Environmental change rate and dispersion pattern modulate the dynamics of evolutionary rescue of the cyanobacterium *Microcystis aeruginosa*.

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Introduction

Evolutionary rescue (ER) occurs when the genetic adaptation allows a population to survive under stress conditions, which *a priori* would cause the disappearance of the population[1]. The dynamic of the ER depends on the size of the population (a proxy of the genetic variability), the previous history of exposure to selective conditions, and the rate of the environmental change[2]. However, more experimental date are necessary to understand the outcomes of the ER.

The aim of this work is to add more knowledge about the ER dynamics creating stressful environments with two different selective agents (salinity and sulphide) and using the toxic cyanobacterium *Microcystis aeruginosa (*Kützing) Kützing as a model organism.

Material and methods

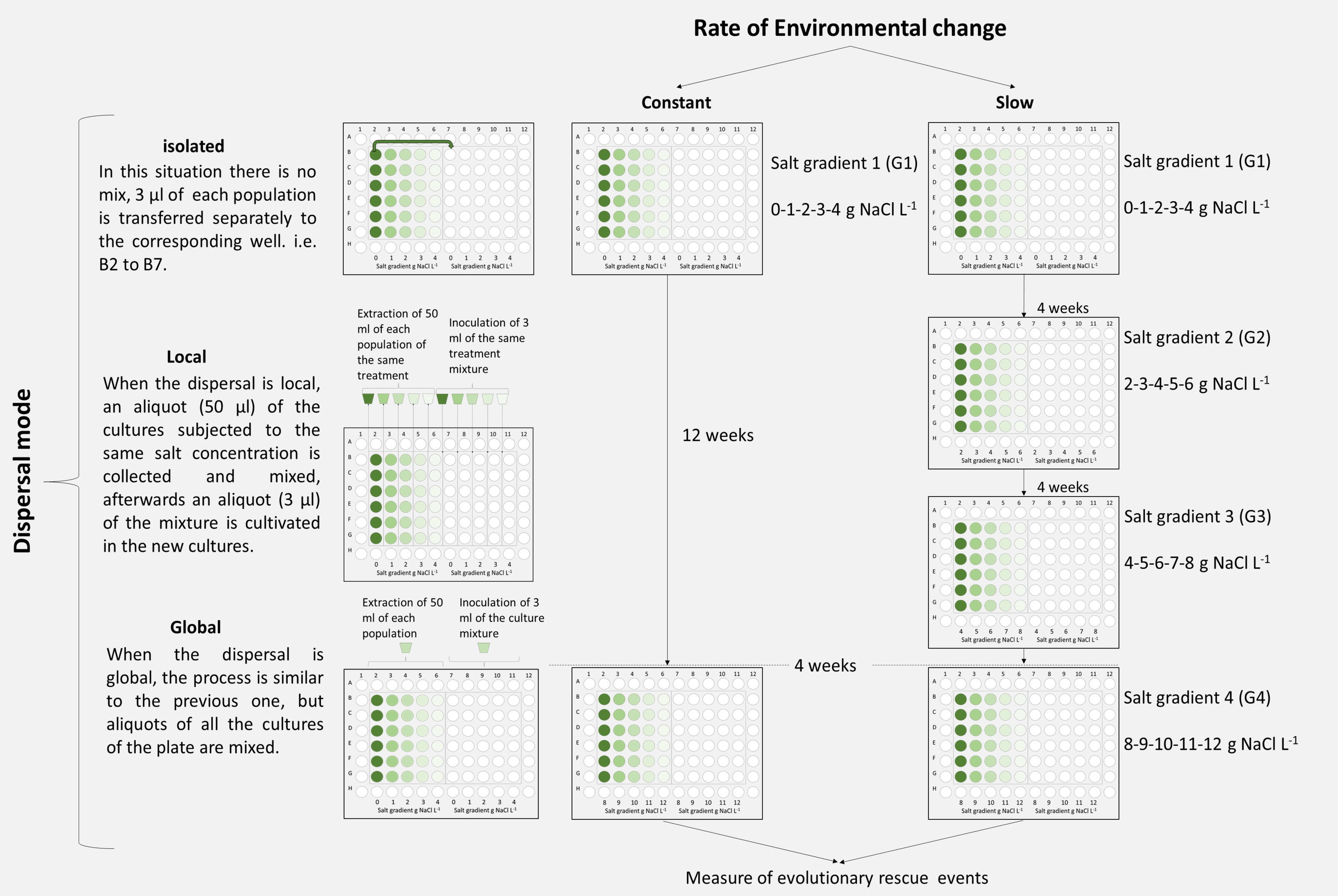

Figure 1. Schematic representation of evolutionary rescue experimental design for salinity. The model consists in two different rates of environmental change (constant and slow) and three dispersal models (isolated, local or global). Details of the experiment with sulphide as a selective agent are shown in the text below.

How can we explore the effects of environmental change on ER events?

To simulate the environmental change, we established four gradients, the initial gradient G_1 included the following concentrations 0-1-2-3-4 g NaCl L^{-1} for salinity and 0-30-60-90 µM for total sulphide [HS^{-2} + S^-]. In the constant environmental change, the population were cultured under G_1 conditions during twelve weeks. The cultures under slow and fast environmental change were cultured under four different gradients of stress (G_1, G_2, G_3, G_4); G_2 included 2-3-4-5-6 g NaCl L^{-1} or 60-90-120-150 µM [HS^{-2} + S^-]; G_3 included 4-5-6-7-8 g NaCl L^{-1} or 120-150-180-210 µM [HS^{-2} + S^-]; and G_4 included 8-9-10-11-12 g NaCl L^{-1} or 150-180-210-240 µM [HS^{-2} + S^-] (salinity and sulphide lethal conditions). On the slow treatment, the populations were maintained four weeks at each gradient, and only two weeks on the fast treatment (applied only to the sulphide ER experiment). The cultures stayed at G_4 treatment for four weeks in all treatments (Fig. 1).

How can we explore the effects of dispersion on ER events?

We established three dispersion modes, creating new cultures each week: 1) isolated (each new culture is inoculated exclusively from the corresponding culture on the past week), 2) local (each culture receives a small contribution from cultures submitted to the same concentration of the selective agent during the last week), and 3) global (each new culture receives a small contribution from all the cultures on the past week). New cultures were created each week (Fig. 1).

When a ER event occurs?

After 12 transfers in the constant (on G_1) and in the slow (on G_3) treatments, and 6 transfers in fast (on G_3) treatment , cultures were transferred to G_4 (in this stage the cultures were exposed to lethal conditions) for four weeks. An ER event was registered if the rate of growth per day at the end of the whole treatment was positive.

Results

Toxicity test: effect of salinity and sulphide on acclimated growth rate

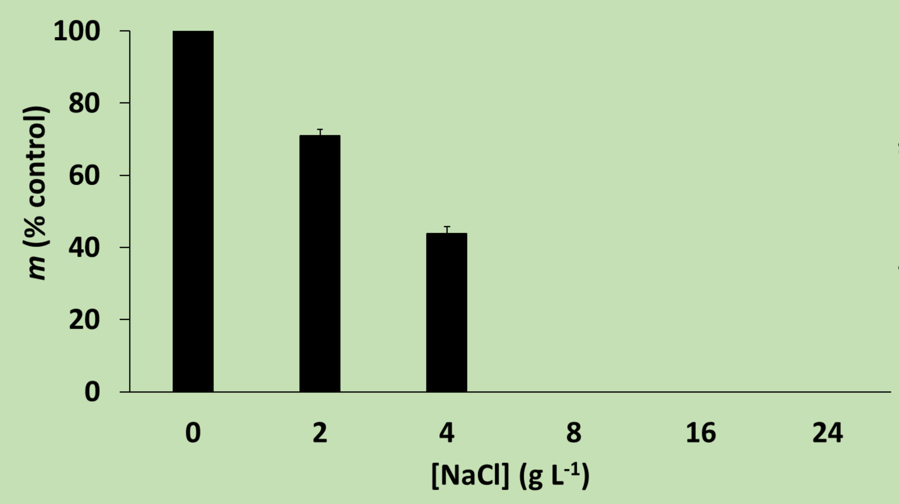

Figure 2. Effect of NaCl on acclimated growth rate (*m*; mean ± SD, *n* = 3) of *M. aeruginosa* wild-type. Lethal dose was found (7 g NaCl L^{-1}).

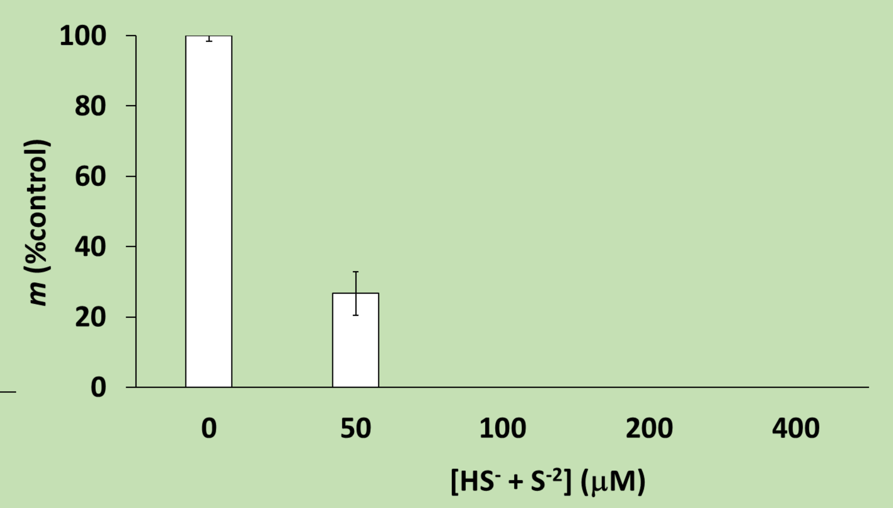

Figure 3. Effect of sulphide on acclimated growth rate (*m*; mean ± SD, *n* = 4) of *M. aeruginosa* wild-type. Lethal dose was found (70 µM [HS^{-2} + S^-]).

Effect of environmental change and dispersion mode on evolutionary rescue

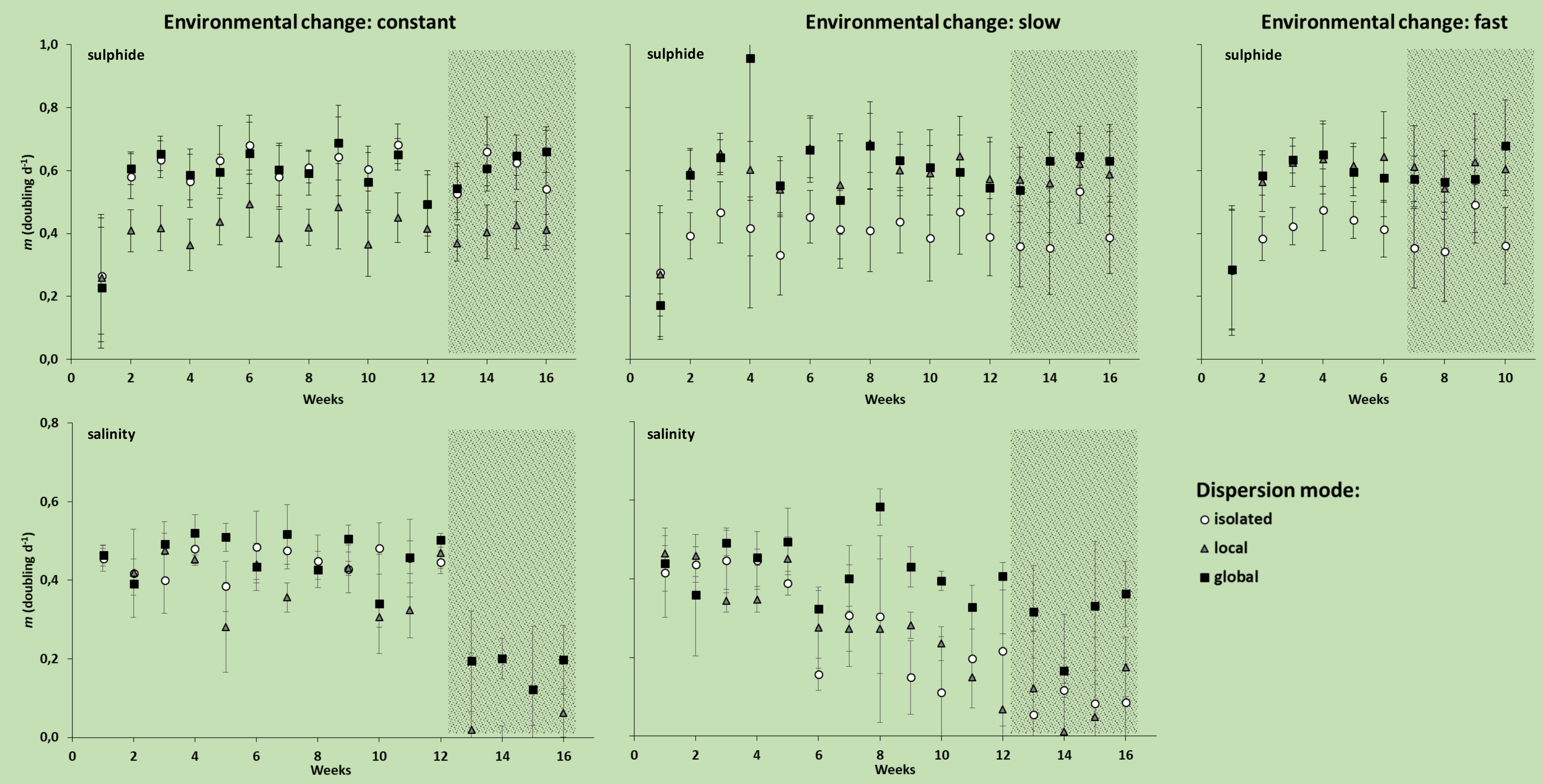

Figure 4. Representation of the acclimated growth rate (*m*) change, during the evolutionary rescue experiment (c_i = 0, c_f = 8 g NaCl L^{-1} and 150 µM [HS^{-2} + S^-] for salinity and sulphide treatments, respectively). the shaded area indicates the lethal dose of each selective agent.

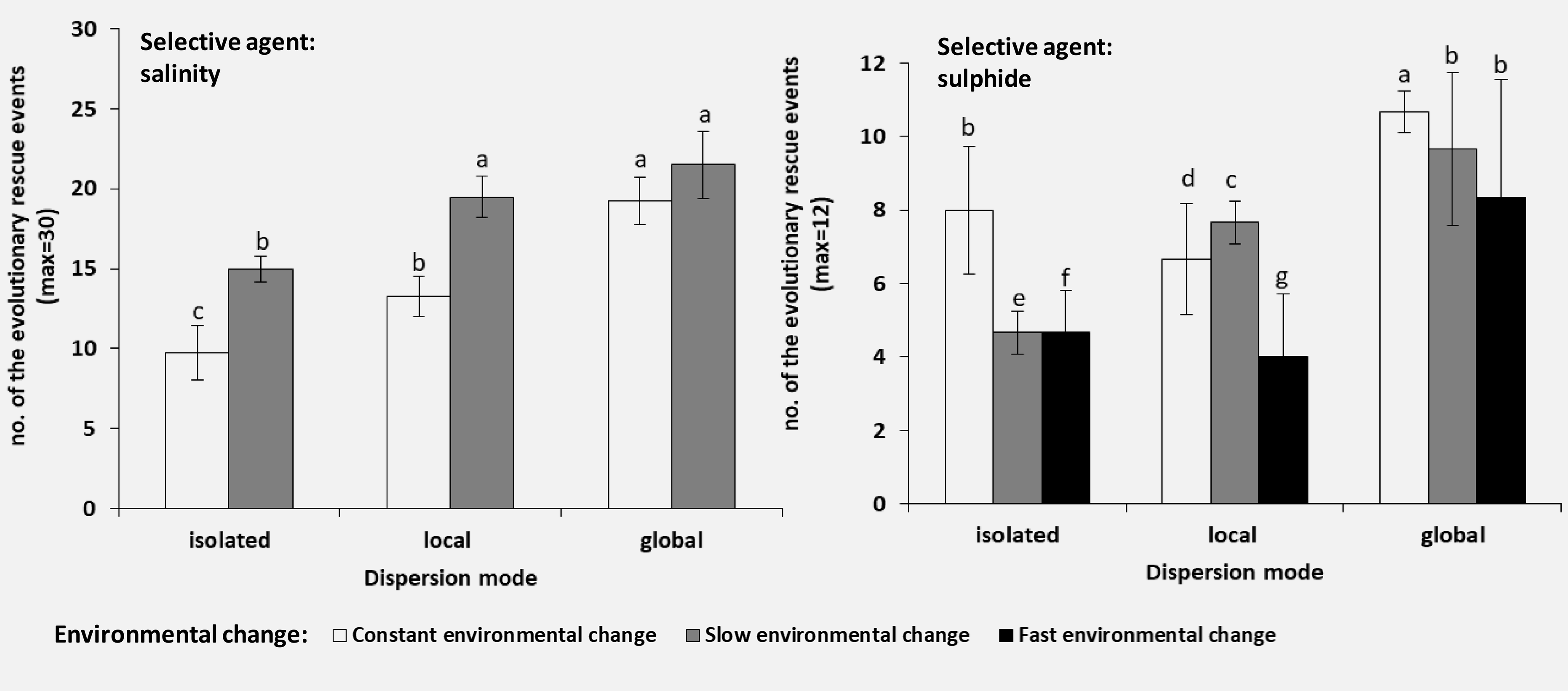

Figure 5. The effect of environmental deterioration change and dispersal mode on the number of evolutionary rescue events (maximum= 30 and 12 for salinity an sulphide, respectively) across the metapopulations after the selective agent lethal conditions. Vertical lines on bars shown SD (*n* = 4 and 3 for salinity and sulphide experiments, respectively). Different letters indicate significant differences (Two-way ANOVA model I, $p < 0.95$) among number of ER events detected by Student-Newman-Keuls (SNK) post-hoc test.

Conclusions

- Dispersal favors ER events for both selective agents (salinity and sulphide).
- ER events increase under constant changes in the populations exposed to sulphide stress. However, ER events were higher when there was previous deterioration (i.e., slow environmental change rate) under salinity stress.
- Evolutionary rescue events in *M. aeruginosa* depend on selective agent, being the probability higher for salinity than for sulphide. Thus, it could be hypothesized that general conclusions in ER studies must take into account the selective agent.

References

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Acknowledgments

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