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Increased number of mucocytes in Aiptasia pallida following heat-induced bleaching

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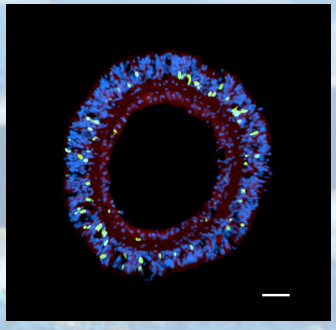
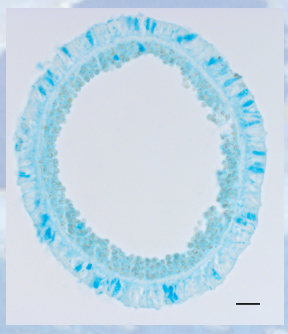
INTRODUCTION

While many researches have emphasized the numerous **histological modifications** occurring in cnidarians during **bleaching** only a few have focused on tissue dynamics during the following **recovery** period. Here, we examine the response of the sea anemone *Aiptasia pallida* to a transient elevation of water temperature combined with high illumination. We focused on **cellular proliferation** and **mucocyte** number directly after bleaching and during the following 8 weeks, when sea anemones have recovered a density of algae similar to pre-bleaching values.

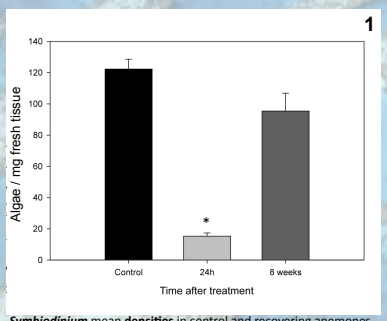


Transversal section of a tentacle of *A. pallida*
EdU (green) and DAPI staining (bar = 50µm)

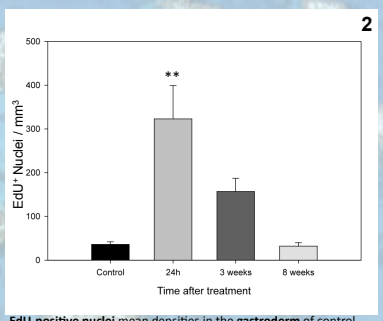


Transversal section of a tentacle of *A. pallida*
Mucocytes staining (bar = 50µm)

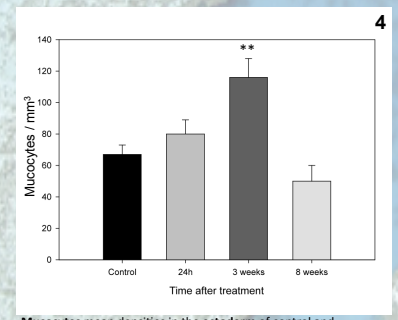
RESULTS



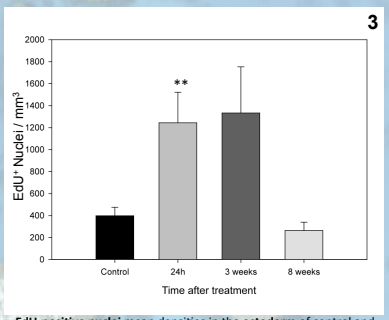
Symbiodinium mean **densities** in control and recovering anemones. (* p < 0,05)



EdU-positive nuclei mean densities in the **gastroderm** of control and recovering anemones. (** p < 0,001)



Mucocytes mean densities in the **ectoderm** of control and recovering anemones. (* p < 0,05)



EdU-positive nuclei mean densities in the **ectoderm** of control and recovering anemones. (** p < 0,001)

Algal Density (1)

Bleaching procedure induced a **tenfold decrease** in algal density. This density returned to **control values after 8 weeks**.

Cell Proliferation (2 - 3)

The number of EdU-positive nuclei showed **a transient increase** following bleaching in both **gastroderm and ectoderm**. This number reached a maximum after 24h and returned to control values after 8 weeks.

Mucocytes (4)

Mucocytes number showed **a transient increase** following bleaching. This number reached a maximum after 3 weeks and returned to control values after 8 weeks.

CONCLUSION

Our results suggest that a **bleaching** event **induces** an increase in **cellular proliferation** in both gastroderm and ectoderm of cnidarians. While the new cells formed in the gastroderm would most likely be host to new *symbiodinium*, the fate of new cells in the ectoderm is still not completely unraveled. Those new cells seem to be, in part, contributing to the increased number of **mucocytes** which could eventually contribute to the prevalence of a temporary heterotrophic state until restoration of the symbiosis.

MATERIAL AND METHODS

Bleaching

Bleaching of ***A. pallida*** was induced by **30h** of exposition to a water temperature of **33°C** and an illumination of **2000 µmol of photons/m²**. Anemones were then allowed to recover in normal condition for 24h, 3 weeks and 8 weeks before being subjected to algal density count or EdU incubation and histological staining.

Incubation

At each time point, control and bleached anemones were incubated for 24h in a solution of 1µM of 5-ethynyl-2'-deoxyuridine (**EdU, a thymidine analogous)** in seawater. This was followed by anaesthesia with $MgCl_2$ and fixation with 4% paraformaldehyde in seawater

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. Slides were also incubated in **Alcian blue** in order to stain mucus. Counting of EdU-positive nuclei and mucocytes (ectoderm) was made in transversal sections of tentacles.

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