Increased mitosis in the bleached gastrodermis of the sea anemone *A. pallida*.

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INTRODUCTION

Today, **coral bleaching** represents a major concern for marine biologists, especially considering the upsurge of this phenomenon possibly linked to **climate change**. Bleached corals, deprived of most of their energy incomes, may show a partial or total mortality, which ultimately lead to shifts in reef communities. Studies focusing on cellular bleaching mechanisms have shown different ways by which **symbiotic algae** (*Symbiodinium*) may be **expelled from gastrodermal host cells**. Among those mechanisms, major emphasis has been put on host cell death, most probably due to both **apoptosis** and **necrosis**. Recovering gastrodermis is then expected to undergo **regeneration** process in order to be **reinfected** by new algae. We describe here this regeneration process in the bleached sea anemone model *A. pallida*.

MATERIALS AND METHODS

BLEACHING:

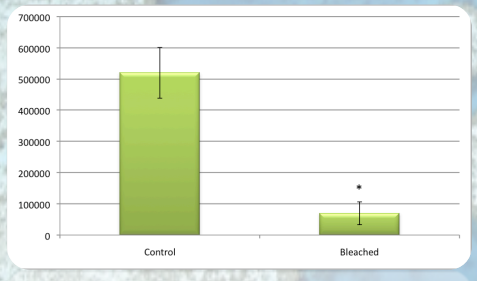
Bleaching was induced by the **cold shock** method. Anemones were subjected to two stress treatments on two successive days. Stress consisted in incubation for 4h at 4°C in the dark. They were then incubated for 48h in the dark at room temperature to complete treatment. *Symbiodinium* density was evaluated 24h later.

EdU INCUBATION:

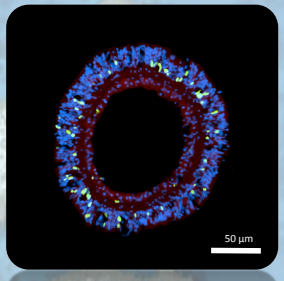
Two groups of bleached anemones were respectively incubated after 24h and one week in a 10µM solution of 5-ethynyl-2'-deoxyuridine (**EdU, a thymidine analogous**). Incubation lasted 4h and was followed by anaesthesia with $MgCl_2$ and fixation with 4% paraformaldehyde in filtered sea water.

HISTOLOGY:

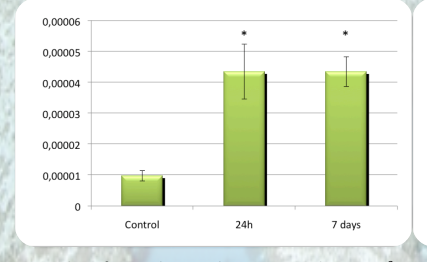
Fixed anemones were imbedded in **paraffin** and cut into 5µm thick slices. Cell multiplication highlighted by EdU incorporation was revealed using the **Click-iT™ method** (Invitrogen) with fluorescence microscopy. Counting of EdU-positive nuclei was made in transversal sections of tentacles.



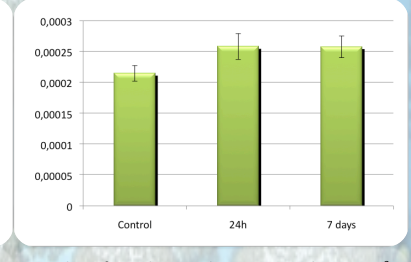
Symbiodinium density (Number of cells/mg of host cell tissues) in control (N=6) and bleached (N=6) anemones. (* $p < 0,05$)



Transversal section of a tentacle showing cell nuclei (DAPI, blue) and EdU-positive nuclei (green).



Number of gastrodermic EdU-positive nuclei per µm³ of ectoderm in the tentacles of control anemones (N=10) and in bleached anemones 24h (N=10) and 7 days (N=10) after cold shock. (* $p < 0,05$)



Number of ectodermic EdU-positive nuclei per µm³ of ectoderm in the tentacles of control anemones (N=10) and in bleached anemones 24h (N=10) and 7 days (N=10) after cold shock.

RESULTS

Considering the effect of bleaching process on the thickness of the gastrodermis, the number of **EdU-positive nuclei** in both gastrodermis and ectoderm was reported to the volume of the ectoderm. While there was only a small non-significant increase of EdU incorporation in the ectoderm, a **significant increase** was reported in the **gastrodermis** compared to control anemones.

CONCLUSIONS

These results highlight a **critical period** for the survival of the bleached cnidarian host during which it has to **regenerate its symbiotic tissues**. The weak trend to increased mitosis in the ectoderm could be explained by a higher production of cnidocysts in an effort to acquire more energy from heterotrophic sources but this hypothesis still needs to be investigated.

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