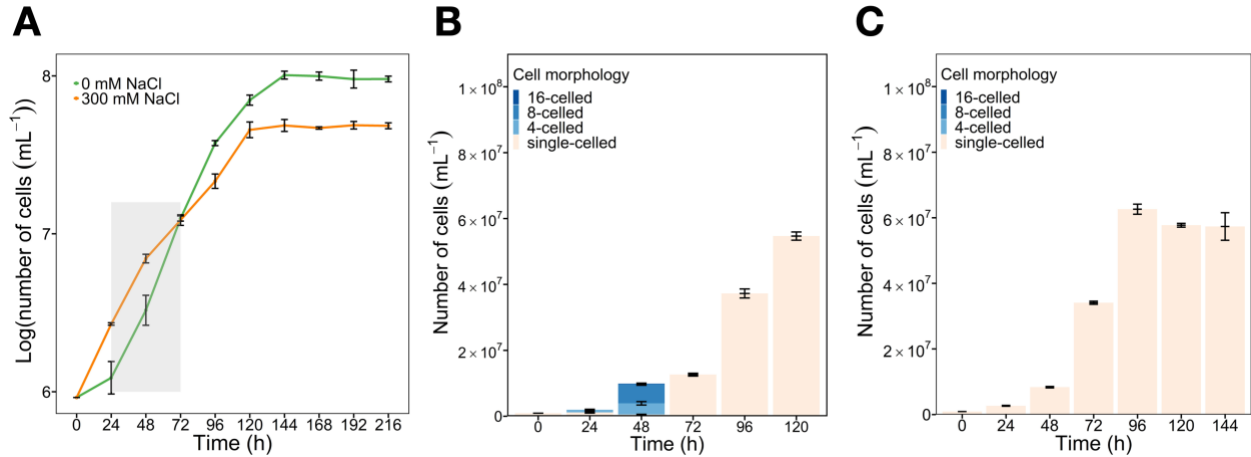


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**Supplemental Information**

**An environmentally induced multicellular  
life cycle of a unicellular cyanobacterium**

**Si Tang, Yuriy Pichugin, and Katrin Hammerschmidt**



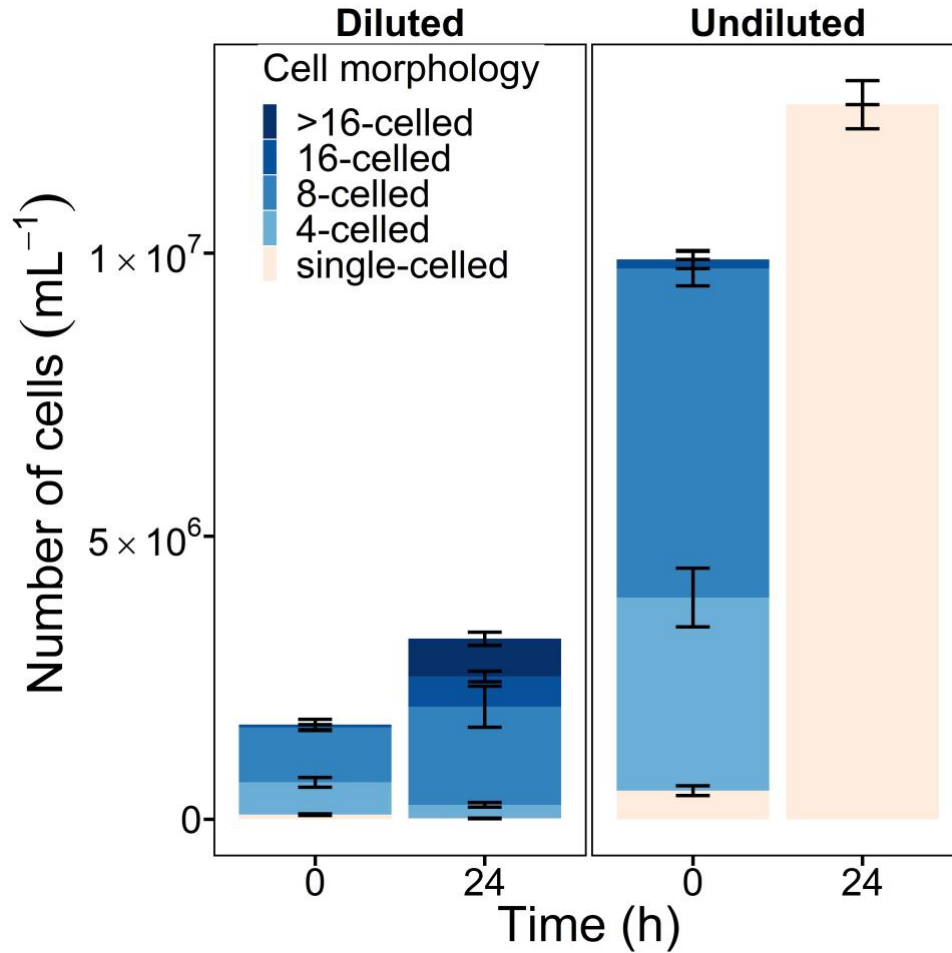
**Figure S1. Growth trajectories and population dynamics of replicate *Cyanothecce* sp. populations in batch cultures over time. Related to Figure 1.**

(A) Comparison of the growth trajectory in batch cultures in 10 mL BG11 medium with 0 mM and 300 mM NaCl over time. The grey area indicates the time period when filaments were observed in medium with 0 mM NaCl. The shortest generation time in freshwater is  $G_{0 \text{ mM}} = 15.2$  h (from 72 h to 96 h), while the shortest generation time in the highest salinity is  $G_{300 \text{ mM}} = 17.5$  h (from 24 h to 48 h).

(B) Population dynamics over the period of 5 days in BG11 *without* added NaCl (populations initiated with  $5 \cdot 10^5$  cells/mL, in 1 mL volume each (24-well plates)).

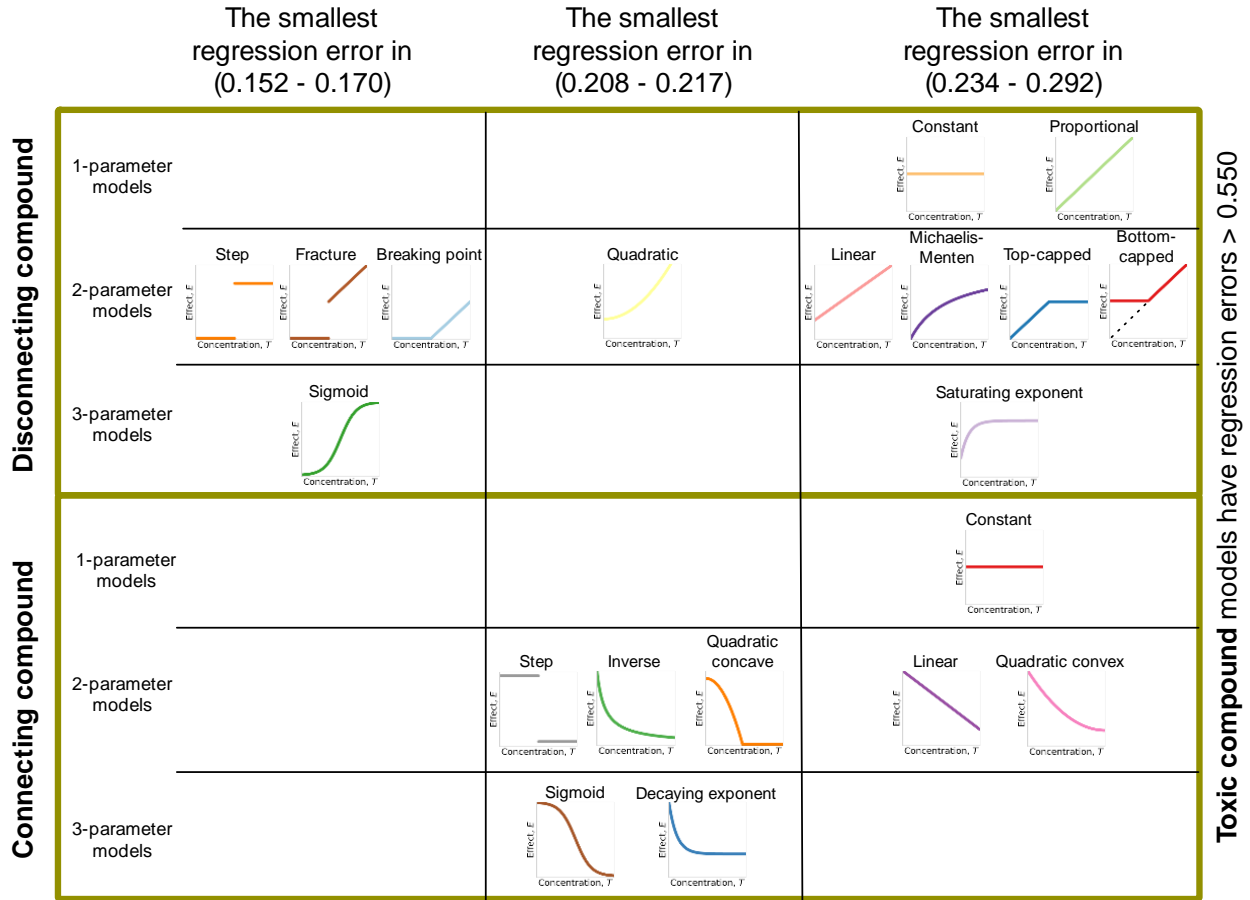
(C) Population dynamics over the period of 6 days in BG11 *with* added NaCl (300 mM) (populations initiated with  $5 \cdot 10^5$  cells/mL, in 1 mL volume each (24-well plates)).

Error bars represent SDs (of each sub-bar for B) ( $n = 3$ ).



**Figure S2. Population composition after the transfer of 72h-old filaments to new medium (left) in contrast to the original population (both in BG11 without added NaCl). Related to Figures 1 and 2.**

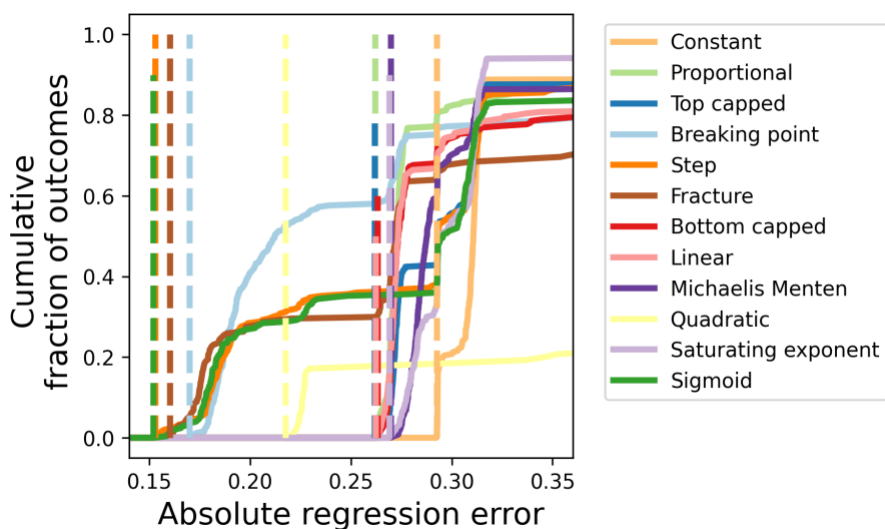
When diluted, filaments kept growing and increased in length, indicated by the observation of filaments of longer than 16 cells in length and by a significantly higher proportion of 8-celled filaments, in contrast to the original culture, where 24 h later only single cells were observed. Error bars represent standard deviation of each sub-bar (n=3).



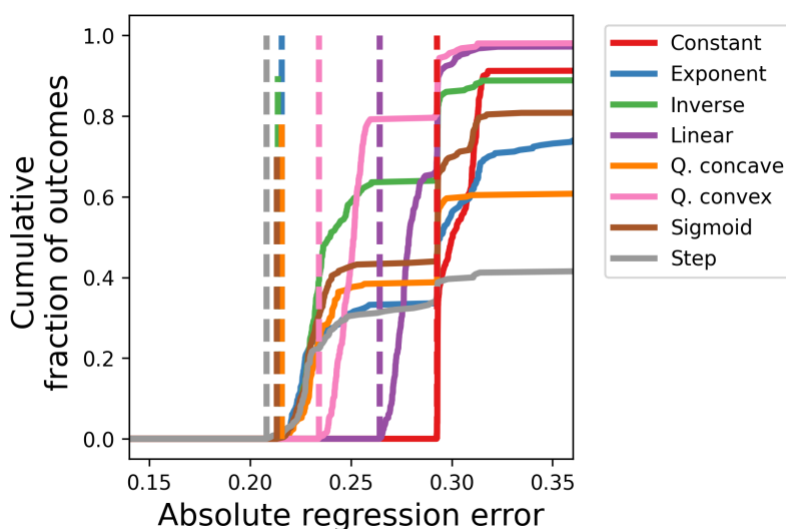
**Figure S3. Models of the acting substance concentration effect in the disconnecting and connecting compound models. Related to Figure 3.**

We consider multiple models of the relationship between acting substance concentration and its effect on the filaments (12 in the disconnecting compound family of models and 8 in the connecting compound family). Disconnecting compound models bring more accurate fit of experimental data than the connecting compound models. The simplest 1-parameteric models yield high regression errors but 3-parameteric models do not bring an advantage over some 2-parameteric ones.

## A Disconnecting compound models



## B Connecting compound models



**Figure S4. Cumulative distribution functions and the minimal regression errors obtained for compound models. Related to Figure 3.**

(A) 12 disconnecting compound models can be classified into two groups: models with a good fit having minimal regression errors below 0.17, and models with worse fit, for which the minimal regression error is above 0.21 (can be increased to 0.26 if quadratic model is dropped), see also Table S2. (B) 8 connecting compound models can be classified into two groups: models with a good fit having minimal regression errors around 0.21, and models with worse fits, for which the minimal regression error is above 0.22, see also Table S3. Plots show sample cumulative distribution functions of regression errors from 250 independent optimizations for each model. Dashed lines represent the minimal regression error in each model.

**Table S1. The morphology of *Cyanothece* sp. is dependent on the composition of the medium. Related to Figure 2.**

Fresh culture medium (BG11) was added to ddH<sub>2</sub>O and both supernatants (filament inhibitor: supernatants from cultures inoculated with 5\*10<sup>6</sup> cells/mL starting cell densities, harvested 24 h after inoculation, and filament fragmentor: supernatants from cultures inoculated with 5\*10<sup>5</sup> cells/mL cell densities immediately after filament fragmentation, harvested at 96 h), creating BG11 ratios from 0 – 100 % with 20 % increments. The emergence of the filamentous morphology was recorded after 48 h, starting with single cells of *Cyanothece* sp. in each dilution treatment. “+” represents filament occurrence; “-” represents no filament occurrence. While 20 % of BG11 in ddH<sub>2</sub>O provided sufficient nutrients for filament formation, 60-80 % of the BG11 was necessary to dilute the filament fragmentor/inhibitor medium before filaments were observed.

	BG11 ratio					
	100%	80%	60%	40%	20%	0%
ddH <sub>2</sub> O	+	+	+	+	+	-
Filament fragmentor	+	+	+	-	-	-
Filament inhibitor	+	+	-	-	-	-

**Table S2. Action law E(T) in models used in the toxic and disconnecting compound model families and the minimal regression errors obtained across 250 independent optimizations. Models with the highest quality fitting are highlighted. Related to Figure 3.**

<b>Model of the acting substance concentration effect</b>	<b>Law of action</b>	<b>The smallest disconnecting compound regression error</b>	<b>The smallest toxic compound regression error</b>
<b>1 parameter models</b>			
Constant	$E(T) = E_0$	0.292	0.590
Proportional	$E(T) = \alpha T$	0.262	0.732
<b>2 parameter models</b>			
Linear	$E(T) = \alpha T + E_0$	0.262	0.596
Step	$E(T) = \begin{cases} 0, & T < T_0 \\ E_0, & T > T_0 \end{cases}$	0.153	0.594
Fracture	$E(T) = \begin{cases} 0, & T < T_0 \\ \alpha T, & T > T_0 \end{cases}$	0.160	0.721
Breaking point	$E(T) = \begin{cases} 0, & T < T_0 \\ \alpha(T - T_0), & T > T_0 \end{cases}$	0.170	0.724
Michaelis-Menten	$E(T) = E_0 \frac{T}{T + T_0}$	0.270	0.598
Quadratic	$E(T) = \left(\frac{T}{T_0}\right)^2 + E_0$	0.217	0.583
Top-capped	$E(T) = \begin{cases} \alpha T, & T < T_0 \\ \alpha T_0, & T > T_0 \end{cases}$	0.262	0.593
Bottom-capped	$E(T) = \begin{cases} \alpha T_0, & T < T_0 \\ \alpha T, & T > T_0 \end{cases}$	0.263	0.613
<b>3 parameter models</b>			
Sigmoid	$E(T) = \frac{E_0}{1 + e^{-\alpha(T-T_0)}}$	0.152	0.583
Saturating exponent	$E(T) = E_{\max} - (E_{\max} - E_{\min})e^{-\alpha T}$	0.269	0.560

**Table S3. Action law E(T) in models used in the connecting compound models family and the minimal regression errors obtained across 250 independent optimizations. Models with the highest quality fitting are highlighted. Related to Figure 3.**

<b>Model of the acting substance concentration effect</b>	<b>Law of action</b>	<b>The smallest connecting compound regression error</b>
1 parameter models		
Constant	$E(T) = E_0$	0.292
2 parameter models		
Linear	$E(T) = \alpha(1 - T) + E_0$	0.264
Step	$E(T) = \begin{cases} E_0, & T < T_0 \\ 0, & T > T_0 \end{cases}$	0.208
Quadratic convex	$E(T) = E_0 + \alpha(1 - T)^2$	0.234
Quadratic concave	$E(T) = \max(0, E_0 - \alpha T^2)$	0.216
Inverse	$E(T) = \frac{E_0}{1 + \frac{T}{T_0}}$	0.214
3 parameter models		
Sigmoid	$E(T) = \frac{E_0}{1 + e^{\alpha(T-T_0)}}$	0.213
Decaying exponent	$E(T) = E_{\max} + (E_{\max} - E_{\min})e^{-\alpha T}$	0.216