

# Allelopathic effects of *Zostera marina* and *Zostera noltii* on the growth of the toxic dinoflagellate *Alexandrium catenella*



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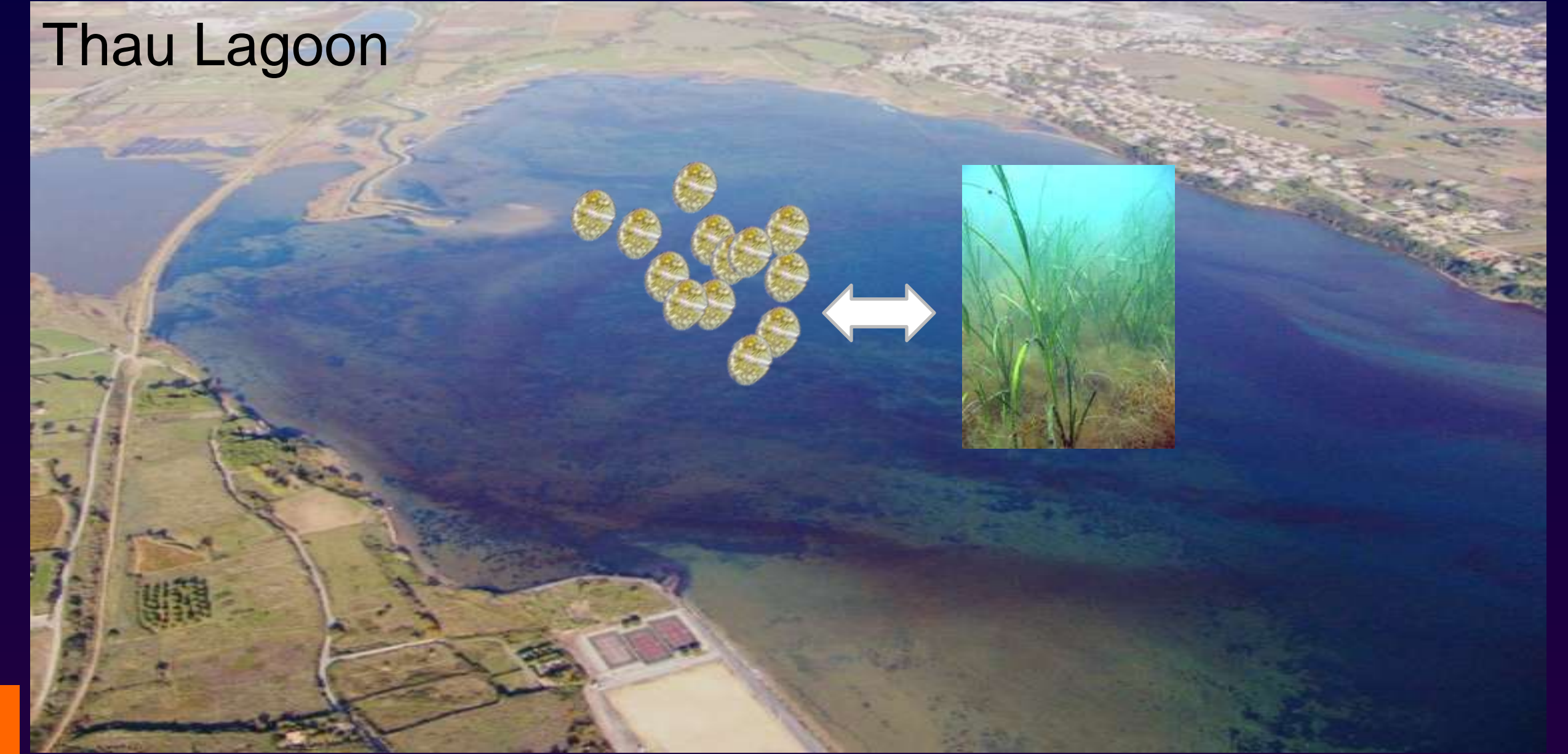
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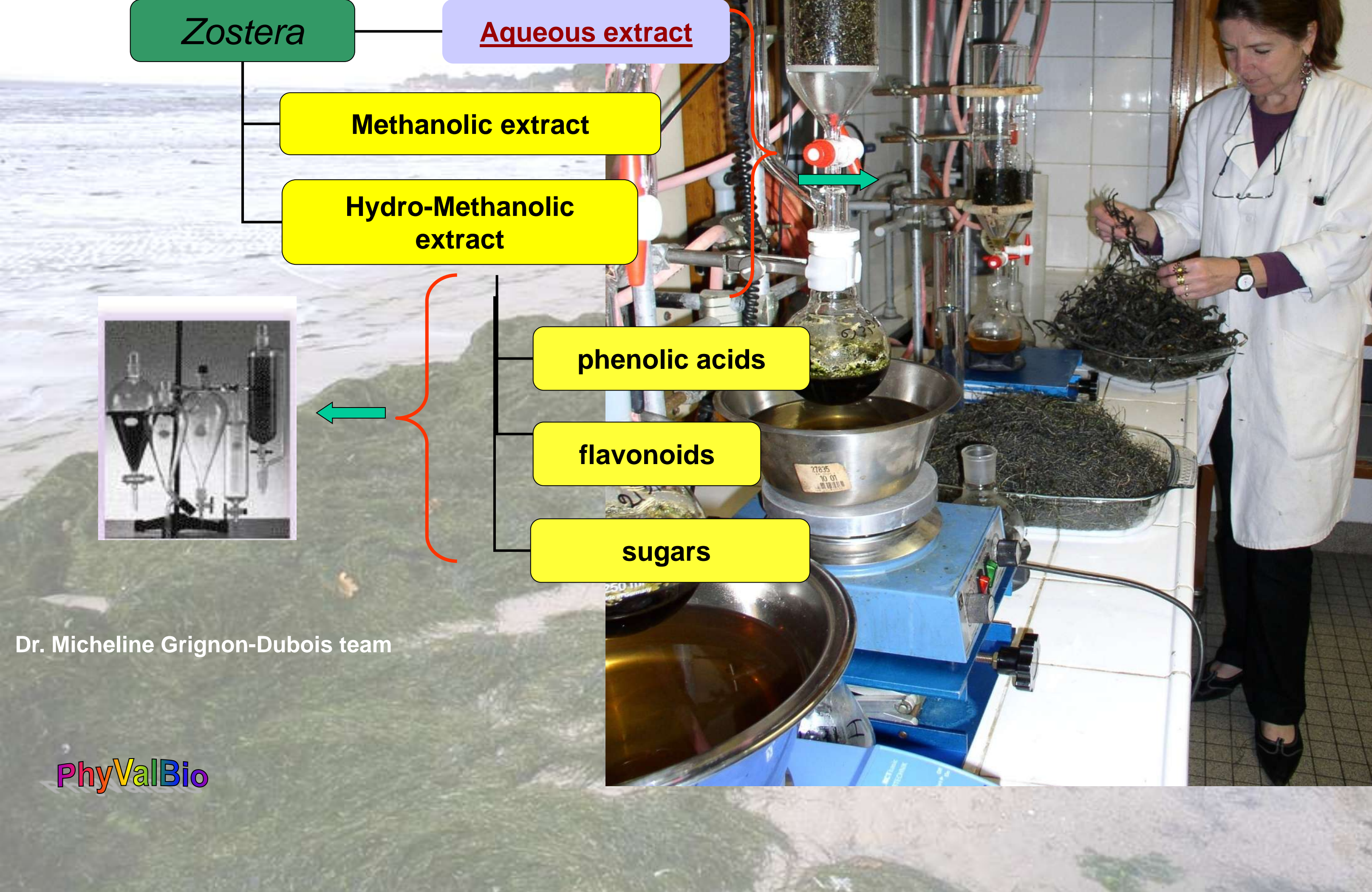
## Introduction

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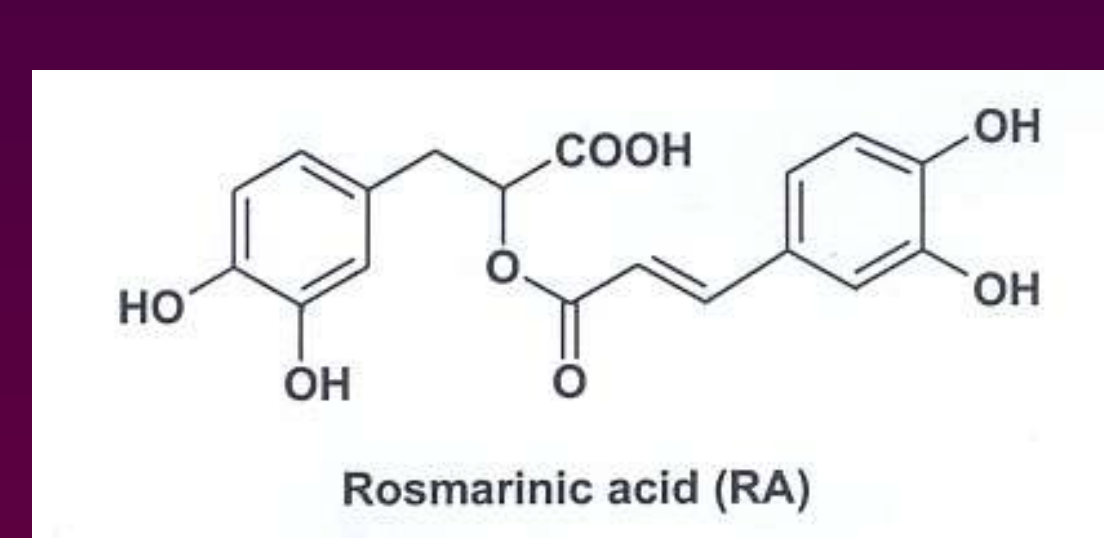
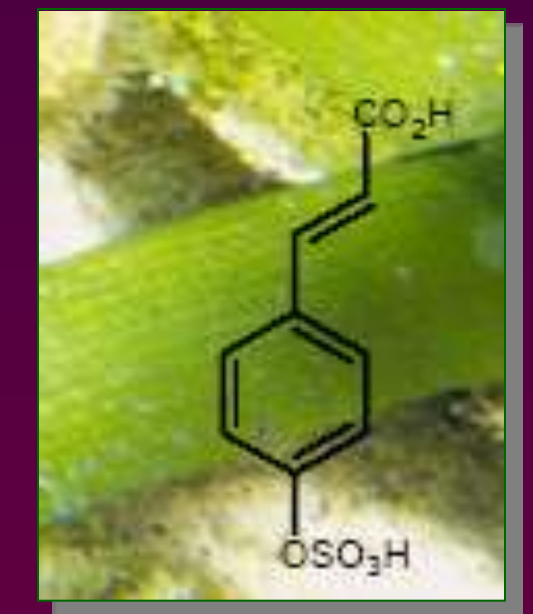
In the Thau lagoon (a French Mediterranean coastal lagoon), a bloom (85000 cellules.l<sup>-1</sup>) of *Alexandrium catenella* occurred for the first time in November 1998. Later similar episodes appeared regularly in spring and autumn reaching high cell concentrations (5\*10<sup>6</sup> cells.l<sup>-1</sup>) that induced the closing of shellfish farms. Allelopathy is a process implying secondary metabolites excreted by plants and micro-organisms which affects the competitive efficiency of the coexistent organisms. *Zostera marina* and *Zostera noltii* produce bioactive molecules as zosteric acid, a sulphated natural product able to prevent settlement of some marine bacteria, algae, barnacles and tube worms at non-toxic concentrations. These seagrass species contain also rosmarinic acid which have been reported to have a number of biological activities *in vitro*, such as antiviral, including human immunodeficiency virus 1, antibacterial, antioxydant, anti-inflammatory and anticarcinogenic. Water-soluble extract of *Z. marina* leaves inhibited the growth of micro-algae and many marine bacteria.



## Preparation of the tested extracts



Dr. Micheline Grignon-Dubois team



Sampling of *Zostera sp.* leaves

Identification

Extraction

Isolation and purification

Isolation and purification

Biological test

Leaves from the seagrasses *Z. noltii* and *Z. marina* collected on the beaches of Arcachon Lagoon over a 3-year period contain high amounts of **zosteric acid**, a natural sulphated phenolic acid and **rosmarinic acid**, a phenolic acid which is an economically important metabolite (Achamlale et al. 2009a, b).

Our observations using photonic microscope showed that cells which stopped to divide in the presence of very low concentrations of the tested extracts presented morphological anomalies and lysis.

## Inhibition of photosynthetic activity

Recent results (no shown here) clearly indicate that methanolic extract (BR.2173.I.S) of *Z. Noltii* have also a significant negative impact on the photosynthetic activity of *A. Catenella* (Phytopam fluorescence measurements during microcosm's experiments).

## Objectives

The present study investigates non-nutrient relationship between two dominant seagrass species and *A. catenella* a toxic dinoflagellate blooming worldwide which is often responsible of paralytic shellfish poisoning events. Aqueous and methanolic crude extracts were prepared from two seagrasses, *Z. marina* and *Z. noltii* which were collected on the beaches of Arcachon lagoon. The potential allelopathic effects of different concentrations of these extracts have been tested on the growth of *A. catenella* (strain collected in Thau in 2003) by using bio-essays.

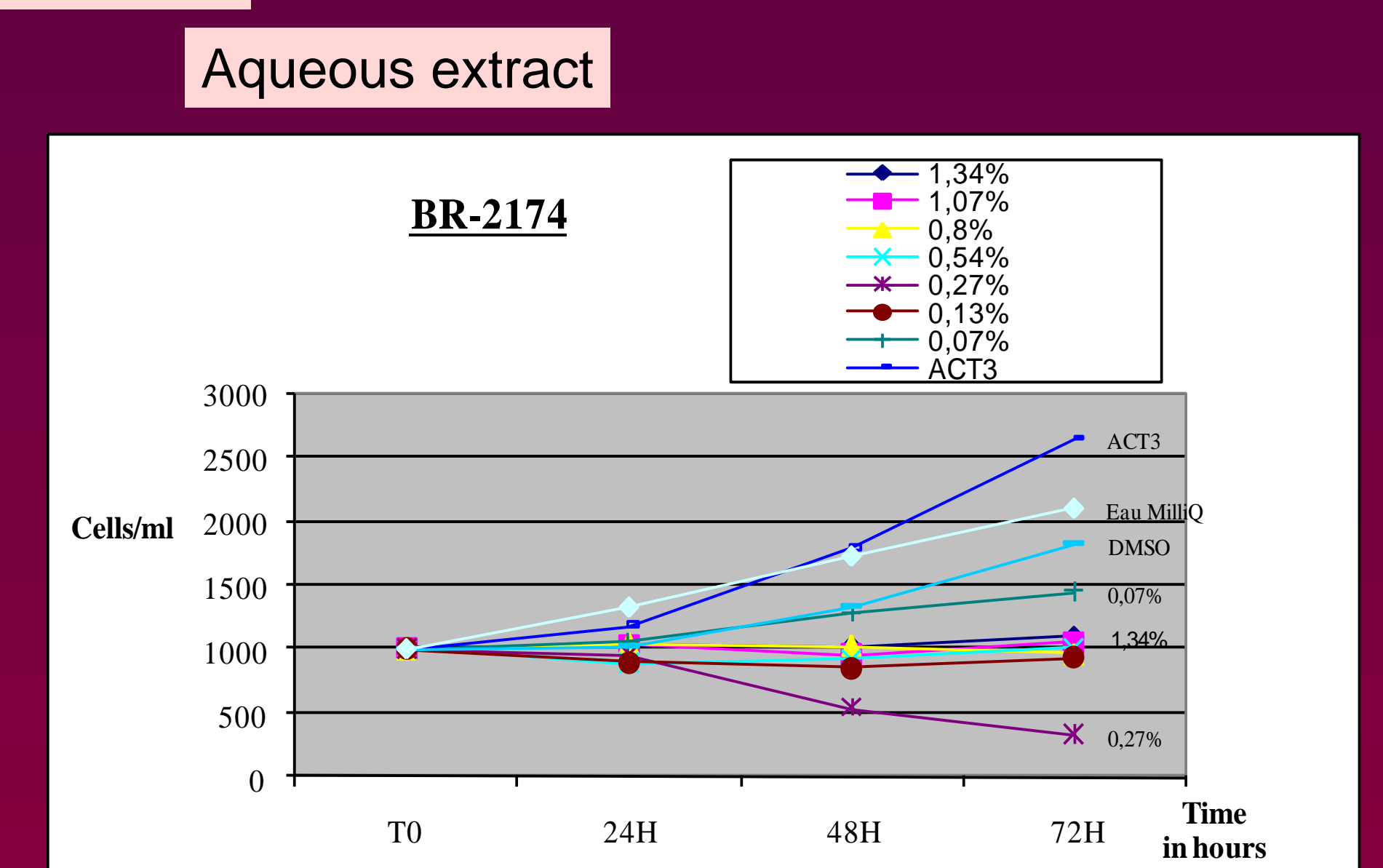
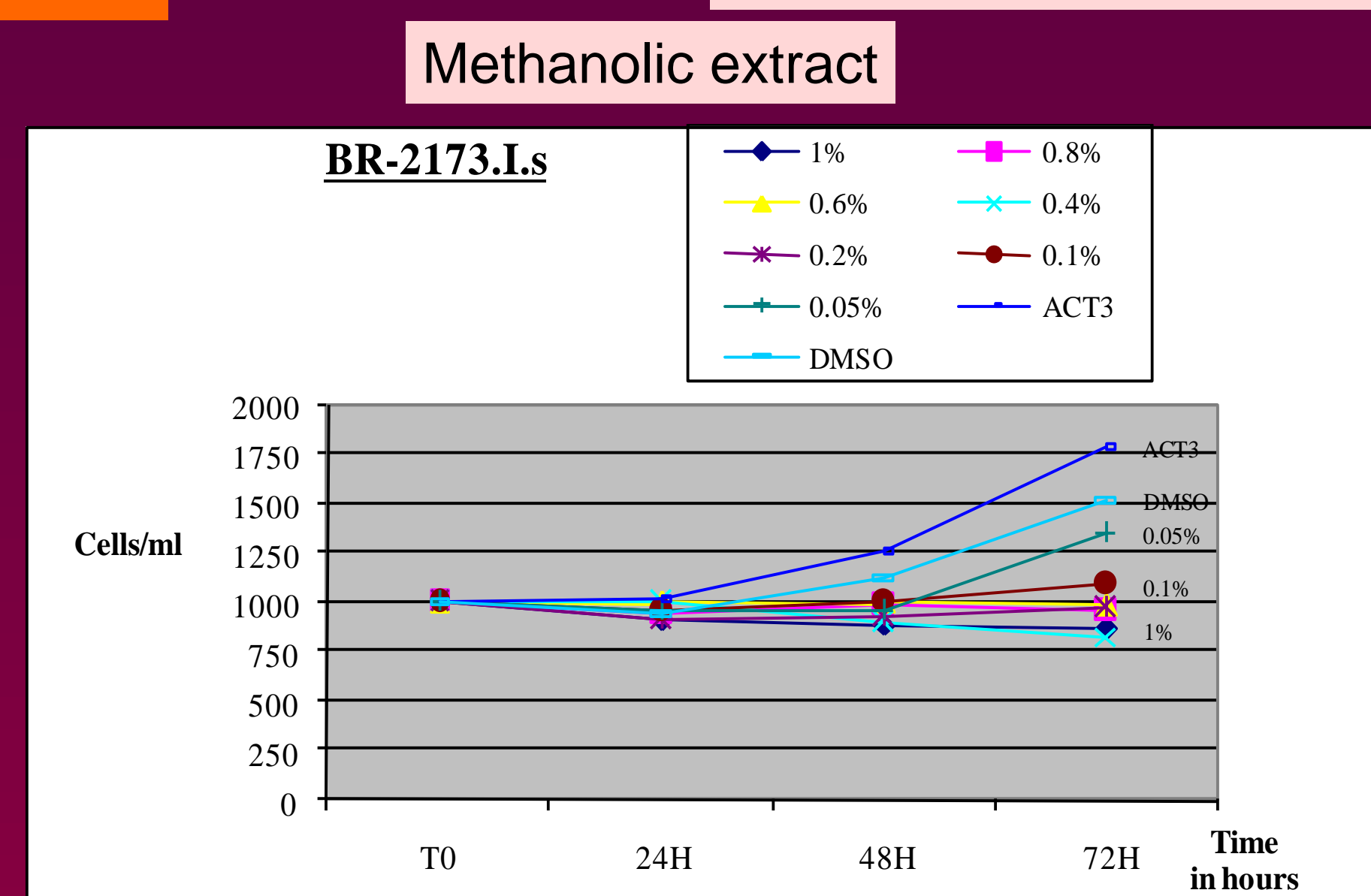
## Methods

*A. catenella* was grown in batch cultures using ESAW medium (Harrison et al. 1980) under appropriate light (100 μmoles.m<sup>-2</sup>.s<sup>-1</sup>) and temperature (20 C) conditions. To test allelopathic effect of *Zostera species*, target species was cultivated in culture medium with different concentrations of the extracts. Nutrients were added at (t0) in order to control the nutrient concentrations in the different tested treatments, including controls.

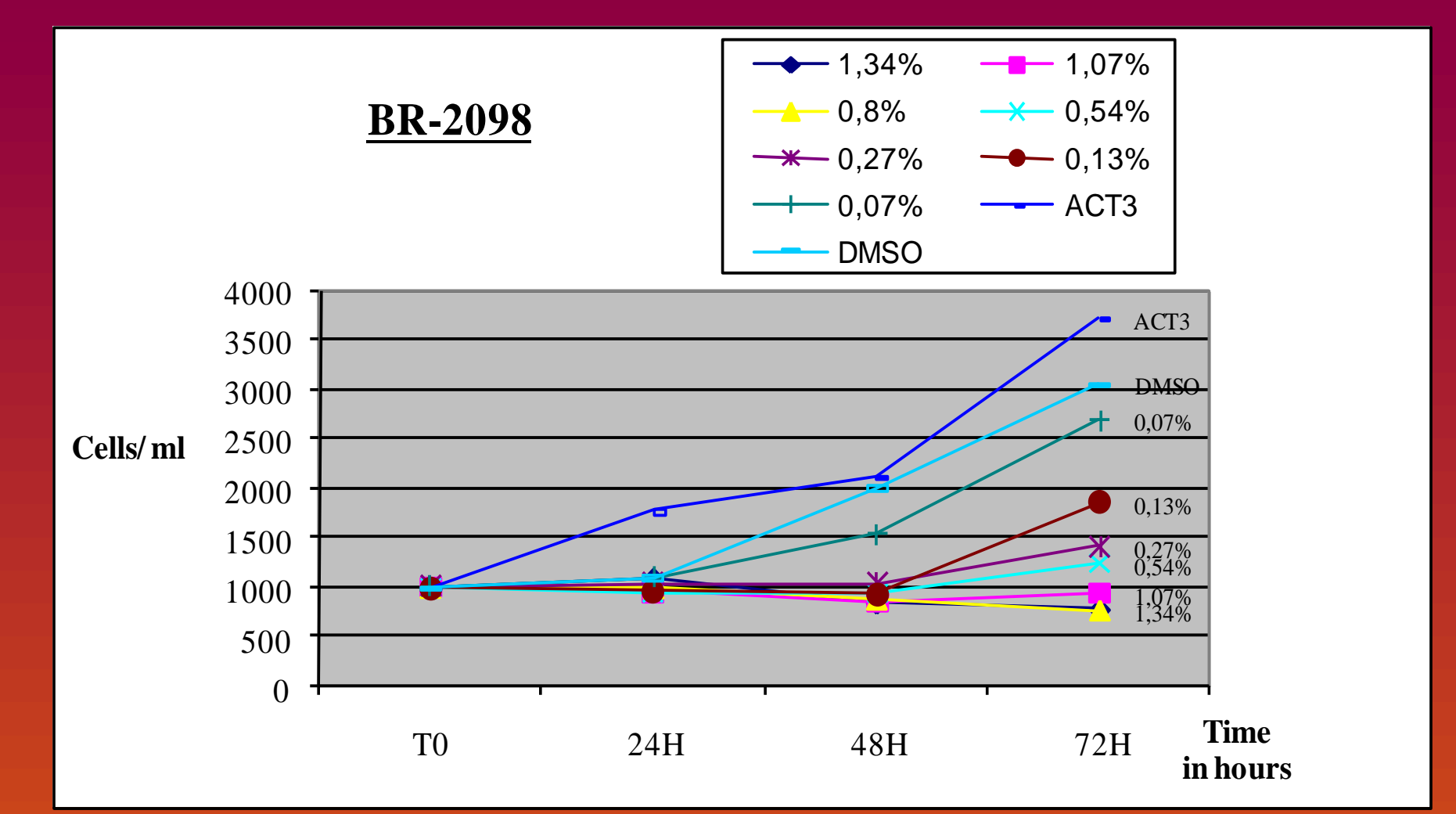
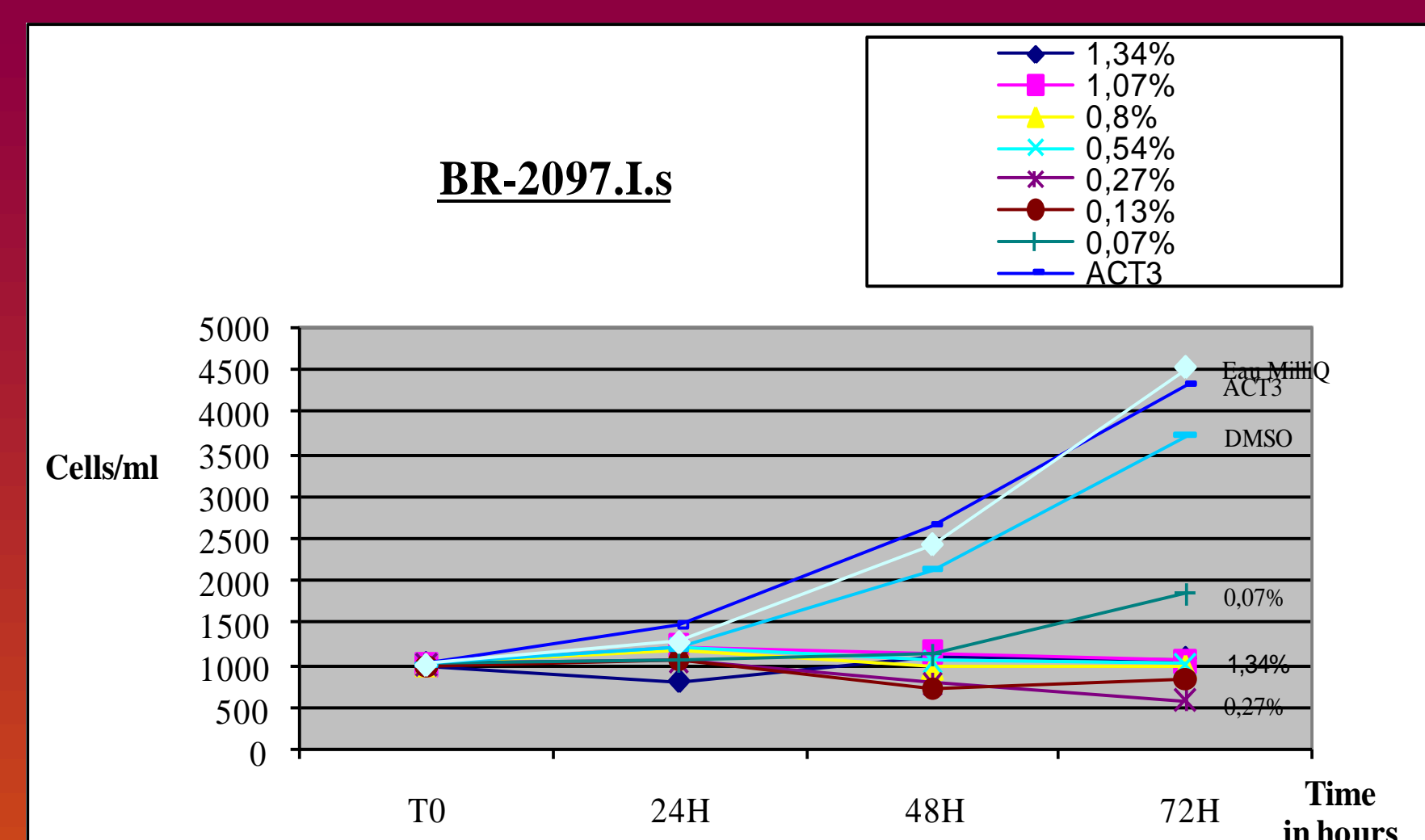
## Results

### Extracts from *Zostera noltii*

### Inhibition of *A. catenella* growth



### Extracts from *Zostera marina*



These results highlighted the strong inhibition effects exerted by natural extracts of *Zostera sp.* on the growth of *A. catenella*, even at very low concentrations. Extracts from leaves of *Zostera species* originated from Thau lagoon will be tested. Additional experiments will be dedicated to test the effect of continuous release of allelochemicals from the fresh seagrass tissues on the growth and the physiological activity of *A. catenella*. Also, the biological effect of purified molecules issued from *Zostera species* will be studied. Because of the severe economic and public health threats caused by harmful microalgae, the allelopathic substances associated to *Zostera detritus* may be considered as potential candidates to mitigate the impact of harmful algal blooms on shellfish aquaculture in lagoons.

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