

An evolutionary approach to detect the maximum potential adaptation of the cyanobacterium *Microcystis aeruginosa* to salinity

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Introduction

Salinity is increasing in many inland waters, on a world-wide scale, due to agricultural practices, droughts, or rise in sea level. In order to understand the effects of increased salinity on freshwater ecosystems, the cosmopolitan toxic cyanobacterium *Microcystis aeruginosa* (Kützing) Kützing was selected as a model. Several studies showed that this species cannot proliferate in salinity $>10\text{-}15\text{ g}\cdot\text{L}^{-1}$ but the limit of adaptation of this species remains to be investigated

The aim of this study was to analyze the maximum genetic adaptation capacity of three strains of *M. aeruginosa* (isolated from a freshwater reservoir with salinity $<0.025\text{ g}\cdot\text{L}^{-1}$, i.e. without previous "evolution salinity history") to the exposure of increased salinity. The effect of velocity of environmental deterioration on maximum adaptation of these strains, was also studied.

Material and methods

How can we explore the maximum adaptation?

By means of the ratchet experiment design (Fig. 1).

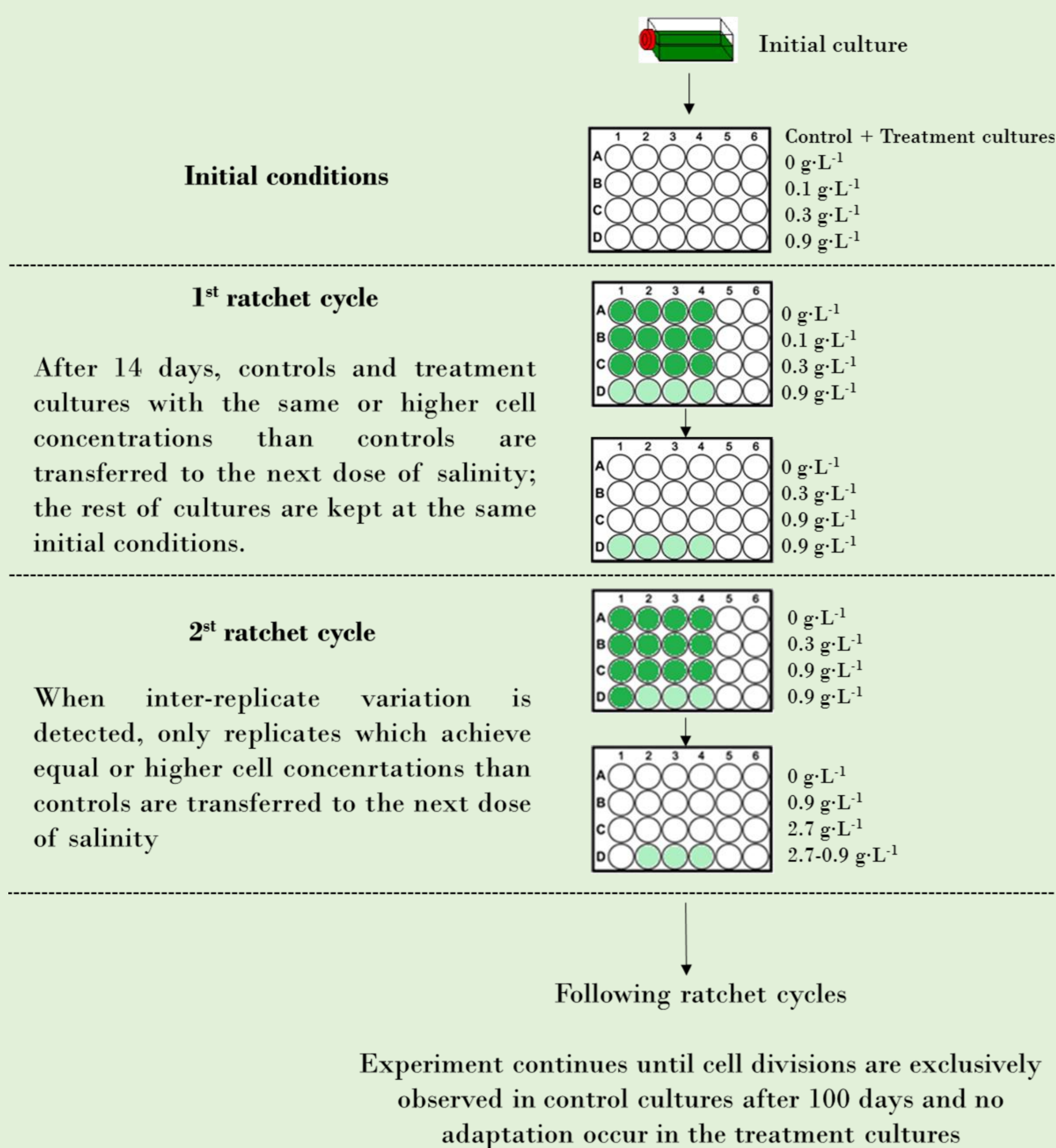


Fig. 1 Schematic representation of the ratchet experimental design.

How can we explore the velocity of environmental deterioration?

In order to analyze the effects of environmental deterioration velocity on maximum potential adaptation, an experimental evolutionary approach (ratchet experiment, Fig. 1) was used⁽²⁾. Two ratchet designs were performed based on "soft" or "intense" exponentially increased salinity. The concentration increment on soft ratchet was by a x1.5 factor while intense ratchet a x3 factor was used. The different velocities were applied when the cultures reached the concentration of $2.7\text{ g}\cdot\text{L}^{-1}$.

How many generations of the sensitive population would be needed to achieve resistance?

The number of generations (g) during the ratchet cycles was computed in accordance with Novick & Szilard (1950)⁽³⁾:

$$g = \text{Log}_e (N_t - N_0) / 0.693,$$

where N_t and N_0 were the number of cells at time t (when the cell density in the culture was similar to that in the control) and time 0, respectively.

Results

Fig. 2 Effect of NaCl on acclimated growth rate (m) of three strains of *Microcystis aeruginosa* wild-type. The values of m were computed after 7 days of exposure to NaCl, in mid-log exponentially growing cells. Vertical lines \pm SD ($n = 3$). Lethal dose was found ($7\text{ g}\cdot\text{L}^{-1}$ in Ma1Vc and Ma5vc and $10\text{ g}\cdot\text{L}^{-1}$ in MaAvc).

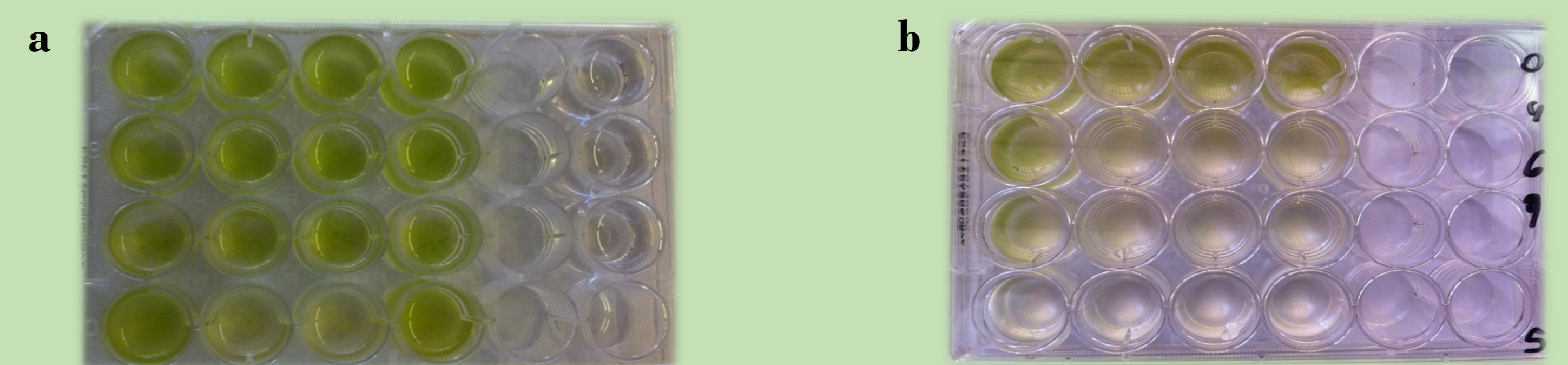
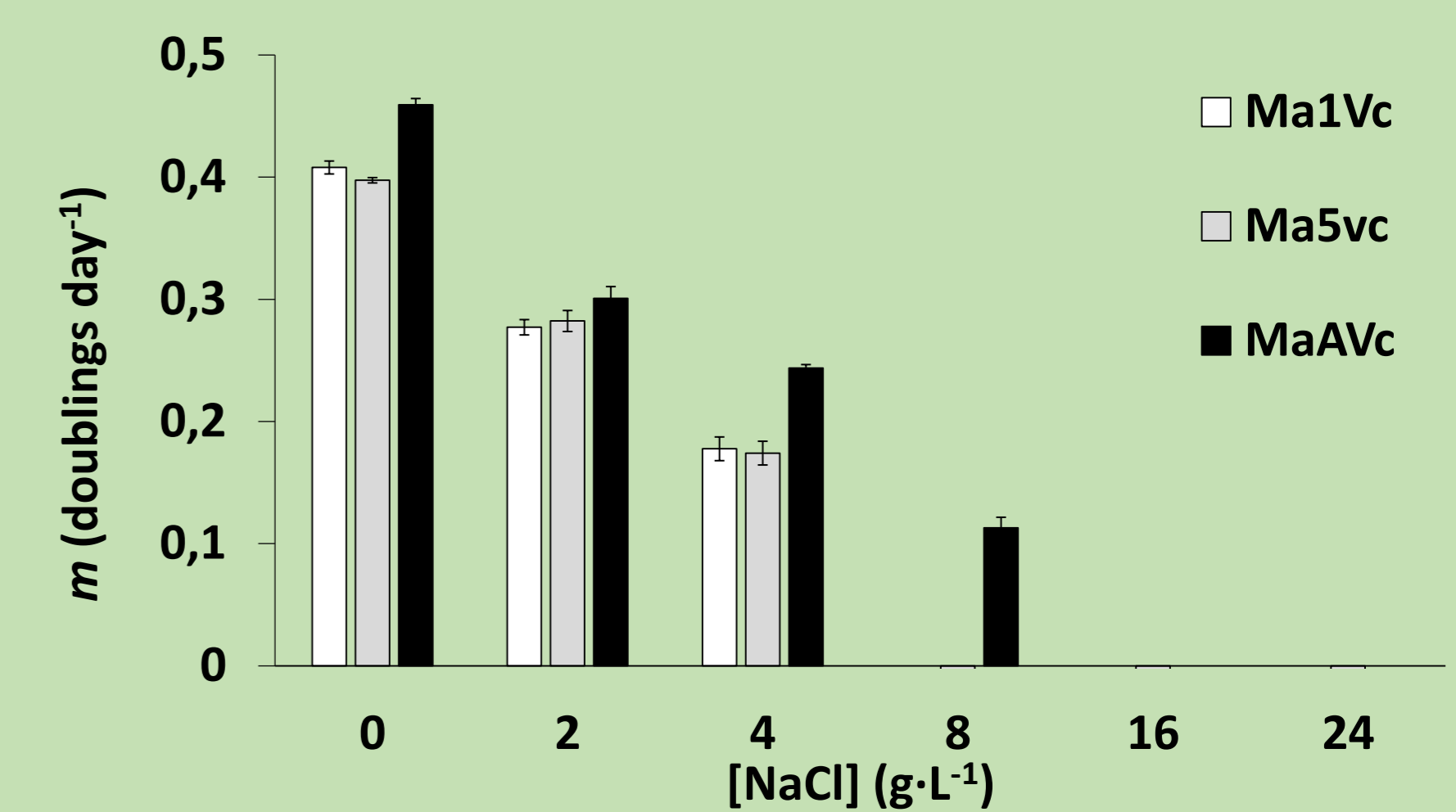


Fig. 3 Example of cultures during the ratchet experiment. a: In this case all cultures are transferred to the next salinity concentration; b: In this case the cultures are not transferred to the next salinity concentration because the cell concentration is lower than control.

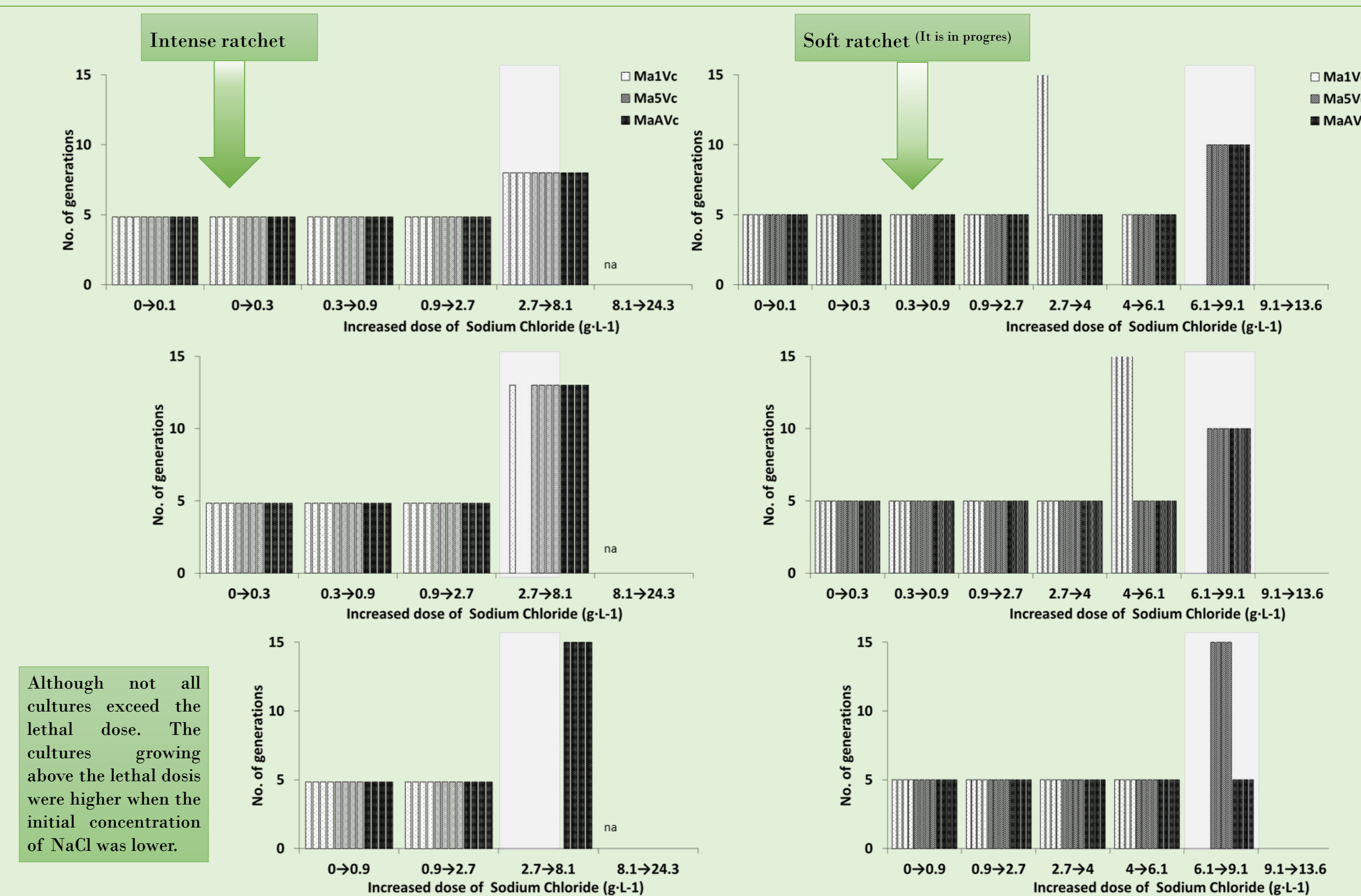


Fig. 4 Number of generations required to grow under increasing doses of NaCl during the ratchet experiment. Four independent cultures (represented by different column patterns) were tested per each strain. Grey texture over a step of increased dose of NaCl shows the concentration interval where initial lethal dose was found (Fig. 2)

Conclusions

- Acclimation to salinity (supported by the gene expression already present in the ancestral populations) was similar in the three strains in both experimental designs.
- A significant enhancement in resistance, supported by the selection of new genetic variants arising by mutations (genetic adaptation), was achieved in all derived populations in both ratchet experiments, and in the three strains.
- The dynamics and the limit of genetic adaptation depends on previous adaptation history (historical contingency), being the maximum adaptation higher when the environmental deterioration is soft in contrast with huge deterioration.

References

1. Tonk, L., Bosch, K., Visser, P. M., & Huisman, J. (2007). Salt tolerance of the harmful cyanobacterium *Microcystis aeruginosa*. *Aquat. Microb. Ecol.*, 46(2), doi: 10.3354/ame046117
2. Rosco, M., López-Rodas, V., González, R., Huertas, I. E., García-Sánchez, M. J., Flores-Moya, A., & Costas, E. (2014). The limit of the genetic adaptation to copper in freshwater phytoplankton. *Oecologia*, 175(4), doi: 10.1007/s00442-014-2963-1
3. Novick, A., & Szilard, L. (1950). Experiments with the chemostat on spontaneous mutations of bacteria. *Proc. Natl. Sci. U.S.A.*, 36(12).

Acknowledgments

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