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

**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 measured 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 analogue). Incubation lasted 4h and was followed by anaesthesia with MgCl₂ 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

**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 cnidocytes in an effort to acquire more energy from heterotrophic sources but this hypothesis still needs to be investigated.

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