Warming-induced shifts in phytoplankton carbon release are species-dependent

Israel Cassiano-Oliveira[a], Inessa Lacativa Bagatini[b], Hugo Sarmento[a]*

[a]Laboratory of Microbial Processes and Biodiversity, Hydrobiology Department, Federal University of São Carlos, São Paulo, Brazil
[b]Laboratory of Phycology, Botany Department, Federal University of São Carlos, São Paulo, Brazil

***Corresponding author:** Laboratory of Microbial Processes and Biodiversity, Hydrobiology Department, Federal University of São Carlos, São Paulo, Brazil
E-mail address: hsarmento@ufscar.br

Supporting Information

Temperature Choice

The control temperature of 24°C was set based on the mean surface lake water temperature in the region where the strains were isolated (Table S1), which is compatible with the standard temperature at the CCMA (23±1°C). The experimental temperature of 28°C represents an increase of 4ºC, defined based on the warming projections for freshwater ecosystems presented in the IPCC's Sixth Assessment Report (in the section 2.5.4) (Parmesan et al., 2022) and in line with recent literature (eg. Yvon-Durocher et al. 2015; Zhang et al. 2018; Feuchtmayr et al. 2019; Urrutia-Cordero et al. 2020; Allen et al. 2021; Vijayaraj et al. 2022).

Acclimation Procedure

We adopted a stepwise approach to acclimate the strains to the warmer experimental temperature of 28°C. The procedure consisted in maintaining the cultures for three weeks at the intermediate temperature of 26°C and then three more weeks at 28°C before the start of the experiments, with weekly addition of sterile fresh media for growth. This way, an acclimated stock culture was obtained to perform the experiments. In a similar way, control cultures were maintained at 24°C for six weeks, with periodic addition of fresh media for growth, thus producing a control stock culture.

By applying this procedure we aimed to minimize abrupt changes in temperature and light, ensuring that the microorganisms would be able to properly deploy physiological and metabolic adjustments. However, as *Cyclotella* sp. does not perform as well at high temperature, the time spent in the 28°C step was prolonged to 61 days.

For starting the experiment cultures, cell counts for the control and acclimated stock cultures were obtained by flow cytometry (as described in the following pages) and used to determine the dilution factor in order to achieve concentrations of 4-5x10^4 cells/mL for *M. aeruginosa* and 2-3x10^4 cells/mL for *Cyclotella* sp for the assays. The diluted acclimated stock culture was used to initiate the acclimated experimental cultures (maintained at 28°C), and the diluted control stock culture was used to initiate the control and non-acclimated experimental cultures (maintained at 24°C and 28°C, respectively).

The experimental cultures were carried out in 10 mL borosilicate screw-cap tubes filled with 6 mL of the diluted microalgae culture. Culture manipulation was always performed in a sterile flow chamber, with sterile materials and a Bunsen burner. These conditions were consistently followed throughout the study.

Table S1. Four-year time series for monthly water surface temperature (°C) recorded at Lobo's reservoir (Itirapina-SP, Brazil). Each value represents a single measurement.

Month	Year 1 (03/2018 - 02/2019)	Year 2 (03/2019 - 02/2020)	Year 3 (03/2020 - 02/2021)	Year 4 (03/2021 - 02/2022)
March	27,61	27,26	27,22	26,68
April	23,69	24,95	24,63	23,33
May	23,17	22,48	21,40	21,37
June	20,56	20,32	21,53	20,43
July	18,97	18,78	21,65	19,10
August	20,00	21,28	21,33	20,36
September	22,37	22,68	22,51	24,01
October	25,60	26,50	25,27	23,74
November	23,99	25,51	26,10	26,38
December	26,37	27,70	28,80	26,38
January	28,34	28,45	27,19	27,90
February	25,38	28,92	28,20	28,64

Global average: 24,27; Global standard deviation: 3,00

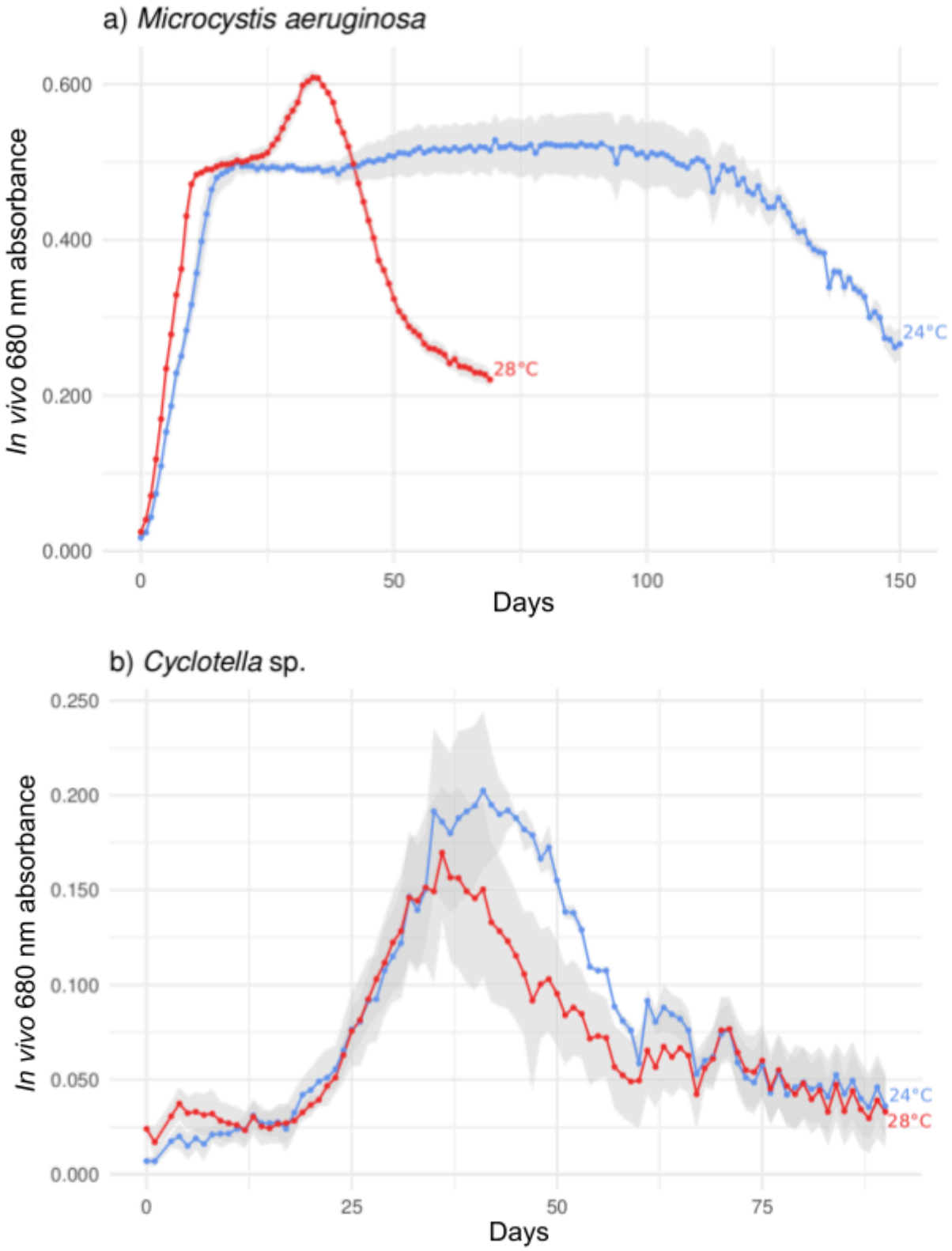

Figure S1. Reference growth curves obtained for *M. aeruginosa* (**a**) and *Cyclotella* sp. (**b**). Lines represent the average absorbance of 3 replicate cultures (except for *Cyclotella* sp. at 24°C), and shadows indicate standard deviation. Red line: 28°C; Blue line: 24°C.

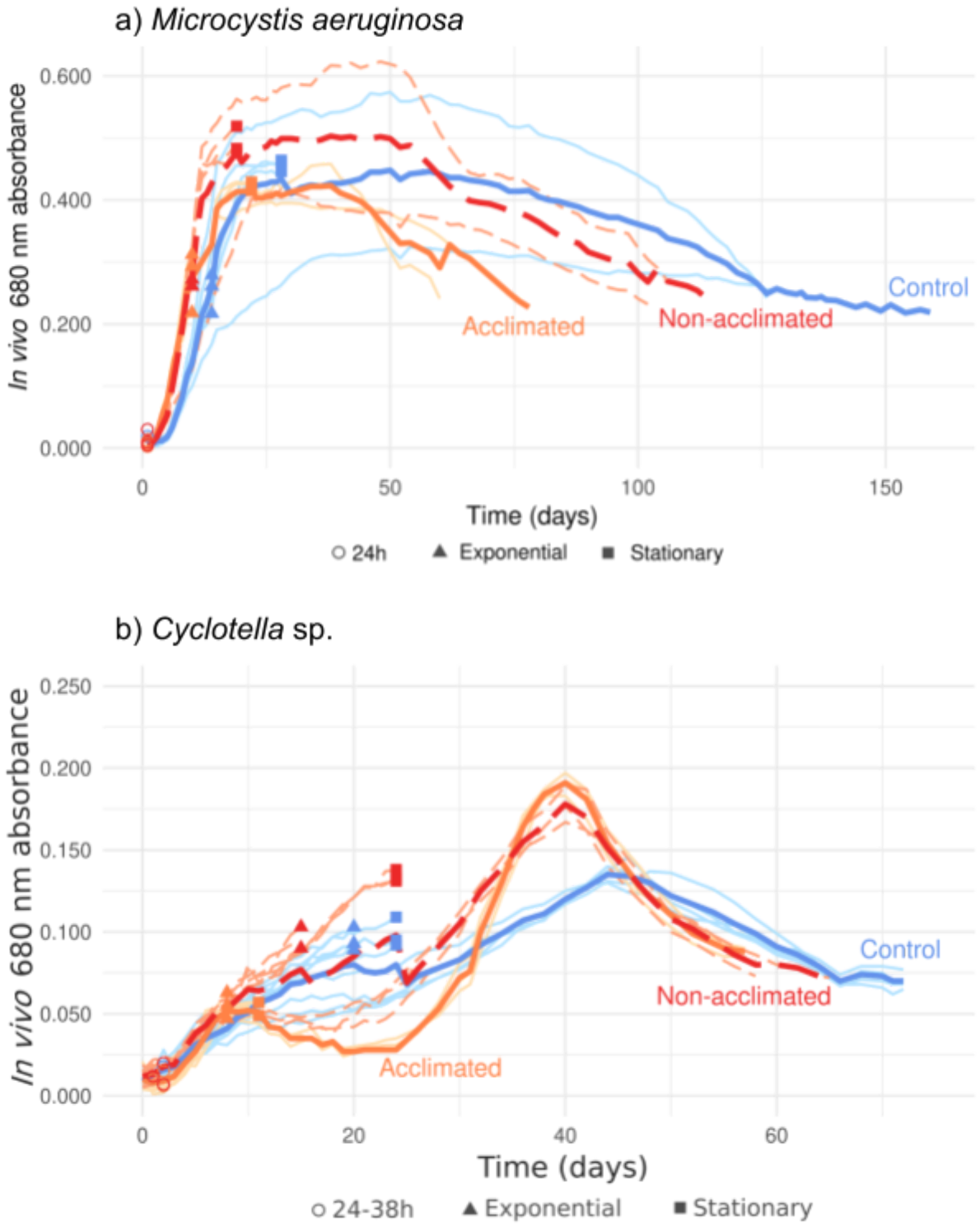

Figure S2. Growth curves obtained for the experiments with *M. aeruginosa* (**a**) and *Cyclotella* sp. (**b**). Thick lines represent the average absorbance for each strain, and the thinner, lighter lines indicate absorbance values for each individual culture. Orange line: acclimated at 28°C; Red line: non acclimated at 28°C; Blue line: control, at 24°C.

Flow Cytometry Procedures

For each sample, a 300 µL aliquot was transferred to a plastic test tube in a UV-sterilized flow chamber, with sterilized materials (121°C, 30 min). In a temperature-controlled room (25°C), each aliquot was dark-incubated with 3 µL of SYTO 13™ fluorescent dye (Thermo Fisher) for 10 to 30 minutes.

Prior to every analysis of sample batches, any air bubbles were purged from the flow cytometer fluidic system; after this, the fluidic system was cleaned with a diluted solution of sodium hypochlorite, followed by a cleanliness check with ultrapure water (figure S3c).

The incubated samples were analyzed on a FACSCalibur™ flow cytometer (BD Biosciences) using the parameters FL-1 (dye fluorescence, green), FL-3 (chlorophyll fluorescence, red) and SSC-H (side scatter, a proxy for particle size), at low speed settings (~10 µL/s, 2 min per sample).

If the cytogram did not show any bacterial populations (figure S3b), the culture was confirmed to remain axenic and proceeded to the next analysis. Also, at the end of the experiments, these cytograms were also used to obtain cell counts for each sample and calculate algae culture concentration in cells per milliliter. On the other hand, if the sample showed contamination by bacteria (figure S3a), the culture was discarded and replaced by another replicate.

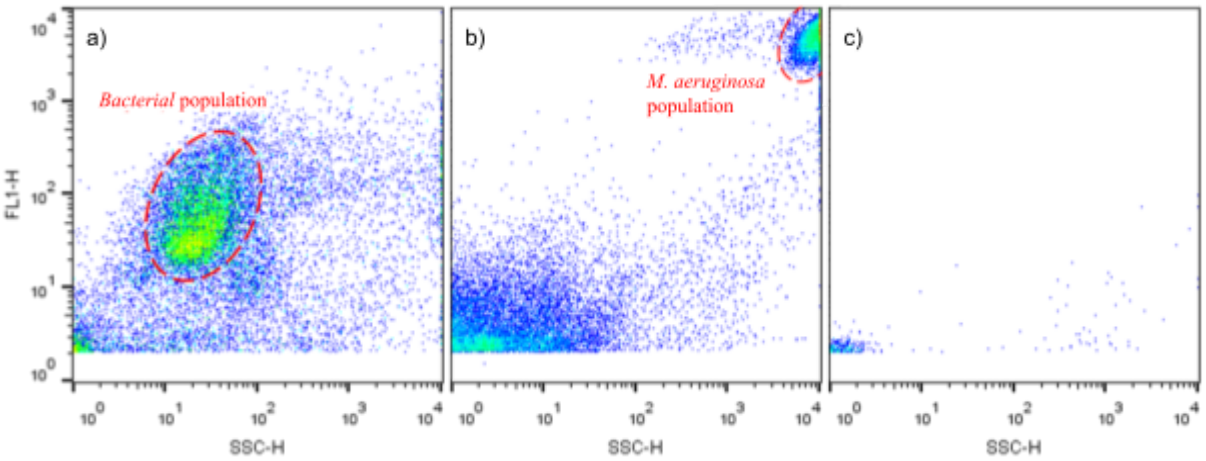

Figure S3. Cytograms of example samples tested for bacteria presence: **a)** freshly collected surface water from Lobo's reservoir; **b)** axenic *M. aeruginosa* culture at exponential growth stage; **c)** ultrapure water. Each dot represents a detected particle, and color indicates dot density. FL1-H: dye fluorescence, arbitrary units; SSC-H: particle size proxy, arbitrary units. Elaborated by the author using FlowJo software (BD Biosciences).

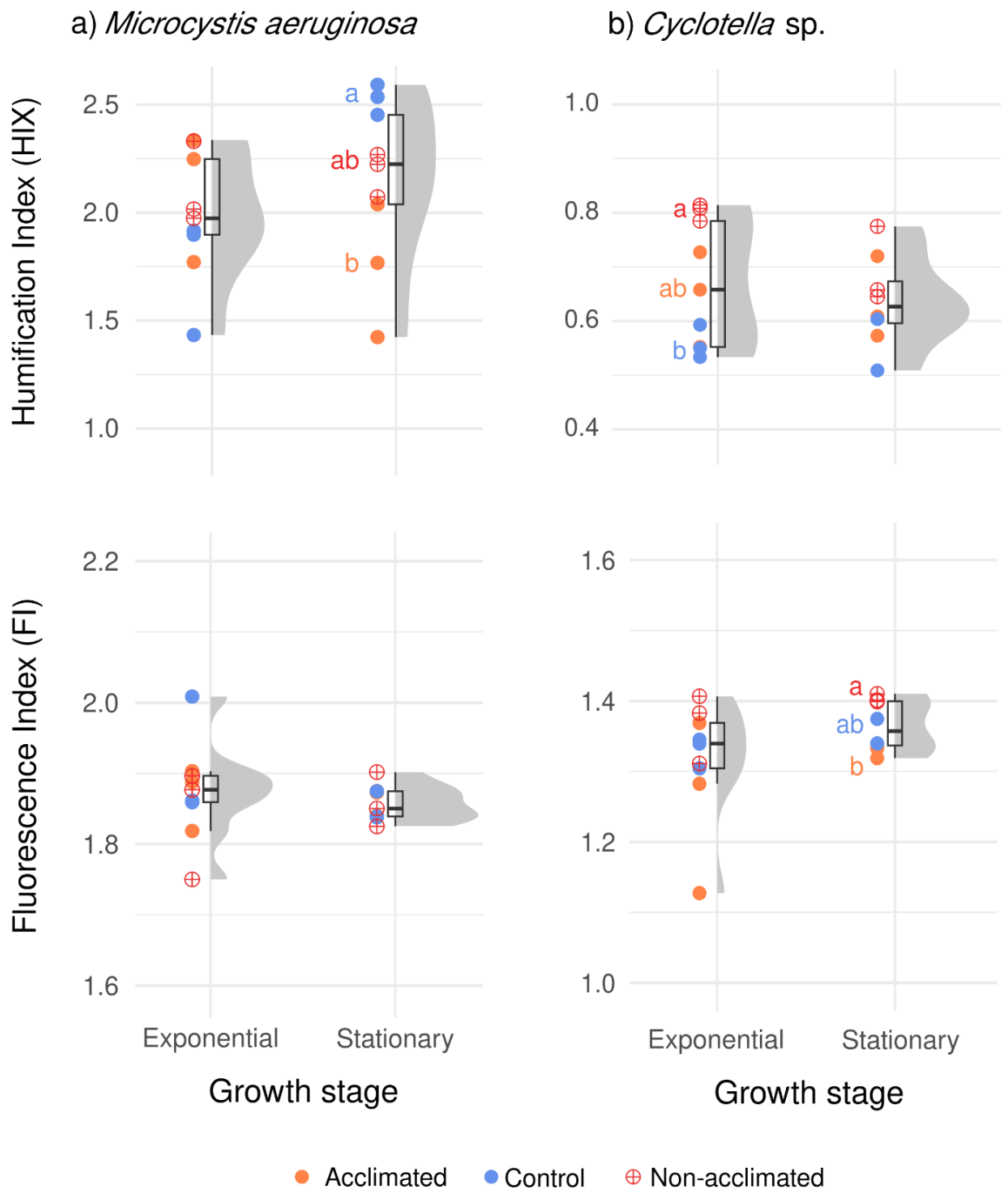

Figure S4. Distribution of Humification Index (HIX) and Fluorescence Index (FI) values calculated for DOMp from both species in the experiments. Letters indicate statistically significant differences between strains at the same growth stage.

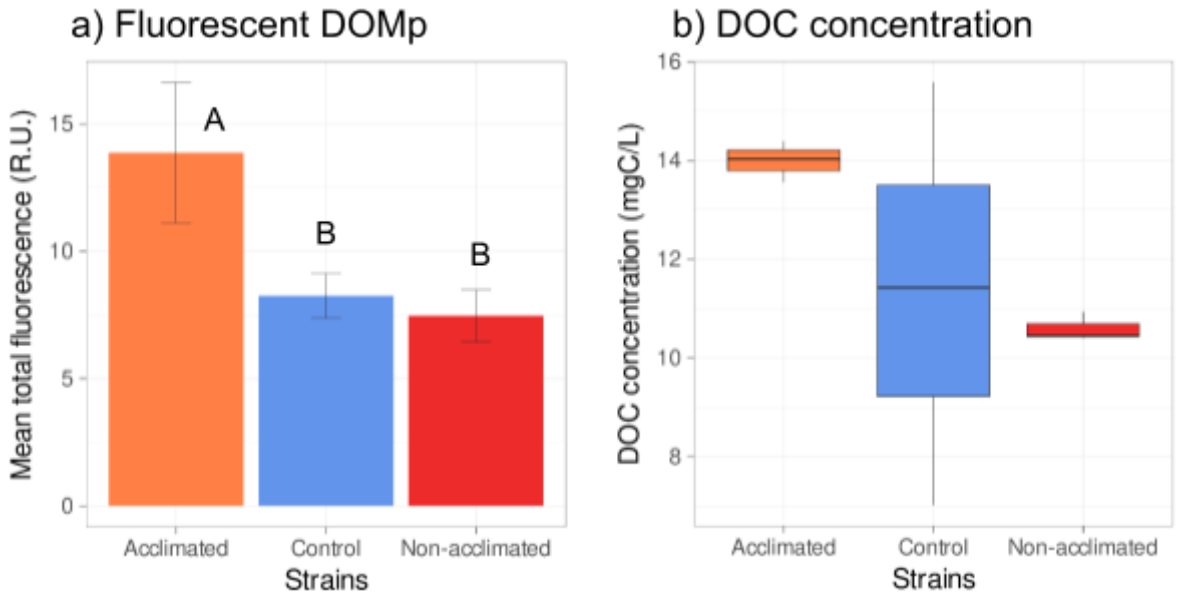

Figure S5. Differences in mean total fluorescence (a) and DOC concentration (b) between strains of *M. aeruginosa.* Statistically significant differences are denoted by distinct letters.

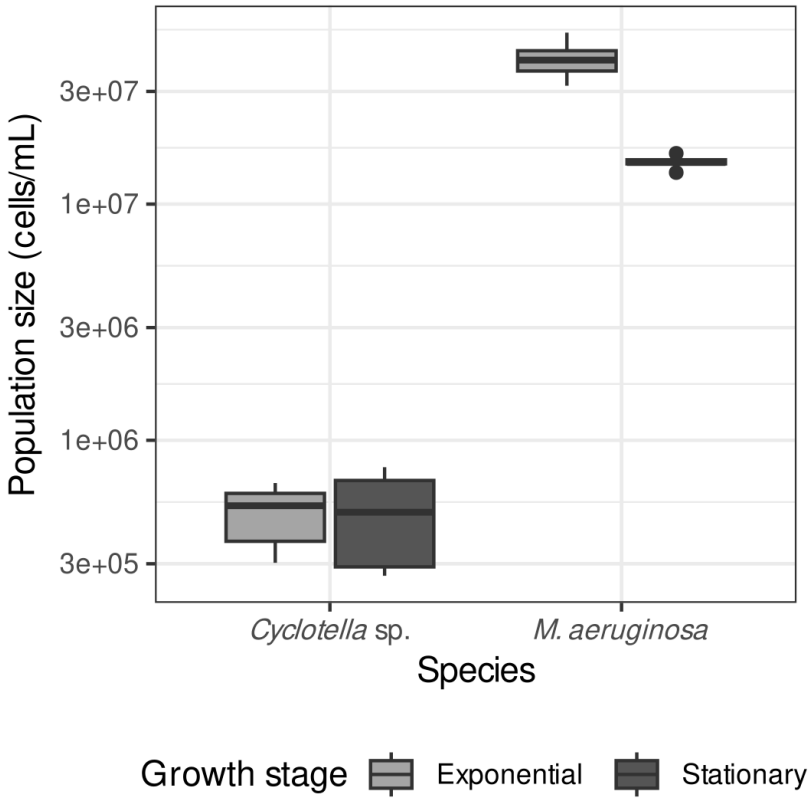

Figure S6. Variation in distribution of cell density values for each species at different growth stages.

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