul, S.; Seubwai, W.; Sangkhamanon, S.; Charoenlappanit, S.; Cha'on, U.; Surapinit, A.; Thongpon, P.; Tanasuka, P.; et al. System-Level Multi-Omics Uncover Proteomic, Peptidomic, and Metabolic Remodeling in Microcystin-LR Exacerbated Chronic Kidney Disease. *Ecotoxicol. Environ. Saf.* **2026**, *317*, 120211. [CrossRef] [PubMed]
71. Kongsintaweesuk, S.; Tunbenjasiri, K.; Pongking, T.; Roytrakul, S.; Charoenlappanit, S.; Anutrakulchai, S.; Pairojkul, C.; Intuyod, K.; Tanasuka, P.; Blair, D.; et al. Microcystin-LR Exacerbates Chronic Kidney Disease in Rats: Insights into Gut Microbiome and Host Proteome Dysregulation. *Life Sci.* **2025**, *378*, 123840. [CrossRef] [PubMed]
72. Le Manach, S.; Khenfech, N.; Huet, H.; Qiao, Q.; Duval, C.; Marie, A.; Bolbach, G.; Clodic, G.; Djediat, C.; Bernard, C.; et al. Gender-Specific Toxicological Effects of Chronic Exposure to Pure Microcystin-LR or Complex *Microcystis aeruginosa* Extracts on Adult Medaka Fish. *Environ. Sci. Technol.* **2016**, *50*, 8324–8334. [CrossRef] [PubMed]
73. Le Manach, S.; Sotton, B.; Huet, H.; Duval, C.; Paris, A.; Marie, A.; Yépremian, C.; Catherine, A.; Mathéron, L.; Vinh, J.; et al. Physiological Effects Caused by Microcystin-Producing and Non-Microcystin Producing *Microcystis aeruginosa* on Medaka Fish: A Proteomic and Metabolomic Study on Liver. *Environ. Pollut.* **2018**, *234*, 523–537. [CrossRef] [PubMed]
74. Li, G.; Yan, W.; Dang, Y.; Li, J.; Liu, C.; Wang, J. The Role of Calcineurin Signaling in Microcystin-LR Triggered Neuronal Toxicity. *Sci. Rep.* **2015**, *5*, 11271. [CrossRef] [PubMed]
75. Li, S.-C.; Gu, L.-H.; Wang, Y.-F.; Wang, L.-M.; Chen, L.; Giesy, J.P.; Tuo, X.; Xu, W.-L.; Wu, Q.-H.; Liu, Y.-Q.; et al. A Proteomic Study on Gastric Impairment in Rats Caused by Microcystin-LR. *Sci. Total Environ.* **2024**, *917*, 169306. [CrossRef] [PubMed]

76. Li, Y.; Xiao, H.; Xie, J.; Liu, L.; Jiang, F.; Liao, J.; Yu, E. TMT Quantitative Proteomics Reveals the Molecular Mechanism Behind Meat Quality Changes in Nile Tilapia Exposed to Environmental Concentrations of Microcystin-LR. *Toxins* **2026**, *18*, 39. [CrossRef] [PubMed]
77. Liu, X.; Zhai, W.-H.; Liu, R.; Liu, B.-L.; Gao, R.-J.; Li, Y.-W.; Xiang, L.; Cai, Q.-Y.; Zhao, H.-M.; Mo, C.-H. Integrated Multi-Omics Analyses of Molecular Pathways Underlying Microcystin-LR Toxicity in the Earthworm Eisenia Fetida. *Ecotoxicol. Environ. Saf.* **2025**, *302*, 118724. [CrossRef] [PubMed]
78. Lyu, K.; Meng, Q.; Zhu, X.; Dai, D.; Zhang, L.; Huang, Y.; Yang, Z. Changes in iTRAQ-Based Proteomic Profiling of the Cladoceran *Daphnia Magna* Exposed to Microcystin-Producing and Microcystin-Free *Microcystis aeruginosa*. *Environ. Sci. Technol.* **2016**, *50*, 4798–4807. [CrossRef] [PubMed]
79. Qiao, Q.; Le Manach, S.; Huet, H.; Duvernois-Berthet, E.; Chaouch, S.; Duval, C.; Sotton, B.; Ponger, L.; Marie, A.; Mathéron, L.; et al. An Integrated Omic Analysis of Hepatic Alteration in Medaka Fish Chronically Exposed to Cyanotoxins with Possible Mechanisms of Reproductive Toxicity. *Environ. Pollut.* **2016**, *219*, 119–131. [CrossRef] [PubMed]
80. Rai, A.K.; Chaturvedi, R.; Kumar, A. Proteomic Evidences for Microcystin-RR-Induced Toxicological Alterations in Mice Liver. *Sci. Rep.* **2018**, *8*, 1310. [CrossRef] [PubMed]
81. Shahmohamadloo, R.S.; Ortiz Almirall, X.; Simmons, D.B.D.; Lumsden, J.S.; Bhavsar, S.P.; Watson-Leung, T.; Eyken, A.V.; Hankins, G.; Hubbs, K.; Konopelko, P.; et al. Cyanotoxins within and Outside of *Microcystis aeruginosa* Cause Adverse Effects in Rainbow Trout (*Oncorhynchus Mykiss*). *Environ. Sci. Technol.* **2021**, *55*, 10422–10431. [CrossRef] [PubMed]
82. Shahmohamadloo, R.S.; Ortiz Almirall, X.; Simmons, D.B.D.; Poirier, D.G.; Bhavsar, S.P.; Sibley, P.K. Fish Tissue Accumulation and Proteomic Response to Microcystins Is Species-Dependent. *Chemosphere* **2022**, *287*, 132028. [CrossRef] [PubMed]
83. Shahmohamadloo, R.S.; Simmons, D.B.D.; Sibley, P.K. Shotgun Proteomics Analysis Reveals Sub-Lethal Effects in *Daphnia Magna* Exposed to Cell-Bound Microcystins Produced by *Microcystis aeruginosa*. *Comp. Biochem. Physiol. Part D Genom. Proteom.* **2020**, *33*, 100656. [CrossRef] [PubMed]
84. Valério, E.; Barreiros, S.; Rodrigues, S.; Turkina, M.V.; Vasconcelos, V.M.; Campos, A. New Insights in Saccharomyces Cerevisiae Response to the Cyanotoxin Microcystin-LR, Revealed by Proteomics and Gene Expression. *Toxins* **2020**, *12*, 667. [CrossRef] [PubMed]
85. Valério, E.; Campos, A.; Osório, H.; Vasconcelos, V. Proteomic and Real-Time PCR Analyses of Saccharomyces Cerevisiae VL3 Exposed to Microcystin-LR Reveals a Set of Protein Alterations Transversal to Several Eukaryotic Models. *Toxicon* **2016**, *112*, 22–28. [CrossRef] [PubMed]
86. Yang, P.; Zhang, P.; Deng, Y.; Liao, Y.; Guo, X.; Sun, M.; Yin, L.; Liu, R. Comprehensive Proteomic and Phosphoproteomic Reveal That Microcystin-LR Contributed to the Malignant Progression of Gastric Cancer by Estrogenic Potency. *Environ. Pollut.* **2023**, *317*, 120744. [CrossRef] [PubMed]
87. Yin, H.; Jiang, H.; Zhao, R.; Zhang, Y.; Fan, X.; Zhai, X.; Yang, X.; Zhang, Y. A Phosphoproteomic Study Uncovers the Importance of PP2A-B56α in Liver Injury Precipitated by NLRP3 Activation during MC-LR Exposure. *Ecotoxicol. Environ. Saf.* **2025**, *302*, 118752. [CrossRef] [PubMed]
88. Yu, Y.; Zhang, Q.; Liu, G.; Deng, Y.; Kang, J.; Zhang, F.; Lu, T.; Sun, L.; Qian, H. Proteomic Analysis of Zebrafish Brain Damage Induced by *Microcystis aeruginosa* Bloom. *Sci. Total Environ.* **2021**, *795*, 148865. [CrossRef] [PubMed]
89. Zhao, S.; Li, G.; Chen, J. A Proteomic Analysis of Prenatal Transfer of Microcystin-LR Induced Neurotoxicity in Rat Offspring. *J. Proteom.* **2015**, *114*, 197–213. [CrossRef] [PubMed]
90. Zhao, S.; Sun, H.; Yan, W.; Xu, D.; Shen, T. A Proteomic Study of the Pulmonary Injury Induced by Microcystin-LR in Mice. *Toxicon* **2018**, *150*, 304–314. [CrossRef] [PubMed]
91. Zhao, S.; Xie, P.; Chen, J.; Liu, L.; Fan, H. A Proteomic Study on Liver Impairment in Rat Pups Induced by Maternal Microcystin-LR Exposure. *Environ. Pollut.* **2016**, *212*, 197–207. [CrossRef] [PubMed]
92. Zhao, Y.; Sokol, H.; Cao, Q.; Zhang, H.; Yan, Y.; Xie, L.; Wang, H. Systemic Inflammatory Response to Daily Exposure to Microcystin-LR and the Underlying Gut Microbial Mechanisms. *J. Hazard. Mater.* **2024**, *480*, 135855. [CrossRef] [PubMed]
93. Zhou, Y.; Sun, M.; Tang, Y.; Chen, Y.; Zhu, C.; Yang, Y.; Wang, C.; Yu, G.; Tang, Z. Responses of the Proteome in Testis of Mice Exposed Chronically to Environmentally Relevant Concentrations of Microcystin-LR. *Ecotoxicol. Environ. Saf.* **2020**, *187*, 109824. [CrossRef] [PubMed]
94. Chan, K.-K.; Tam, N.F.-Y.; Ng, C.; Kwok, C.S.-N.; Xu, S.J.-L.; Sze, E.T.-P.; Lee, F.W.-F. Proteome Response of Meretrix Bivalves Hepatopancreas Exposed to Paralytic Shellfish Toxins Producing Dinoflagellate Gymnodinium Catenatum. *J. Mar. Sci. Eng.* **2021**, *9*, 1039. [CrossRef]
95. Chen, X.; Sun, Y.; Huang, H.; Liu, W.; Hu, P.; Huang, X.; Zou, F.; Liu, J. Uncovering the Proteome Response of Murine Neuroblastoma Cells against Low-Dose Exposure to Saxitoxin. *Toxicol. Mech. Methods* **2018**, *28*, 335–344. [CrossRef] [PubMed]
96. Even, Y.; Pousse, E.; Chapperon, C.; Artigaud, S.; Hégaret, H.; Bernay, B.; Pichereau, V.; Flye-Sainte-Marie, J.; Jean, F. Physiological and Comparative Proteomic Analyzes Reveal Immune Defense Response of the King Scallop *Pecten maximus* in Presence of Paralytic Shellfish Toxin (PST) from *Alexandrium minutum*. *Harmful Algae* **2022**, *115*, 102231. [CrossRef] [PubMed]

97. Liu, X.; Wang, F.; Yu, H.; Liu, C.; Xia, J.; Ma, Y.; Chen, B.; Liu, S. Differential Proteomic Analysis of Low-Dose Chronic Paralytic Shellfish Poisoning. *Mar. Drugs* **2024**, *22*, 108. [CrossRef] [PubMed]
98. Sun, Q.; Chen, X.; Liu, W.; Li, S.; Zhou, Y.; Yang, X.; Liu, J. Effects of Long-Term Low Dose Saxitoxin Exposure on Nerve Damage in Mice. *Aging* **2021**, *13*, 17211–17226. [CrossRef] [PubMed]
99. Vicentini, M.; Calado, S.L.M.; Pessati, J.B.K.; Perussolo, M.C.; Lirola, J.R.; Marcondes, F.R.; Nascimento, N.D.; Beghetto, C.L.; Vilar, M.C.P.; Mela, M.; et al. Temperature Rise and Its Influence on the Toxic Effects Caused by Cyanotoxins in a Neotropical Catfish. *Environ. Pollut.* **2024**, *353*, 124166. [CrossRef] [PubMed]
100. Doucette, A.A.; Nickerson, J.L. Developing Front-end Devices for Improved Sample Preparation in MS-based Proteome Analysis. *J. Mass Spectrom.* **2020**, *55*, e4494. [CrossRef] [PubMed]
101. Wang, P.; Bouwman, F.G.; Mariman, E.C.M. Generally Detected Proteins in Comparative Proteomics—A Matter of Cellular Stress Response? *Proteomics* **2009**, *9*, 2955–2966. [CrossRef] [PubMed]
102. Petrak, J.; Ivanek, R.; Toman, O.; Cmejla, R.; Cmejlova, J.; Vyoral, D.; Zivny, J.; Vulpe, C.D. Déjà vu in Proteomics. A Hit Parade of Repeatedly Identified Differentially Expressed Proteins. *Proteomics* **2008**, *8*, 1744–1749. [CrossRef] [PubMed]
103. Groh, K.J.; Suter, M.J.-F. Stressor-Induced Proteome Alterations in Zebrafish: A Meta-Analysis of Response Patterns. *Aquat. Toxicol.* **2015**, *159*, 1–12. [CrossRef] [PubMed]
104. Chora, S.; McDonagh, B.; Sheehan, D.; Starita-Geribaldi, M.; Roméo, M.; Bebianno, M.J. Ubiquitination and Carbonylation as Markers of Oxidative-Stress in Ruditapes Decussatus. *Mar. Environ. Res.* **2008**, *66*, 95–97. [CrossRef] [PubMed]
105. Kwon, J.-E.; Jo, S.-H.; Song, W.-S.; Lee, J.-S.; Jeon, H.-J.; Park, J.-H.; Kim, Y.-R.; Baek, J.-H.; Kim, M.-G.; Kwon, S.-Y.; et al. Investigation of Metabolic Crosstalk between Host and Pathogenic Clostridioides Difficile via Multiomics Approaches. *Front. Bioeng. Biotechnol.* **2022**, *10*, 971739. [CrossRef] [PubMed]
106. Polaki, S.; Thatipaka, S.D.R.; Paila, R.V. Impact of Sublethal Copper Exposure on Penaeus Indicus Post-Larvae: Biochemical and Proteomic Insights. *Aquac. Int.* **2025**, *33*, 16. [CrossRef]
107. Devi, S.; Kim, J.-J.; Singh, A.P.; Kumar, S.; Dubey, A.K.; Singh, S.K.; Singh, R.S.; Kumar, V. Proteotoxicity: A Fatal Consequence of Environmental Pollutants-Induced Impairments in Protein Clearance Machinery. *J. Pers. Med.* **2021**, *11*, 69. [CrossRef] [PubMed]
108. Han, N.-C.; Bullwinkle, T.J.; Loeb, K.F.; Faull, K.F.; Mohler, K.; Rinehart, J.; Ibba, M. The Mechanism of β-N-Methylamino-l-Alanine Inhibition of tRNA Aminoacylation and Its Impact on Misincorporation. *J. Biol. Chem.* **2020**, *295*, 1402–1410. [CrossRef]
109. Wang, Z.-Q.; Zhang, C.-C. A tRNA t6A Modification System Contributes to the Sensitivity towards the Toxin β-N-Methylamino-L-Alanine (BMAA) in the Cyanobacterium Anabaena Sp. PCC 7120. *Aquat. Toxicol.* **2022**, *245*, 106121. [CrossRef] [PubMed]
110. Fulda, S.; Gorman, A.M.; Hori, O.; Samali, A. Cellular Stress Responses: Cell Survival and Cell Death. *Int. J. Cell Biol.* **2010**, *2010*, 214074. [CrossRef] [PubMed]
111. Kültz, D. MOLECULAR AND EVOLUTIONARY BASIS OF THE CELLULAR STRESS RESPONSE. *Annu. Rev. Physiol.* **2005**, *67*, 225–257. [CrossRef] [PubMed]
112. Welten, R.D.; Meneely, J.P.; Elliott, C.T. A Comparative Review of the Effect of Microcystin-LR on the Proteome. *Expo. Health* **2020**, *12*, 111–129. [CrossRef]
113. Tricco, A.C.; Lillie, E.; Zarin, W.; O'Brien, K.K.; Colquhoun, H.; Levac, D.; Moher, D.; Peters, M.D.J.; Horsley, T.; Weeks, L.; et al. PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. *Ann. Intern. Med.* **2018**, *169*, 467–473. [CrossRef] [PubMed]
114. The UniProt Consortium; Bateman, A.; Martin, M.-J.; Orchard, S.; Magrane, M.; Adesina, A.; Ahmad, S.; Bowler-Barnett, E.H.; Bye-A-Jee, H.; Carpentier, D.; et al. UniProt: The Universal Protein Knowledgebase in 2025. *Nucleic Acids Res.* **2025**, *53*, D609–D617. [CrossRef] [PubMed]
115. Uhlén, M.; Fagerberg, L.; Hallström, B.M.; Lindskog, C.; Oksvold, P.; Mardinoglu, A.; Sivertsson, Å.; Kampf, C.; Sjöstedt, E.; Asplund, A.; et al. Tissue-Based Map of the Human Proteome. *Science* **2015**, *347*, 1260419. [CrossRef] [PubMed]
116. Zhang, J.; Chen, J.; Xia, Z. Microcystin-LR Exhibits Immunomodulatory Role in Mouse Primary Hepatocytes Through Activation of the NF-κB and MAPK Signaling Pathways. *Toxicol. Sci.* **2013**, *136*, 86–96. [CrossRef] [PubMed]
117. Wuerger, L.T.D.; Kudiabor, F.; Alarcan, J.; Templin, M.; Poetz, O.; Sieg, H.; Braeuning, A. Okadaic Acid Activates JAK/STAT Signaling to Affect Xenobiotic Metabolism in HepaRG Cells. *Cells* **2023**, *12*, 770. [CrossRef] [PubMed]
118. He, J.; Xu, W.-L.; Chen, F.; Chen, J.; Xie, P. Microcystin-LR Disrupts Bile Acid Homeostasis, Driving Cholestatic Liver Injury and Gallstones via FXR/SHP Antagonism. *Environ. Sci. Technol.* **2026**, *60*, 8958–8970. [CrossRef] [PubMed]
119. Liu, W.; Yang, M.; Wen, X.; Long, S.; Hu, H.; Pan, Y.; Zou, T.; Zhan, C.; Yang, F. Lipophagy Suppression: A Novel Mechanism for Developmental Disruption by Nanoplastics/MC-LR in Zebrafish. *Environ. Int.* **2026**, *207*, 110004. [CrossRef] [PubMed]
120. Chen, Z.; Li, M.; Jiang, J.; Mo, Y.; Li, D.; Li, H.; Yang, W. DSTs May Affect the Swimming Ability of Marine Medaka by Interfering with the Neurotransmitter System and Calcium Signaling. *Environ. Pollut.* **2026**, *405*, 128547. [CrossRef] [PubMed]
121. Barbosa, V.; Santos, M.; Anacleto, P.; Maulvault, A.L.; Pousão-Ferreira, P.; Costa, P.R.; Marques, A. Paralytic Shellfish Toxins and Ocean Warming: Bioaccumulation and Ecotoxicological Responses in Juvenile Gilthead Seabream (*Sparus aurata*). *Toxins* **2019**, *11*, 408. [CrossRef] [PubMed]

122. Clinton, M.; Król, E.; Sepúlveda, D.; Andersen, N.R.; Brierley, A.S.; Ferrier, D.E.K.; Hansen, P.J.; Lorenzen, N.; Martin, S.A.M. Gill Transcriptomic Responses to Toxin-Producing Alga Prymnesium Parvum in Rainbow Trout. *Front. Immunol.* **2021**, *12*, 794593. [CrossRef] [PubMed]
123. Hidayat, A.S.; Lefebvre, K.A.; MacDonald, J.; Bammler, T.; Aluru, N. Symptomatic and Asymptomatic Domoic Acid Exposure in Zebrafish (*Danio rerio*) Revealed Distinct Non-Overlapping Gene Expression Patterns in the Brain. *Aquat. Toxicol.* **2022**, *252*, 106310. [CrossRef] [PubMed]
124. Guinle, C.; Núñez-Vázquez, E.J.; Fernández-Herrera, L.J.; Corona-Rojas, D.A.; Tovar-Ramírez, D. Toxicogenomic Effects of Dissolved Saxitoxin on the Early Life Stages of the Longfin Yellowtail (*Seriola rivoliana*). *Mar. Drugs* **2023**, *21*, 597. [CrossRef] [PubMed]
125. Fröhlich, K.; Fahrner, M.; Brombacher, E.; Seredynska, A.; Maldacker, M.; Kreutz, C.; Schmidt, A.; Schilling, O. Data-Independent Acquisition: A Milestone and Prospect in Clinical Mass Spectrometry–Based Proteomics. *Mol. Cell. Proteom.* **2024**, *23*, 100800. [CrossRef] [PubMed]
126. Kitata, R.B.; Yang, J.; Chen, Y. Advances in Data-independent Acquisition Mass Spectrometry towards Comprehensive Digital Proteome Landscape. *Mass Spectrom. Rev.* **2023**, *42*, 2324–2348. [CrossRef] [PubMed]
127. Lou, R.; Shui, W. Acquisition and Analysis of DIA-Based Proteomic Data: A Comprehensive Survey in 2023. *Mol. Cell. Proteom.* **2024**, *23*, 100712. [CrossRef] [PubMed]
128. Yang, Y.; Qiao, L. Data-independent Acquisition Proteomics Methods for Analyzing Post-translational Modifications. *Proteomics* **2023**, *23*, 2200046. [CrossRef] [PubMed]

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