1 Seasonal dynamics are the major driver of microbial 2 diversity and composition in intensive freshwater 3 aquaculture

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24 Highlights

- 25 • We present three years of monthly microbiome data from an aquaculture
- 26 facility.
- 27 • The microbiome changes seasonally, likely driven by temperature and
- 28 rainwater runoff.
- 29 • Summer blooms of toxin-producing cyanobacteria are repeatedly observed.
- 30 • Fish food may impact microbiome through changes in nutrient ratios.

31

32

33 Graphical abstract

34

35

36 **Abstract**

37 Aquaculture facilities such as fishponds are one of the most anthropogenically
38 impacted freshwater ecosystems. The high fish biomass reared in aquaculture is
39 associated with an intensive input into the water of fish-feed and fish excrements.
40 This nutrients load may affect the microbial community in the water, which in turn
41 can impact the fish health. To determine to what extent aquaculture practices and
42 natural seasonal cycles affect the microbial populations, we characterized the
43 microbiome of an inter-connected aquaculture system at monthly resolution, over
44 three years. The system comprised two fishponds, where fish are grown, and a
45 “control” operational water reservoir in which fish are not actively stocked. Clear
46 natural seasonal cycles of temperature and inorganic nutrients concentration, as well
47 as recurring cyanobacterial blooms during summer, were observed in both the
48 fishponds and the reservoir. The structure of the aquatic bacterial communities in the
49 system, characterized using 16S rRNA sequencing, was explained primarily by the
50 natural seasonality, whereas aquaculture-related parameters had only a minor
51 explanatory power. However, the cyanobacterial blooms were characterized by
52 different cyanobacterial clades dominating at each fishpond, possibly in response to
53 distinct nitrogen and phosphate ratios. In turn, nutrient ratios may have been by the
54 magnitude of fish feed input. Taken together, our results show that, even in strongly
55 anthropogenically impacted aquatic ecosystems, the structure of bacterial
56 communities is mainly driven by the natural seasonality, with more subtle effects if
57 aquaculture-related factors.

58

59 1. Introduction

60 Freshwater environments come in many shapes and forms, from pristine mountain
61 lakes to highly polluted wastewater treatment facilities, each hosting a complex and
62 dynamic microbial community (“microbiome”; Bautista-de los Santos et al., 2019;
63 Bruno et al., 2018; Zorz et al., 2019). Such aquatic microbiomes are strongly affected
64 by environmental conditions within the water body (e.g., temperature and nutrients
65 availability), and in turn shape its biogeochemistry, for example by processing and
66 recycling organic and inorganic matter (Hach et al., 2020; Liu et al., 2017; Rowe et
67 al., 2018). Aquatic microbial communities may also affect other organisms living
68 within the water body, as well as human communities in contact with the water. For
69 example, the eggs and larvae of some aquatic organisms are inoculated by microbes
70 from the surrounding water, in turn affecting the health and fitness of these organisms
71 (Giatsis et al., 2016; Grześkowiak et al., 2012; Pérez-Sánchez et al., 2014; Song et al.,
72 2014). Similarly, aquatic microbiomes may serve as pools of various pathogens, and
73 some aquatic microorganisms (primarily prokaryotic and eukaryotic microalgae) can
74 produce toxins (Paerl et al., 2018; Wurtsbaugh et al., 2019). Given the importance of
75 freshwater microbiomes to water quality and environmental health worldwide,
76 significant effort has been invested in understanding the interplay between
77 environmental conditions and microbiome structure and function (reviewed by Orland
78 et al., 2019).

79 Freshwater aquaculture facilities such as fishponds are an example of an aquatic
80 ecosystem that is significantly impacted by anthropogenic activity. The growth of
81 human population and the concomitant increase in the demand for animal-based
82 protein have resulted in a rapid expansion of aquaculture worldwide, a process also

83 termed a “blue revolution” (reviewed by Garlock et al., 2020). The aquaculture
84 industry has been growing at an average rate of ca. 6-8% since the 1970s, and now
85 supplies more fish and seafood biomass than natural catch (Ahmed and Thompson,
86 2019; Garlock et al., 2020). As a result, fishponds (as an ecosystem) are becoming
87 more abundant, and their interaction with surrounding environments is increasing.

88

89 Modern intensive aquaculture practices rely on growing large stocks (i.e., high
90 biomass) of fish or other aquatic organisms in a limited area. This requires large
91 amounts of fish-feed that often leads (together with fish excrement) to hyper-
92 eutrophic conditions (Ahmed and Thompson, 2019; Gowen, 1994; Talbot and Hole,
93 1994). Additionally, aquaculture is often performed in monoculture (i.e., a single type
94 of organism is grown) or in polyculture of a limited number of organisms, and the
95 combination of ultra-dense populations and hyper-eutrophic conditions (i.e., poor
96 water quality) can lead to disease outbreaks (Ahmed and Thompson, 2019; Glibert et
97 al., 2002; Mennerat et al., 2010). As a result, aquaculture often supports fish health by
98 using drugs and antibiotics, as well as algacides in the case of algal blooms.

99 However, despite the ubiquity of such aquaculture husbandry practices, and their
100 potential impact on aquatic microbial populations, little is known about the temporal
101 dynamics of the microbiome in fishponds, and how it responds to natural and
102 anthropogenic perturbations (Patil et al., 2020; Rodriguez-Brito et al., 2010).

103 To address these questions, we characterized, over three years, temporal microbiome
104 dynamics in two fishponds connected to a semi-natural operational water reservoir,
105 which recycles the fishpond water (Fig. 1, see detailed description below). Our aim
106 was to identify factors that dictate the water microbiome composition of these highly

anthropogenically impacted ecosystems. These included the quality of the water, phytoplankton populations, as well as to the types of fish present and their feeding regimen. We hypothesized that specific intensive fishery practices in each fishpond (e.g., the types of fish raised and their feeding regime) would shape the microbiomes of the fishponds, resulting in different populations between the fishponds, as well as between them and the reservoir. Our results, however, point to seasonality rather than specific aquaculture practices as the major force driving the microbial population structure.

2. Materials and Methods

2.1 Sampling and analysis of environmental data

Monthly samples were collected at the reservoir and fishponds D1 and V2 (when they contained water) from 2013-2015 (Table S1). Each pond was sampled at a constant location from the edge of the water body and included measurement of dissolved oxygen, temperature and pH, using field probes (Eutech instruments, Singapore). At each location, five liters of surface water were collected using plastic jerrycans (specific to each location) that were washed with double distilled water and with 70% ethanol prior to sampling. Within one hour the samples were transferred in darkness to the lab and processed according to the methodology described below.

For measurement of pigments, water was filtered through GF/C filters (glass fiber filter, 13 mm, nominal pore size 1.2 μm ; Whatman plc, Maidstone, UK) and placed into 1.5 mL sterile Eppendorf tubes. The water was filtered until the filters were clogged, and the volume was recorded (30-150 mL). For DNA extraction and microcystin measurements, water was filtered through GF/F filters (glass fiber filter, 13 mm, nominal pore size 0.7 μm ; Whatman plc, Maidstone, UK). The filters were

placed into 1.5 mL sterile tubes, and for the purpose of DNA extraction were covered with 1 mL of storage buffer (40 mM EDTA, 50 mM Tris-HCl, 0.75 M Sucrose) and stored at -80 °C until analyzed. The filtrate from the GF/F filters was collected for measurement of dissolved inorganic nutrients, and were maintained at -20 °C until analysis.

2.2 Inorganic nutrients measurements

The concentrations measurements of PO_4^{+} , NH_4^{+} , NO_3^{-} and NO_2^{-} in fishponds and in the water reservoir were measured using the AA3 Segmented Flow Multi-Chemistry Analyzer (SEAL Analytical, Germany), following the manufacturer protocols (Ammonia: Method no. G-327-05 Rev. 7 - Fluorescent method; Nitrate and Nitrite: Method no. G-172-96 Rev. 17 and Phosphate: Method no. G-297-03 Rev. 5).

Inorganic nutrients in the rainwater runoff were measured using a Lachat Autoanalyzer (Lachat Instruments, Milwaukee, WI, USA) by the manufacturer's protocol.

2.3 Photosynthetic pigment analysis

Pigments were extracted for 3 hours in absolute methanol in the dark. Pigment extractions were immediately clarified with syringe filters (Acrodisc CR, 13 mm, 0.2 µm PTFE membranes; Pall Life Sciences, New York, NY, USA) and transferred to glass ultra-performance liquid chromatography (UPLC) vials. The samples were preheated to 30 °C, and 10 µl were injected into an ACQUITY UPLC system (Waters Corporation, Milford, MA, USA) equipped with a C8 column (1.7 µm particle size, 2.1 mm internal diameter, 50 mm column length, ACQUITY UPLC BEH) heated to 50 °C and a guard column. The protocol was based on the LOV method (Hooker and McClain, 2000), with some modifications: the mobile phase gradient consisted of a

155 mixture of 70:30 methanol: 0.5M ammonium acetate as solvent A and 100% methanol
156 as solvent B. Peaks were monitored at 440 nm and their absorbance spectra was
157 determined using a photodiode array (PDA) detector. Known standards of
158 Chlorophyll a, Chlorophyll b, Chlorophyll c₂, Zeaxanthin, β -carotene, Diatoxanthin,
159 Dincoxanthin, Fucoxanthin and Peridinin were separated before each run for further
160 identification and quantification. All standards were purchased from the DHI
161 Laboratory (Hørsholm Denmark). Pigments were identified by retention time and
162 spectrum absorbance (obtained by PDA detector reads at 350-700 nm). Pigment
163 concentrations were adjusted to 1 mL extraction volume and filtration volume.

164 *2.4 Fish biomass and supplied food mass estimation*

165 Fish biomass and the fish-feed input were estimated for the sampling time-points
166 based on the limited records kept at the Dor Aquaculture Research Unit (DARU).
167 When the fishponds were established for the growing season, the total number and
168 average biomass of the larvae or juvenile fish was recorded. Once or twice a month,
169 during the outgrowth period, a representative sample of fish was weighed and
170 returned to the water. The number of fish in the water was estimated and the amount
171 of fish-feed supplied was recorded. Over the course of the last several weeks of the
172 growing period, fish are often harvested in batches, resulting in a gradual decrease in
173 the number of fish in the fishpond. During each harvesting event, a representative
174 sample of the removed fish was weighed. At the end of the growing period (as the
175 water is removed and the fishponds dried until the next growing season), the final
176 number of fish and their weight were recorded. Based on these measurements, we
177 calculated the fish biomass and used a fitted model in Microsoft Excel (Table S2, tabs
178 "D1_calculations" and V2_calculations") to estimate the fish biomass when our
179 samples were taken, which was always during the outgrowth period. A similar

180 approach was applied to estimate the amount of food added to the fishponds. A
181 second order polynomial model provided a good fit to most of the data (R^2 above
182 0.96; Table S2, tabs "D1_calculations" and V2_calculations"). We would like to
183 emphasize that these models are not designed to be predictive or mechanistic and are
184 limited to interpolating the data to provide estimates of the biomass and feed mass
185 during the sampling times.

186 *2.5 Microcystin extraction and measurement*

187 Cyanotoxins (microcystins or nodularins) in phytoplankton biomass were measured
188 immunologically. Biomass collected on filters was subjected to methanolic extraction
189 (3 hours incubation in 1 mL 100% Methanol), followed by 15 minutes sonication. The
190 upper 500 μ L were dried under vacuum for 2 hours in a speedvac, resuspended in 50
191 μ L of 75% methanol and further dilution to 4% methanol. A subsample of 50 μ L of
192 that solution was used for quantification by the Microcystins/Nodularins (ADDA)
193 Elisa Kit (Eurofins Abraxis Inc, Warminster, PA, USA). This protocol led to a
194 concentration factor of 5-100 (depending on the initial filtered volume), bringing the
195 samples within the range of sensitivity of the measuring kit ($0.1 \mu\text{g L}^{-1}$). In some
196 cases, the ELISA results were above the linear range of the kit, therefore, these
197 samples (marked in Fig. 2, Fig. S1, and Table S2) should be considered as likely
198 underestimates.

199 *2.6 DNA isolation and 16S rRNA amplicon sequencing*

200 The DNA extraction was performed using a combination of manual treatments and
201 robotic extraction with the QiaCube robot (Biomedical Core Facility, Technion,
202 Haifa, Israel). Briefly, filters were thawed, centrifuged for 10 minutes at $15,000 \times g$
203 and the storage buffer was removed. Lysis buffer from the DNeasy Blood & Tissue

204 Kit (Qiagen, Hilden, Germany) was added and the samples were mechanically
205 pounded with two 3 mm sterile stainless steel beads at the speed of 30 Hz for 1.5
206 minutes using a TissueLyser LT (Qiagen, Hilden, Germany). After addition of 30 μ l
207 lysozyme the tubes were incubated at 37 °C for 30 minutes, followed by the addition
208 of 25 mL proteinase K and 200 μ L of the buffer AL with an additional incubation for
209 1 hour at 56 °C on a shaker. Finally, the tubes were centrifuged for 10 minutes at
210 5,000 $\times$ g and the upper liquid was transferred to a new 2 mL tube for extraction by the
211 QiaCube robot, according to manufacturer's instructions (Qiagen, Hilden, Germany).
212 The DNA quantity was assessed using Picogreen (Quant-iT PicoGreen dsDNA
213 reagent; Invitrogen, Carlsbad, CA, USA), and the quality (apparent size and
214 degradation products) was assessed using a TapeStation (Agilent Technologies, Santa
215 Clara, CA, USA).

216 Polymerase chain reaction (PCR) reactions for amplifying 16S rRNA gene fragments
217 were performed in triplicate for each DNA sample, using the following primers that
218 target the V3-V4 region: forward CS1_341F (Muyzer et al., 1993) (5'-ACA CTG
219 ACG ACA TGG TTC TAC ANN NNC CTA CGG GAG GCA GCA G-3') and
220 reverse CS3_806R (Caporaso et al., 2011) (5'-TAC GGT AGC AGA GAC TTG GTC
221 TGG ACT ACH VGG GTW TCT AAT-3'). The PCR reactions were performed in a
222 final volume of 25 μ l, with 10 ng of DNA template. The PCR protocol was as
223 follows: initial denaturation stage at 95 °C for 5 minutes, followed by 28 cycles at 95
224 °C for 30 seconds, 50 °C for 30 seconds, and 72 °C for 60 seconds, with a final
225 extension step at 72 °C for 5 minutes, using BIOLINE 2x MyTaq Red Mix (Sigma-
226 Aldrich, St. Louis, MO, USA), and the PCR reaction was performed in a
227 TProfessional Basic Gradient thermocycler (Biometra, Göttingen, Germany).
228 Following the first PCR, triplicate samples were pooled, and the products were sent to

the DNA Services Facility of the University of Illinois, Chicago, where a second PCR was performed to incorporate barcodes and sequencing adapters (CS1 and CS3). Sequencing was performed using a 2×250 base pair format on a MiSeq flow cell (V3 chemistry, Illumina, USA). The 2013-2014 and 2015 samples were sequenced at different times and on two separate sequencing lanes (see results). The raw sequencing reads are available as BioProject PRJNA688121 at the NCBI SRA database.

2.7 Bioinformatics and statistical analyses

The raw paired-end reads were primer-trimmed using cutadapt (Martin, 2011), and subsequent analyses of the generated sequences were conducted using R (v3.6.3; <http://www.Rproject.org/>) in RStudio (v1.2.5033; <http://www.rstudio.com/>) as described below. The trimmed libraries were processed using DADA2 (Callahan et al., 2016) (v1.14.1), following the suggested tutorial (<https://benjjneb.github.io/dada2/tutorial.html>). Initially, chimeras and singletons were filtered out, and subsequently, amplicon sequence variants (ASVs) were taxonomically classified against the Silva reference database (release 138) (Quast et al., 2012). The ASVs that were taxonomically unclassified to the domain level, or not assigned to bacterial or archaeal lineages, were excluded from further analysis. Furthermore, all ASVs which were taxonomically assigned to mitochondria and chloroplast were removed from the dataset.

Sample data matrices were managed using the R package ‘phyloseq’ (v1.28.0; McMurdie and Holmes, 2013) and plots were generated using R package ‘ggplot2’ (v3.3.0; Wickham, 2016). Calculation and visualization of shared ASVs was conducted using R package ‘venn’ (v1.9; Dusa, 2020). Statistical tests were conducted

253 using R package ‘vegan’ (v2.5.6; Oksanen et al., 2019). The sample rarefaction
254 analyses were conducted using R package ‘iNEXT’ (v2.0.20; Hsieh et al., 2019). All
255 beta-diversity and RDA analyses were conducted on a transformed ASV table using a
256 geometric mean. The fold-change in abundance of each ASV between the water layers
257 was calculated using the R package ‘DEseq2’ (v1.24.0; Love et al., 2014). The
258 method applies a generalized exact binomial test on variance stabilized ASV
259 abundance. The results were filtered by significance, after correction for multiple-
260 testing according to Benjamini and Hochberg (1995) with an adjusted p-value <0.1.

261 Scripts for the molecular data processing and statistical analyses can be accessed at
262 https://github.com/edfadeev/Dor_ponds_16S

263 **3. Results**

264 *3.1 Description of the study site*

265 The Dor Aquaculture Research Unit (DARU - 32°36'25.4"N 34°55'54.7"E) is situated
266 on the coastal plain of Israel (Fig. 1). DARU belongs to the Ministry of Agriculture
267 and Rural development of Israel and operates as a research unit and a semi-
268 commercial outgrowth facility. The research at DARU is performed in approximately
269 80 miniature ponds (0.03 hectare), and focuses on genetically-informed breeding
270 programs, increasing fish health by testing new immunization methods and
271 developing healthier and more ecologically-sound methods of intensive fish rearing
272 (Patil et al., 2016). In addition, DARU has two larger ponds, termed D1 and V2 (1.8
273 and 1.6 hectares each, with 18,000 and 12,000 m³ of water, respectively), which are
274 used for semi-commercial outgrowth of Common carp (*Cyprinus carpio*) and Silver
275 carp (*Hypophthalmichthys molitrix*). All the ponds are connected to an external, one-
276 hectare, operational reservoir (ca. 8,000 m³), which is used to regulate the water levels

277 and temperature in the fishponds. Water is supplied to the reservoir from a local well
278 and from floodwater during the winter months. The ponds and the reservoir are
279 connected by a series of channels (Fig. 1).

280 During the three-year study period, fishpond D1 was stocked with Common carp,
281 whereas fishpond V2 was stocked with Silver carp in 2013 and with Common carp in
282 2014-2015 (Table S1). The outgrowing period of the Common carp is typically about
283 5-7 months whereas that of a Silver carp is shorter (ca. 2-3 months), both occurring
284 during the spring and summer months (i.e., March-September). When no fish are
285 grown the fishponds are kept dry, with the exception of occasional flood-water
286 puddles at the deepest part of the fishpond. At the initiation of the outgrowth period,
287 two-thirds of the fishponds' volume is filled with water pumped from the operational
288 reservoir, and one-third with fresh groundwater from a local well. During the growing
289 period, the water temperature is regulated by adding cold water from the well, or
290 warmer water from the reservoir. At the end of the growing period, water from the
291 fishponds is transferred through connecting channels to the operational reservoir, and
292 then via a natural stream to the Mediterranean Sea. The reservoir contains water
293 throughout the entire year, and was sampled almost monthly, and primarily during the
294 outgrowth period in fishponds D1 and V2.

295

296 *3.2 Water quality parameters change seasonally in the reservoir*

297 We first focus on the operational reservoir, which contains water throughout the entire
298 year and provides a baseline for the system over the three years of sampling (Fig. 2).
299 As expected from a water body situated in a typical Mediterranean climate (cold and
300 wet winters and hot and dry summers), and despite the capacity at DARU to regulate

301 water temperature to some extent by adding well-water, the temperature showed a
 302 clear seasonal cycle, ranging from 10-20 °C during the rainy season (November-April;
 303 further addressed as ‘wet season’) to 20-30 °C during the dry season (May-October;
 304 further addressed as ‘dry season’; Fig. 2a). Inorganic nutrient concentrations were
 305 typically low during the summer months, began rising in autumn and reached the
 306 highest concentrations during the winter months, although some variability was
 307 observed from year to year (Fig. 2a). Importantly, we had expected that the nutrients
 308 in the reservoir would be higher during the fish outgrowth period (spring and
 309 summer), when intensive fish feeding occurs, as a potential result of water from the
 310 fishponds reaching the reservoir (Fig. S1a). The observation that nutrient
 311 concentrations peaked during winter prompted us to search for other, non-aquaculture
 312 related, processes that might have provided nutrient input to the reservoir. One such
 313 process is rainwater runoff from agricultural fields and banana plantations
 314 surrounding DARU (Fig. 1b). Indeed, runoff water sampled during one rain event
 315 (Feb 12, 2015) had high concentrations of inorganic nutrients (270-390, 90-140 and
 316 1200-5600 µg L⁻¹ of ammonia, nitrite and nitrate, respectively). These concentrations
 317 are within the range of the highest concentrations measured in the reservoir over the
 318 time-series.

319

320 *3.3 Water quality parameters, fish biomass and feeding differed between the two* 321 *fishponds*

322 Similar to the reservoir, the temperature changed seasonally in the two fishponds, D1
 323 and V2, and dissolved inorganic nitrogen concentrations were typically higher during
 324 the winter months (even when fish had not been stocked yet; Fig. S1). However, there

were major differences between the fishponds, and between them and the reservoir. The reservoir waters consisted of up to tenfold higher concentrations of inorganic nitrogen (N; $2017 \pm 263 \mu\text{g L}^{-1}$, $n=23$), compared to the fishponds D1 ($619 \pm 301 \mu\text{g L}^{-1}$, $n=19$) and V2 ($464 \pm 222 \mu\text{g L}^{-1}$, $n=20$; Kruskal-Wallis test, Chi square=17.61, $df=1$, $p<0.001$). On the other hand, both fishponds reached up to tenfold higher concentrations of soluble reactive phosphate (P; D1 - $59 \pm 25 \mu\text{g L}^{-1}$, $n=17$; V2 - $91 \pm 21 \mu\text{g L}^{-1}$, $n=19$), compared to the reservoir ($8 \pm 2 \mu\text{g L}^{-1}$, $n=22$; Kruskal-Wallis test, Chi square=17.66, $df=1$, $p<0.001$). The N:P ratio also strongly differed between the ponds. In both the reservoir and the fishpond D1 the median N:P ratio (i.e., the ratio in half of the sampled time points) was equal or above the Redfield ratio of 16N:1P (ca. 275 and ca. 16, respectively). In contrast, in fishpond V2 the median N:P ratio was close to 1. This similarity pattern between the water bodies was also observed in the chlorophyll *a* (chl-*a*) concentrations. In the reservoir and in fishpond D1, the chl-*a* was lower ($0.04 \pm 0.01 \text{ mg L}^{-1}$; and $0.03 \pm 0.01 \text{ mg L}^{-1}$, $n=29$, $n=16$ respectively) compared to the fishpond V2 ($0.12 \pm 0.03 \text{ mg L}^{-1}$).

Over the three years of the study, the fish biomass differed between the two fishponds, D1 and V2 (Fig. S2; see materials and methods for the estimation of biomass and feed from routine aquaculture measurements). During the times where fish were maintained in both ponds, the total estimated fish biomass was always higher in fishpond D1 than at fishpond V2 (Fig. S2). However, the estimated feed input was similar between the two fishponds. Some fish also live in the reservoir, however, they are not actively stocked nor provided with food, and thus the biomass there was assumed to be negligible compared to the densely populated fishponds.

348

349 3.4 Seasonal dynamics are observed in reservoir and the fishponds microbiome 350 structures

351 To obtain an overview of the microbial populations in the reservoir and the fishponds,
352 we sequenced 16S rRNA gene amplicons using Illumina technology. The final dataset
353 consisted of 1,840,160 sequences from 74 samples that were assigned to 5,236 ASVs
354 Amplicon Sequence Variants (Table S2). Rarefaction curves reached a plateau in all
355 of the sampled communities (Fig. S3). An estimated asymptotic extrapolation of the
356 curves to double the amount of sequences (i.e., theoretical deeper sequencing) showed
357 only few additional ASVs, thus suggesting that our sequencing effort was sufficient to
358 represent most of the bacterial diversity. Although identical extraction and
359 amplification protocols were used, we observed a strong difference in the number of
360 sequences per sample and the number of observed ASVs between 2013-2014 and
361 2015, which were sequenced separately (Fig. S3). Therefore, despite the overall
362 similarity in community composition at high taxonomic classifications (e.g., class;
363 Fig. 2b and Fig. S4), further statistical analyses were mainly performed on the 2013-
364 2014 samples, with separated complementary analysis of the 2015 samples.

365 Overall, the bacterial communities of all three water bodies were strongly dominated
366 by the classes *Actinobacteria*, *Alphaproteobacteria*, *Bacteroidia*,
367 *Gammaproteobacteria*, and a prominent bloom of *Cyanobacteria* was observed
368 during the summer months (Fig. 2 and Fig. S1). Richness and diversity indices did not
369 show significant differences between the bacterial communities of the different water
370 bodies (Table S2 tab "Alpha_diversity"; Kruskal-Wallis test, $p > 0.05$). The
371 communities of the reservoir and the fishponds shared 497 ASVs (one-fifth of total
372 observed ASVs; Fig. S4), that comprised ca. 30-80% of the sequences in the bacterial
373 communities. Each water body contained an additional ca. 500 unique ASVs (one

third of the ASVs observed in each water body), which constituted up to 15% of the total ASVs. The rest of the ASVs were shared between two of the three water bodies.

We had expected to observe major differences between the fishponds, where high fish biomass is grown, and the reservoir, in which fish are not actively stocked. Indeed, significant differences in the bacterial community composition of the two water bodies were revealed (Fig. S5a; PERMANOVA test, $F_{2,42}=1.55$, $R^2=0.06$, $p<0.01$), however, seasonal effects on the microbiome appeared to be a stronger explanatory variable (PERMANOVA test, $F_{1,42}=5.5$, $R^2=0.10$, $p<0.001$). This suggests similar seasonal dynamics of the microbial population in all three water bodies. Furthermore, a separate analysis of the bacterial communities in 2015 revealed similar pattern of a significant dissimilarity between the water bodies (PERMANOVA test, $F_{2,20}=1.66$, $R^2=0.12$, $p<0.01$), which was lower than between the seasons (PERMANOVA test, $F_{2,20}=2.45$, $R^2=0.09$, $p<0.001$).

3.5 Specific ASVs enriched in each water body

Using comparative sequence enrichment tests, we identified the bacterial genera that had significantly different relative sequence abundance between wet and dry seasons in all water bodies combined. The ASVs within each genus were defined as enriched when they had a $\log_2$ fold change >1 and an adjusted p value <0.1 . We found that 84 and 74 ASVs were significantly enriched in the bacterial communities of dry and wet seasons, respectively (Fig. 3). In both seasons the sequences of these enriched ASVs comprised 20-60% of the bacterial communities (Fig. 3). In the wet season, the enriched ASVs of the genera *Limnohabitans* (class *Gammaproteobacteria*; total of 11 ASVs) comprised $8\pm1\%$ of the bacterial communities. In the dry season, a quarter of

the enriched ASV (total of 23) were of *Cyanobacteria*. Enriched ASVs of the cyanobacterial genera *Cyanobium* (15 ASVs) comprised $8 \pm 1\%$ of the bacterial communities. Furthermore, there were 2 significantly enriched ASVs from the genus *Microcystis*, which comprised $5 \pm 2\%$ of the bacterial communities. Thus, the cyanobacterial blooms are a pervasive phenomenon underlying the differences in community composition between the wet and dry seasons. These blooms were associated with the presence of cyanobacterial toxins in the water (microcystins or nodularins, Fig. 2a and Fig. S1).

3.6 The effect of physicochemical conditions on the bacterial community dynamics

In order to identify whether the observed seasonal changes in the bacterial communities are associated with natural physicochemical dynamics or with aquaculture-related dynamics we performed redundancy analyses (RDA) constrained by various measured and estimated parameters. Temperature, inorganic nutrient concentrations (phosphate, ammonia, nitrate and nitrite), as well as N:P ratio and microcystin concentrations, showed the highest explanatory power, explaining 15% of the total variation in the bacterial communities. These parameters separated the bacterial communities of wet and dry seasons, in accordance with the unconstrained dissimilarity analysis (Fig. 4a). The wet season bacterial communities were associated with lower water temperatures and higher concentrations of N, whereas the bacterial communities of the dry season were associated with higher temperatures and to some extent with higher microcystin concentrations.

Interestingly, in 2013 we observed association between the bacterial communities of the dry season in fishpond V2 with higher P concentrations. The concentrations of P

in all sampled water bodies strongly correlated to concentrations of chlorophyll *a* and other photosynthetic pigments (Fig. S7), and thus may represent distinct phytoplankton bloom conditions. An RDA constrained by concentrations of photosynthetic pigments explained 8% of the total bacterial community variation, and revealed a similar separation pattern between wet and dry seasons (Fig. 4b). In this analysis, the dry season bacterial communities of fishpond V2 corresponded with higher concentrations of the cyanobacteria-related pigment zeaxanthin. Aquaculture-related estimated parameters, total fish biomass and fish-feed weight, explained only 4% of the total variation in the bacterial communities and did not reveal a clear separation between the fishponds and the untreated reservoir in an RDA ordination (Fig. S6).

In addition to the effect of environmental parameters on the microbial community structure, a strong association was identified between specific ASVs and environmental parameters. Two clusters of cyanobacterial ASVs were identified, one of which seemed to be associated with higher P concentrations and the other with higher microcystin concentrations (Fig. 5a). These cyanobacterial ASVs were mostly associated with the species *Cyanobium* PCC-6307 (15/23 ASVs), with no clear differences between the two clusters. As expected, the *Cyanobacteria* enriched ASVs corresponded to higher concentrations of cyanobacterial pigment zeaxanthin (Fig. 5b).

4. Discussion

Aquaculture facilities are anthropogenically altered hypertrophic ecosystems. Densely stocked aquaculture fishponds are supplemented with kilograms of fish-feed daily, which in most cases is not fully consumed. The remaining fish-feed, combined with

446 fish excretions, leads to an increase in concentrations of nutrients in the water. In
 447 addition, the fishponds often receive various pharmaceuticals (Bricknell and Dalmo,
 448 2005; Gudding and Van Muiswinkel, 2013; Sommerset et al., 2005) including
 449 antibiotics (Cháfer-Pericás et al., 2010; Kirchhelle, 2018; Lalumera et al., 2004). The
 450 resulting conditions, which are often hypereutrophic, are very different from natural
 451 freshwater environments (Klippel et al., 2020). At our study site, we examined two
 452 intensive aquaculture fishponds that are connected to an operational reservoir, which
 453 does not contain fish biomass and is not subjected directly to the intense aquaculture
 454 practices. At the initiation of this study, we expected the water quality in the
 455 fishponds, as well as the fishpond microbiomes, to differ significantly from those in
 456 the more pristine reservoir. Indeed, the nutrient concentrations in the fishponds were
 457 different from those in the reservoir, characterized by higher P and lower N
 458 concentrations, leading to differences in the N:P ratio. Therefore, it was surprising
 459 that the bacterial communities of the three water bodies were not very different, and
 460 their dynamics were primarily dictated, somewhat predictably, by seasonal (non-
 461 anthropogenic) parameters. Similar dynamics were observed recently in the Lake
 462 Taihu, where seasonality was shown to “overwhelm” net-pens aquaculture activity
 463 (Zeng et al., 2019). Our results extend this observation to a system where the water
 464 bodies are separate (linked by limited water flow), and differences in water quality
 465 (nutrients, chl-*a*) between the water bodies are much more pronounced.

466

467 *4.1 Temperature and rainwater runoff as potential seasonal drivers of water quality*
 468 *and bacterial diversity.*

469 What are the potential environmental drivers of the observed seasonal patterns? We
 470 identified temperature as a significant explanatory variable of bacterial community

composition, and indeed several studies have highlighted the importance of temperature as a driver of microbiome composition in aquatic environments (McFall-Ngai et al., 2013; Sunagawa et al., 2015). This includes aquaculture facilities in Lake Taihu as well as a semi-intensive aquaculture systems for growing sea bass and sea bream in Ria de Aveiro estuarine lagoon, Portugal (Duarte et al., 2019; Zeng et al., 2019). Temperature can also impact the gut microbiomes of fish and other aquatic organisms, such as tadpoles, which could also affect the structure of the fishpond microbiome (Kohl and Yahn, 2016; Kokou et al., 2018).

An additional seasonal factor that could affect water quality and subsequently the aquatic microbiome, is rainfall. During significant rain events, DARU receives runoff from the surrounding agricultural fields, carrying sediments, nutrients, fertilizers, pesticides and other soil-based compounds (Kraemer et al., 2020; Mallin et al., 2009; Maltby et al., 1995; Marmen et al., 2020; Page et al., 1995). While we lack a quantitative budget of the water and nutrient inputs into the reservoir and fishponds, our results suggest that such runoff may be important at DARU. Importantly, rainwater collected through runoff is often used as a source of water for aquaculture, and the availability of such water is likely to be reduced due to climate change (Ahmed et al., 2018). Our results highlight the need for understanding rainwater-driven links between aquaculture facilities and their surrounding terrestrial environments, through input of microorganisms, nutrients and potential contaminants (e.g., fertilizers and pesticides).

492

493 *4.2 Aquaculture practices may affect the bacterial diversity via nutrient* 494 *concentrations and N:P ratios*

495 Despite the importance of seasonality in determining the microbial population at
496 DARU, our results also revealed differences in the water quality and bacterial
497 community composition between the two fishponds, and between them and the
498 reservoir. The constantly full reservoir showed much higher concentrations of N,
499 compared to the fishponds that are emptied annually and are refilled mostly with new
500 underground water. It is therefore possible that seasonality, for example in rainwater
501 runoff, exerts a stronger influence on the “older” waters of the reservoir. On the other
502 hand, both fishponds revealed tenfold higher P concentrations, compared to the
503 reservoir. The much higher concentrations of P in the fishponds are likely a result of
504 the nutritional input into them, as the fish-feed used had an estimated 8% N (50%
505 protein times 16% N in protein) compared to 1.5% PO₄ (N:P ratio of 5.3). Indeed, the
506 concentration of P in the water increased during the fish-stocking season.

507 The differences in the timing, amplitude, and potential source of nutrient input into
508 the system led to pronounced differences in the N:P ratio between the three water
509 bodies. The ratio between inorganic N and P is often used to infer the limiting nutrient
510 for phytoplankton growth (Deutsch and Weber, 2012). Based on this logic, the very
511 high N:P ratios in the reservoir suggest that primary production of this freshwater
512 body is limited by availability of P. Fishpond D1 was likely also P-limited, whereas
513 fishpond V2 was likely mostly N limited. The differences in nutrient concentrations,
514 and potentially in the identity of the limiting nutrient, may be due with the
515 aquaculture-related mismatch of the similar fish-feed input despite the severalfold
516 lower fish biomass in fishpond V2, compared to fishpond D1, which was especially
517 pronounced in the seasons of 2013. Notably, chl-*a* concentrations were typically

518 higher in fishpond V2 compared to D1 and the reservoir. It is tempting to speculate
519 that the higher chl-*a* concentrations in fishpond V2 are a result of the surplus P in this
520 water body, although other causes such as differences in zooplankton or fish grazing
521 cannot be ruled out. The bacterial communities of fishpond V2 during the dry season
522 of 2013, where chl-*a* was high, also showed a strong association with higher
523 concentrations of P. Therefore, we hypothesize that characteristics (e.g., magnitude)
524 of the phytoplankton bloom, including also cyanobacteria, may be influenced by
525 aquaculture-associated processes, in turn affecting the microbial communities.

526

527 *4.3 Cyanobacterial blooms as major seasonal occurrences in aquaculture*

528 Perhaps the most clearly observed seasonal change in the bacterial community
529 structure were the summer cyanobacterial blooms. As a group, cyanobacteria
530 generally exhibit optimal growth rates at relatively high temperatures, usually in
531 excess of 25 °C (Paerl and Huisman, 2009; Robarts and Zohary, 1987) and the
532 formation of cyanobacterial blooms is encouraged by warm and calm weather
533 (Kanoshina et al., 2003). These conditions are prevalent during the summer months in
534 Israel. Certain cyanobacteria can produce metabolites that cause undesirable flavors to
535 the fish products (Lee et al., 2017; Lindholm-Lehto and Vielma, 2019; Mares et al.,
536 2009; Paerl and Tucker, 1995; Schrader and Dennis, 2005; Tucker and Schrader,
537 2020), and/or various toxins that can kill both invertebrates and fish (Oberemm et al.,
538 1999; Smith et al., 2008). Over the course of this study, cyanotoxins (microcystins or
539 nodularins) were repeatedly observed in the particulate fraction of the reservoir and
540 the fishponds during summer, at concentrations that, at times, were above the
541 recommended limit for drinking water of 1 µg microcystins L⁻¹ (according to

542 guidelines set by the World Health Organization (WHO, 2019). Microcystins have
 543 repeatedly been documented in aquaculture ponds worldwide, at concentrations
 544 similar to, or higher than, observed here (Ahmed et al., 2008; Chia et al., 2009). In a
 545 survey of 485 catfish production ponds across the Southeastern USA, microcystins
 546 were detected in 47% of the ponds, with ca. 10% of the ponds containing microcystin
 547 concentrations were above the World Health Organization limit (Zimba and Grimm,
 548 2003). These concentrations may cause damage to fish tissues, as demonstrated in a
 549 survey of fishponds in Serbia (Drobac et al., 2016). Additionally, there is evidence
 550 that microcystins affect human epidermal skin cells (Kozdęba et al., 2014), and that
 551 exposure to non-lethal doses ($0.5 \mu\text{g L}^{-1}$) may contribute to the development of
 552 hepatocellular carcinoma (Pearson et al., 2010; Yoshizawa et al., 1990; Yu, 1995).
 553 Given that many aquaculture workers are often not aware of the presence of toxins in
 554 the water or their potentially harmful impact (based on personal communication with
 555 aquaculture workers around Israel), future research assessing the effects of chronic
 556 exposure, followed if needed by appropriate communication and training, are
 557 important.

558
 559 While microcystins or nodularins were observed in the water of the reservoir every
 560 summer, the cyanobacteria that produced the blooms differed from year to year, and
 561 even between consecutive months of the same year (Fig. S8). Indeed, some
 562 cyanobacterial ASVs seemed to be highly specific, appearing only ephemerally, for
 563 example an ASV related to *Sphaerospermopsis torques-reginae*, which was present at
 564 high relative sequence abundance in fishpond V2 during August 2015, but was almost
 565 completely absent at other times. More generally, four main families contributed to
 566 these blooms: *Microcystaceae* (genus *Microcystis*), *Phormidiaceae* (genus

567 *Planktothrix*), *Oscillatoriaceae* (an ASV related to *Planktothrocoides raciborskii*) and
568 *Cyanobiaceae* (genus *Cyanobium*; Fig. S8). There appeared to be consistent
569 differences between the ponds in the composition of the cyanobacterial community.
570 For example, while the reservoir and fishpond V2 were often characterized by high
571 relative sequence abundances of *Cyanobium*, this clade was much less common in
572 fishpond D1, which was usually dominated by *Microcystis*.

573 *Microcystis* are one of the most common bloom-forming, and potentially toxin-
574 producing, cyanobacteria (Manach et al., 2018). Blooms of *Microcystis*, including
575 toxic species, are a well-known issue in Israel, particularly for growers of the
576 Common carp in the northern coastal region and the Beit-Shean valley (e.g., Elkobi-
577 Peer and Carmeli, 2015). In fact, in our previous work, we detected the presence of
578 *Microcystis* with the potential of microcystins production in all sampled fishponds in
579 Israel (Marmen et al., 2016). Such blooms are also common elsewhere in the world
580 (Berdalet et al., 2018; Paerl and Paul, 2012; Zimba et al., 2001). However, in this
581 study there were times during which, based on the 16S rRNA amplicons, *Microcystis*
582 was almost absent, yet microcystins were present in the water. For example, a major
583 bloom of *Planktothrocoides raciborskii* was observed in the reservoir during July
584 2013, with relatively high microcystin concentrations (Fig. 2, Fig. S8). However,
585 during this bloom <1% of the 16S reads were associated with *Microcystis*. At this
586 time, it is possible that the microcystins were produced by a *Planktothrix* that
587 comprised ca. 8% of the 16S rRNA reads (Christiansen et al., 2003).

588 Finally, the cyanobacterial clade that differed between the wet and dry periods was
589 *Cyanobium*. Relatively little is known about this clade of pico-cyanobacteria
590 (Komárek et al., 1999; Pagels et al., 2020), despite its observed dominance in

591 cyanobacterial blooms, including toxic ones (Li et al., 2020). They are typically found
592 as free-living single cells, but can produce colonies in response to grazing pressure
593 (Huber et al., 2017; Jezberová and Komárková, 2007). They have also been shown to
594 produce allelopathic substances, although there is currently no evidence that they can
595 produce microcystins (Costa et al., 2015; Kovács et al., 2018). Multiple ASVs of this
596 clade were observed, and more research is needed in order to determine which factors
597 govern the dynamics of this microorganism in the DARU system or in freshwater
598 environments in general.

599

600 *4.4 Are heterotrophic bacteria associated with cyanobacterial blooms or aquaculture*
601 *practices?*

602 In this study, we identified major changes in the bacterial community structure
603 between the wet and dry seasons, and smaller changes potentially associated with the
604 aquaculture activity. While cyanobacteria were clearly associated with the dry season,
605 several other genera of bacteria were enriched in the dry or wet seasons. Notably, due
606 to the fractional (relative) nature of 16S rRNA amplicon sequencing, changes in
607 relative sequence abundance of an ASV cannot be equated with actual changes in the
608 cell numbers of the relevant organisms (McLaren et al., 2019). The increase in
609 cyanobacterial sequences, and the enrichment of cyanobacterial ASVs during the dry
610 season, are likely due to blooms of these organisms, since increases were observed in
611 both zeaxanthin, a pigment associated with cyanobacteria (Zhang et al., 2018), and
612 cyanotoxins. For this reason, it is unclear whether the enrichment of genera such as
613 *Polynucleobacter*, *Limnohabitans* and *Hydrogenophaga* in the wet season represents
614 bona-fide blooms of these organisms, or whether this is a result of the increase in

615 relative sequence abundance of cyanobacteria. In contrast, enrichment of ASVs in the
616 dry season may be easier to interpret as bona-fide biological patterns in abundance. In
617 this regard, the enrichment of nine ASVs belonging to the family *Saprospiraceae* clade
618 during the dry season is intriguing, as this clade is thought to be associated with the
619 hydrolysis and utilization of complex carbon sources as well as with active predation
620 on bacteria and algae, including *Microcystis* (Ashton and Robarts, 1987).
621 Additionally, both *Saprospiraceae* and another group enriched in the dry season, the
622 genus *Haliscomenobacter*, has been suggested to be associated with the presence of
623 both cyanobacterial blooms and antibiotic resistance genes in Lake Taihu (Zhang et
624 al., 2020a). While antibiotics are not used directly in the three sampled water bodies,
625 they are used prophylactically at DARU at the beginning of the fish fattening cycle
626 (beginning of dry season), including in additional fishponds connected to the system
627 (Patil et al., 2016; Patil et al., 2020).

628
629 It has previously been suggested that the microorganisms in the aquaculture water
630 may have a direct effect on the health of the fish (Assefa and Abunna, 2018; Kim and
631 Lee, 2017). For example, environmental microorganisms can form the initial inocula
632 for fish eggs or larvae, and pathogens can be transferred through the water, especially
633 when fish biomass/density is very high. Therefore, significant effort has been invested
634 in studying the aquatic microbiome in some aquaculture types, with the aim of using
635 these measurements to understand fish health, to identify biomarkers/early markers of
636 emerging diseases, or to explore the use of probiotics (Dawood et al., 2019; Kolda et
637 al., 2020; Kuebutornye et al., 2019; Zhang et al., 2020b). In our dataset, we identify
638 several bacterial lineages known as fish pathogens, including the genera *Aeromonas*,
639 *Vibrio* and *Enterobacter*, in agreement with previous studies from DARU (Patil et al.,

2016; Patil et al., 2020). However, due to the limited phylogenetic resolution of partially sequenced 16S rRNA gene, it is usually not possible to identify whether the observed taxa are associated specifically with pathogenic strains. For such analysis, one would need to analyze additional markers, for example genes related directly with pathogenicity. Nevertheless, we did identify one potentially interesting pattern, whereby *Vibrio*-related ASVs were found almost only in fishpond D1, and specifically during May and June of 2013 and 2014 (but not 2015), during which the *Vibrio* comprised 3-12% of the bacterial community. During these three years, fishpond D1 was used for Common carp outgrowth, however, no major cases of pathogen outbreaks were observed in the fishpond. Interestingly, during 2014 and 2015, fishpond V2 was used for outgrowth of Common carp larvae, but we haven't detected *Vibrio* presence there. Thus, further study is needed to identify potential reasons for the strong association of *Vibrio* with a specific fishpond and time, and to what extent this is associated with changes in fish health.

5. Conclusions

As human impact on aquatic environments increases, it is imperative to understand how microbial communities will respond to anthropogenic stressors, and to differentiate between these responses and natural ecosystem dynamics. The aquaculture unit at DARU represents a unique semi-controlled system where water bodies under strong anthropogenic pressure – densely stocked fishponds – are tightly connected with a more “pristine” water body (the reservoir). Our results show that, even in this highly modified aquatic ecosystem, natural seasonality (e.g. temperature and rainwater runoff) is the main driver of the bacterial diversity. Perhaps the clearest manifestation of the natural cycle is the presence of cyanobacterial blooms during

summer. However, the cyanobacterial bloom dynamics, which at times also included toxin-producing organisms, seem to be affected by aquaculture practices, such as fish feeding. Thus, based on the results presented here, we suggest that nutrient dynamics and possibly elemental ratios in freshwater environments, including in aquaculture, should be taken into account in the management of such ecosystems.

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.

Author contributions

Sophi Marmen: Conceptualization, Data curation, Investigation, Formal analysis, Visualization, Writing – original draft; review and editing. **Eduard Fadeev:** Data curation, Formal analysis, Visualization, Writing – original draft; review and editing. **Ashraf Al Ashhab:** Data curation, Formal analysis, Visualization, Writing – original draft; review and editing. **Ayana Benet-Perelberg:** Conceptualization, Investigation, Writing –review and editing. **Alon Naor:** Investigation, Writing –review and editing. **Hemant J. Patil:** Investigation, Writing –review and editing. **Eddie Cytryn:** Conceptualization, Funding Acquisition, Writing –review and editing. **Diti Viner-Mozzini:** Investigation, Writing –review and editing. **Assaf Sukenik:** Conceptualization, Funding Acquisition, Writing –review and editing. **Maya Lalzar:** Data curation, Formal analysis, Supervision, Writing –review and editing. **Daniel Sher:** Conceptualization, Project administration, Investigation, Formal analysis, Visualization, Writing –review and editing, Supervision, Funding Acquisition.

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695 **References**

- 696 Ahmed M, Hiller S, Luckas B. Microcystis aeruginosa bloom and the occurrence of
697 microcystins (heptapeptides hepatotoxins) from an aquaculture pond in
698 Gazipur, Bangladesh. Turkish Journal of Fisheries and Aquatic Sciences 2008;
699 8: 37-41.
- 700 Ahmed N, Thompson S. The blue dimensions of aquaculture: A global synthesis.
701 Science of The Total Environment 2019; 652: 851-861.
- 702 Ahmed N, Ward JD, Thompson S, Saint CP, Diana JS. Blue-Green Water Nexus in
703 Aquaculture for Resilience to Climate Change. Reviews in Fisheries Science
704 & Aquaculture 2018; 26: 139-154.
- 705 Alderman D, Hastings T. Antibiotic use in aquaculture: development of antibiotic
706 resistance–potential for consumer health risks. International journal of food
707 science & technology 1998; 33: 139-155.
- 708 Ashton PJ, Robarts RD. Apparent predation of Microcystis Aeruginosa Kütz. emend
709 elenkin by a saprospira-like bacterium in a hypertrophic lake (Hartbeespoort

710 Dam, south Africa). Journal of the Limnological Society of Southern Africa
711 1987; 13: 44-47.

712 Assefa A, Abunna F. Maintenance of Fish Health in Aquaculture: Review of
713 Epidemiological Approaches for Prevention and Control of Infectious Disease
714 of Fish. Veterinary Medicine International 2018; 2018: 5432497.

715 Bautista-de los Santos QM, Chavarria KA, Nelson KL. Understanding the impacts of
716 intermittent supply on the drinking water microbiome. Current Opinion in
717 Biotechnology 2019; 57: 167-174.

718 Baxaa M, Musil M, Kummel M, Hanzlík B, Pechar L. Dissolved oxygen deficits in a
719 shallow eutrophic aquatic ecosystem (fishpond) – Sediment oxygen demand
720 and water column respiration alternately drive the oxygen regime. Science of
721 The Total Environment 2020.

722 Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and
723 Powerful Approach to Multiple Testing. Journal of the Royal Statistical
724 Society: Series B (Methodological) 1995; 57: 289-300.

725 Berdalet E, Kudela RM, Banas NS, Bresnan E, Burford MA, Davidson K, et al.
726 GlobalHAB: Fostering International Coordination on Harmful Algal Bloom
727 Research in Aquatic Systems. In: Glibert PM, Berdalet E, Burford MA,
728 Pitcher GC, Zhou M, editors. Global Ecology and Oceanography of Harmful
729 Algal Blooms. Springer International Publishing, Cham, 2018, pp. 425-447.

730 Bricknell I, Dalmo RA. The use of immunostimulants in fish larval aquaculture. Fish
731 & shellfish immunology 2005; 19: 457-472.

732 Bruno A, Sandionigi A, Bernasconi M, Panio A, Labra M, Casiraghi M. Changes in
733 the Drinking Water Microbiome: Effects of Water Treatments Along the Flow

734 of Two Drinking Water Treatment Plants in a Urbanized Area, Milan (Italy).
735 Frontiers in Microbiology 2018; 9.

736 Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2:
737 High-resolution sample inference from Illumina amplicon data. Nature
738 Methods 2016; 13: 581-583.

739 Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ,
740 Fierer N and Knight R. Global patterns of 16S rRNA diversity at a depth of
741 millions of sequences per sample. PNAS 2011; 108: 4516-4522

742 Cháfer-Pericás C, Maquieira Á, Puchades R, Company B, Miralles J, Moreno A.
743 Multiresidue determination of antibiotics in aquaculture fish samples by
744 HPLC–MS/MS. Aquaculture Research 2010; 41: e217-e225.

745 Chia A, Oniye S, Ladan Z, Lado Z, Pila A, Inekwe V, et al. A survey for the presence
746 of microcystins in aquaculture ponds in Zaria, Northern-Nigeria: Possible
747 public health implication. African Journal of Biotechnology 2009; 8.

748 Christiansen G, Fastner J, Erhard M, Börner T and Dittmann E. Microcystin
749 Biosynthesis in Planktothrix: Genes, Evolution, and Manipulation. Journal of
750 Bacteriology 2003; 185: 564-572

751 Costa MS, Costa M, Ramos V, Leão PN, Barreiro A, Vasconcelos V, et al.
752 Picocyanobacteria from a clade of marine Cyanobium revealed bioactive
753 potential against microalgae, bacteria, and marine invertebrates. J Toxicol
754 Environ Health A 2015; 78: 432-42.

755 Dawood MAO, Koshio S, Abdel-Daim MM, Van Doan H. Probiotic application for
756 sustainable aquaculture. Reviews in Aquaculture 2019; 11: 907-924.

757 Deutsch C, Weber T. Nutrient ratios as a tracer and driver of ocean biogeochemistry.
758 Annual Review of Marine Science 2012; 4: 113-141.

759 Dokulil MT, Teubner K. Cyanobacterial dominance in lakes. *Hydrobiologia* 2000;
760 438: 1-12.

761 Drobac D, Tokodi N, Lujic J, Marinović Z, Subakov-Simić G, Dulić T, Važić T,
762 Nybom S, Meriluoto J, Codd GA and Svirčev Z. Cyanobacteria and
763 cyanotoxins in fishponds and their effects on fish tissue. *Harmful Algae* 2016;
764 55: 66-76

765 Duarte LN, Coelho FJ, Cleary DF, Bonifácio D, Martins P, Gomes NC. Bacterial and
766 microeukaryotic plankton communities in a semi-intensive aquaculture system
767 of sea bass (*Dicentrarchus labrax*): A seasonal survey. *Aquaculture* 2019; 503:
768 59-69.

769 Dusa A. Draw Venn Diagrams, 2020. <https://CRAN.R-project.org/package=venn>

770 Elkobi-Peer S, Carmeli S. New prenylated aeruginosin, microphycin, anabaenopeptin
771 and micropeptin analogues from a *Microcystis* bloom material collected in
772 Kibbutz Kfar Blum, Israel. *Marine drugs* 2015; 13: 2347-2375.

773 Garlock T, Asche F, Anderson J, Bjørndal T, Kumar G, Lorenzen K, et al. A Global
774 Blue Revolution: Aquaculture Growth Across Regions, Species, and
775 Countries. *Reviews in Fisheries Science & Aquaculture* 2020; 28: 107-116.

776 Giatsis C, Sipkema D, Ramiro-Garcia J, Bacanu GM, Abernathy J, Verreth J, et al.
777 Probiotic legacy effects on gut microbial assembly in tilapia larvae. *Scientific*
778 *Reports* 2016; 6: 33965.

779 Glibert PM, Landsberg JH, Evans JJ, Al-Sarawi MA, Faraj M, Al-Jarallah MA, et al.
780 A fish kill of massive proportion in Kuwait Bay, Arabian Gulf, 2001: the roles
781 of bacterial disease, harmful algae, and eutrophication. *Harmful Algae* 2002;
782 1: 215-231.

783 Gowen RJ. Managing eutrophication associated with aquaculture development.
784 Journal of Applied Ichthyology 1994; 10: 242-257.

785 Grasshoff K, Kremling K, Ehrhardt M. Methods of seawater analysis: John Wiley &
786 Sons, 2009.

787 Grzeškowiak Ł, Collado MC, Salminen S. Evaluation of aggregation abilities between
788 commensal fish bacteria and pathogens. Aquaculture 2012; 356-357: 412-414.

789 Gudding R, Van Muiswinkel WB. A history of fish vaccination: science-based disease
790 prevention in aquaculture. Fish & shellfish immunology 2013; 35: 1683-1688.

791 Hach PF, Marchant HK, Krupke A, Riedel T, Meier DV, Lavik G, et al. Rapid
792 microbial diversification of dissolved organic matter in oceanic surface waters
793 leads to carbon sequestration. Scientific Reports 2020; 10: 13025.

794 Havens KE. Cyanobacteria blooms: effects on aquatic ecosystems. Springer, 2008, pp.
795 733-747.

796 Hooker SB, McClain CR. The calibration and validation of SeaWiFS data. Progress in
797 Oceanography 2000; 45: 427-465.

798 Hsieh T, Ma K, Chao A. iNEXT: iNterpolation and EXTrapolation for species
799 diversity. R package version 2.0. 19. Fecha de consulta: 13 de marzo de 2019,
800 2019.

801 Huber P, Diovisalvi N, Ferraro M, Metz S, Lagomarsino L, Llames ME, et al.
802 Phenotypic plasticity in freshwater picocyanobacteria. Environ Microbiol
803 2017; 19: 1120-1133.

804 Jezberová J, Komárková J. Morphological transformation in a freshwater Cyanobium
805 sp. induced by grazers. Environ Microbiol 2007; 9: 1858-62.

806 Kanoshina I, Lips U, Leppänen J-M. The influence of weather conditions
807 (temperature and wind) on cyanobacterial bloom development in the Gulf of
808 Finland (Baltic Sea). *Harmful Algae* 2003; 2: 29-41.

809 Kim JY, Lee J-L. Correlation of Total Bacterial and *Vibrio* spp. Populations between
810 Fish and Water in the Aquaculture System. *Frontiers in Marine Science* 2017;
811 4.

812 Kirchhelle, C. Pharming animals: a global history of antibiotics in food production
813 (1935–2017). *Palgrave Communications* 2018; 4: 1-13

814 Klippel G, Macêdo RL, Branco CWC. Comparison of different trophic state indices
815 applied to tropical reservoirs. *Lakes & Reservoirs: Science, Policy and*
816 *Management for Sustainable Use* 2020; 25: 214-229.

817 Kohl KD, Yahn J. Effects of environmental temperature on the gut microbial
818 communities of tadpoles. *Environmental Microbiology* 2016; 18: 1561-1565.

819 Kokou F, Sasson G, Nitzan T, Doron-Faigenboim A, Harpaz S, Cnaani A, et al. Host
820 genetic selection for cold tolerance shapes microbiome composition and
821 modulates its response to temperature. *Elife* 2018; 7: e36398.

822 Kolda A, Gavrilović A, Jug-Dujaković J, Ljubešić Z, El-Matbouli M, Lillehaug A, et
823 al. Profiling of bacterial assemblages in the marine cage farm environment,
824 with implications on fish, human and ecosystem health. *Ecological Indicators*
825 2020; 118: 106785.

826 Komárek J, Kopecký J, Cepák V. Generic characters of the simplest cyanoprokaryotes
827 *Cyanobium*, *Cyanobacterium* and *Synechococcus*. *Cryptogamie Algologie*
828 1999; 20: 209-222.

829 Kovács AW, Tóth VR, Pálffy K. The effects of interspecific interactions between
830 bloom forming cyanobacteria and *Scenedesmus quadricauda* (chlorophyta) on
831 their photophysiology. *Acta Biol Hung* 2018; 69: 210-223.

832 Kozdęba M, Borowczyk J, Zimoląg E, Wasylewski M, Dziga D, Madeja Z, et al.
833 Microcystin-LR affects properties of human epidermal skin cells crucial for
834 regenerative processes. *Toxicon* 2014; 80: 38-46.

835 Kraemer SA, Barbosa da Costa N, Shapiro BJ, Fradette M, Huot Y, Walsh DA. A
836 large-scale assessment of lakes reveals a pervasive signal of land use on
837 bacterial communities. *The ISME Journal* 2020; 14: 3011-3023.

838 Kuebutornye FKA, Abarike ED, Lu Y. A review on the application of *Bacillus* as
839 probiotics in aquaculture. *Fish & Shellfish Immunology* 2019; 87: 820-828.

840 Lalumera GM, Calamari D, Galli P, Castiglioni S, Crosa G, Fanelli R. Preliminary
841 investigation on the environmental occurrence and effects of antibiotics used
842 in aquaculture in Italy. *Chemosphere* 2004; 54: 661-668.

843 Li H, Barber M, Lu J, Goel R. Microbial community successions and their dynamic
844 functions during harmful cyanobacterial blooms in a freshwater lake. *Water*
845 *Research* 2020; 185: 116292

846 Lee J, Rai PK, Jeon YJ, Kim K-H, Kwon EE. The role of algae and cyanobacteria in
847 the production and release of odorants in water. *Environmental Pollution*
848 2017; 227: 252-262.

849 Lindholm-Lehto PC, Vielma J. Controlling of geosmin and 2-methylisoborneol
850 induced off-flavours in recirculating aquaculture system farmed fish—A
851 review. *Aquaculture Research* 2019; 50: 9-28.

852 Liu J, Wu Y, Wu C, Muylaert K, Vyverman W, Yu H-Q, et al. Advanced nutrient
853 removal from surface water by a consortium of attached microalgae and
854 bacteria: A review. *Bioresource Technology* 2017; 241: 1127-1137.

855 Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion
856 for RNA-seq data with DESeq2. *Genome Biology* 2014; 15: 550.

857 Mallin MA, Johnson VL, Ensign SH. Comparative impacts of stormwater runoff on
858 water quality of an urban, a suburban, and a rural stream. *Environmental*
859 *monitoring and assessment* 2009; 159: 475-491.

860 Maltby L, Forrow DM, Boxall AB, Calow P, Betton CI. The effects of motorway
861 runoff on freshwater ecosystems: 1. Field study. *Environmental Toxicology*
862 *and Chemistry: An International Journal* 1995; 14: 1079-1092.

863 Manach S.L, Sotton B, Huet H, Duval C, Paris A, Marie A, Yépreman C, Catherine
864 A, Mathéron L, Vinh J, Edery M and Marie B. Physiological effects caused by
865 microcystin-producing and non-microcystin producing *Microcystis aeruginosa*
866 on medaka fish: A proteomic and metabolomic study on liver. *Environmental*
867 *Pollution* 2018; 523-537

868 Mares J, Palikova M, Kopp R, Navratil S, Pikula J. Changes in the nutritional
869 parameters of muscles of the common carp (*Cyprinus carpio*) and the silver
870 carp (*Hypophthalmichthys molitrix*) following environmental exposure to
871 cyanobacterial water bloom. *Aquaculture Research* 2009; 40: 148-156.

872 Marmen S, Aharonovich D, Grossowicz M, Blank L, Yacobi YZ, Sher DJ.
873 Distribution and habitat specificity of potentially-toxic *Microcystis* across
874 climate, land, and water use gradients. *Frontiers in microbiology* 2016; 7: 271.

875 Marmen S, Blank L, Al-Ashhab A, Malik A, Ganzert L, Lalzar M, et al. The role of
876 land use types and water chemical properties in structuring the microbiomes of
877 a connected lake system. *Frontiers in microbiology* 2020; 11: 89.

878 Martin M. Cutadapt removes adapter sequences from high-throughput sequencing
879 reads. *EMBnet.journal* 2011; 10-12.

880 McFall-Ngai M, Hadfield MG, Bosch TC, Carey HV, Domazet-Lošo T, Douglas AE,
881 et al. Animals in a bacterial world, a new imperative for the life sciences.
882 *Proceedings of the National Academy of Sciences* 2013; 110: 3229-3236.

883 McLaren MR, Willis AD, Callaha BJ. Consistent and correctable bias in metagenomic
884 sequencing experiments. *eLife* 2019;8:e46923.

885 McMurdie PJ, Holmes S. phyloseq: An R Package for Reproducible Interactive
886 Analysis and Graphics of Microbiome Census Data. *PLOS ONE* 2013; 8:
887 e61217.

888 Mennerat A, Nilsen F, Ebert D, Skorpung A. Intensive Farming: Evolutionary
889 Implications for Parasites and Pathogens. *Evolutionary Biology* 2010; 37: 59-
890 67.

891 Muyzer G, De Waal EC and Uitterlinden G. Profiling of complex microbial
892 populations by denaturing gradient gel electrophoresis analysis of polymerase
893 chain reaction-amplified genes coding for 16S rRNA. *Applied and*
894 *Environmental Microbiology* 1993; 59: 695-700

895 Nir I, Barak H, Kramarsky-Winter E, Kushmaro A. Seasonal diversity of the bacterial
896 communities associated with petroglyphs sites from the Negev Desert, Israel.
897 *Annals of Microbiology* 2019; 69: 1079-1086.

898 Oberemm A, Becker J, Codd G, Steinberg C. Effects of cyanobacterial toxins and
899 aqueous crude extracts of cyanobacteria on the development of fish and

900 amphibians. *Environmental Toxicology: An International Journal* 1999; 14:
901 77-88.

902 Oksanen J, Blanchet F, Friendly M, Kindt R, Legendre P, McGlinn D, et al. *vegan*:
903 Community Ecology Package. R package version 2.5. 4. 2019, 2019.

904 Orland C, Emilson EJS, Basiliko N, Mykytczuk NCS, Gunn JM, Tanentzap AJ.
905 Microbiome functioning depends on individual and interactive effects of the
906 environment and community structure. *The ISME Journal* 2019; 13: 1-11.

907 Paerl H. Nutrient and other environmental controls of harmful cyanobacterial blooms
908 along the freshwater–marine continuum. *Cyanobacterial harmful algal blooms*:
909 State of the science and research needs. Springer, 2008, pp. 217-237.

910 Paerl HW, Huisman J. Climate change: a catalyst for global expansion of harmful
911 cyanobacterial blooms. *Environmental microbiology reports* 2009; 1: 27-37.

912 Paerl HW, Otten TG, Kudela R. Mitigating the Expansion of Harmful Algal Blooms
913 Across the Freshwater-to-Marine Continuum. *Environmental Science &*
914 Technology 2018; 52: 5519-5529.

915 Paerl HW, Paul VJ. Climate change: Links to global expansion of harmful
916 cyanobacteria. *Water Research* 2012; 46: 1349-1363.

917 Paerl HW, Tucker CS. Ecology of blue-green algae in aquaculture ponds. *Journal of*
918 the World Aquaculture Society 1995; 26: 109-131.

919 Page HM, Petty RL, Meade DE. Influence of watershed runoff on nutrient dynamics
920 in a southern California salt marsh. *Estuarine, Coastal and Shelf Science* 1995;
921 41: 163-180.

922 Pagels F, Salvaterra D, Amaro HM, Lopes G, Sousa-Pinto I, Vasconcelos V, et al.
923 Bioactive potential of *Cyanobium* sp. pigment-rich extracts. *Journal of*
924 Applied Phycology 2020; 32: 3031-3040.

925 Patil HJ, Benet-Perelberg A, Naor A, Smirnov M, Ofek T, Nasser A, et al. Evidence
926 of Increased Antibiotic Resistance in Phylogenetically-Diverse *Aeromonas*
927 Isolates from Semi-Intensive Fish Ponds Treated with Antibiotics. *Frontiers in*
928 *Microbiology* 2016; 7.

929 Patil HJ, Gatica J, Zolti A, Benet-Perelberg A, Naor A, Dror B, et al. Temporal
930 Resistome and Microbial Community Dynamics in an Intensive Aquaculture
931 Facility with Prophylactic Antimicrobial Treatment. *Microorganisms* 2020; 8:
932 1984.

933 Pearson L, Mihali T, Moffitt M, Kellmann R, Neilan B. On the chemistry, toxicology
934 and genetics of the cyanobacterial toxins, microcystin, nodularin, saxitoxin
935 and cylindrospermopsin. *Marine drugs* 2010; 8: 1650-1680.

936 Pérez-Sánchez T, Ruiz-Zarzuela I, de Blas I, Balcázar JL. Probiotics in aquaculture: a
937 current assessment. *Reviews in Aquaculture* 2014; 6: 133-146.

938 Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA
939 ribosomal RNA gene database project: improved data processing and web-
940 based tools. *Nucleic Acids Research* 2012; 41: D590-D596.

941 Robarts RD, Zohary T. Temperature effects on photosynthetic capacity, respiration,
942 and growth rates of bloom-forming cyanobacteria. *New Zealand Journal of*
943 *Marine and Freshwater Research* 1987; 21: 391-399.

944 Rodriguez-Brito B, Li L, Wegley L, Furlan M, Angly F, Breitbart M, et al. Viral and
945 microbial community dynamics in four aquatic environments. *The ISME*
946 *Journal* 2010; 4: 739-751.

947 Rowe OF, Dinasquet J, Paczkowska J, Figueroa D, Riemann L, Andersson A. Major
948 differences in dissolved organic matter characteristics and bacterial processing

949 over an extensive brackish water gradient, the Baltic Sea. *Marine Chemistry*
950 2018; 202: 27-36.

951 Schrader KK, Dennis ME. Cyanobacteria and earthy/musty compounds found in
952 commercial catfish (*Ictalurus punctatus*) ponds in the Mississippi Delta and
953 Mississippi–Alabama Blackland Prairie. *Water Research* 2005; 39: 2807-2814.

954 Smith JL, Boyer GL, Zimba PV. A review of cyanobacterial odorous and bioactive
955 metabolites: impacts and management alternatives in aquaculture. *Aquaculture*
956 2008; 280: 5-20.

957 Sommerset I, Krossøy B, Biering E, Frost P. Vaccines for fish in aquaculture. *Expert*
958 review of vaccines 2005; 4: 89-101.

959 Song SK, Beck BR, Kim D, Park J, Kim J, Kim HD, et al. Prebiotics as
960 immunostimulants in aquaculture: A review. *Fish & Shellfish Immunology*
961 2014; 40: 40-48.

962 Sunagawa S, Coelho LP, Chaffron S, Kultima JR, Labadie K, Salazar G, et al.
963 Structure and function of the global ocean microbiome. *Science* 2015; 348.

964 Talbot C, Hole R. Fish diets and the control of eutrophication resulting from
965 aquaculture. *Journal of Applied Ichthyology* 1994; 10: 258-270.

966 Tucker CS, Schrader KK. Off-flavors in pond-grown ictalurid catfish: Causes and
967 management options. *Journal of the World Aquaculture Society* 2020; 51: 7-
968 92.

969 Wickham H. *ggplot2: elegant graphics for data analysis*: springer, 2016.

970 World Health Organization, 2019

971 Wurtsbaugh WA, Paerl HW, Dodds WK. Nutrients, eutrophication and harmful algal
972 blooms along the freshwater to marine continuum. *WIREs Water* 2019; 6:
973 e1373.

974 Yoshizawa S, Matsushima R, Watanabe MF, Harada K-i, Ichihara A, Carmichael
975 WW, et al. Inhibition of protein phosphatases by microcystis and nodularin
976 associated with hepatotoxicity. Journal of cancer research and clinical
977 oncology 1990; 116: 609-614.

978 Yu SZ. Primary prevention of hepatocellular carcinoma. Journal of gastroenterology
979 and hepatology 1995; 10: 674-682.

980 Zeng J, Lin Y, Zhao D, Huang R, Xu H, Jiao C. Seasonality overwhelms aquacultural
981 activity in determining the composition and assembly of the bacterial
982 community in Lake Taihu, China. Science of The Total Environment 2019;
983 683: 427-435.

984 Zhang Q, Zhang Z, Lu T, Peijnenburg WJGM, Gillings M, Yang X, et al.
985 Cyanobacterial blooms contribute to the diversity of antibiotic-resistance
986 genes in aquatic ecosystems. Communications Biology 2020a; 3: 737.

987 Zhang X, Xu X, Shen Y, Li L, Wang R, Li J. Using GFP as a biomarker to visualize
988 the process of bacterial infection in black carp (*Mylopharyngodon piceus*).
989 Aquaculture Reports 2020b; 18: 100530.

990 Zhang Y, Liu Z, Sun J, Xue C, Mao X. Biotechnological production of zeaxanthin by
991 microorganisms. Trends in Food Science & Technology 2018; 71: 225-234.

992 Zimba PV, Grimm CC. A synoptic survey of musty/muddy odor metabolites and
993 microcystin toxin occurrence and concentration in southeastern USA channel
994 catfish (*Ictalurus punctatus* Rafinesque) production ponds. Aquaculture 2003;
995 218: 81-87.

996 Zimba PV, Khoo L, Gaunt PS, Brittain S, Carmichael WW. Confirmation of catfish,
997 *Ictalurus punctatus* (Rafinesque), mortality from *Microcystis* toxins. Journal of
998 Fish Diseases 2001; 24: 41-47.

999 Zorz JK, Sharp C, Kleiner M, Gordon PMK, Pon RT, Dong X, et al. A shared core
1000 microbiome in soda lakes separated by large distances. Nature
1001 Communications 2019; 10: 4230.

Figures

Fig. 1. The Dor Aquaculture Research Unit (DARU). a) Mean annual precipitation map of Israel, showing DARU on the coastal region which has a Mediterranean climate. The red square marks the location of panel b. b) The coastal plain between the Carmel mountains and the sea, with the location of DARU (panel c) shown as a red square. DARU is situated in a predominantly agricultural region with banana plantations and various fields. c) DARU. The arrows show the flow direction between the D1 and V2 fishponds and the Reservoir. The mean annual precipitation map of Israel was derived from the digital elevation model (DEM) using ArcGIS (ESRI, Redlands, CA, USA).

Fig. 2. Seasonal dynamics in the Reservoir between 2013 and 2015. (a) Monthly measured physicochemical properties of the water. The different photosynthetic pigments are colored according to the legend. Nitrogen represents the total concentration of nitrate and nitrite. In microcystins, samples marked with 'x' represent potentially underestimated concentration. For visualization purposes, microcystins measurements of Oct. 2014 and Aug. 2015 (1.6 and $10.4 \mu\text{g L}^{-1}$, respectively) were omitted from the figure. (b) Sequence proportion overview of bacterial communities on a class level. The classes represented by colors according to the legend, all classes with sequence proportions below 2% were classified as "Other classes".

1024

1025 **Fig. 3.** Differential enrichment analysis comparing ASV sequence abundances during
1026 dry season (left) and wet season (right). The x-axis of the central plot represents the
1027 log₂ fold change for all significantly enriched ASVs within each labeled genera.
1028 Positive and negative values represent enrichment during wet and dry seasons,
1029 respectively. Each point and bars represents the mean and the standard deviation of
1030 the ASVs log₂ fold change in each genera. Only taxonomic families with at least two
1031 significantly enriched (adjusted p<0.1) ASVs, and an absolute log₂ fold change value
1032 of 1, were included in the figure. The peripheral barplots represent the respective
1033 sequence proportions of the enriched ASVs within the bacterial communities of the
1034 different pools.

1035

1036 **Fig. 4.** RDA ordination of bacterial community composition constrained by
1037 physicochemical parameters (a) and photosynthetic pigments (b). Colors represent the
1038 seasons (dry – April to October; Wet –November to March) and shapes represent the
1039 different ponds. The percentage on the X and the Y axis represent the proportion of
1040 explained variance. The environmental variables are: T - temperature, N - inorganic
1041 nitrogen, P - inorganic phosphate, A - ammonia, M -microcystins, R - the N:P ratio.
1042 The pigments are: Ca- chlorophyll *a*, Cb- chlorophyll *b*, b - β -carotene, D -
1043 Dinoxanthin, F - fucoxanthin, Z - Zeaxanthin. Special conditions: (*) – no fish in the
1044 fishpond, (#) – decomposed corpse of a donkey at the edge of the reservoir, (+) –
1045 winter puddle in a fishpond, outside of the culturing season.

1046

1047 **Fig. 5.** RDA ordination of bacterial ASVs constrained by physicochemical parameters
 1048 (a) and photosynthetic pigments (b). The filled points represent significantly enriched
 1049 ASVs, colored according to their taxonomic class. The percentage on the X and the Y
 1050 axis represent the proportion of explained variance. The environmental variables are:
 1051 T - temperature, N - inorganic nitrogen, P - inorganic phosphate, A - ammonia, M -
 1052 microcystins, R - the N:P ratio. The pigments are: Ca- chlorophyll *a*, Cb- chlorophyll
 1053 *b*, b - β -carotene, D - Dinokanthin, F - fucoxanthin, Z - Zeaxanthin.

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