Supporting information

Metabolic changes in fish induced by cyanobacterial exposures in mesocosms: an integrative approach combining proteomic and metabolomic analyses.

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2 supplementary tables
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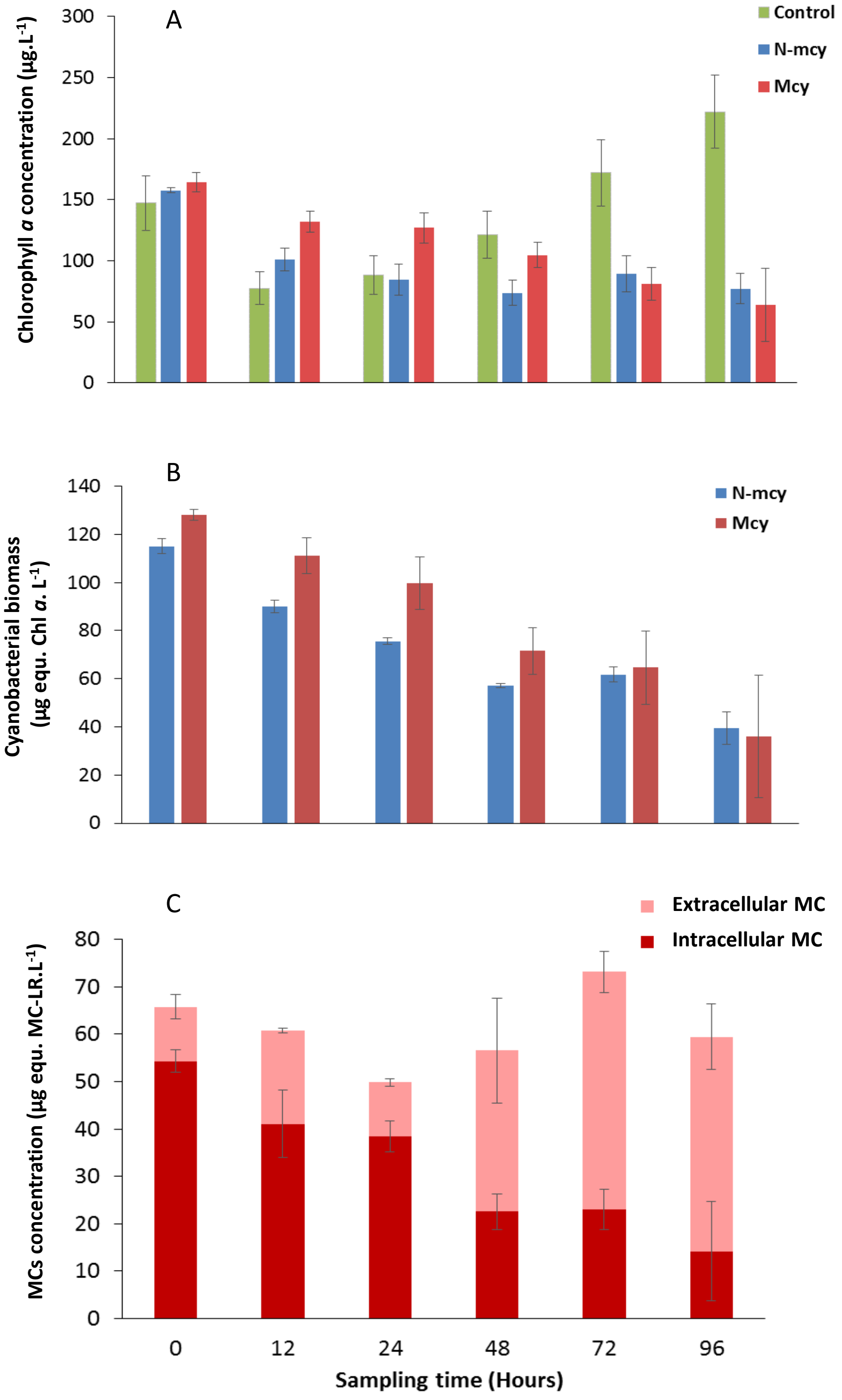
Supplementary Table 1: Complete list of proteins that are significantly dysregulated by cyanobacterial treatments compared to control in female livers.

			Log2(Fold-change)	
Name	Function	Accession Number	N-mcy	Mcy
Calmodulin 3b	Calcium binding	I6L4R5	0.3	0.2
Serum lectin isoform 3	Carbohydrate binding	H2LG01	0.6	0.4
Alpha-amylase	Carbohydrate metabolism	H2N0D4	0.2	0.3
Asialoglycoprotein receptor 2-like	Carbohydrate metabolism	H2MXH8	0.0	0.4
Chitinase acidic 1	Carbohydrate metabolism	H2M494	1.3	0.4
Enolase 1a	Carbohydrate metabolism	H2LLZ8	0.3	0.2
Glutamine-fructose-6-P transaminase 1	Carbohydrate metabolism	H2M6R6	-0.3	-0.3
Glycerol-3-phosphate dehydrogenase 1a	Carbohydrate metabolism	H2MAL3	0.2	0.3
Sialic acid acetylesterase	Carbohydrate metabolism	H2MHU5	0.2	-0.3
Scavenger receptor B2	Cell adhesion	H2L3X8	0.5	0.2
N-myc downstream regulated 1a	Cell proliferation	H2LER5	0.1	0.3
Ubiquitin-like enzyme 7	Cellular process	H2LED8	-0.3	-0.2
Glutathione peroxidase 3	Detoxification	M1H6G2	0.3	0.3
Glutathione S-transferase 1.2	Detoxification	H2M5R1	-0.4	-0.1
Glutathione S-transferase 2	Detoxification	H2LN28	0.3	0.3
Glutathione S-transferase kappa 1	Detoxification	H2M9B4	0.0	0.3
Glutathione S-transferase rho	Detoxification	H2MF28	-0.3	-0.1
Glutathione S-transferase theta 1a	Detoxification	H2N2A9	0.0	0.7
Glutathione S-transferase theta 1a	Detoxification	H2MWI7	-0.5	0.3
Collagen alpha 1b	Extracellular matrix component	H2M6N2	0.1	1.9
Collagen type I alpha 2	Extracellular matrix component	H2LHF2	0.2	1.3
Collagen, type I, alpha 1a	Extracellular matrix component	A8QX86	0.0	1.4
Hydroxysteroid (17-beta) dehydrogenase 4	Gonad development	H2LEQ2	0.2	0.3
Heme binding protein 2	Heme transport	H2MFD7	0.4	0.3
Heme binding protein 2-like	Heme transport	H2LNC6	0.4	0.3
Hemoglobin alpha	Heme transport	H2LKL6	0.3	0.2
Hemoglobin alpha 1	Heme transport	H2LD06	0.5	0.2
hemoglobin beta 1	Heme transport	Q8JIM9	0.3	0.0
Hemopexin WP65	Heme transport	Q8JIP9	0.7	0.0
Myoblogin	Heme transport	I1SRL0	2.4	0.5
Myosin heavy chain 6	Heme transport	C6L8J0	1.2	0.0
Myosin light chain 10	Heme transport	B9ZZQ5	1.2	0.0
D-dopachrome tautomerase	Immunity	H2M5M8	0.3	0.4
Transferrin-a	Ion transport	H2MPL0	0.6	0.2
Annexin A1a	Lipid metabolism	O93446	-0.1	-0.3
Apolipoprotein A-Ia	Lipid metabolism	H2MLX9	0.3	0.1
Apolipoprotein A-II	Lipid metabolism	H2MG18	0.4	0.1
Apolipoprotein Bb	Lipid metabolism	H2MBD1	0.4	0.3
Apolipoprotein Eb	Lipid metabolism	H2MG05	0.4	0.4
Fatty acid binding protein 11a	Lipid metabolism	H2M559	-0.4	-0.4
Fatty acid binding protein 3	Lipid metabolism	H2M7N9	0.5	0.4
Fatty acid binding protein 7a	Lipid metabolism	H2ME97	0.0	-0.3
Fatty acid synthase	Lipid metabolism	A0A0F7G9Q9	0.5	0.2
Prostaglandin D2 synthase 21kDa	Lipid metabolism	H2LQH0	1.0	0.5
Prostaglandin D2 synthase b	Lipid metabolism	H2LSA0	0.6	0.3
Signal sequence receptor alpha	Membrane component	H2LDF6	0.3	0.2
Signal sequence receptor delta	Membrane component	H2LS18	-0.1	-0.3
Transmembrane protein 214	Membrane component	H2MTM5	-0.1	-0.3
3-oxoacid CoA transferase 1a	Metabolism	H2LGX0	-0.4	-0.1
Acetyl-CoA acyltransferase 2	Metabolism	H2LBC1	-0.4	-0.3
Aconitate 3-methyltransferase	Metabolism	H2MV50	0.3	0.3
Acyl-CoA dehydrogenase	Metabolism	H2LR71	-0.4	-0.3
Acyl-CoA oxidase 1	Metabolism	H2L5A2	0.1	0.3
Aldehyde dehydrogenase 9A1b	Metabolism	H2M419	-0.3	-0.1
Aspartate dehydrogenase	Metabolism	H2M788	0.0	-0.4
Biliverdin reductase B	Metabolism	H2L8G7	0.0	0.4
Disulfide isomerase family A2	Metabolism	H2LX92	-0.1	0.5
Disulfide isomerase family A4	Metabolism	H2LSQ4	-0.1	-0.3
Phosphohistidine phosphatase-like	Metabolism	H2MQA7	-0.3	-0.1
Retinol binding protein 1a	Metabolism	H2MAD5	0.4	0.4
Retinol dehydrogenase 11-like	Metabolism	H2LY96	-0.3	-0.3
UDP glucuronosyltransferase 1b	Metabolism	H2M746	-0.3	-0.2
UDP glucuronosyltransferase 5c2	Metabolism	H2MD16	-0.4	-0.2
Catechol-O-methyltransferase 1	Methylation	H2LXJ9	0.1	0.3
Glycine N-methyltransferase	Methylation	H2MY01	0.4	0.3
Phosphoethanolamine methyltransferase	Methylation	H2LCT1	-0.5	-0.3
ES1 protein homolog	Mitochondrion	H2M702	-0.4	-0.2
Histone H1	Nucleosome	H2M8F1	0.4	0.3
Histone H1	Nucleosome	H2LU02	0.0	0.3
Nucleic acid binding protein a	Nucleosome	H2M789	-0.3	-0.2
Adenylate kinase 2	Nucleotide metabolic process	H2LT42	-0.2	-0.3
Adenylate kinase 4	Nucleotide metabolic process	H2M295	-0.3	-0.2
Nucleoside diphosphate kinase 2b	Nucleotide metabolic process	H2MW80	0.6	-0.1
Carbonic anhydrase	One-carbon metabolic pathway	H2LWI8	0.4	0.2
Carbonic anhydrase II	One-carbon metabolic pathway	H2MBN7	0.4	0.3
Methionine adenosyltransferase alpha 11b	One-carbon metabolic pathway	H2LPD1	-0.6	-0.2
Choriogenin H	Ovogenesis	Q8JHT8	-0.1	-0.4
Choriogenin L	Ovogenesis	H2M2R4	-0.1	-0.4
Vitelline membrane outer layer 1a	Ovogenesis	H2LLD6	0.5	0.2
Vitellogenin 1 precursor	Ovogenesis	H2LNM8	0.0	-0.5
Vitellogenin 3	Ovogenesis	H2LQ37	0.0	-0.3
Vitellogenin II precursor	Ovogenesis	H2LP13	-0.5	-0.7
Calnexin	Protein folding	H2MWK1	-0.4	-0.5
Sec23 homolog A	Protein folding	H2MDD7	-0.3	-0.3
Alpha-2-macroglobulin 1	Proteolysis	A0A0F7G9U8	0.3	0.0
Coagulation factor XI	Proteolysis	H2MEU2	0.8	0.4
Complement component 1q	Proteolysis	H2MLW8	1.0	0.3
Complement component 3	Proteolysis	H2MVB4	0.4	0.4
Complement component 9	Proteolysis	H2LCR1	0.3	0.2
Complement factor H	Proteolysis	H2MXP3	0.5	0.3
Kallikrein	Proteolysis	H2MEZ4	0.3	0.2
Metallo-endopeptidase M12	Proteolysis	H2L3F5	0.7	0.4
Proteasome beta 3	Proteolysis	H2MZ13	-0.2	-0.3
Serine protease 59	Proteolysis	A4UWM7	0.3	0.2
Serpin peptidase inhibitor A	Proteolysis	H2MMF6	0.7	0.2
Alcohol dehydrogenase 8a	Redox homeostasis	H2MZR4	0.3	0.3
Aldo-keto reductase family 7A3	Redox homeostasis	H2LHN4	0.3	0.5
Cytochrome C oxidase 2	Redox homeostasis	C6L6H1	-0.5	-0.3
Cytochrome c oxidase VIb2	Redox homeostasis	H2LVT0	0.3	0.1
Cytochrome P450 2A like	Redox homeostasis	H2L6R3	-0.4	0.0
Peroxiredoxin 1	Redox homeostasis	H2M443	0.3	0.4
Spectrin beta chain 1	Redox homeostasis	H2MFE5	0.3	0.3
Glutaminyl-tRNA synthetase	Translation	H2M4U8	-0.3	-0.2
Glycyl-tRNA synthetase	Translation	H2M0E5	-0.3	-0.1
Leucyl-tRNA synthetase b	Translation	H2L846	-0.2	-0.3
Nuclear ribonucleoprotein A0	Translation	H2L3F3	0.3	0.3
Ribosomal protein L18a	Translation	H2N0P8	-0.2	-0.3
Ribosomal protein L28	Translation	H2LK88	-0.2	-0.3
Ribosomal protein L3	Translation	H2L7K6	-0.3	-0.2
Ribosomal protein L5	Translation	H2LK51	-0.3	-0.1
Ribosomal protein P2-like	Translation	H2LMY7	0.3	0.0
Ribosomal protein S16	Translation	H2MNR2	-0.2	-0.3
Ribosomal protein S23	Translation	H2MSQ6	-0.4	-0.3
Ribosomal protein S27a	Translation	H2LBD5	0.3	0.3
Ribosomal protein S9	Translation	H2M6L4	-0.4	-0.3

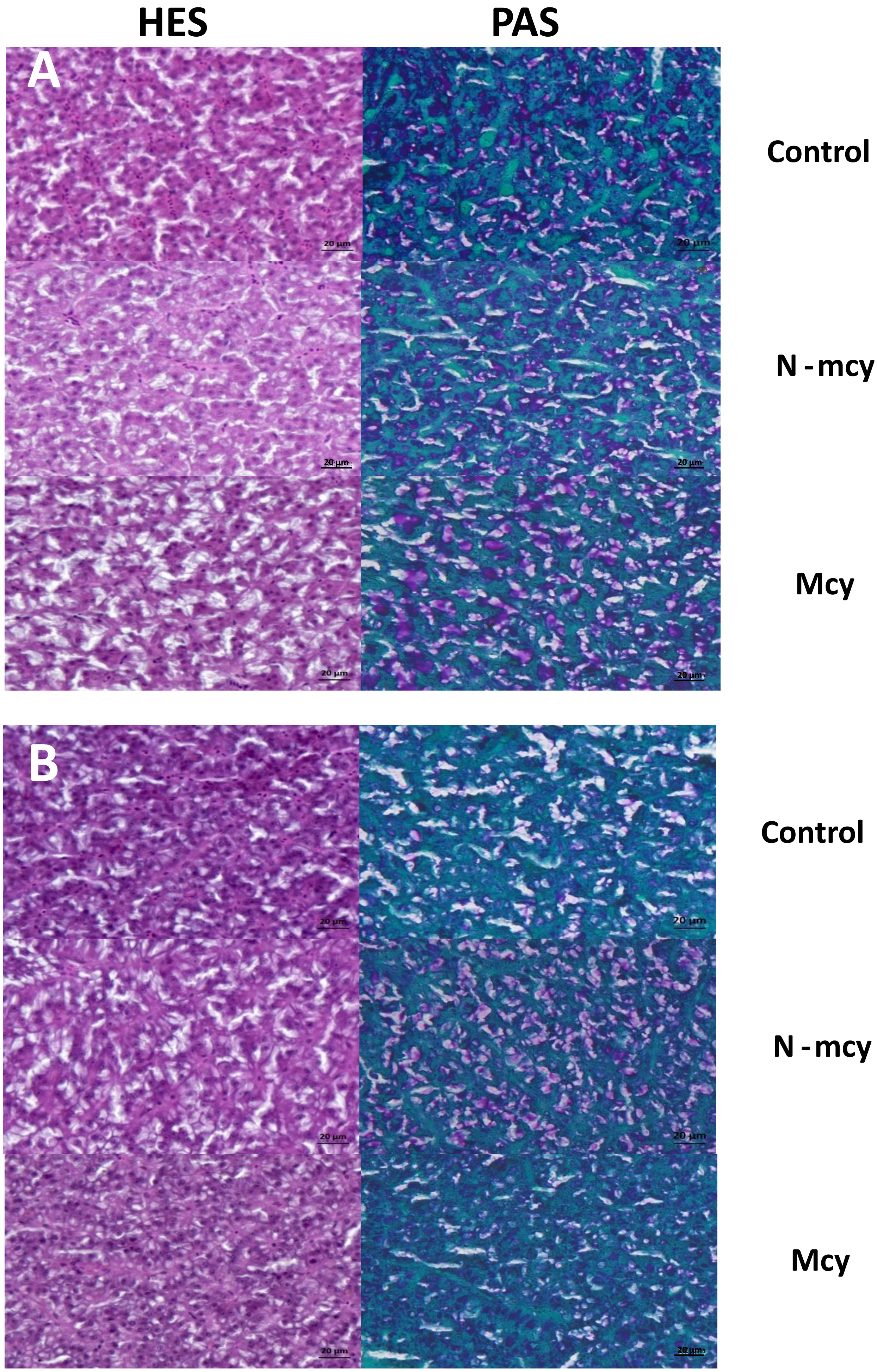
Supplementary Table 2: Complete list of proteins that are significantly dysregulated by cyanobacterial treatments compared to control in male livers.

			Log2(Fold-change)	
Name	Function	Accession Number	N-mcy	Mcy
Glucose-6-phosphate dehydrogenase	Carbohydrate metabolism	H2LRX4	-0.3	-0.1
HSP70 binding protein 21	Cellular process	H2LB83	0.3	0.1
Ubiquitin like	Cellular process	H2TNX7	-0.1	0.3
Glutathione dehydrogenase III	Detoxification	Q6R5I9	0.1	-0.4
Glutathione S-transferase theta 1a	Detoxification	H2MWI7	-0.2	0.4
Collagen alpha 1b	Extracellular matrix component	H2M6N2	1.2	0.6
Myosin heavy chain alpha 6	Heme transport	H2MLX5	-0.2	1.1
Hemoglobin beta 1	Heme transport	Q8JIM9	-0.3	-0.1
Hemoglobin alpha	Heme transport	H2LKL6	-0.4	-0.1
Prostaglandin D2 synthase b	Lipid metabolism	H2LSA0	0.5	0.6
Fatty acid binding protein 3	Lipid metabolism	H2M7N9	0.4	0.2
Prostaglandin D2 synthase 21kDa	Lipid metabolism	H2LQH0	0.3	0.4
Neutral cholesterol ester hydrolase 1a	Lipid metabolism	H2M8K0	-0.2	-0.3
Fatty acid binding protein 10a	Lipid metabolism	A0A0F7G9G7	-0.2	-0.3
NADH dehydrogenase 1 beta 7	Metabolism	H2MIX8	0.3	0.3
Succinate dehydrogenase flavoprotein A	Metabolism	H2LNX5	0.1	-0.3
Acyl-CoA oxidase 1	Metabolism	H2L5A2	-0.1	-0.3
Dimethylarginine dimethylaminohydrolase 1	Metabolism	H2M4Q8	-0.3	-0.3
Retinol saturase	Metabolism	H2LCC0	-0.3	-0.3
Serine protease 59	Proteolysis	A4UWM7	0.7	0.2
Tripeptidyl peptidase I	Proteolysis	H2MLP4	0.3	0.3
Dipeptidyl-peptidase 7	Proteolysis	H2MTU3	0.3	0.2
Alpha-trypsin inhibitor 3b	Proteolysis	H2LAK3	0.3	0.3
Cystatin C	Proteolysis	H2M908	0.3	0.4
Complement factor H	Proteolysis	H2MXP3	0.1	0.3
Spectrin beta 2	Redox homeostasis	H2LT16	0.2	0.7
Cytochrome P450 2W1	Redox homeostasis	H2MLQ7	0.0	0.3
Monoamine oxidase	Redox homeostasis	H2MF43	-0.1	-0.3
Hydroxyacid oxidase	Redox homeostasis	H2MY51	-0.3	0.0
Ribosomal protein P2 like	Translation	H2LMY7	0.4	0.4

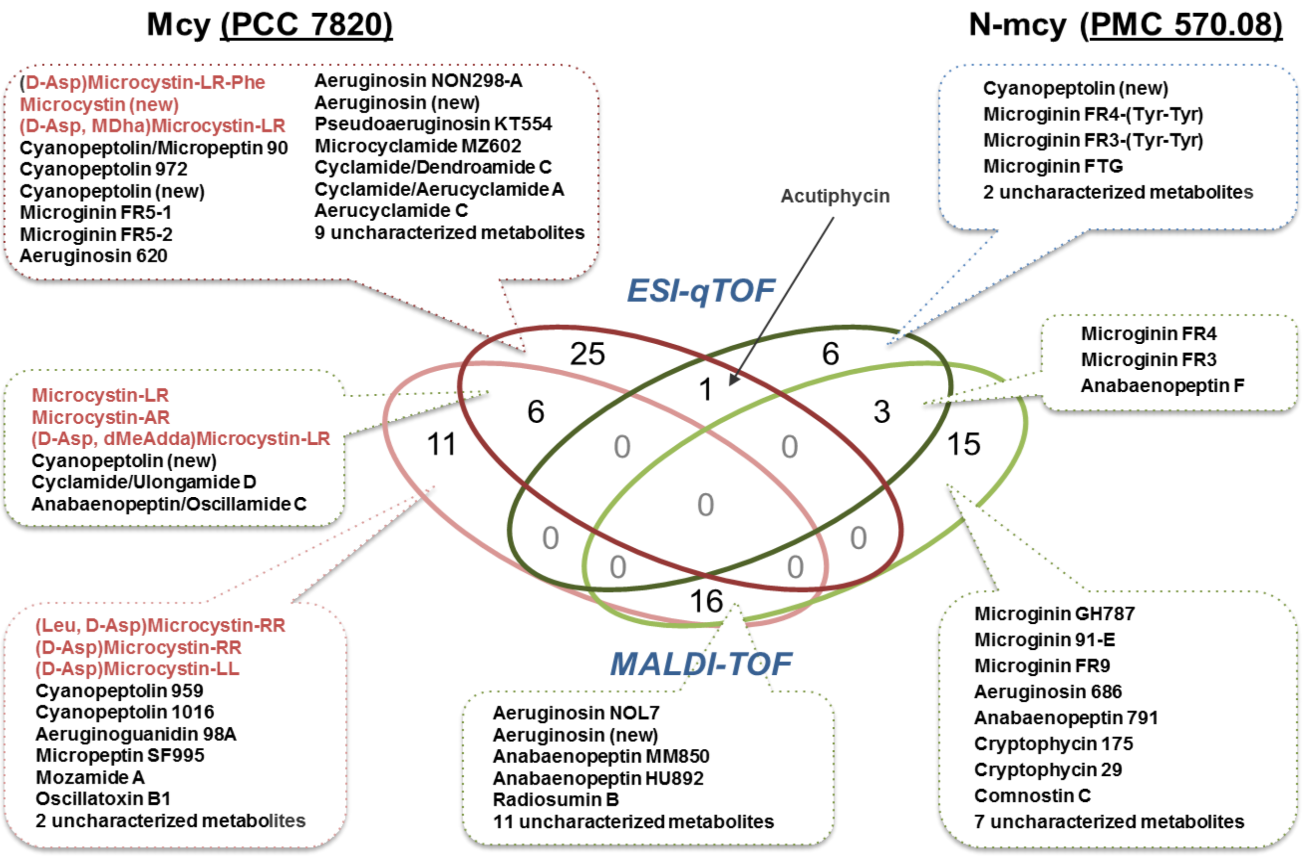
Supplementary Figure S1: Monitoring of experimental parameters linked to the presence of cyanobacteria. (A) Total Chlorophyll-*a* concentrations (µg L^{-1}). (B) Estimated cyanobacterial concentrations (µg eq. chl *a* L^{-1}) measured by BBE probe. (C) Intracellular and extracellular microcystins concentrations (µg eq. MC-LR L^{-1}) measured by ELISA in the treatment containing the MC-producing cyanobacterial strain (no MCs were detected in the other treatments). All data are represented as mean ± standard deviation (SD) measured in tanks (n=3/treatment).



Supplementary figure S2: Light microscopy transverse sections of medaka livers, stained with hematoxylin–eosin–saffron (HES) and Periodic acid–Schiff (PAS). (A) Male and (B) Female medaka.



Supplementary figure S3: Venn diagram of the chemodiversity revealed by LC-ESI-Q-TOF-MS and MALDI-TOF in experimental cyanobacterial strains.



Mcy (PCC 7820)

N-mcy (PMC 570.08)

(D-Asp)Microcystin-LR-Phe
Microcystin (new)
(D-Asp, MDha)Microcystin-LR
Cyanopeptolin/Micropeptin 90
Cyanopeptolin 972
Cyanopeptolin (new)
Microginin FR5-1
Microginin FR5-2
Aeruginosin 620
Aeruginosin NON298-A
Aeruginosin (new)
Pseudoaeruginosin KT554
Microcyclamide MZ602
Cyclamide/Dendroamide C
Cyclamide/Aerucyclamide A
Aerucyclamide C
9 uncharacterized metabolites

Cyanopeptolin (new)
Microginin FR4-(Tyr-Tyr)
Microginin FR3-(Tyr-Tyr)
Microginin FTG
2 uncharacterized metabolites

Acutiphycin

Microcystin-LR
Microcystin-AR
(D-Asp, dMeAdda)Microcystin-LR
Cyanopeptolin (new)
Cyclamide/Ulongamide D
Anabaenopeptin/Oscillamide C

Microginin FR4
Microginin FR3
Anabaenopeptin F

(Leu, D-Asp)Microcystin-RR
(D-Asp)Microcystin-RR
(D-Asp)Microcystin-LL
Cyanopeptolin 959
Cyanopeptolin 1016
Aeruginoguanidin 98A
Micropeptin SF995
Mozamide A
Oscillatoxin B1
2 uncharacterized metabolites

Aeruginosin NOL7
Aeruginosin (new)
Anabaenopeptin MM850
Anabaenopeptin HU892
Radiosumin B
11 uncharacterized metabolites

Microginin GH787
Microginin 91-E
Microginin FR9
Aeruginosin 686
Anabaenopeptin 791
Cryptophycin 175
Cryptophycin 29
Comnostin C
7 uncharacterized metabolites