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A test for microbiome-mediated rescue via host phenotypic plasticity in *Daphnia*

René S. Shahmohamadloo[1]*, Amir R. Gabidulin[1], Ellie R. Andrews[1], John M. Fryxell[2], Seth M. Rudman[1]*

[1] School of Biological Sciences, Washington State University, Vancouver, WA, United States
[2] Department of Integrative Biology, University of Guelph, Guelph, ON, Canada

***Corresponding authors:**
René S. Shahmohamadloo, School of Biological Sciences, Washington State University, 14204 NE Salmon Creek Ave, Vancouver, WA 98686, USA. Email: rene.shahmohamadloo@wsu.edu
Seth M. Rudman, School of Biological Sciences, Washington State University, 14204 NE Salmon Creek Ave, Vancouver, WA 98686, USA. Email: seth.rudman@wsu.edu

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Running title: Testing Microbiome-Mediated Rescue in Daphnia

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One sentence summary: *Daphnia* exhibits considerable plasticity in individual and population-level responses to a cosmopolitan stressor, yet shifts in microbiome composition are not correlated with the magnitude of this plasticity.

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44 **Abstract:** Phenotypic plasticity is a primary mechanism by which organismal phenotypes shift in
45 response to the environment. Host-associated microbiomes often exhibit considerable shifts in
46 response to environmental variation and these shifts could facilitate host phenotypic plasticity,
47 adaptation, or rescue populations from extinction. However, it is unclear how much shifts in
48 microbiome composition contribute to host phenotypic plasticity, limiting our knowledge of the
49 underlying mechanisms of plasticity and, ultimately, the fate of populations inhabiting changing
50 environments. In this study, we examined phenotypic responses and microbiome composition in 20
51 genetically distinct *Daphnia magna* clones exposed to non-toxic and toxic diets containing *Microcystis*, a
52 cosmopolitan cyanobacteria and common stressor for *Daphnia*. *Daphnia* exhibited significant plasticity
53 in survival, reproduction, and population growth rates in response to *Microcystis* exposure. However,
54 the effects of *Microcystis* exposure on the *Daphnia* microbiome were limited, with the primary effect
55 being differences in abundance observed across five bacterial families. Moreover, there was no
56 significant correlation between the magnitude of microbiome shifts and host phenotypic plasticity.
57 Our results suggest that microbiome composition played a negligible role in driving host phenotypic
58 plasticity or microbiome-mediated rescue.
59
60 **Introduction**
61
62 Projected biodiversity losses from anthropogenic global change this century (Urban et al., 2016)
63 necessitate an understanding of the magnitude and mechanisms of adaptive phenotypic responses to
64 environmental change (Bellard et al., 2012; Lavergne et al., 2010). Although organisms can rapidly
65 evolve in response to global change (Hendry & Kinnison, 1999; Hoffmann & Sgrò, 2011; Rudman et
66 al., 2022), environmentally induced phenotypic plasticity—defined as the capacity for a given genotype
67 to express different phenotypes when faced with a different environmental challenge (West-Eberhard,
68 2003)—is a primary mechanism by which organisms respond to environmental changes (Ghalambor
69 et al., 2007; Merilä & Hendry, 2014). This plasticity can be adaptive, meaning it can enhance an
70 organism's fitness in response to environmental change, and such adaptive plasticity is a key
71 component of reducing phenotype-environment mismatches that can cause extinction (Bell & Collins,
72 2008; Bell & Gonzalez, 2009). Yet, the mechanisms that produce adaptive phenotypic plasticity are
73 not always well understood.
74
75 The microbiome impacts nearly all aspects of host phenotype (Akbar et al., 2022; Berg et al., 2020;
76 Flandroy et al., 2018; Moran & Baumann, 2000) and extends the host's genetic repertoire
77 (Decaestecker et al., 2024). Host-associated microbiomes both show sensitivity to environmental
78 changes and have demonstrated diverse impacts on host fitness (Decaestecker et al., 2024). The
79 composition of associated microbial communities can influence host performance and relative fitness
80 (Gould et al., 2018), and intraspecific variation in microbiome composition has been observed to
81 affect host physiology and performance in various taxa (Bolnick et al., 2014; Rudman et al., 2019; J.
82 Wang et al., 2015). This variation and its impact on host phenotypes have spurred considerable
83 speculation about the microbiome's crucial role in host population persistence(White et al., 2023) and
84 in extending host adaptive capacities (Henry et al., 2021).
85
86 Microbiome-mediated plasticity—defined as changes in microbiome composition or function that
87 influence host phenotype—has been proposed as a general mechanism that facilitates evolutionary
88 adaptation by reducing mismatches between host phenotype and the environment (Henry et al., 2021;
89 Kolodny & Schulenburg, 2020). Experimental work on sea anemones suggest that microbiome-
90 mediated plasticity can enhance thermal acclimation and that the benefits of such acclimation can be
91 transmitted to the next generation, suggesting microbiota as a mechanism of rapid adaptation to

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92 environmental changes (Baldassarre et al., 2022). Coral microbiomes too may shift to protect against
93 extreme challenges like heat stress and pathogens, underpinning the health and resilience of reef
94 ecosystems (Bourne et al., 2016; Webster & Reusch, 2017). Research using *Caenorhabditis elegans* in a
95 novel environment—though with limited population-level replication—also suggests that adaptation
96 to new conditions can be jointly influenced by host genetics and microbiome composition,
97 highlighting the complex interactions between host and microbiome in shaping evolutionary
98 trajectories (Petersen et al., 2023).
99
100 Cases where microbiome-mediated plasticity impact host population growth and persistence under
101 challenging environments have received particular attention. This has led to the concept of
102 microbiome rescue—defined here as changes in microbiome abundance, composition, or activity that
103 improves host fitness and decreases the likelihood of extinction (Mueller et al., 2020)—and speculation
104 that it may be an important process for the maintenance of biodiversity in rapidly changing
105 environments (Mueller et al., 2020; Shade, 2023). Microbiomes can play a critical role in helping hosts
106 cope with various environmental challenges, including toxicants, by potentially modulating
107 detoxification mechanisms and stress responses (Adamovsky et al., 2018; Kikuchi et al., 2012; G.-H.
108 Wang et al., 2020). However, while these studies highlight the potential for microbiomes to influence
109 host responses to stress, they do not provide direct evidence of microbiome rescue in populations.
110 Limited empirical evidence remains linking shifts in microbiome composition to significant impacts
111 on host population dynamics outside of obligate symbiosis, leaving considerable uncertainty about the
112 strength of any such beneficial effects or the potential for rescue (Mason, 2020).
113
114 Empirical tests of the contributions of shifts in the microbiome to adaptive plasticity are needed to
115 determine whether microbiomes facilitate population persistence or rescue in response to
116 environmental stress. *Daphnia* (water fleas) exhibit observable and quantifiable phenotypic changes in
117 response to environmental fluctuations that are crucial to population dynamics, including alterations
118 in survival rates, morphology, and reproductive strategies (Boersma et al., 1998). The interaction
119 between *Daphnia* and harmful algal blooms (HABs) of the cyanobacterium *Microcystis*—a global
120 environmental challenge (Harke et al., 2016)—is both important for host fitness and environmental
121 health (Hairston et al., 2001; Isanta-Navarro et al., 2021; Shahmohamadloo, Poirier, et al., 2020;
122 Shahmohamadloo, Simmons, et al., 2020). Microbiome transplant experiments have demonstrated
123 that both host genotype and gut microbiota can mediate tolerance in *Daphnia* to *Microcystis* (Macke et
124 al., 2017); host genotypic variation in tolerance disappears when *Daphnia* are made germ-free and
125 inoculated with a standardized microbiome (Macke et al., 2017). In another reciprocal transplant
126 experiment, *Daphnia* performed better when receiving a microbiome from their source region when
127 exposed to toxic *Microcystis*, indicating microbiome-mediated local adaptation in stress tolerance
128 (Houwenhuyse et al., 2021). This effect was most pronounced when donor microbiomes were pre-
129 exposed to toxic cyanobacteria, and it also depended on the pond and genotype of the *Daphnia*
130 (Houwenhuyse et al., 2021). More broadly, the *Daphnia* microbiome exhibits considerable plasticity in
131 both diversity and composition, influenced by the environmental bacterial community and host
132 genotype, among other potential environmental drivers (Callens et al., 2020; Hegg et al., 2021;
133 Houwenhuyse et al., 2021).
134
135 Prior work on *Daphnia-Microcystis* interactions has been instrumental in revealing the mechanisms by
136 which microbiomes can impact hosts, but understanding the implications of these interactions for
137 host plasticity and population dynamics requires different approaches. First, determining the extent
138 of *Daphnia* microbiome shifts, and whether these shifts are repeated and deterministic, is critical to
139 understanding how robust the effects of microbiome changes may be on host phenotypes (Härer &

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140 Rennison, 2022; Stuart et al., 2017). Second, explicitly testing whether the magnitude or direction of
141 microbiome shifts are correlated with overall phenotypic plasticity is key to understanding the
142 contribution of microbiome change to host phenotypic response (Kolodny & Schulenburg, 2020).
143 Finally, measuring the magnitude of an observed shift on host fitness due specifically to microbiome
144 alteration is critical in evaluating the microbiome rescue hypothesis (Mueller et al., 2020; Shade, 2023).
145 Doing so requires measurements of the relationship between microbiome shifts and parameters
146 central to population dynamics. Together these data can provide a test of the role of microbiome shifts
147 in host phenotypic plasticity and population dynamics in changing environments.
148
149 To address key questions regarding the role of the microbiome in facilitating host plasticity and rescue,
150 we conducted an algal toxicity and microbiome study on 20 genetically distinct clones of *Daphnia magna*
151 collected from a single lake. We first catalog the impacts of *Microcystis* exposure on *Daphnia* phenotypes
152 and population growth rate for each genotype. We then tested the following questions: 1) What is the
153 magnitude of *Daphnia* phenotypic response to *Microcystis* exposure? 2) Does *Microcystis* exposure alter
154 *Daphnia* microbiome composition and abundance and, if so, are these changes parallel and
155 deterministic across different genotypes, under varying treatments? 3) Is the magnitude and/or
156 direction of shifts in the microbiome in response to *Microcystis* correlated with the magnitude of
157 adaptive phenotypic plasticity in *Daphnia*? To answer these questions, we conducted a 21-d chronic
158 toxicity exposure experiment across two common gardens (non-toxic, *Chlorella*-only; and toxic, 3:1
159 ratio of *Chlorella* to *Microcystis*) using 20 *D. magna* clones. Clonal replication allows for an assessment of
160 the effects of microbiome shifts across multiple genetic backgrounds and enables projections of
161 population-level responses. We use these fitness-associated phenotypes in each of 20 clones to
162 parameterize population projection models and test for the effects of shifts in the microbiome on host
163 plasticity.
164
165 **Methods**
166
167 *Daphnia magna field collection and culturing*
168
169 In late spring and early summer, 20 genotypes of *D. magna* were obtained from 'Langerodevijver'
170 (LRV; 50° 49' 42.08", 04° 38' 20.60"), a small lake situated within the nature reserve of Doode Bemde,
171 Vlaams-Brabant, Belgium (Orsini et al., 2012). LRV, with a surface area of 140,000 m^2 and a maximum
172 depth of 1 m, features a single basin and experiences seasonal HABs of *Microcystis*, a common
173 occurrence in lakes worldwide. Additionally, LRV harbors a large population of *D. magna*.
174 Parthenogenetic lines of each genotype were maintained for over five years (approximately 300
175 generations) in continuous cultures at 20 ºC, utilizing UV-filtered dechlorinated municipal tap water
176 enriched with 2 mg C L^{-1} of the green alga *C. vulgaris* (strain CPCC 90; Canadian Phycological Culture
177 Centre, Waterloo, ON, Canada). Culturing of *C. vulgaris* was carried out using COMBO medium
178 (Kilham et al., 1998). Filters with a pore size of 0.22 μm were placed at both the input and output of
179 the aeration system to prevent any bacterial contamination.
180
181 *Microcystis aeruginosa culturing*
182
183 In accordance with our previously outlined methodology (Shahmohamadloo et al., 2019), *M. aeruginosa*
184 (strain CPCC 300; Canadian Phycological Culture Centre, Waterloo, ON, Canada) was cultivated in
185 BG-11 media and maintained in a growth chamber under sterile conditions at a constant temperature
186 of 21 ± 1 ºC, illuminated with cool-white fluorescent light at an intensity of 600 ± 15 lx, and subjected
187 to a photoperiod of 16:8 h light:dark. The culture was allowed to grow undisturbed for at least one

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188 month before being prepared for the 21-d chronic study. *M. aeruginosa* CPCC 300 is known to produce
189 microcystin-LR (CAS: 101043-37-2, $C_{49}H_{74}N_{10}O_{12}$) and its desmethylated form [D-Asp³]-microcystin-
190 LR (CAS: 120011-66-7, $C_{48}H_{72}N_{10}O_{12}$), which are prevalent in freshwater ecosystems and exhibit
191 toxicity to zooplankton (Chorus & Welker, 2021; Harke et al., 2016).
192
193 To facilitate testing on *D. magna*, an aliquot of the stock was inoculated into 100% COMBO medium
194 two weeks prior to test initiation, where it was cultured until reaching a cell density of $1.25 \pm 0.02 \times$
195 10^7 cells mL^{-1}. This medium was chosen because it supports the growth of both algae and
196 cyanobacteria while remaining non-hazardous to zooplankton (Kilham et al., 1998). Filters with a pore
197 size of 0.22 μm were placed at both the input and output of the aeration system to prevent any bacterial
198 contamination.
199
200 *Gut microbiome experiment*
201
202 We evaluated shifts in the microbiome to *M. aeruginosa* using 20 genotypes of *D. magna*. Phenotypic
203 responses measured include survival, reproduction (number of offspring produced), and the timing
204 and number of broods.
205
206 In preparation for this investigation, we individually housed one adult female *D. magna* per genotype
207 in separate 50-mL glass tubes containing COMBO medium and *C. vulgaris* at a concentration of 2 mg
208 C L-1. Daily monitoring was conducted to observe reproduction. Neonates of *D. magna* born within
209 24 h were gathered from each genotype and individually placed into 50-mL glass tubes, following the
210 previously described procedure. This process resulted in 10 replicates per genotype and a total of 200
211 tubes. These 200 *D. magna*, representing 20 genotypes, served as the founding mothers for this study.
212 All *D. magna* were maintained under constant environmental conditions, including a temperature of
213 21 ± 1 ºC, cool-white fluorescent light at an intensity of 600 ± 15 lx, and a photoperiod of 16:8 h
214 light:dark.
215
216 A 21-day chronic toxicity study was performed following previously described methods
217 (Houwenhuyse et al., 2021; OECD, 2012). *D. magna* neonates from each of the 20 genotypes, born
218 within a 24-hour period, were collected. Groups of 15 neonates were placed in 1-L glass jars containing
219 750 mL of UV-filtered water, with triplicates for each genotype. Each jar was then assigned to its
220 respective feeding treatment. Two common gardens were included: *Chlorella*-only (non-toxic diet) and
221 3:1 *Chlorella*:*Microcystis* (toxic diet). Following these ratios, all *D. magna* were fed 2 mg C L^{-1},
222 corresponding to 3×10^6 cells total, consistent with previous studies exposing daphnids to dietary
223 combinations of green algae and cyanobacteria (Isanta-Navarro et al., 2021; Rohrlack et al., 2005;
224 Shahmohamadloo, Poirier, et al., 2020). In total, 1,800 animals were used for this study (i.e., 3 replicates
225 per genotype, 900 animals per treatment).
226
227 As this was a semi-static test, solutions were refreshed 3 × per week on Mondays, Wednesdays, and
228 Fridays. This process involved transferring *D. magna* from the old to the new glass jar and providing
229 *D. magna* with a food supply consisting of 3×10^6 cells (i.e., the 3:1 treatment received 2×10^6 *C.*
230 *vulgaris* cells and 1×10^6 *M. aeruginosa* cells, corresponding to 2 mg C L^{-1}). Throughout this period, daily
231 survival rates, reproductive output, and the timing and number of broods were recorded to evaluate
232 potential interactions between genotype and treatment effects. The study was conducted under 400–
233 800 lx cool-white fluorescent light at a temperature of 20 ± 1 °C with a 16:8 light:dark cycle.
234

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235 At 21 days, *D. magna* replicates were transferred to sterile-filtered tap water for 24 h to eliminate food
236 particles from the gut and environmental bacteria from the carapace and filter apparatus
237 (Houwenhuyse et al., 2021). Subsequently, *D. magna* guts were extracted using dissection needles under
238 a stereomicroscope and transferred into an Eppendorf tube filled with 10 µL sterile milliQ water,
239 after which they were stored in −80 °C for future DNA extraction.
240
241 *Library preparation and sequencing*
242
243 To analyze the gut microbial communities of *D. magna* at the conclusion of the 21-d experiment, DNA
244 extractions were performed using the NucleoSpin Soil DNA kit (Macherey-Nagel, Düren, Germany).
245 Prior to bead beating samples according to kit instructions, each *D. magna* gut was pressed using a
246 stainless steel probe. DNA was eluted using 50 µL of 5 mM Tris/HCl at pH 8.5. Subsequent DNA
247 quantification was conducted with 5 µL per sample of eluted DNA on a Qubit (Invitrogen,
248 Massachusetts, United States) using a dsDNA broad-range assay. Samples that had low DNA
249 concentrations were vacufuged before sequencing to increase concentration. Isolated DNA was
250 quantified (Equalbit 1x dsDNA HS Assay kit) and amplified using primers covering the V3-V4
251 hypervariable 16s rRNA region. Library quality was assessed and libraries were dual indexed before
252 Equimolar pooling based on QC values. Pooled libraries were sequenced on an Illumina MiSeq with
253 a 250 bp read length configuration to a depth of 0.3M reads for each sample.
254
255 Data were imported using demux single end forward reads with initialization of primer trimming
256 followed by high-resolution DADA2 (Callahan et al., 2016) filtering at truncation quality 20 (if
257 nucleotide of quality 20 is detected, the sequence from then on is truncated). Reads were then filtered
258 using default QIIME2 parameters (Bolyen et al., 2018). We retained an average of 16941 reads per
259 sample (Quantile range: 1% = 7237, 25% = 14943, 50% = 17006, 75% = 19835, 100% = 25629).
260 Taxonomy was assigned using greenegene full length 16s rRNA backbone database with a scikit-learn
261 naive Bayes machine-learning classifier (Bokulich et al., 2018). Before transitioning into the *phyloseq*
262 package in R (R Core Team, 2022), we generated an unrooted phylogenetic tree to improve the
263 accuracy of the downstream analysis. In *phyloseq*, the combined dataset was first filtered by taxa that
264 were represented with less than 5 total taxonomy classifications in the whole sample set, then rarefied
265 at a depth of 20,000 ASV. Data was aggregated to bacterial class for analyses of relative abundance
266 and differential abundance analysis graphs (Figure 3).
267
268 *Water sampling for analysis*
269
270 Water samples were collected from each treatment at the beginning of the test, during solution
271 changes, and at the conclusion to measure cell concentrations and standard water parameters. These
272 samples were quick-frozen at -80 °C and subsequently analyzed for cyanobacterial toxins, which have
273 been reported previously (Shahmohamadloo et al., 2023). Briefly, water parameters recorded before
274 and after solution renewals remained within the specified test criteria. No indications of hypoxia-
275 induced stress on *D. magna* behavior were observed. Mortality and immobilization were initially
276 detected within 48 hours after exposure to the toxic treatments and persisted throughout the 21-d test
277 period. This outcome was anticipated, given that the selected toxic concentrations can range from
278 sublethal (Shahmohamadloo, Poirier, et al., 2020) to lethal (Ferrão-Filho et al., 2000; Lürling & van
279 der Grinten, 2003) in *Daphnia* laboratory assays, and are commonly encountered in freshwater
280 ecosystems affected by HABs (Chorus & Welker, 2021).
281

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282 *Statistical analysis*
283
284 All analyses were completed in R version 4.2.2 (R Core Team, 2022). To test the magnitude of clonal
285 variation across treatments, we used a Linear Mixed Effects (LME) model for each *D. magna*
286 phenotypic response with 'clone' treated as a random effect and 'treatment' treated as a fixed effect.
287 We constructed Leslie matrices for each clone in the toxic and non-toxic exposure treatments. The
288 population growth rate (λ) and exponential rate of increase (*r*) based on the Euler-Lotka equation were
289 additionally calculated to estimate the reproductive output of *D. magna* that were exposed to toxic and
290 non-toxic diets. These calculations were constructed using the full 21-d data on *D. magna*. The
291 difference in neonate production between toxic and non-toxic diets were calculated for each 'clone'
292 and 'treatment' combination and plotted.
293
294 Downstream microbiome analyses were conducted in the *phyloseq* package in R. We calculated
295 Shannon diversity index and tested for the effects of algal treatment and clone on α-diversity using
296 the *lme4* package in R. Bray-Curtis and Weighted Unifrac ß-diversity metrics were used to assess the
297 multivariate effects of algal treatment and clone in a permutational multivariate analysis
298 (PERMANOVA) adonis2 function from the *vegan* package in R. ß-diversity distance matrices were
299 treated as response variables with 'treatment' + 'clone' treated as fixed effects and the number of
300 sampled guts in each replicate treated as a random effect. To identify bacterial differences on the
301 family level between samples associated with algal treatment, we used a differential abundance analysis
302 from the *DESeq2* package in R, where ASVs representing less than 1% of the reads were discarded
303 and p-values were adjusted using Benjamini-Hochberg procedure (Benjamini & Hochberg, 1995). To
304 support our findings from *DESeq2*, we additionally employed the *phylofactor* package in R as an
305 alternative which utilizes phylogenetic data to assess the differences in microbial community
306 composition (Washburne et al., 2017).
307
308 We tested for relationships between shifts in the gut microbiome composition and host plasticity
309 across algal treatments. We used the *multivarvector* package in R (Härer & Rennison, 2022) to generate
310 multivariate vectors and angles by connecting the population means of Principal Component Analysis
311 (PCA) scores with clones across different algal treatments (20 total vectors and angles) as an empirical
312 measure of microbiome shifts in response to *Microcystis* exposure. The relationship between the
313 magnitude of plasticity in microbial composition and host performance, *r* and delta mean neonate
314 production, was assessed using the goodness of fit of R^2 extracted from a linear model.
315
316 To assess the degree of parallelism in the microbiome shift associated with algal treatment, we used
317 *multivarvector* package, generating the angular value of the parallelism of replicates given toxic and non-
318 toxic diets to then assess the parallel, non-parallel, or anti-parallel relationships of our data. In addition
319 to *multivarvector,* we implemented betadisper from *vegan* to assess the multivariate homogeneity of
320 groups dispersions on our ß-diversity metrics.
321
322 **Results**
323
324 *Daphnia magna plasticity in life-history traits and population projection*
325
326 Exposure to the toxic diet (3:1 Chlorella:Microcystis) had widespread plastic effects on *Daphnia,*
327 including a significant decrease in survival ($F_{1,19}$ = 10.81, p = 0.0039; Figure 1a). *Daphnia* from the
328 non-toxic diet had a 81.5 ± 1.3% survival rate compared to *Daphnia* from the toxic diet who had a
329 68.13 ± 3.2% survival rate to the cessation of the study at 21 days. Similarly, we observed a significant

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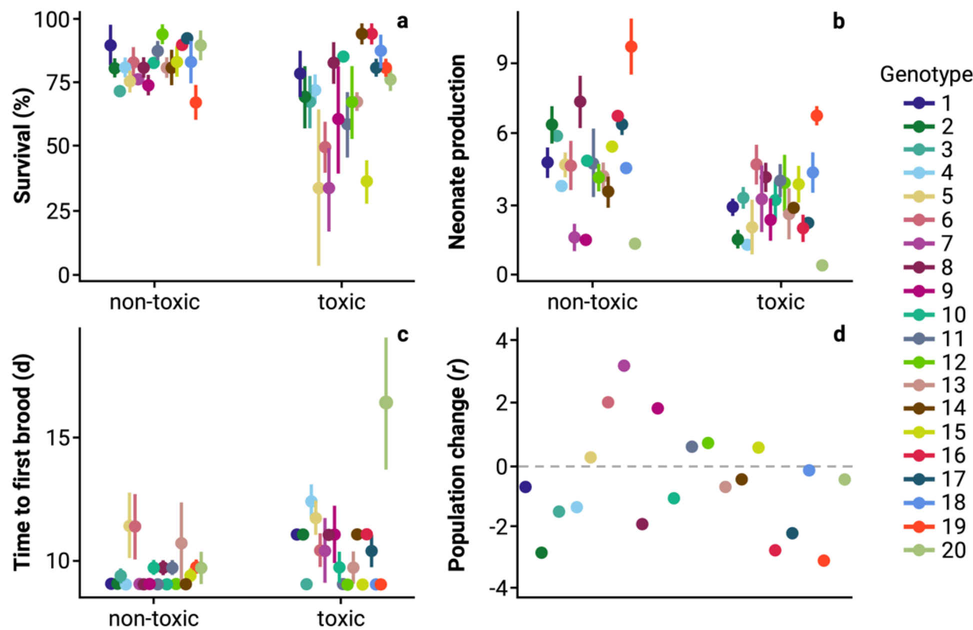
330 effect on neonate production per *Daphnia* ($F_{1,19}$ = 19.56, p = 0.00029; Figure 1b); *Daphnia* reared on
331 non-toxic diets produced 4.76 ± 0.29 neonates per *Daphnia* compared to 3.02 ± 0.23 neonates per
332 *Daphnia* for those reared on toxic diets. Lastly, we observed a significant delay in time to first brood
333 ($F_{1,19}$ = 6.66, p = 0.018; Figure 1c); *Daphnia* reared on non-toxic diets reproduced at 9.52 ± 0.15 days
334 compared to 10.53 ± 0.26 days for those reared on toxic diets. We did not observe significant effects
335 on the total number of broods per *Daphnia* ($F_{1,19}$ = 0.25, p = 0.62) by 21 days.
336
337 To make inferences on the effects of *Microcystis* exposure on population dynamics we constructed
338 Leslie matrices for each clone on both non-toxic and toxic diets. We then calculated the exponential
339 rate of increase (*r*) for each *Daphnia* clonal population on each diet and the mean difference in *r*
340 between toxic and non-toxic for each 'clone' and 'treatment' combination to determine whether
341 *Microcystis* exposure would have a net positive ($r > 0$), neutral ($r = 0$), or negative ($r < 0$) effect relative
342 to rearing on a control diet (Figure 1d). *Microcystis* exposure negatively impacted 13/20 clones and had
343 positive effects on 7/20 clones (Figure 1d). The mean difference in population growth rate between
344 toxic (2.38) and non-toxic (2.88) for all clones was -0.49, indicating the overall effect of *Microcystis*
345 exposure on *Daphnia* population growth was negative.
346



347
348 **Figure 1.** Phenotypic variation among the 20 *Daphnia magna* clones at the end of the 21-d gut
349 microbiome chronic experiment across two treatments: non-toxic diet (Chlorella-only) and toxic diet
350 (3:1 Chlorella:Microcystis). Phenotypes measured were a) survival (%), b) mean number of neonates
351 produced per *D. magna*, and c) mean time to first brood per *D. magna* (d). Panel d) is the exponential
352 rate of population change (*r*) between treatments across each of 20 *D. magna* clonal populations at the
353 end of the 21-d gut microbiome chronic experiment. A positive population growth response is $r > 0$
354 whereas a negative population growth response is $r < 0$.
355
356 *Testing for effects of toxic exposure on Daphnia microbiomes*
357
358 We found no significant effect of algal treatment (F_{38} = 0.763, P = 0.388) or *Daphnia* clone (F_{38} =
359 0.265, P = 0.609) on microbiome α-diversity (Figure 2). Effects of algal treatment and clone were
360 likewise modest on ß-diversity; we observed no significant effect of algal treatment (F_1 = 1.53, P =

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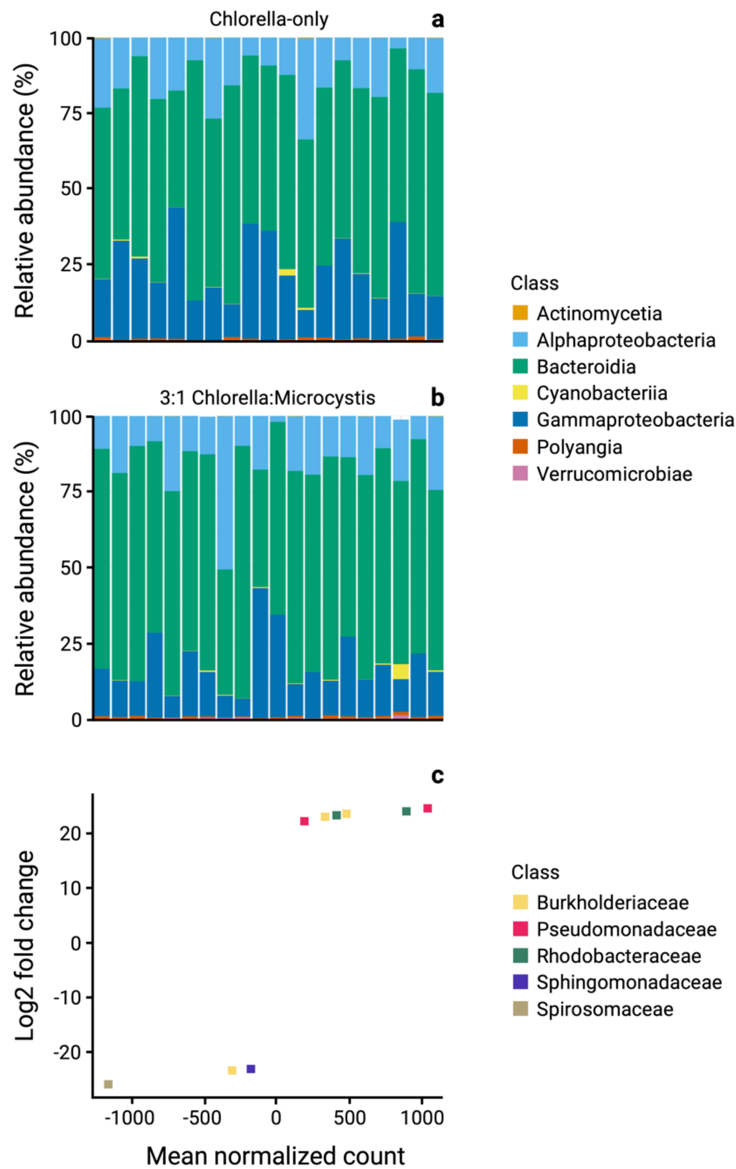
361 0.195) or clone (F_{19} = 1.22, P = 0.173) using Weighted UniFrac (Figure 3a) and a marginally significant
362 effect of clone (F_{19} = 1.17, P = 0.049), but not algal treatment (F_1 = 0.96, P = 0.505), when assessed
363 using Bray-Curtis (Figure 3b). Using a permutation test for homogeneity of multivariate dispersions,
364 we likewise saw no significant difference in variance in community composition associated with algal
365 treatments (Weighted UniFrac: F_1 = 0.601, P = 0.443; and Bray-Curtis: F_1 = 0.009, P = 0.152).
366



367
368 **Figure 2.** The average relative abundance of bacterial classes in the gut microbiomes of 20 *Daphnia*
369 *magna* clones across a) non-toxic (Chlorella-only) and b) toxic (3:1 Chlorella:Microcystis) treatments at
370 the end of the 21-d gut microbiome chronic experiment, color coated based on bacterial classes. c)
371 The *DESeq2* differential abundance analysis, color coated based on family.
372

373 Although ß-diversity metrics demonstrated no significant community wide differences in microbiome
374 composition, there were particular microbial groups that differed in their abundance across algal diets.
375 A *DESeq2* based analysis of differential read abundance found five differentially abundant bacterial

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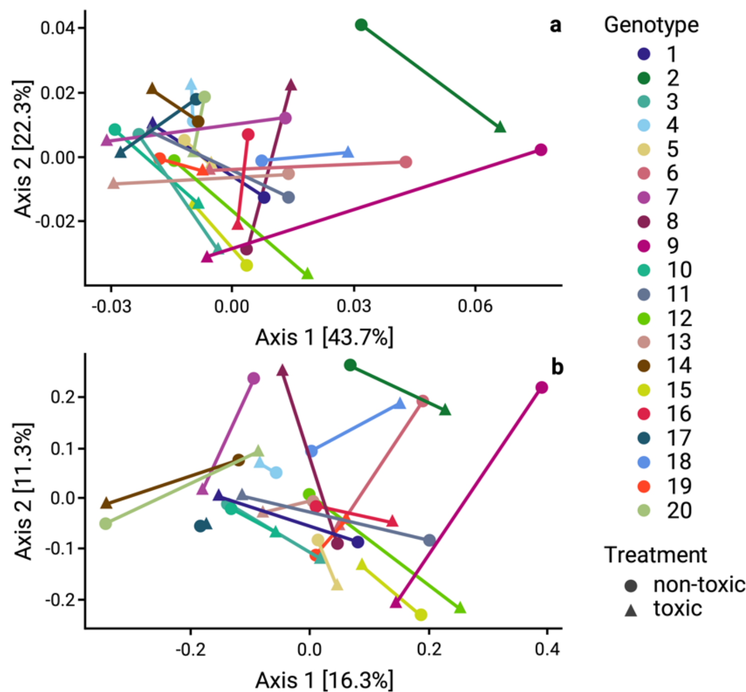
376 families (Figure 2c): *Spirosomaceae* (log2FC = -25.57, P < 0.001), *Sphingomonadaceae* (log2FC = -23.02, P
377 < 0.001), *Rhodobacteraceae* (log2FC = 23.03, P < 0.001), *Pseudomonadaceae* (log2FC = 23.57, P < 0.001),
378 and *Burkholderiaceae* (log2FC = 23.67, P < 0.001). A *Phylofactor* based analysis identified only
379 *Spirosomaceae* (F_2 = 11.28, P = 0.001) as a differentially abundant taxa.
380
381 To determine whether diet treatments produced parallel shifts in microbiome composition, we
382 employed *multivarvector* (Härer & Rennison, 2022). Treating each clone as a unique contrast, we found
383 the shift in microbiome composition associated with non-toxic and toxic diet exposure was non-
384 parallel (student's t-test with a null hypothesis of 90° (non-parallel) for both Weighted UniFrac (t_{19} =
385 -0.231, df = 19, P = 0.82, Mean = 89.73) and Bray-Curtis (t_{19} = 0.34, df = 19, P = 0.74, Mean = 90.14)
386 indicated a non-parallel relationship).
387



388
389 **Figure 3.** Principal Component of Analysis (PCoA) in a) Weighted UniFrac, and b) Bray-Curtis
390 distances in relation to the gut microbiome across the 20 *Daphnia magna* clones subjected to non-toxic
391 (Chlorella-only; ▲) and toxic (3:1 Chlorella:Microcystis; ●) treatments. Each clone is connected by a
392 line to show the shift in host response across treatments.
393
394 *Magnitude of microbial plasticity and degree of parallelism*
395
396 We next tested whether the magnitude of microbiome shifts that occurred with exposure to toxic
397 diets, as calculated in multivariate space, was associated with the magnitude of host plasticity across
398 diets. To do so, we tested for a correlation between the magnitude of the microbiome shift in
399 multivariate space and the magnitude of change in *r* across diet treatments for each of the 20 clones.
400 We observed no significant relationship between the degree of shifts in the microbiome and the
401 difference in *r* across diet treatments in either Weighted Unifrac (F_{18} = 0.083, P = 0.776, R^2 = 0.005;
402 Figure 4a) or Bray Curtis (F_{18} = 0.009, P = 0.923, R^2 = 0.0005; Figure 4b).
403

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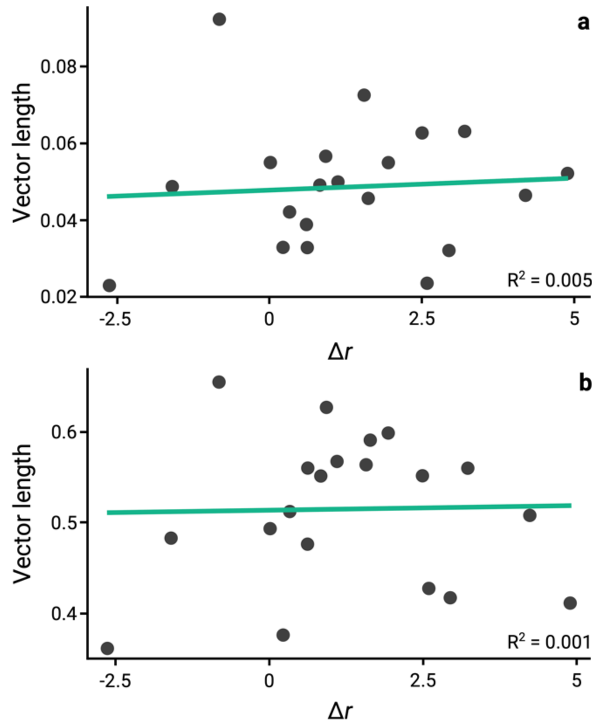

404
405 **Figure 4.** Magnitude of microbial plasticity in a) Weighted UniFrac, and b) Bray-Curtis distances in
406 relation to the difference in difference in population growth rates (*r*) across the 20 *Daphnia magna*
407 clones subjected to non-toxic (Chlorella-only) and toxic (3:1 Chlorella:Microcystis) treatments. The
408 mean vector is represented by the bluegreen line.
409
410 **Discussion**
411
412 *Relationship between phenotypic responses, shifts in the microbiome, and population-level effects*
413
414 Overall patterns of host phenotypic plasticity associated with *Microcystis* dietary exposure were
415 considerable, with mean shifts in survival, number of offspring produced, and time to first brood
416 generally trending toward a negative response across the 20 *Daphnia* clones (Figure 1a-c). When these
417 phenotypes were combined to parameterize a population projection, dietary exposure to *Microcystis*
418 produced considerable clonal variation and led to an overall decrease in population growth rates
419 (Figure 1d). This response aligns with prior work (Shahmohamadloo et al., 2024), which has shown
420 significant mortality in *Daphnia* due to *Microcystis* (Chislock et al., 2013; Harke et al., 2016; Isanta-
421 Navarro et al., 2021; Shahmohamadloo, Poirier, et al., 2020), along with evidence of adaptive
422 responses to *Microcystis* blooms (Chislock et al., 2013; Hairston et al., 2001; Sarnelle & Wilson, 2005).
423
424 In contrast to these pronounced effects on host phenotypic plasticity, *Microcystis* exposure produced
425 only modest shifts in the microbiome (Figure 2a-c), with no parallel changes across *Daphnia* clones
426 (Figure 3). Furthermore, the magnitude of microbiome shifts was not correlated with the magnitude
427 of phenotypic plasticity observed across clones (Figure 4). These findings stand in stark contrast to
428 prior work focused on axenic rearing and microbiome transplants which has suggested the select few
429 community members that do dominate the *Daphnia* gut microbiome (Freese & Schink, 2011)—of
430 which we identified five microbial families that significantly differed (Figure 2c)—have a prominent
431 influence on host fitness including survival, growth, and reproduction when diets feature *Microcystis*

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432 (Callens et al., 2018; Macke et al., 2017; Peerakietkhajorn et al., 2016; Sison-Mangus et al., 2015). Our
433 results, however, suggest that exposure to a common environmental stressor did not lead to
434 microbiome-mediated plasticity with adaptive phenotypic or positive demographic effects that could
435 stabilize population growth rates and decrease extinction risk.
436
437 *Microbiomes and adaptive plasticity revisited*
438
439 The role of the microbiome in host responses to environmental stress has been a topic of considerable
440 interest (Henry et al., 2021; Kolodny & Schulenburg, 2020; Rocca et al., 2018; Zilber-Rosenberg &
441 Rosenberg, 2008). Spurred by early work documenting shifts in microbiome composition associated
442 with environmental stress (Engel et al., 2012; Motta et al., 2018), there has been considerable
443 speculation about the role of the microbiome in host acclimation and adaptation (Mueller et al., 2020;
444 Rudman et al., 2019). Our findings raise questions about the circumstances and generality of
445 microbiomes as a mechanism underlying adaptive plasticity in hosts. We show that the *Daphnia*
446 microbiome only exhibits significant shifts in the abundance of five bacterial families. Though it has
447 been proposed that shifts in microbiome composition play a crucial role in influencing the fitness of
448 hosts and stabilizing growth rates to reduce the probability of extinction (Kolodny & Schulenburg,
449 2020; Mueller et al., 2020; Shade, 2023), we find no correlation between the degree of shift in the
450 microbiome and host adaptive plasticity (Figure 3) or population growth rate (Figure 1d).
451
452 Prior work demonstrating a role of adaptive plasticity has largely studied the contribution of
453 microbiomes without host genetic diversity (Baldassarre et al., 2022) or through complete microbiome
454 transplants (Callens et al., 2018, 2020; Houwenhuyse et al., 2021; Macke et al., 2017, 2020). While this
455 work illustrates how influential host-microbiome interactions can be in specific and tightly controlled
456 circumstances, these types of approaches could lead to overestimates of the importance of shifts in
457 the microbiome for host phenotypic responses in more natural settings (Hegg et al., 2021).
458
459 An important caveat of our work is that it was conducted under laboratory conditions, where *D. magna*
460 gut bacterial communities can differ significantly from those in natural environments. Recent work
461 shows that microbiome diversity and composition can change rapidly after transfer to the lab, with
462 ongoing shifts even after two years (Houwenhuyse et al., 2023). Despite these differences, key bacterial
463 classes (Hegg et al., 2021; Houwenhuyse et al., 2023)*Burkholderiaceae*, *Pseudomonadaceae*, and
464 *Sphingomonadaceae* were dominant across field and lab rearing environments and present in high
465 abundances in our study. Our test tests for parallelism (Figure 4) revealed that their relative
466 abundances were not repeatedly changed by exposure to *Microcystis*, aligning with prior work under
467 semi-natural conditions showing no evidence that natural variation in microbiome diversity or
468 composition was associated with tolerance to cyanobacteria (Hegg et al., 2021). Future work that
469 extends our general experimental framework to field conditions is needed to determine whether hosts
470 can modulate microbiome composition through horizontal acquisitions, thereby producing adaptive
471 plasticity and positive effects on population growth.
472
473 While it is possible that certain traits or environments might benefit from microbiome-mediate rescue
474 (Mueller et al., 2020; Shade, 2023) or 'microbiome flexibility' (Voolstra & Ziegler, 2020), our findings
475 suggest this was not the case here. Moreover, field exposures similarly recover limited evidence for
476 *Daphnia* microbiome shifts in response to *Microcystis*, which does not indicate a strong pathway for
477 adaptive plasticity via microbiome shifts or microbiome rescue (Hegg et al., 2021). Although concepts
478 like microbiome plasticity and rescue were initially motivated by findings in organisms with strong

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479 host-microbiome symbiosis, the authors explicitly posited that these phenomena are likely to be broad
480 and widespread (Mueller et al., 2020; Shade, 2023).
481
482 In the absence of microbiome rescue or flexibility operating to reduce phenotype-environment
483 mismatches, other mechanisms, such as phenotypic plasticity and adaptation from standing genetic
484 variation, play crucial roles in shaping the phenotypic diversity observed in natural populations (Barrett
485 & Schluter, 2008; Rennison et al., 2019; Rudman et al., 2022; West-Eberhard, 2003). These
486 mechanisms shape the phenotypic response of organisms to environmental challenges (Ghalambor et
487 al., 2007; Price et al., 2003; West-Eberhard, 2003). It is plausible that microbiome-driven adaptive
488 plasticity is also a component of adaptive plasticity but operates on longer timescales (Kolodny &
489 Schulenburg, 2020) or requires transgenerational effects (Baldassarre et al., 2022), as suggested by the
490 microbiota-mediated transgenerational acclimatization concept (Webster & Reusch, 2017). These
491 effects might be limited to systems with high microbiome heritability (Doolittle & Booth, 2017) and
492 may not be as general as initially proposed. Advancing microbiome studies beyond tightly controlled
493 mechanistic investigations to include genetically, environmentally, and phenotypically diverse
494 populations will be critical for understanding the role of microbiomes in organismal responses to
495 environmental change (Greyson-Gaito et al., 2020).
496
497 *Quantitative and qualitative replication of host-microbiome studies*
498
499 Despite observing significant shifts in *Daphnia* life-history traits and population growth associated with
500 *Microcystis* exposure, we observed no community-level differences in gut microbiome composition
501 between *Daphnia* reared on toxic and non-toxic diets. This result aligns with findings from outdoor
502 mesocosms, which demonstrated that despite significant seasonal changes in *D. magna* gut microbiome
503 diversity and composition there was surprisingly no evidence linking natural variation in microbiome
504 diversity or composition with exposure to *Microcystis* (Hegg et al., 2021).
505
506 Our results suggest that earlier mechanistic studies—which used axenic cultures and reciprocal
507 microbiome transplants to test the effects of microbiome composition on *Microcystis* toxin tolerance
508 (Houwenhuyse et al., 2021; Macke et al., 2017, 2020)—may not adequately capture the dynamics of
509 host-microbiome interactions in natural settings, and prompts further investigation into the ecological
510 relevance of such mechanistic findings (Greyson-Gaito et al., 2020). These differences highlight major
511 questions about reproducibility and repeatability in microbiome research, due both to underlying
512 stochasticity and inherent differences between experimental environments (Schloss, 2018). In
513 planning this study, we consulted authors on the prior work and they generously shared
514 methodological details which we made efforts to replicate. However, there are experimental
515 differences that could contribute to the considerable variation seen between studies. These include:
516 previous studies did not detail the concentration of *Microcystis* cells or amount of microcystin toxins,
517 and hence exposures may not have been comparable (Orr et al., 2018); biological variation in *Daphnia*
518 test populations and *Microcystis* strains; and inherent differences in lab microbiomes. Whether these
519 differences are sufficient to lead to qualitatively different conclusions about the role of microbiome
520 composition in host fitness is beyond our scope to determine. The differences observed highlight the
521 need to consider not only genetic and environmental factors but also methodological consistency
522 when interpreting microbiome research results. Given the observed differences between studies and
523 the inherent repeatability challenges in microbiome research, our findings should be interpreted within
524 the context of these broader uncertainties. While our results contribute valuable insights, they also
525 underscore the importance of continued investigation and methodological rigor in this field.
526

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527 *Broader implications*
528
529 Our findings provide an empirical test of the contribution of the microbiome to host plasticity and
530 population dynamics. Although significant phenotypic changes were observed in *Daphnia* exposed to
531 *Microcystis*, there was no correlation between this response and the degree of change in the composition
532 of the microbiome or any pattern observed indicative of microbiome-mediated rescue. These results
533 suggest that the microbiome's role in host plasticity may be more context-dependent than previously
534 thought. However, given the scope of this single study, further research is needed to explore how
535 shifts in the microbiome influence reaction norms in fitness associated phenotypes across species and
536 environmental contexts. Future studies in natural conditions, where hosts can acquire a wider range
537 of microbes, will be critical to developing a more comprehensive understanding of how host-
538 microbiome interactions shape natural populations. Such investigations may help resolve longstanding
539 questions about the microbiome's potential to direct host evolution and serve as a mechanism of
540 rescue in populations facing novel environmental challenges.
541
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