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Benjamin Marie, Maiwenn Le Meur, Charlotte Duval, Manon Quiquand, Emilie Lance, et al.. The threat is in the details – critical gap in ecotoxicological assessment of Microcystis blooms revealed by critical distinctions of genotype effects induced on Medaka fish. *Environmental Pollution*, 2025, 387, pp.127344. <10.1016/j.envpol.2025.127344>. <hal-05349474>

HAL Id: hal-05349474

<https://hal.science/hal-05349474v1>

Submitted on 5 Nov 2025

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The threat is in the details – critical gap in ecotoxicological assessment of *Microcystis* blooms revealed by critical distinctions of genotype effects induced on Medaka fish[☆]

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ARTICLE INFO

Keywords:

Ecotoxicology
Cyanobacteria
Cyanotoxins
Microbiote
Metabolomics
Multi-omics

ABSTRACT

Microcystis spp., prolific bloom-forming freshwater cyanobacteria, produce a wide range of bioactive secondary metabolites. While microcystins (MCs) are well-characterized hepatotoxins, the ecotoxicological roles of other *Microcystis*-derived compounds remain poorly understood. This study evaluates the toxic potential of four *Microcystis* strains - two MC-producing (PMC 728.11, 807.12) and two non-MC-producing (PMC 810.12, 826.12) - in Medaka fish (*Oryzias latipes*) at different life stages. Embryos and larvae exposed to extracts from non-MC-producing strains exhibited pronounced developmental toxicity and malformations, linked to the presence of microginins, aeruginosins, and microcyclamides. MC-rich strain PMC 728.11 induced significant post-hatch toxicity. In adult females exposed under microcosm conditions to environmentally relevant concentrations, strain-specific disruptions were observed in gut microbiota composition and tissue metabolomes. Notably, PMC 728.11 caused microbial dysbiosis, while PMC 826.12 impaired digestive functions, enhancing susceptibility to toxicant uptake. The remaining strains produced milder effects. Our results demonstrate that *Microcystis* toxicity extends beyond MCs, driven by complex and variable metabolite mixtures. This biochemical diversity poses challenges for bloom risk assessment and raises concerns over unrecognized environmental hazards. These findings advocate for broader monitoring of cyanobacterial metabolites and their mechanistic impacts in aquatic toxicology and environmental health.

1. Introduction

One of the most significant stressors for aquatic fauna in ponds and lakes is the occurrence of cyanobacterial blooms, which are increasing in frequency and intensity due to anthropogenic impacts (Huisman et al., 2018; Erratt et al., 2023). Despite their high productivity and nutritional potential (Cerri et al., 2021), cyanobacterial blooms are unsuitable as animal feed due to their harmful effects (Chen et al., 2021). These blooms can disrupt aquatic food webs, deplete dissolved oxygen through bacterial decomposition of biomass, outcompete other photosynthetic organisms, and directly threaten aquatic life through the production of toxic compounds (Whitton, 2012).

Freshwater bloom-forming cyanobacteria are known to produce a wide array of bioactive metabolites, some with toxic properties (Demay

et al., 2019). Over 2300 such compounds have been identified across various cyanobacterial genera, including peptides, alkaloids, terpenes, macrolides, and lipopolysaccharides (Jones et al., 2021). These metabolites can be released into the water during bloom senescence, posing risks to both aquatic organisms and humans (Codd et al., 2005).

Among freshwater bloom-forming cyanobacteria, *Microcystis* is one of the most prolific and widespread genera, occurring on all continents and in nearly every country (Harke et al., 2016). It thrives under a broad range of physico-chemical conditions and often dominates phytoplankton communities. This ecological success is attributed to several key functional traits, including genetic diversity among co-existing clones/strains that harbor distinct gene sets (Dick et al., 2021). Notably, many *Microcystis* genotypes produce a variety of bioactive metabolites, including the hepatotoxic microcystins (MCs), which pose

[☆] This paper has been recommended for acceptance by Wen Chen.

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well-established local risks for water used in drinking, recreation, agriculture, and fisheries (Codd et al., 2005; Sivonen et al., 1999).

Genomic analyses have identified up to 18 distinct biosynthetic gene clusters (BGCs) in *Microcystis*, including those encoding non-ribosomal peptide synthetases (NRPS), polyketide synthases (PKS), and enzymes for ribosomally synthesized and post-translationally modified peptides (RiPPs) or terpene biosynthesis (Cai et al., 2023). These BGCs correspond to known metabolites such as microcystins, aeruginosins, cyanopeptolins, microginins, anabaenopeptins, cyanobactins, and microviridins, as well as many still-uncharacterized compounds (Humbert et al., 2013; Yancey et al., 2024). Some studies suggest that even non-MC-producing *Microcystis* strains can exert ecotoxicological effects on aquatic organisms, including fish, which play crucial roles in ecosystem functioning (Marie, 2020; de Almeida et al., 2024; Banerjee et al., 2021).

MC-LR, one of the most studied cyanotoxins produced by *Microcystis*, is a potent hepatotoxin that accumulates in the liver and impairs fish physiology and reproduction even at low chronic exposures (Qiao et al., 2016; Qiao et al., 2019). However, Saraf and colleagues (2018) demonstrated in zebrafish embryos that MCs alone do not account for the full spectrum of toxicity observed with *Microcystis* strains, suggesting additive or synergistic effects from other metabolites. Similarly, yellowtail tetra embryos exhibited developmental toxicity when exposed to *Microcystis* biomass regardless of MC production (Fernandes et al., 2019). Studies have also shown that non-MC-producing *Microcystis* strains can induce high embryotoxicity due to metabolites like microginins and cyanopeptolins (de Almeida Torres et al., 2023), while purified microcyclamide has shown toxicity levels comparable to MC-LR in zebrafish embryos (Freitas et al., 2023). Moreover, several investigations have revealed that non-MC-producing strains cause distinct, yet intense, tissue and physiological disturbances in adult fish (Le Manach et al., 2018; Sotton et al., 2017a,b). Recent findings also report impacts on cell viability and reproductive gene expression in fish exposed to these strains (Harshaw et al., 2024).

Given that fish primarily encounter cyanotoxins through ingestion, the gut microbiome has recently emerged as a critical focus in ecotoxicological studies of *Microcystis* (Duperron et al., 2020; Duan et al., 2020; Gallet et al., 2023). Some recent work has thus linked exposure to *Microcystis* biomass with reduced fish appetite and subsequent neuroendocrine disruption (Niu et al., 2024; Long et al., 2024). In addition, small bioactive molecules such as terpenes, possibly derived from retinoid-like compounds in *M. aeruginosa* biomass, may contribute to teratogenic effects (Jonas et al., 2015). Together, these findings support the hypothesis that the broader chemical diversity of *Microcystis* metabolites may present greater ecotoxicological threats than MCs alone, and therefore warrants further investigation (Janssen et al., 2019).

Based on these observations, we hypothesize that different *Microcystis* strains presenting distinct genetic background associated with distinct corteges of bioactive molecules might induce different ecotoxicological threats. Thus, the present study aims to investigate the diversity of toxic mechanisms associated with *Microcystis*, focusing on the genetic and chemical variability among bloom-forming genotypes and their respective toxicological effects on the model fish *Oryzias latipes* (Medaka). Four *Microcystis* strains were selected based on phylogenetic diversity and distinct metabolite profiles to rationally assess the effects of MCs and other bioactive cyanopeptides (e.g., cyanopeptolins, aeruginosins, microginins, microcyclamides). A comprehensive suite of bioassays was used to evaluate toxicity at both individual (mortality, malformations) and sub-individual (metabolomic and microbiota alterations) levels. By simulating environmentally relevant bloom conditions, this study provides ecologically meaningful insights into the complexity and variability of *Microcystis*-induced effects on fish health. Overall, the findings emphasize the multifaceted risks associated with *Microcystis* blooms and highlight the importance of considering chemical diversity in ecological risk assessments.

2. Material and methods

2.1. *Microcystis* strain materials

Four *Microcystis* strains - PMC 728.11, PMC 807.12, PMC 810.12, and PMC 826.12 - were obtained from the Paris Muséum Collection (PMC) (Hamlouli et al., 2022). Strain PMC 728.11 was originally isolated from Lake Juanon near Valence, France (Halary et al., 2020), while strains PMC 807.12, PMC 810.12, and PMC 826.12 were collected during a bloom event in a recreational pond in Champs-sur-Marne (Île-de-France, France) (Gaëtan et al., 2023). These strains were specifically selected based on their phylogenetic diversity, representing four genetically distinct clades of *Microcystis* that can co-occur within a single bloom. Each strain is characterized by the production of a unique profile of bioactive metabolites. Details on genome sequencing and biosynthetic gene cluster analysis are provided in the Supporting Information (Text S1).

2.2. Medaka fish embryo experimentations

All experiments were conducted using *Oryzias latipes* (Cab lineage), maintained at the Medaka facility of the Muséum National d'Histoire Naturelle (MNHN, Paris, France) under standard laboratory conditions, in compliance with European animal welfare regulations. Freshly fertilized eggs were incubated in 24-well plates containing *Microcystis* strain extracts (14 mg mL⁻¹ dry biomass equivalent in 1 % methanol). After 24 h, the exposure medium was fully replaced. Embryo survival, developmental arrest, and morphological abnormalities (e.g., head and tail malformations) were assessed at 48 h post-exposure (see Fig. S1).

Additionally, a separate batch of embryos was incubated in Yamamoto's medium in 6-cm Petri dishes, with daily medium renewal until hatching (8 days post-fertilization, dpf). Hatched larvae were then exposed for 24 h in 12-well plates to the same extract concentration. Survival rates were recorded after 24 h. Full methodological details are available in Text S2.

2.3. Adult female medaka exposure

Experiments on adult fish complied with European Directive 2010/63/EU and were approved by the ethics committee CEEA Cuvier n°68 (authorization APAFiS#19316–2019032913284201 v1). Groups of four 6-month-old female Medaka were housed in 10-L aquaria and exposed for 4 days to control water or to water containing environmentally relevant concentrations (100 µg.L⁻¹ chlorophyll-a) of one of the four *Microcystis* cultures (PMC 728.11, 807.12, 810.12, or 826.12) (Foucault et al., 2022). Additional methodological details are provided in Text S3.

2.4. Fish metabolite extraction and metabolomic analysis

Tissues (liver, gut, muscle), faeces, and tank water samples were homogenized in cold extraction solvent (75:25 methanol:water; 1 mL per 100 mg tissue) on ice (Colas et al., 2020). Residual pellets were stored at -80 °C for later DNA extraction (Duperron et al., 2023). Metabolite profiling was conducted using ultra-high-performance liquid chromatography (UHPLC; ELUTE, Bruker) coupled to a high-resolution electrospray ionization mass spectrometer (ESI-Qq-TOF Compact, Bruker). Analyses were performed in positive ion mode with MS/MS acquisition over 50–1500 *m/z* using collision-induced dissociation (CID).

Raw data were processed with MetaboScape 4.0. Metabolite annotation was performed using mass and isotopic patterns matched to the CyanometDB 2.0 database (Jones et al., 2021) and further validated via MS² fragmentation profiles using the GNPS platform (Wang et al., 2016). Detailed methods are presented in Text S4.

2.5. Microbiome 16S rRNA analysis

DNA was extracted from gut, faeces, and tank water pellets. The V3–V4 region of the 16S rRNA gene was amplified and sequenced on an Illumina MiSeq platform as previously described (Gallet et al., 2023). Sequence data were analyzed using the QIIME 2 (version 2020.11) pipeline (Bolyen et al., 2018), with rarefaction to 6282 reads per sample. Additional procedural details are outlined in Text S5.

2.6. Data treatment

Metabolomic data were processed using the MetaboAnalyst 5.0 platform (Pang et al., 2021). Microbiome data were analyzed for alpha and beta diversity using the MicrobiomeAnalyst 2.0 web server (Lu et al., 2023). Multi-omics integration was conducted using the DIABLO tool (Singh et al., 2019) from the mixOmics R package (Rohart et al., 2017). Further methodological specifics are available in Text S6.

3. Results and discussion

3.1. Diversity of the four selected *Microcystis* strains

From the monoclonal *Microcystis* strains available in culture collections, we selected four strains that represent the genetic diversity of *Microcystis*, particularly focusing on genotypes known to co-occur during the same bloom event (Fig. 1). Under light microscopy (Fig. 1A), the strains displayed individual cell development characteristic of the species *Microcystis aeruginosa*, without distinct morphological features allowing for differentiation.

Recent studies have shown that the various morpho-species within *Microcystis* spp. actually belong to a single cosmopolitan genus composed of multiple genotypes distinguishable at the genomic level, though not corresponding to previously described colony morphologies or morpho-species classifications. A phylogenomic analysis confirmed that the four selected strains belong to four genetically distinct clades that are evenly distributed across the *Microcystis* phylogenetic tree (Fig. 1B).

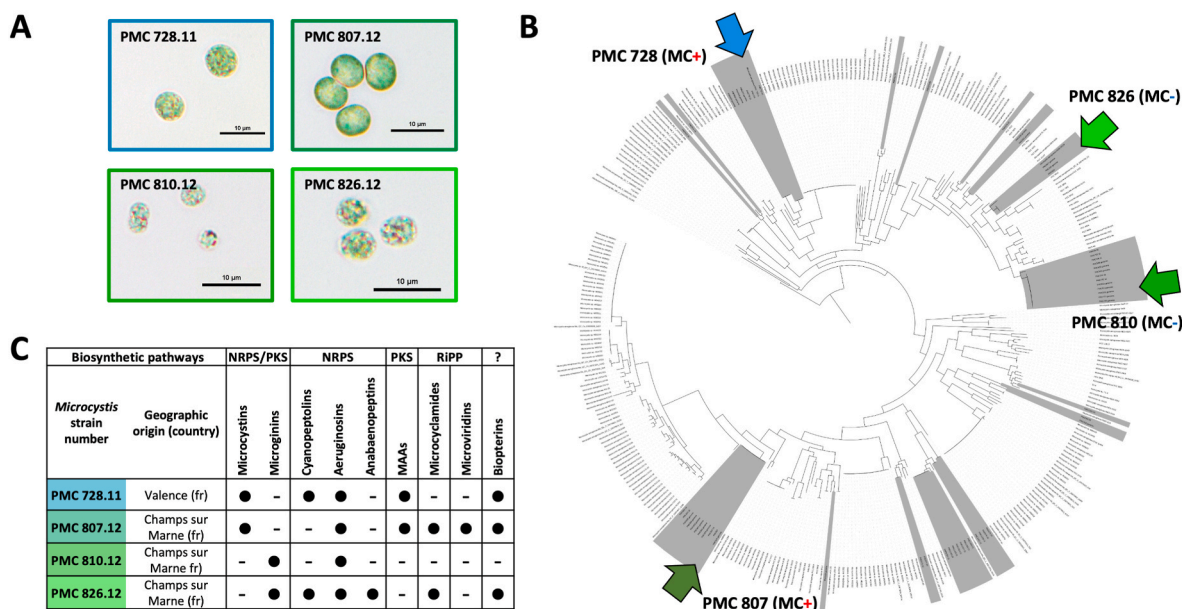


Fig. 1. Diversity of the four *Microcystis* strains selected for the present study, regarding their respective morphology under a light microscope (A), their phylogenetic position within the main genetic clades regarding the whole phylogenomics of known *Microcystis* genomes, with strains available from the Paris Museum Collection (PMC, MNHN) underlined in grey (B), their chemical composition determined by direct detection of metabolite chemical families in 75 % methanol extracts using mass spectrometry (Supplementary Fig. S1), which were further validated by exploring their BGC with AntiSmash 6.0 (C). The PMC *Microcystis* strains collected from various French lakes are highlighted in grey. Dark spots indicate the production of different accessory metabolite families.

Further analyses of their accessory genomes and metabolite profiles revealed a broad spectrum of bioactive - and in some cases, potentially toxic - secondary metabolites (Supplementary Table S1–S4). Genome mining and metabolomic profiling demonstrated that strains PMC 728.11 and PMC 807.12 produce microcystins (MCs), aeruginosins, and mycosporin-like amino acids, among others. In contrast, strains PMC 810.12 and PMC 826.12 produce microgrinins, aeruginosins, and, to some extent, microcyclamides, along with various other compound families (Fig. 1C; Supplementary Fig. S3). Collectively, these findings underscore the extensive chemical diversity that can exist within the distinct *Microcystis* genotypes (Humbert et al., 2013; Le Manach et al., 2019) and highlight the importance of considering the intra-specific diversity when assessing the (eco)toxicological risks associated with *Microcystis* blooms.

3.2. Toxicity on medaka fish embryos and larvae

We performed two separate toxicological bioassays using Medaka (*Oryzias latipes*) embryos (0–2 days post-fertilization, dpf) and larvae (8–9 dpf) to evaluate developmental toxicity at different life stages (Fig. 2). These tests targeted distinct developmental processes and allowed us to assess both lethal and sub-lethal effects.

In embryos (0–2 dpf), exposure to the PMC 810.12 extract induced significant mortality or developmental arrest. By contrast, extracts from PMC 728.11 and PMC 807.12 showed only minor toxicity, and PMC 826.12 caused no mortality relative to the solvent control. However, the PMC 826.12 extract did result in pronounced teratogenic effects, including tail torsion and head malformation, which were observed in 100 % of exposed embryos (n = 40), when the other three extracts induced no comparable malformations. Larval assays revealed distinct patterns of toxicity. Exposure to PMC 728.11 and PMC 810.12 extracts resulted in rapid mortality –100 % of larvae died within 1–2 h. In contrast, the PMC 807.12 extract caused low toxicity (25 % mortality), while PMC 826.12 showed no observable effect (n = 24).

The apparent discrepancy in toxicity between embryos and larvae - particularly for PMC 728.11 - may be attributed to the different condition states of these developmental stages. Embryos are less

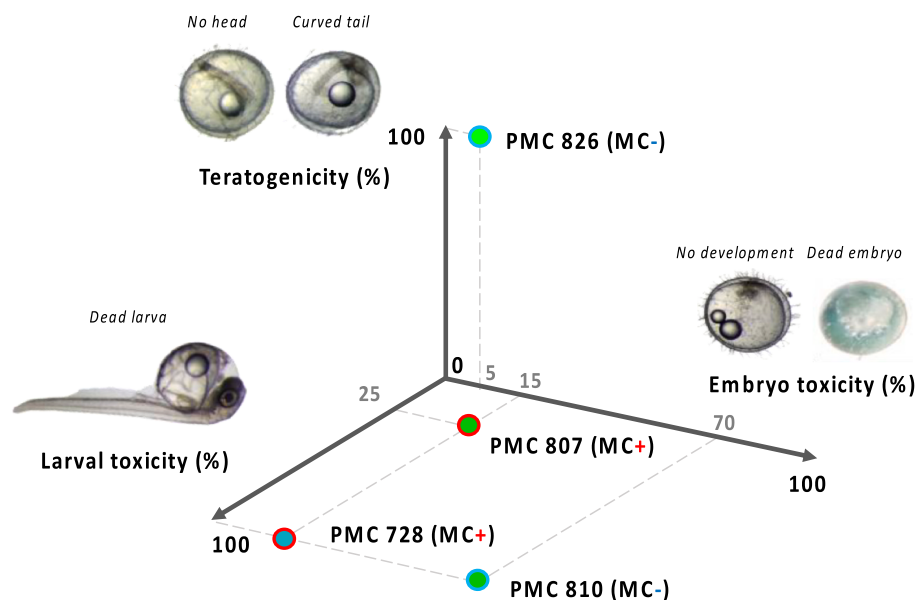


Fig. 2. Synthetic results of toxicity tests conducted on the embryos and larvae of the Medaka fish using four *Microcystis* extracts. The results are expressed as the % age of embryo death or developmental arrest, occurrence of embryo malformations on tail or head, and death of larvae. The red, orange and blue colours present the respective MC content rate of each extract. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

differentiated, and early-stage toxicity likely reflects cytotoxicity affecting cell proliferation or survival. In contrast, larvae possess functional organs and differentiated cell types, which may present a wider array of molecular targets for toxic compounds. This could explain the greater susceptibility of larvae to certain extracts (Gopala Krishnan et al., 2008). An alternative explanation involves the protective function of the chorion, which can act as a barrier preventing large or hydrophilic toxins from reaching embryonic targets (Mandrell et al., 2012; Yang et al., 2020). Large bioactive compounds such as microcystins (MCs) - cyclic heptapeptides - are believed not to be able to pass through the chorion, potentially explaining their reduced toxicity in embryos compared to hatched larvae (Liu et al., 2002).

Interestingly, semi-quantitative MC analyses performed by LC-MS revealed that the PMC 728.11 extract contained above than 10-fold higher levels of MCs compared to PMC 807.12, while PMC 810.12 and PMC 826.12 extracts contained no MC. This observation suggests that other compounds - besides MCs - may account for the embryo lethality observed with PMC 810.12 and the teratogenic effects caused by PMC 826.12. These findings emphasize the wide variability in toxicological responses driven by the production of distinct bioactive metabolite profiles among different *Microcystis* genotypes (Fig. 2).

Several efforts have been made to perform efficient bioassays using model fish embryos to evaluate cyanobacteria toxicity based on the OECD 236 fish embryo acute toxicity test (OCDE, 2013; Oberemm et al., 1997; Oxendine et al., 2006; Berry et al., 2007; Palíková et al., 2007). Considering the various toxicological effects observed in these fish embryonic tests, these works illustrate the potential impacts of *Microcystis* on survival, cellular damage, heart rate, hatching success, body length, yolk edema occurrence behavior, and with the occurrence of severe developmental perturbations and defects (Davidović et al., 2023; Li et al., 2021; Saraf et al., 2018). Although these exposure concentrations are relatively high, they may reflect acute environmental scenarios, such as bloom senescence, when massive cell lysis releases large quantities of intracellular toxins into aquatic systems.

Compounds such as retinoic acids and carotenoid glycosides - previously identified in some *Microcystis* strains (Wu et al., 2012; Jonas et al., 2015; Priebojová et al., 2018) - are known to induce teratogenic effects in fish embryos (Jaja-Chimedza et al., 2017). However, our chemical analysis of the four strain extracts (Supplementary Fig. S3) did

not detect such compounds, highlighting the need for further research to identify the specific metabolite(s) responsible for the teratogenic effects seen with PMC 826.12. Based on our data, candidate *Microcystis* toxic compounds include various cyanobacterial peptides (e.g., microcystins, cyanopeptolins, microginins, microcyclamides), as well as smaller molecules such as retinoic acids or di-tert-butylphenol (Jonas et al., 2015; Fernandes et al., 2019; Almeida et al., 2023; Freitas et al., 2023; Wang et al., 2024). Nevertheless, it is likely that additional toxic compounds produced by *Microcystis* remain to be characterized.

3.3. Adult medaka fish exposure and sublethal effects on the microbiome and metabolome

Six-month-old adult female *Medaka* fish were exposed for four days to live cultures of four *Microcystis* strains at a standardized concentration of $100 \mu\text{g.L}^{-1}$ chlorophyll *a* - a level representative of bloom conditions commonly encountered in temperate freshwater environments during summer months (Marie and Gallet, 2022). This exposure level was selected based on prior experiments with strain PMC 728.11 (Gallet et al., 2023), aiming to induce detectable yet ecologically relevant molecular alterations in the gut microbiome and metabolome (Le Manach et al., 2018). To capture early molecular responses while limiting acclimation and indirect side effects, a short but sufficient exposure duration was used (Foucault et al., 2022). The objective was to compare the strain-specific effects at the level of individual fish (Duperron et al., 2023).

Thus, although no mortality or observable physiological or behavioral changes occurred, significant alterations in both microbiome composition and metabolomic profiles were detected, serving as early indicators of early biological stress (Figs. 3–5; Supplementary Fig. S4–S15; Supplementary Table S5–S8).

High-throughput sequencing of the V3–V4 region of the 16S rRNA gene produced 1,223,933 quality-filtered paired-end reads (mean: 20,398 reads/sample; Supplementary Fig. S4). Microbiome species richness increased in all *Microcystis*-exposed groups, particularly in fish exposed to PMC 826.12 (Supplementary Fig. S5A). At the genus level, dominant taxa included *Cetobacterium*, *Gemmobacter*, *Hyphomicrobium*, *ZOR0006* (Firmicutes), *Aeromonas*, *Rhodococcus*, *Microbacterium*, *Legionella*, and *Terrimicrobium* (Supplementary Fig. S5B–C).

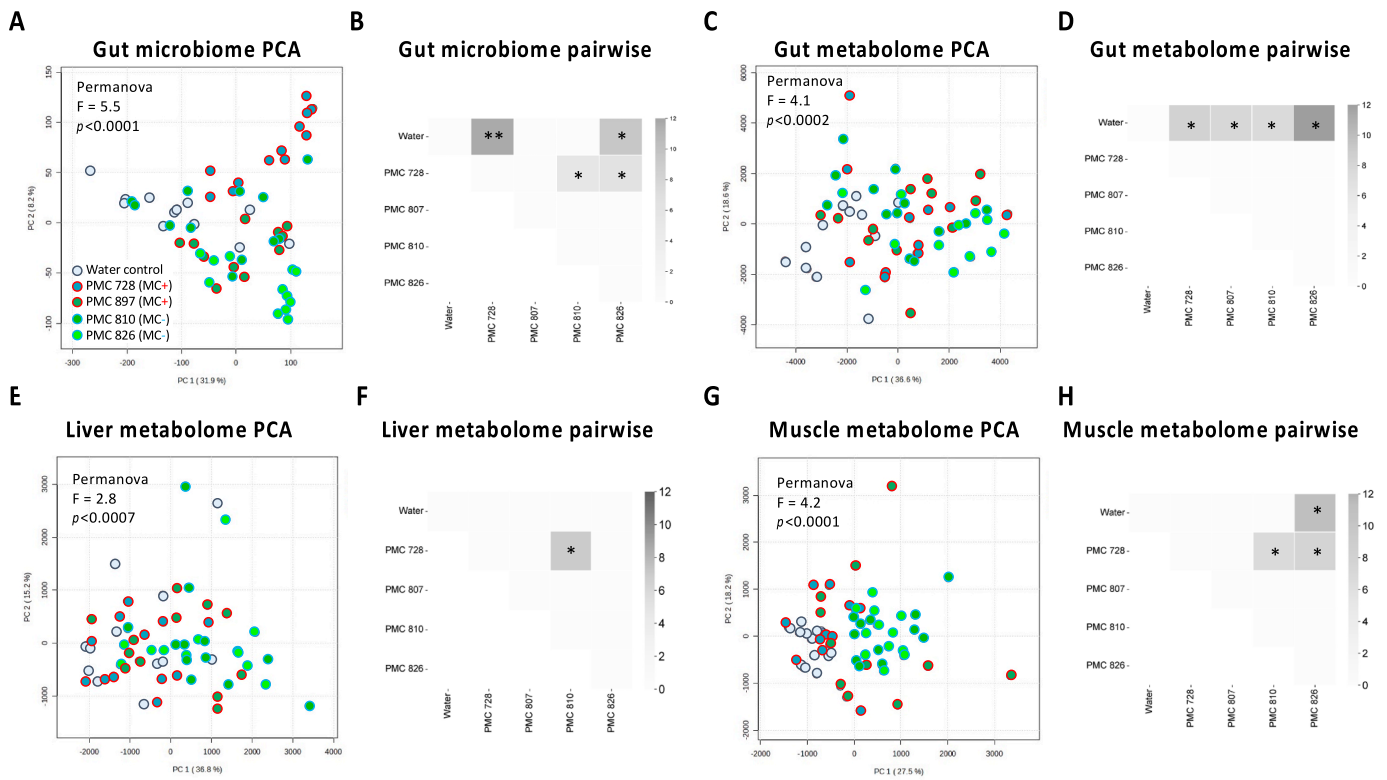


Fig. 3. Unsupervised multivariate analyses of the gut microbiome taxonomic composition (A–B), the gut metabolome composition (C–D), the liver metabolome composition (E–F), and the muscle metabolome composition (G–H). This includes individual plots of principal component analyses (PCA), Permanova (A, C, E and G) and pairwise Adonis analyses (B, D, F and H). * and ** indicate p-values <0.01 and 0.001, respectively.

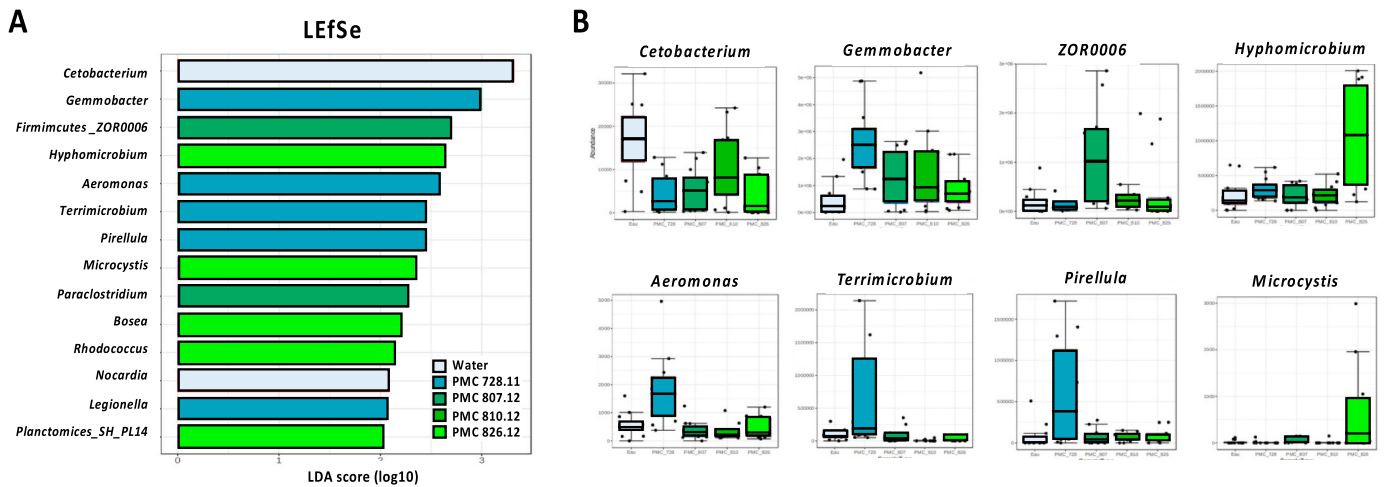


Fig. 4. Effects of exposing young female adult Medaka fish to the different *Microcystis* strains for 4 days on the taxonomic composition (performed here at the family level) of the gut microbiome. This was compared to control fish exposed only to water. The results are displayed on a barplot of LefSe discriminant analysis (Kruskal-Wallis sum rank test $p < 0.01$ and LDA score (\log_{10}) > 2) (A) along with respective boxplots showing the 8 most dysregulated genera (B).

Unsupervised ordinations (Bray–Curtis PCoA and sum-scaled PCA) showed distinct clustering of microbial communities by treatment group (Supplementary Fig. S6; Fig. 3A). PERMANOVA results confirmed significant group differences ($p < 0.0001$), especially between the control and PMC 728.11 or PMC 826.12 exposures (pairwise Adonis, $p < 0.01$ and $p < 0.001$, respectively; Fig. 3B).

Metabolomic profiling via LC-MS detected 1,268, 1,389, and 1994 analytes in gut, liver, and muscle tissues, respectively. Among these, 204 (gut), 149 (liver), and 126 (muscle) were tentatively annotated as LPCs, PEs, amino acids, nucleotides, bile acids, polysaccharides, or glutathione

derivatives using GNPS-supported MS/MS molecular networking (Supplementary Fig. S7). Heatmaps revealed 57, 20, and 50 metabolites with significant group differences (ANOVA, $p < 0.01$) in gut, liver, and muscle tissues, respectively (Supplementary Fig. S8). Hierarchical clustering illustrated clear strain-specific metabolomic profiles, particularly in gut and muscle of fish exposed to PMC 826.12. PCA revealed pronounced group separations of metabolomes (Fig. 3C–H). Notably, gut metabolomes of all *Microcystis*-exposed fish differed significantly from controls (PERMANOVA $p < 0.001$; pairwise Adonis $p < 0.01$; Fig. 3D). Muscle metabolomes in the PMC 826.12 and PMC 810.12

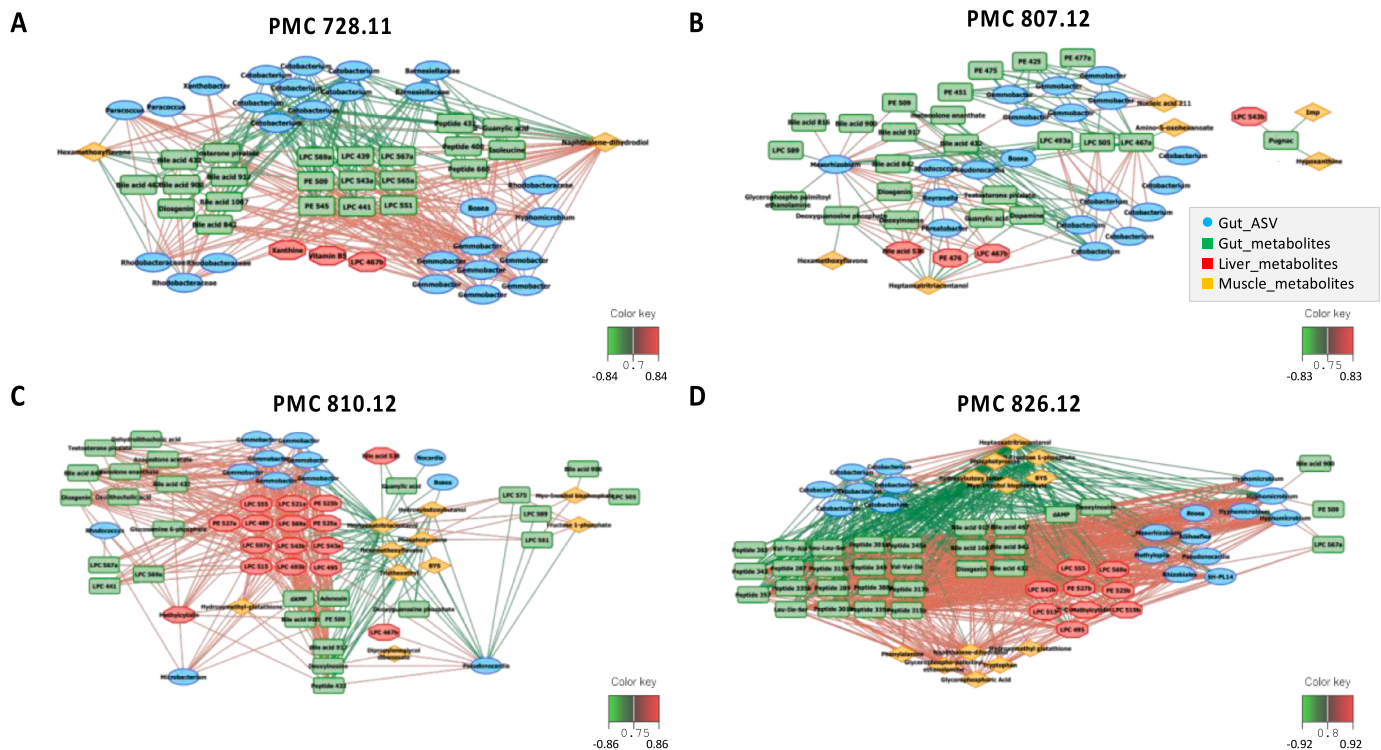


Fig. 5. Relevance network analyses performed with MixOmics illustrating the most correlated metabolites (green, red, and orange for gut, liver and muscle metabolome) and gut ASVs (in blue) that discriminate among the four treatments compared to the control water treatment considered separately with A representing PMC 728.11, B representing PMC 807.12, C representing PMC 810.12 and D representing PMC 826.12. Only variables (ASVs and metabolites) with correlation values above ± 0.7 , 0.75 , 0.75 and 0.8 , respectively, are displayed to highlight the most significant features for each treatment. Pearson correlations between covariate metabolites and ASVs are represented by colored segments (green indicating a negative association, red indicating a positive association). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

groups also differed significantly from controls (pairwise Adonis $p < 0.01$; Fig. 3H), while liver metabolomes only showed significant divergence between PMC 728.11 and PMC 810.12 (Fig. 3F). Gut and muscle samples showed the most marked differences (F-values 4.1 and 4.2, respectively), whereas liver F-values were lower (~ 2.8), indicating more subtle metabolic shifts. Interestingly, gut microbiome composition showed an even stronger effect ($F = 5.5$). Among the tested strains, PMC 826.12 and PMC 728.11 induced the most significant and marked metabolomic changes (Fig. 3). Overall, these microbiome and metabolome shifts underscore the strain-specific nature of *Microcystis*-induced responses.

Additional differential analyses were conducted at the levels of ASV or genus and of the different metabolite to better characterize these effects (Figs. 4 and 5; Supplementary Fig. S9).

LefSe analysis identified taxa with significantly altered abundance across groups (Fig. 4A). The most consistently dysregulated taxon was *Cetobacterium*, which declined markedly in all *Microcystis*-exposed groups (Fig. 4B). As a key producer of vitamin B12 and a core member of healthy fish microbiota (Tsuc hi ya et al., 2008; Ma et al., 2019; Duval et al., 2022), its decline may indicate dysbiosis. Notably, *Cetobacterium*—a potential gut resident taxa (Supplementary Figs. S10–S11) — was previously found to present stable abundance in fish exposed to purified microcystin-LR or to extracts of *Microcystis* strains (Duperron et al., 2023),⁶⁷ suggesting that the direct exposure to live cultures induces broader effects. In contrast, *Gemmobacter* — another current bacterium of the gut microbiota (Supplementary Fig. S11) (Sheu et al., 2013; Siriypagouder et al., 2018) — increased in abundance across all treatments, especially in fish exposed to PMC 728.11 (Fig. 4B). Whereas *Gemmobacter* are not so far considered as directly pathogenic on their own, their rise coincided with increases in potentially pathogenic taxa such as *Aeromonas* and *Legionella*, a pattern also observed

previously in similar experiments (Gallet et al., 2023) and might correspond to a signature of dysbiotic conditions (de Almeida Torres et al., 2023). Similar increases in opportunistic pathogens associated with dysbiotic bacterial signatures have been reported in zebrafish exposed to *M. aeruginosa* (Qian et al., 2019).

In addition, ZOR0006 (Firmicutes), a carbohydrate-degrading commensal, exhibited variable responses: it decreased with PMC 728.11 — as previously observed (Gallet et al., 2023; Foucault et al., 2022) — and PMC 826.12 exposures, but sharply increased in the PMC 807.12 group. This suggests, without being demonstrative, that PMC 807.12 may promote beneficial commensals, in contrast to the other strains. Unexpectedly, the gut microbiota of fish exposed to PMC 826.12 also showed high relative abundances of *Hyphomicrobium* and *Microcystis* ASVs (Fig. 4). While detection of *Microcystis* DNA within the gut after exposure is not surprising, its presence only in PMC 826.12-treated fish suggests the occurrence of incomplete digestion or survival of the cells during gut passage induced by this specific strain. Previous studies reported the absence of *Microcystis* sequences post-exposures to PMC 728.11, suggesting the occurrence of effective degradation (Gallet et al., 2023; Foucault et al., 2022). This may reflect differences in digestibility between strains. Additional analyses, including PLS-DA (Supplementary Fig. S12), are discussed further in Supplementary Text S7.

3.4. Multi-omic integration of the fish molecular responsiveness to *Microcystis* exposure

A comprehensive integration of the dataset — including gut microbiome profiles and gut, liver, and muscle metabolomes of fish exposed to four *Microcystis* strains and a water control — allowed for the construction of a correlation network (Supplementary Fig. S13). This multi-omic analysis highlights the global interplay between microbiome and

metabolome features in response to *Microcystis* bloom conditions (Supplementary Fig. S14). While this approach does not establish causality, it effectively reveals coordinated variations between gut bacteria and metabolites across different organs.

Key findings from this integrative analysis include: 1/negative correlations between seven *Cetobacterium* ASVs and nine cholic acids in the gut; 2/negative correlations between seven *Gemmobacter* ASVs and various gut lipids, including LPCs and PEs; 3/positive correlations between *Microcystis* and *Hyphomicrobium* ASVs and 20 gut peptides. These patterns suggest systemic perturbations in the fish gut environment under *Microcystis* stress exposure. For instance, the depletion of *Cetobacterium* may be compensated by increased cholic acid production to facilitate digestion. Conversely, *Gemmobacter*, potentially pathogenic, appears to be associated with altered lipid and peptide profiles. The presence of undigested *Microcystis* cells may contribute to these changes, affecting key metabolic markers such as phosphotyrosine, dAMP, and fructose 1-phosphate.

When analyzing each exposure condition separately (*i.e.*, each *Microcystis* strain vs. control), distinct and strain-specific correlation networks emerge (Fig. 5A–D): PMC 728.11 exposure (Fig. 5A) is characterized by numerous negative correlations between *Cetobacterium* ASVs and several gut LPCs and cholic acids, alongside positive correlations between *Gemmobacter* ASVs and various LPCs, PEs, and peptides. Notably, some muscle metabolites, such as naphthalene-dihydrothiol and hexamethoxyflavone, occupy central nodes within the network, showing broad associations with both microbial and metabolite features across multiple tissues.

Differently, PMC 807.12 exposure (Fig. 5B) results in a less complex network with fewer correlations and features involved, indicating a comparatively subdued molecular response. As a limitation of the present global omic approach, these observations of the faint specific effects induced by the PMC 807.12 strain exposure does not provide evidence on the potential beneficial metabolic role of potential carbohydrate-degrading commensal bacteria that would require targeted analyses and be the subject of further investigations.

PMC 810.12 exposure (Fig. 5C) shows positive correlations between seven *Gemmobacter* ASVs and multiple gut metabolites, including three LPCs and eight cholic acids, as well as ten LPCs and three PEs from the liver. This points to a coordinated metabolic shift across organs.

PMC 826.12 exposure (Fig. 5D) reveals a distinct and highly structured correlation network. Here, seven *Cetobacterium* ASVs are negatively correlated with 19 gut peptides and six cholic acids, while *Hyphomicrobium* ASVs show positive correlations with these same metabolites, in addition to six LPCs and two PEs from the liver. Moreover, *Hyphomicrobium* is linked to various amino acids, saccharides, and nucleic acids from muscle tissue, as well as to other gut bacterial genera such as *Bosea*, *Pseudonocardia*, and *Rhizobiales*.

These results underscore the complex and strain-specific interactions between the gut microbiome and host metabolism in response to *Microcystis* exposure. They highlight the importance of considering both microbial and metabolic dimensions when assessing ecological toxicity and organismal health under bloom conditions. However, as a limitation, one should also keep in mind that in the present study several technical aspects could lead to slight variations in the respective proportion of the *Microcystis* metabolite cocktails to whose fish embryo/larvae and adult Medaka were exposed. These include the cyanobacterial culture media (Z8 and BG11, respectively), the selectivity of extraction procedure (75 % methanol) and the physiological condition of the cultures that were presently maintained in the exponential growing state. So far, even if we assume they might not be critical, we may still do not fully evaluate the effects of those variations on the final toxicological effects observed.

A precision ecotoxicology approach that integrates multiple biological compartments at the individual level offers unprecedented insights into organismal responses to environmental stressors (Brooks et al., 2024). In this study, it enabled the comparison of strain-specific effects

of different *Microcystis* isolates on both physiological impairments and gut microbiota dysbiosis (Marie, 2020). Furthermore, a microbiome-aware ecotoxicology - accounting for the holobiont concept - emphasizes the critical role of the microbiota as a dynamic interface between the host organism and its environment (Duperron et al., 2020). Disruptions to the gut microbial community are often accompanied by metabolic variations in the gut metabolome. These shifts suggest the onset of dysbiosis, with possible physiological consequences that may extend beyond the gut and affect other organs. Although the liver metabolome presently displayed only modest changes - likely due to the short exposure duration (four days) - the muscle metabolomes exhibited more pronounced and distinct molecular signatures. These molecular signatures observed in the muscle may serve as valuable biomarkers of fish stress.

Our findings confirm that the *Microcystis* strain PMC 728.11 produces distinct accessory metabolites that are likely involved in the observed alterations of the gut microbiota (Gallet et al., 2023; Foucault et al., 2022). Notably, PMC 728.11 produces over 10 times more microcystins (MCs) than PMC 807.12, which may partly explain the magnitude of its effects. Among the secondary metabolites identified, microcyclamides (also described as aerucyclamides) are known to possess antimicrobial properties (Martins et al., 2015; Portmann et al., 2008) and may directly affect susceptible bacterial taxa in the intestinal lumen, as previously suggested (Gallet et al., 2023).

Conversely, the PMC 826.12 strain induces markedly different and more severe early disruptions at both the microbiome and metabolome levels. This strain is associated with a significant decline in *Cetobacterium* and a striking increase in *Hyphomicrobium* and *Microcystis*, alongside elevated levels of various peptides and cholic acids in the gut, as well as increased concentrations of LPCs and PEs in the liver and several small metabolites in the muscle. These findings underscore substantial and organ-specific molecular responses triggered by distinct *Microcystis* strains, affecting both the microbiome and metabolome. Additionally, the data suggest that digestive processes may be disrupted in fish exposed to PMC 826.12 (Supplementary Fig. S15–S16), although the exact mechanisms and responsible compounds remain unidentified. These aspects are further discussed in Text S8.

With regard to the transfer of cyanobacterial metabolites across fish compartments (gut, liver, muscle, and faeces; Supplementary Fig. S17), semi-quantitative LC-MS analysis revealed that fish exposed to PMC 826.12 harbored markedly higher levels of cyanobacteria-specific metabolites - including cyanopeptides such as microcyclamides, microginins, anabaenopeptins, cyanopeptolins, and microcystbiopterins - in their gut, liver, and muscle tissues compared to those exposed to the other three strains. Furthermore, faeces from these fish showed significantly elevated levels of these metabolites, suggesting a higher degree of organismal exposure and reduced biodegradation during the digestive transient.

Together, these findings suggest that the disruption of potentially protective gut bacterial taxa may impair cyanopeptide biodegradation within the digestive tract. This disruption likely results in greater systemic transfer of toxic or bioactive metabolites to internal organs, as well as increased excretion in faeces.

3.5. Ecotoxicological implications for natural *Microcystis* blooms

By comparing the deleterious effects induced by distinct strains of *Microcystis* - one of the most prevalent harmful cyanobacteria (Harke et al., 2016) - we demonstrate how the chemical diversity of bioactive metabolites can differentially impact aquatic organisms repeatedly exposed to blooms throughout their life cycle. Our multi-endpoint approach evaluates both acute effects on embryos and larvae, as well as environmentally relevant dose effects on adult medaka fish, revealing a broad spectrum of potential perturbations specifically induced by different *Microcystis* chemotypes.

Notably, we observed significant toxicity and teratogenicity in

chorion-bearing embryos following exposure to extracts of the non-microcystin (MC) producing strains PMC 810.12 and PMC 826.12, respectively. In contrast, the MC-producing strain PMC 728.11 induced specific toxicity in chorion-free larvae. Additionally, PMC 728.11 and PMC 826.12 (and to a lesser extent PMC 810.12) caused marked dysregulation of the gut microbiome and metabolome in adult fish. Metabolites produced by the PMC 826.12 strain, in particular, appear to inhibit gut digestive processes, resulting in reduced biodegradation of cyanobacterial compounds. Conversely, the PMC 807.12 strain, which contains MC levels over ten times lower than PMC 728.11, exhibited minimal embryonic and larval toxicity, low cyanobacterial metabolite accumulation, and a notable enrichment of *ZOR0006* - a potential commensal-to-mutualistic gut bacterium.²⁹

Collectively, these findings illustrate that microcystins are not the sole toxic metabolites of concern in *Microcystis* blooms. Various other bioactive compounds may be responsible for the diverse strain-specific effects observed. The larval toxicity induced by PMC 728.11 likely stems from its high MC content, which is presumed not to penetrate the chorion barrier in early embryos. In contrast, the toxicity associated with PMC 810.12 in both embryos and larvae is likely due to other compounds capable of crossing the chorion. This strain produces a wide array of secondary metabolites - including microginins, cyanopeptolins, aeruginosins, anabaenopeptins, microcyclamides, and biopterins - as well as unidentified metabolites revealed through genome mining and metabolomics. These compounds, often of lower molecular weight and polarity than MCs or cyanopeptolins, may be more permeable through the chorion.⁷ Alternatively, although not yet demonstrated, active transport mechanisms on the embryo surface may facilitate uptake (Qiao et al., 2019; Singo et al., 2017). In addition, the teratogenic effects induced by PMC 826.12 in early embryos do not appear to be linked to known retinoid family compounds, as none were detected in metabolomic analyses. However, although our joint metabolomic/genome mining approach allows a rapid cross-validated annotation of the main metabolite classes produced by the different *Microcystis* strains, it does not deliver a full list of firmly identified metabolites, that still requires standard molecule validation based on reference material which remain to be produced.

To deepen our understanding, long-term chronic exposure studies using environmentally relevant concentrations of various *Microcystis* strains or genotypes are needed. Such studies would provide better insight into their effects on fish life-history traits, including developmental success, growth, behavior, and overall ecological fitness (Falushynska et al., 2021; Cai et al., 2023). Considering that natural blooms often consist of diverse *Microcystis* genotypes (Briand et al., 2009; Pérez-Carrascal et al., 2019) - each producing a unique metabolic cocktail (Lifshits and Carmeli, 2012) - they likely present multiple and strain-specific risks to fish and other aquatic organisms (Maric et al., 2020). However, accurately predicting the ecological consequences of *Microcystis* blooms on different fish populations and ecological guilds remains challenging (Drovac et al., 2021; de Almeida et al., 2024). Both MC-producing and non-MC-producing genotypes have demonstrated deleterious, albeit sometimes mechanistically distinct, effects (Le Manach et al., 2018; Sotton et al., 2017b). This highlights the necessity of broadening ecotoxicological assessments beyond microcystins to include other potentially harmful metabolites (Janssen, 2019).

Moreover, the lack of a consistent correlation between cyanobacterial biomass, MC content, and fish tissue contamination (Drovac et al., 2016) suggests more complex interactions than previously assumed. It is increasingly important to recognize that natural blooms may produce bioactive compounds - including inhibitors of digestive enzymes - in addition to traditional cyanotoxins such as MCs. These compounds may impair digestion of cyanobacterial cells within the fish gut, reducing toxin degradation, but also impacting the absorption across the intestinal epithelium, and ultimately influencing the systemic accumulation of cyanotoxins within tissues.

4. Conclusion

This study underscores the importance of considering the intrinsic genotypic diversity of natural *Microcystis* blooms - and their associated metabolite profiles - when evaluating ecotoxicological risks. As the complexity of cyanobacterial metabolite production continues to be revealed, it becomes increasingly clear that a one-size-fits-all assessment centered solely on microcystins is insufficient.

Emerging ecotoxicological challenges now call for a deeper understanding of several key aspects: 1/Deciphering mixture toxicity, the need to unravel the effects of complex cocktails of cyanobacterial bioactive metabolites and to predict their combined toxicological impacts; 2/Assessing cumulative exposure, recognizing that repeated or successive bloom events may exert additive or synergistic effects on fish physiology, potentially leading to chronic health impairments; 3/Evaluating species-specific and community-level impacts, identifying how diverse fish species respond to different metabolite profiles and assessing the broader consequences on fish guilds and overall aquatic ecosystem functioning.

Together, these considerations highlight the need for a more nuanced, integrative ecotoxicology framework - one that moves beyond microcystin-centric models to account for the full spectrum of cyanobacterial secondary metabolites and their multifaceted ecological effects.

CRedit authorship contribution statement

Benjamin Marie: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Maiwenn Le Meur:** Writing – review & editing, Writing – original draft, Formal analysis. **Charlotte Duval:** Writing – review & editing, Resources, Methodology, Formal analysis, Data curation. **Manon Quiquand:** Writing – review & editing, Writing – original draft, Validation, Resources, Formal analysis. **Emilie Lance:** Writing – review & editing, Validation, Supervision, Funding acquisition, Data curation, Conceptualization. **Sébastien Duperron:** Writing – review & editing, Validation, Supervision, Methodology, Data curation, Conceptualization.

Associated contents

Data are available upon request by the corresponding author.

Competing interests

The authors declare no conflict of interest.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the ANR MC-Tox project, grant ANR CE34-SJ 11008-22 of the French Agence Nationale de la Recherche, and the Tox Model project financed by grant ANSES 22-EST-111 of the Agence National de sécurité Sanitaire de l'alimentation, de l'Environnement et du travail. Maiwenn Le Meur PhD is funded by a DIM One-Health 2.0 grant provided by the Ile-de-France region. The Paris Muséum Collection (PMC) is funded by the Muséum National d'Histoire Naturelle. The mass spectrometry analyses were acquired at the Plateau technique de spectrométrie de masse bio-organique, Muséum National d'Histoire Naturelle, Paris, France. We thank Eglantine Soubrand and Pierre Foucault for their technical assistance. This work benefitted from

the French GDR "Aquatic Ecotoxicology" and RT eTOX frameworks.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2025.127344>.

Data availability

Data will be made available on request.

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