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Pierre Foucault, Alison Gallet, Charlotte Duval, Benjamin Marie, Sébastien Duperron. Gut microbiota and holobiont metabolome composition of the Medaka fish (*Oryzias latipes*) are affected by a short exposure to the cyanobacterium *Microcystis aeruginosa*. *Aquatic Toxicology*, 2022, 253, pp.106329. <10.1016/j.aquatox.2022.106329>. <hal-04314150>

HAL Id: hal-04314150

<https://hal.science/hal-04314150v1>

Submitted on 29 Nov 2023

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1 **Gut microbiota and holobiont metabolome composition of the Medaka fish (*Oryzias***
2 ***latipes*) are affected by a short exposure to the cyanobacterium *Microcystis aeruginosa***

3 Pierre Foucault¹, Alison Gallet¹, Charlotte Duval¹, Benjamin Marie¹, Sébastien Duperron^{1*}

4

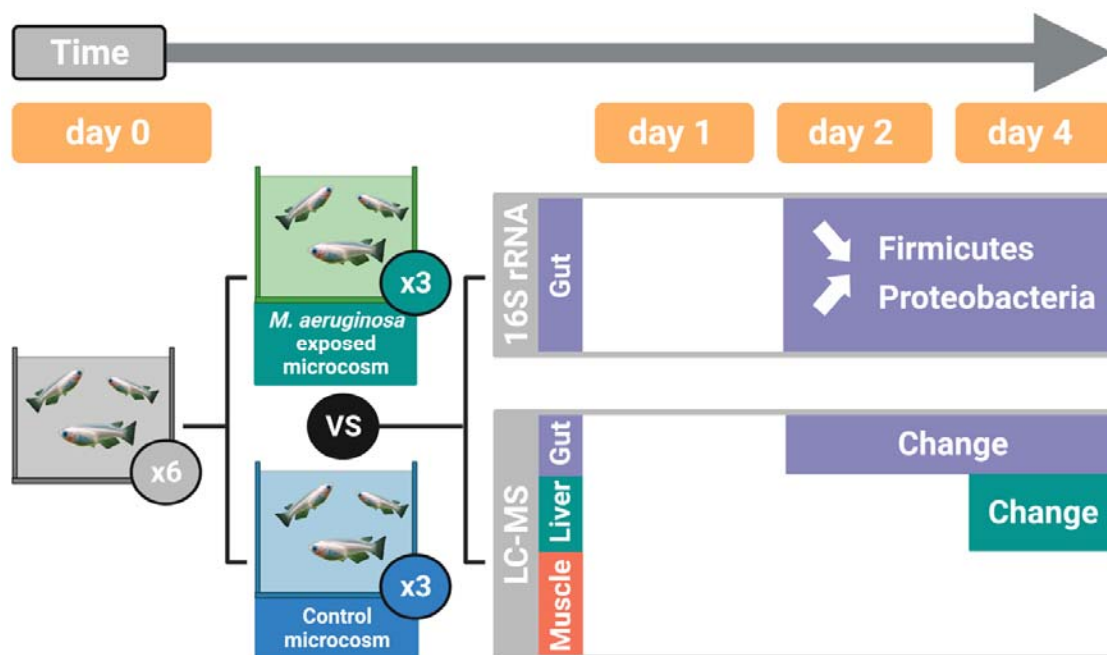
5 ¹UMR7245 Molécules de Communication et Adaptation des Micro-organismes, Muséum

6 National d'Histoire Naturelle, CNRS, Paris, France

7

8 *Corresponding author: sebastien.duperron@mnhn.fr

9 **Graphical abstract**



10

11 **Highlights:**

- 12 - A 2-day exposure to a simulated *M. aeruginosa* bloom is enough to sharply decrease
13 the Firmicute/Proteobacteria ratio in the gut of *Oryzias latipes* fish.
14 - The exposure induced changes in metabolome composition after 2 days in the gut and
15 4 days in the liver.
16 - The gut bacterial microbiota response occurred faster than metabolome's; we
17 hypothesize that changes in gut microbiota may drive the gut metabolome
18 compositional changes.

19 **Abstract**

20 Blooms of toxic cyanobacteria are a common stress encountered by aquatic fauna.
21 Evidence indicates that long-lasting blooms affect fauna-associated microbiota. Because of
22 their multiple roles, host-associated microbes are nowadays considered relevant to
23 ecotoxicology, yet the respective timing of microbiota versus functional changes in holobionts
24 response needs to be clarified. The response of gut microbiota and holobiont's metabolome to
25 exposure to a dense culture of *Microcystis aeruginosa* was investigated as a microcosm-
26 simulated bloom in the model fish species *Oryzias latipes* (medaka). Both gut microbiota and
27 gut metabolome displayed significant composition changes after only 2 days of exposure. A
28 dominant symbiont, member of the Firmicutes, plummeted whereas various genera of
29 Proteobacteria and Actinobacteriota increased in relative abundance. Changes in microbiota
30 composition occurred earlier and faster compared to metabolome composition, suggesting that
31 the microbiota drives the holobiont's response. Liver and muscle metabolome were much less
32 affected than guts, supporting that gut and associated microbiota are in the front row upon
33 exposure. This study highlights that even short cyanobacterial blooms, that are increasingly
34 frequent, trigger changes in microbiota composition and holobiont metabolome. It emphasizes
35 the relevance of multi-omics approaches to explore organism's response to an
36 ecotoxicological stress.

37 **Keywords:** Microbiota; Ecotoxicology; Multi-omics; Time-series; Cyanobacteria; Holobiont

38 1. Introduction

39 One of the most common yet intense stress encountered by fauna in ponds and lakes is
40 the occurrence of algal bloom involving noxious cyanobacteria, which are increasing in
41 relation to anthropogenic effects¹. Adverse effects of blooms, ranging from specific organ
42 toxicity (e.g. hepatotoxicity and cardiotoxicity) to reproduction alteration, are increasingly
43 documented in various animal models²⁻⁵. On the other hand, effects of blooms on associated
44 microbiota are barely known. Beyond their role in holobiont development and functioning
45 (nutrition, immunity, protection, behavior...), host-associated microbial communities are now
46 considered fully relevant to ecotoxicology⁶⁻⁹. Indeed, communities are often located at the
47 interface between a host and its environment. They react to and interact with contaminants,
48 with outcomes ranging from inactivation to potentialization¹⁰. Because of their relative
49 limited diversity of gut microorganisms, notably compared to endotherms, and of their
50 patrimonial and economic value, teleost fish are particularly relevant vertebrate models for

51 aquatic microbiome-aware ecotoxicology⁷. Among these, the medaka *Oryzias latipes* and the
52 zebrafish *Danio rerio* are commonly employed for ecotoxicological studies.

53 Recently, 28-day exposure experiments conducted in microcosms on the medaka
54 revealed that extracts of *Microcystis aeruginosa* containing microcystin-LR (MC-LR) among
55 other bioactive metabolites could trigger dysbiosis in gut microbiota¹¹. Since then, similar
56 effects were shown in the zebrafish^{12,13}. A recent study used microcosm-simulated *M.*
57 *aeruginosa* blooms by exposing fish to high density of a cyanobacterial culture. Results
58 revealed dose-dependent alterations of the composition of medaka gut bacterial communities
59 and the holobiont metabolome, including a sharp decrease in abundance of a significant
60 Firmicutes symbiont¹⁴. Altogether, these studies demonstrate that long exposures to
61 cyanobacteria, together with their subsequent metabolites affect fish microbiota, and at least
62 one of those studies also suggests an alteration of holobiont functions correlated with changes
63 in microbiota composition¹⁴. In nature though, cyanobacterial blooms are often shorter than
64 the typical 20-28 days exposure used in ecotoxicological studies and documenting the early
65 dynamics of changes is the key to understanding holobiont response in natural relevant
66 settings.

67 In this study, we investigated the early response of the medaka gut microbiota and
68 organ metabolomes (gut, liver and muscle) to a 4-day exposure to high yet environmentally
69 relevant density of *M. aeruginosa*, the main species responsible for blooms in temperate lakes
70 and ponds. Compositions of the gut bacterial communities and gut, liver and muscle
71 metabolomes are monitored using 16S rRNA gene sequencing and LC-MS metabolomics,
72 respectively. Alteration dynamics of these compartments are then compared to highlight the
73 key role of gut microbiota in the holobiont functional response. This study is the first attempt
74 to address the initial short-term response of fish gut microbiota and holobiont metabolome to
75 a simulated cyanobacterial bloom.

76

77 **2. Material and methods**

78

79 **2.1 Exposure experiments**

80 Experiments were performed in six aquaria (10-liters each, 3 control and 3 exposed,
81 assigned randomly), each containing 15 specimens of 6-months old adult male medaka.
82 Aquaria were stabilized for one month and fishes acclimatized for one week prior to exposure.
83 At day 0, nine fish in total were sampled randomly across the six aquaria and 25 mL of water

84 from each aquarium were pooled as controls. Fish were then exposed for 4 days to water, or
85 water containing a strong but environmentally relevant density of the live non-axenic mono-
86 clonal *M. aeruginosa* strain PMC 728.11¹⁵ of the Paris Museum Collection¹⁶ to simulate a
87 bloom ($100 \mu\text{g}\cdot\text{L}^{-1}$ Chl *a*). The strain was cultured accordingly to anterior publication^{11,14}
88 (details in the supplementary information). *M. aeruginosa* concentrations were estimated after
89 performing Chlorophyll *a* extractions and absorbance measurements as a proxy using a
90 spectrophotometer¹⁷ (Cary 60 UV-Vis, Agilent). Culture was sampled for DNA (1 mL) and
91 metabolome (50 mL) analyses on days 0 and 2. The *M. aeruginosa* level in exposed aquaria
92 was adjusted on days 0 and 2 to maintain exposure level. Three fish per aquarium were
93 sampled on days 1 and 2 and four per aquarium at day 4 (3 in one aquarium), as well as water
94 samples.

95 Water parameters were monitored on days 0, 2 and 4 (pH, temperature, conductivity,
96 nitrates and nitrites), feces were removed daily by aspiration, and half of the water was
97 replaced with freshwater (2/3 osmosis (RiOs 5, Merck Millipore) and 1/3 filtered) containing
98 or not, adjusted amount of *Microcystis* cells. Fish were exposed to constant temperature (25.4
99 ± 1 °C), pH (7.61 ± 0.1) and conductivity ($246 \pm 12.4 \mu\text{S}\cdot\text{cm}^{-1}$), to low levels of nitrates and
100 nitrites (Table S1) to a 12h:12h light/dark cycle. They were fed twice daily (~3-5% of the fish
101 biomass per day) with Nutra HP 0.3 (Crude protein 57, Crude fat 17, N.F.E 7.5, Ash 10,
102 Crude fiber 0.5, Phosphorus 1.7, Vitamins A, D3, E; Skretting, Norway). Total microcystines
103 (MCs) levels were quantified in duplicates on days 0 and 4 (details in the supplementary
104 information).

105

106 **2.2 Metabolites extraction and characterization**

107 Metabolites were extracted from flash-frozen dissected medaka guts, livers, muscles,
108 and from lyophilized *M. aeruginosa* cultures. Mechanical extraction (GLH850 OMNI; 25 000
109 $\text{r}\cdot\text{min}^{-1}$; 30s) followed by sonication (Sonics Vibra-Cell VCX 13; 60% amplitude; 30s) were
110 performed on weighted samples suspended in the extraction solvent (75-25% UHPLC
111 methanol-water, 1 mL per 100 mg of tissue or per 10 mg of lyophilized culture, on ice). After
112 centrifugation (10 min; 4 °C; 15,300 g), gut pellets were dried and used for subsequent DNA
113 extraction¹⁴.

114 Supernatants containing metabolite extracts were analyzed by Ultra high-performance
115 liquid chromatography (UHPLC, Elute Bruker) using a Polar Avance II 2,5 pore C18 column
116 ($300 \mu\text{L}\cdot\text{min}^{-1}$, Thermo) coupled with a high-resolution mass spectrometer (ESI-Qq-TOF,
117 Compact Bruker) at 2 Hz speed on positive simple MS mode. Feature peak lists were

118 generated from MS spectra within a retention time window of 1-15 minutes and a filtering of
119 5000 counts using MetaboScape 4.0 software (Bruker). The peak lists consisted of the area-
120 under-the-peaks of extracted analytes from the three tissues (medaka's gut, liver and muscles)
121 sampled on days 0, 1, 2, 4 and the *M. aeruginosa* lyophilized culture. A log transformation
122 was applied to metabolomics datasets. Principal Component Analysis (PCA) were performed
123 using the mixOmics¹⁸ (v6.14.1) R package.

124

125 **2.3 DNA extraction, sequencing and analysis of the V4-V5 region of bacterial 16S rRNA**

126 DNA was extracted from the pellets of the guts and *M. aeruginosa* culture, and from
127 0.22 μm filters for aquarium water. Extractions were performed using the ZymoBIOMICS
128 DNA Mini-prep kit with a FastPrep 5G beat beater disruption (DNA Matrix; 4x30s; 6m.s⁻¹)
129 following manufacturer's instructions. An extraction-blank control sample was also
130 performed. The V4-V5 region of the 16S rRNA encoding gene was amplified using primers
131 515R and 926F¹⁹ and sequenced on an Illumina MiSeq 250x2 bp platform (Biomnigene,
132 Besançon, France). Reads were deposited into the Sequence Read Archive (SRA) database
133 (accession number PRJNA836730 (samples SRR19170691-SRR19170767; Table S2).

134 Sequence analysis including primer removal and quality control was performed using the
135 QIIME2-2021.2 pipeline²⁰. Forward and reverse reads were trimmed at 250 and 200 bp,
136 respectively. Amplicon Sequence Variants (ASVs) were obtained with DADA2 (default
137 parameters) and affiliated with the SILVA 138-99 database. Diversity metrics were computed
138 with the phyloseq²¹ (v1.34.0) R package. Statistical analyses were performed using R
139 packages vegan²² (v2.5-7) and RVAideMemoire²³ (v0.9-79). All values are displayed as
140 median \pm standard deviation.

141

142 **2.4 MEBA analysis**

143 A Multivariate Empirical Bayesian Analysis^{24,25} (MEBA) was performed to discriminate
144 differentially abundant taxa through time and between treatments. Relative abundance tables
145 at the Phylum and Genus taxonomic levels were obtained from phyloseq and analyzed using
146 the MEBA plugin from Metaboanalyst 5.0 (<https://www.metaboanalyst.ca>), with no data
147 transformation and all by-default parameters. Taxa with a MEBA T² score superior to 5 were
148 consider discriminant and further statistically analyzed.

149

150 **2.5 ASV-metabolites correlative networks**

151 A correlative network analysis was performed with DIABLO²⁶, a multi-omics
152 framework created by the MixOmics team¹⁸, to analyze putative correlated dynamics between
153 gut microbiota ASVs and gut metabolites. Briefly, the *block.plsda()* function performs a
154 Pattern Latent Structure Discriminant Analysis which provides a reduced-dimension space
155 with covariance-maximizing axes for each dataset (named “block”). A correlation score for
156 the given blocks was computed with the *plotDiablo()* function.

157

158 **2.6 Comparative dynamics of microbiota and metabolome**

159 The composition change dynamics in microbiota versus metabolome were investigated
160 by comparing trajectories of centroids using a newly developed method: MOTA (Multivariate
161 Omics Trajectory Analysis). Microbiota and metabolome datasets were log transformed and
162 analyzed separately but in a similar way. Distances between PCAs' centroid coordinates were
163 computed to create a trajectory between each sampling day for the two treatments and
164 displayed as the fraction of the total length achieved at each day (from 0% to 100% between
165 day 0 to 4). Trajectories were plotted for the 16S rRNA versus metabolomic data, allowing
166 comparison of their respective dynamics in the two treatments (details in the supplementary
167 information).

168

169 **3. Results and discussion**

170

171 Concentrations of *M. aeruginosa* varied between 31 and 126 $\mu\text{g.L}^{-1}$ Chl *a* in exposed
172 aquaria, well above values recently documented to alter microbiota composition after a 28-
173 day exposure¹⁴, and are thus appropriate to investigate short-term effect and early response of
174 the holobiont. In these aquaria, MCs levels were high at day 1 and increased at day 4 (12 ± 5
175 to $21 \pm 9 \mu\text{g eq. L}^{-1}$ MC-LR, respectively; Table S1). These conditions, above values reported
176 from extracts used previously¹¹, are expected to produce major changes in a 14-day exposure
177 and are thus appropriate to evaluate short term effects.

178

179 **3.1 Rapid shift in community compositions during exposure to *Microcystis aeruginosa***

180 Analysis of bacterial community compositions based on the V4-V5 region of 16S
181 rRNA-encoding gene from 67 fish guts, 7 waters, 2 *M. aeruginosa* cultures and 1 extraction
182 blank samples yielded 3,702,081 raw reads, of which 73.4% were retained after sequence
183 assembly, denoising and chimera removal. Samples displayed 21,134 to 59,009 reads ($31,638$
184 $\pm 7,000$), except the extraction blank (520 reads; Table S3). After taxonomic assignment and

185 removal of eukaryotes, mitochondria and chloroplasts, sequences clustered into 936 ASVs. Of
186 these, 856 were considered as abundant (they represented at least 1% of reads in at least 1
187 sample). Rarefaction curves reached saturation confirming that our sequencing effort was
188 sufficient to describe most of the bacterial diversity (not shown). Alpha diversity indices did
189 not indicate major changes among microcosm compartments (culture, water and fish guts) or
190 between dates and treatments (Fig S1; Table S4). Although bacterial ASVs richness was
191 higher in fish guts than other compartments and bacterial ASVs richness and evenness
192 increased during the experiment in both exposed and unexposed fish guts, differences were
193 not statistically significant.

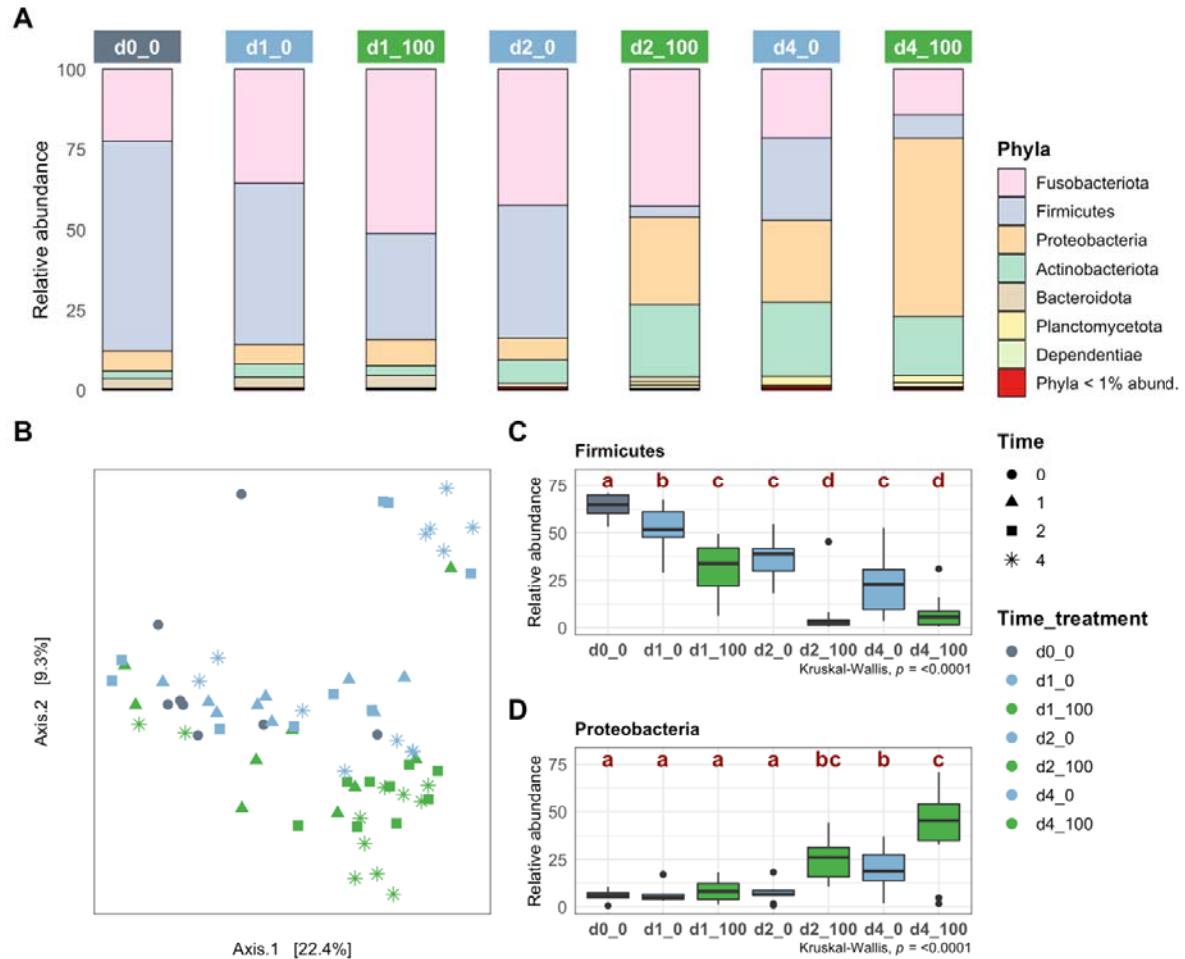
194 Community compositions were significantly different in fish guts compared to water
195 and culture samples (Unweighted UniFrac; Permanova $p < 0.05$; Table S5). Among fish guts,
196 composition differences were significant (Unweighted UniFrac; Permanova $p < 0.05$; Table
197 S5). Visually, samples from different time points are scattered along the first PCoA axis and
198 the two treatments are well separated on the second axis of the PCoA (Fig 1B). Indeed, the
199 microbiota of fish exposed to high density of *M. aeruginosa*, or non-exposed, displayed
200 compositions different from d0 at both d2 and d4. In addition, significant differences between
201 the two treatments were observed at d2 and d4 (Unweighted UniFrac; Pairwise.Permanova
202 $p < 0.05$; Table S5), indicating that gut community compositions were affected by exposure
203 duration (day 0 versus 2 and 4) and treatment (water versus *M. aeruginosa* exposed). Intra-
204 group variances were not significantly different, allowing group comparison (Permdisp
205 $p > 0.05$; Table S5).

206 Firmicutes was the dominant phylum in the fish gut bacterial communities at day 0
207 (64.7 % \pm 6 of reads; Fig. 1A) and was discriminant among time and treatment (MEBA T^2
208 score $>$ 5; Table S6). In *M. aeruginosa*-exposed fish, Firmicutes decreased significantly
209 between day 0 and 1 (64.7 \pm 6% to 33.7 \pm 14.4%; Wilcoxon $p < 0.05$; Fig 1C; Table S6), then
210 day 2 (to 2.91 \pm 14.24 %; Wilcoxon $p < 0.05$; Table S6) after when then remained stable at day
211 4 (Wilcoxon $p > 0.05$; Table S6). Firmicutes decreased less dramatically in unexposed fish.
212 Significant decreases were observed on days 2 and 4 (38.8 \pm 12.3% and 22.78 \pm 16.49%
213 respectively; Wilcoxon $p < 0.05$; Table S6). The gap between exposed and unexposed fish on
214 days 2 and 4 was significant, supporting that the treatment itself further decreased Firmicutes
215 abundance on top of the effect observed in unexposed fish. Among discriminant taxa, this
216 decreasing pattern was only observed with Firmicutes and its main genus *ZOR0006* (Table
217 S6). A similar decrease of *ZOR0006* was observed after 28 days exposure to even moderate
218 levels of *M. aeruginosa*¹⁴. Based on its genome content¹⁴, *ZOR0006*, a dominant resident in

219 guts of healthy medaka¹⁴, can perform lactate pyruvate interconversion, a function essential to
220 the gut homeostasis as lactate generally inhibits the growth of most pathogens while pyruvate
221 stimulates it²⁷⁻²⁹. Lactate can also be involved in the repair of the gut epithelium, yet high
222 levels can also be associated with inflammatory bowel disease in humans^{30,31}. The decrease of
223 Firmicutes has been considered an indicator of dysbiosis²⁴, here suggesting that exposure to
224 *M. aeruginosa* triggers a strong dysbiosis as early as 2 days. The moderate decrease perceived
225 in unexposed specimens could be a consequence of the stress associated with specimen
226 handling³².

227

228 Contrariwise, Proteobacteria were also discriminant (MEBA T² score>5), but their
229 abundances increased significantly from day 2 in *M. aeruginosa*-exposed fish. It was even
230 more intense at day 4 (18.70 ±11.34 to 45.21 ±21.65%; Fig 1D; Wilcoxon $p<0.05$; Table S6),
231 while it was significant only at day 4 for unexposed fish (Wilcoxon $p<0.05$; Table S6). As for
232 the decreasing pattern, the gap between the unexposed and exposed samples on days 2 and 4
233 was significant (Wilcoxon $p<0.05$; Table S6), indicating that the treatment influenced the
234 abundance of the Proteobacteria on top of the effect observed in unexposed fish. Four
235 discriminant genera (*Xanthobacter*, *Reyranella* and *Devosia* belonging to Proteobacteria and
236 *Nocardioides*, phylum Actinobacteriota) displayed a similar pattern (MEBA T² score>5;
237 Table S6). Other discriminant taxa abundances were not significantly altered between
238 treatments on both days 2 and 4 (Table S6).



239
240

Fig. 1: Composition and diversity of fish gut bacterial microbiota among time and treatment.

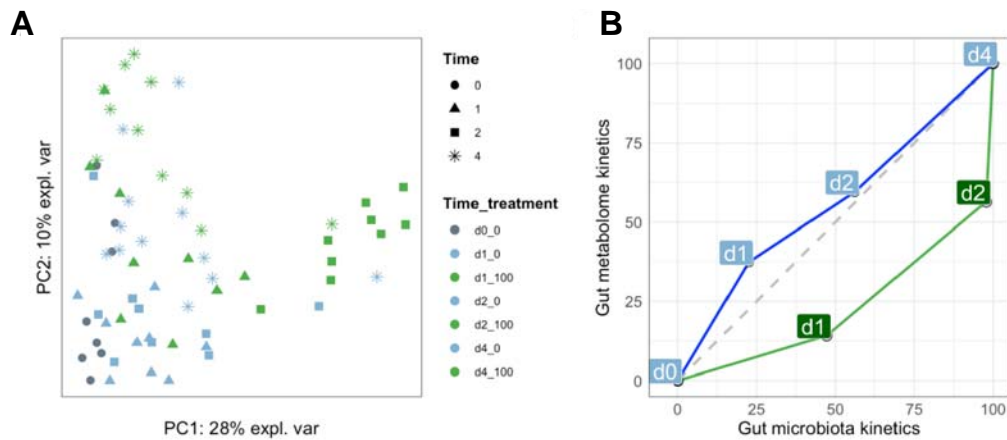
241 **A:** Gut microbiota composition at the phylum level (median values of samples belonging to the same
242 time_treatment group). **B:** Principal Coordinates Analysis (Unweighted UniFrac distance). **C-D:**
243 Relative abundance variations over time in both treatments, observed in Firmicutes (**C**) and
244 Proteobacteria (**D**); letters refer to Benjamini-Hochberg (BH) adjusted Wilcoxon post-hoc test
245 significance.

246

247 3.2 Exposure to *Microcystis aeruginosa* induces changes mostly in gut metabolome 248 composition

249 Mass spectrometry distinguishes 921, 2,521 and 4,190 metabolites present in gut,
250 liver, and muscle samples, respectively. The PCA analysis visually separates gut
251 metabolomes of fish exposed to *M. aeruginosa* for 2 and 4 days, away from other samples
252 (Fig. 2A). Metabolome compositions were significantly altered on days 2 and 4 for exposed
253 fish and, to a lesser extent, unexposed fish (Permanova $p < 0.05$; Table S5). The metabolite
254 compositions between treatments on days 2 and 4 was different (Permanova $p < 0.05$; Table

255 S5), suggesting an additional effect of the treatment to the changes observed in unexposed
256 fish. This assumption is further supported by the observation that 574 of the 916 observed in
257 gut metabolites displayed differential abundances among dates in exposed specimens versus
258 only 152 in unexposed fish (Anova $p < 0.05$; Fig S2A, B). Liver metabolome composition is
259 significantly different between day 4 and other dates, and at this day between treatments,
260 suggesting an effect of both the duration of the experiment and the *M. aeruginosa* exposure
261 after 4 days (Permanova $p < 0.05$; Table S5). Intra-group variances were not significantly
262 different, allowing group comparison (Permdisp $p > 0.05$; Table S5). Finally, composition of
263 muscle metabolomes did not display any significant variation (Permanova $p > 0.05$; Table S5).
264 Thus, the gut functional status appears altered earlier than the livers, while the muscles
265 functional status is not affected. This is not surprising given that cyanobacteria enter fish
266 through oral ingestion and their bio-active metabolites transfer through the intestine, which
267 could buffer their effects before other organs are affected, and thus protect the host during
268 short blooms^{4,33}.



269
270 **Fig. 2:** Dissimilarity between fish gut metabolome samples among time and treatment and
271 composition change dynamic comparison between gut microbiota and metabolome.

272 **A:** Principal Component Analysis of gut metabolite compositions of exposed and unexposed fish over
273 time. **B:** Trajectory of the composition change in microbiota versus metabolome expressed as
274 percentages of the total trajectory achieved on days 0, 1, 2 and 4 on each axis.

275

276 3.3 Do changes in gut bacterial communities drive functional changes in the holobiont?

277 In unexposed fish, the percentages of the total distance achieved by gut metabolome and
278 microbiota compositions are similar on days 1 (22 and 37%) and 2 (56 and 59%), resulting in
279 a trajectory that is close to the 1:1 diagonal (Fig. 2B), possibly reflecting an overall drift of the
280 system due to the aforementioned handling stress³². The shape of the trajectory is different in

281 exposed fish. Indeed, the gut metabolome achieved 14% of the total distance at day 1 versus
282 47% for the microbiota. At day 2, cumulative values are 56% for the metabolome and 98%
283 for the microbiota, suggesting that most of the change ultimately observed in the microbiota
284 composition is achieved after only 2 days. This holds true also when accounting for a higher
285 percentage of the total explained variance (Fig S3). The trajectory thus shifts towards the gut
286 microbiota axis for exposed samples, suggesting a faster response of the gut microbiota
287 compared to the gut metabolome. The gut microbiota bacterial ASVs and gut metabolites
288 datasets were well correlated at both day 2 and 4 (correlation score: 0.91). These results
289 suggests that changes in microbiota might be preceding, and possibly driving observed
290 metabolome changes, a hypothesis congruent with the localization of the gut microbes at the
291 interface between host and its environment (digestive lumen), and thus their role as a primary
292 barrier to contaminants^{7,10}. Although it is tempting to infer causality here, further
293 confirmation is needed since the gut metabolome contains both host and microbiota-derived
294 metabolites, which could amplify the metabolome variations when bacterial communities
295 change a lot.

296 **4. Conclusion**

297

298 Fish gut bacterial community composition and metabolome are affected within the
299 first two days upon exposure to *M. aeruginosa*. Thus, even short cyanobacterial blooms can
300 trigger drastic variations in bacterial phyla abundances, with consequences for the functioning
301 of the gut. This way, iterative short bloom events could lead to major shifts in gut microbiota
302 compositions and its associated functions, for example by progressively eliminating some
303 sensitive resident symbiont lineages, exemplified here by the Firmicutes *ZOR0006*. Although
304 sex-specific responses to perturbations have been observed in ecotoxicological³⁴ and gut
305 microbiota³⁵ studies, only male fish were used here to first test the existence of a short-term
306 response. Further experiments are now needed to address alterations in female fish. This study
307 highlights the relevance of time-series exposure experiments and complementarity of omics
308 approaches, using metabolomics as a proxy of the integrated functional response, to address
309 short and long-term responses of holobionts to ecotoxicological stress, and the respective
310 roles played by host and microbes.

311

312 **Author's contributions**

313 A.G., B.M. and S.D. conceived the study. A.G., C.D. and B.M. and S.D. conceived the
314 experiment. P.F., A.G. and C.D. conducted the experiment. B.M. and S.D. took part in the

315 experiment. P.F., A.G. and C.D. conducted molecular data processing. P.F. B.M., and S.D.
316 analyzed data. P.F., B.M. and S.D. wrote the manuscript. All authors contributed and agreed
317 on the contents.

318

319 **Funding**

320 The study was funded by MNHN through a grant to P.F. (UMR 7245) and ATM grant 3 M
321 awarded to S.D. and B.M.. A.G. is funded through a Ph.D. grant from Ecole Doctorale 227
322 “Sciences de la Nature et de l’Homme”, MNHN.

323

324 **Notes:**

325 Experimental procedures were carried out in accordance with European legislation on animal
326 experimentation (European Union Directive 2010/63/EU) and were approved for ethical
327 contentment by an independent ethical council (CEEA Cuvier n°68) and authorized by the
328 French government under reference number APAFiS#19316-2019032913284201 v1. Fish
329 were anesthetized in 0.1% tricaine methanesulfonate (MS-222; Sigma, St. Louis, MO)
330 buffered with 0.1% NaHCO₃ prior to sacrifice.

331

332 **Acknowledgements**

333 Kandiah Santhirakumar helped managing fish maintenance, Claude Yéprémian advised on
334 cyanobacterial cultivation. We thank the Amagen platform for providing medaka fish, and the
335 PtSMB platform of MNHN for metabolomics.

336

337 **Data availability**

338 All R scripts are available on Github upon publication (<https://github.com/PierreFoucault/>).
339 All microbial communities’ samples sequencing reads were deposited into the Sequence Read
340 Archive (SRA) database (accession number PRJNA836730 (samples SRR19170691 to
341 SRR19170767; Table S1).

342

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491

492 **Supplementary information: supplementary Material and methods, Fig S1 and Fig S2**

493 **Supplementary tables are summarized in a single supplementary file containing the**
494 **following:**

495 Table S1: Parameters monitoring from the microcosm experiment

496 Table S2: SRA accession numbers

497 Table S3: Sample IDs, raw reads counts, Sequencing depth and read filtering

- 498 Table S4: Gut microbiota samples median alpha-diversity indices and significativity group
499 Table S5: Gut microbiota samples alpha and beta diversity metrics; and gut, liver and muscles
500 group comparison scores and significance levels
501 Table S6: Gut microbiota phylum and genera MEBA Hoteling T^2 , Kruskal Wallis and
502 Wilcoxon post-hoc significativity levels (ns: not significant; * <0.05 ; ** <0.01 ; *** <0.001)
503 Tables S7, S8 and Table S9: Gut, liver and muscle metabolites log-normalized count tables