Integrated field and laboratory approaches to examine the role of herbicide residues in the decline of eelgrass (*Zostera* sp.) meadows

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Context:

- ✓ Seagrasses are keystone marine flowering plants… which have undergone a major worldwide decline of 7% per year since 1990 (Wayott *et al.*, 2009)
- ✓ Various factors are involved in this decrease; herbicide residues (from agriculture or from antifouling paints) are suspected to play a role in some situations
- ✓ We are particularly interested in one seagrass species: the eelgrass *Zostera noltii* Hornem. (Fig. 1)

Questions addressed:

- ✓ What is the level of environmental contamination by herbicides in eelgrass meadows?
- ✓ Is there a link between contamination level and *in situ* eelgrass condition?
- ✓ What are the effects of these herbicides on eelgrass grown in laboratory conditions?

Approaches currently developped:

In the field: *French lagoons (Arcachon Bay and Etang de Vaccarès in Camargue)*

Eelgrass condition ⟷ **Correlation ?** ⟷ **Nature and concentration of herbicides**

Eelgrass condition

Parameters monitored:

- ✓ Shoot density & leaf morphometry
- ✓ Rhizome carbohydrate reserves
- ✓ Stress markers

Fig. 1: The eelgrass *Zostera noltii*

Other physicochemical parameters (that can influence seagrass condition)

In sediments:

- ✓ Grain size
- ✓ Organic matter
- ✓ Redox potential
- ✓ Nutrients (P and N)

In water column:

- ✓ pH
- ✓ Salinity
- ✓ Turbidity
- ✓ Temperature
- ✓ Dissolved oxygen
- ✓ Nutrients (P and N)

Nature and concentration of herbicides

In sediments:

- ✓ Punctual sampling
- ✓ Accelerated solvent extraction (ASE)

In water column:

- ✓ POCIS integrative sampling (Fig. 2)

Fig. 2: Polar Organic Chemical Integrative Samplers (POCIS) in water, providing average concentrations of chemicals over 3-weeks deployment periods.

Herbicides are analyzed by gas and liquid chromatography coupled to a mass spectrophotometer (GC -LC/MS/MS).

Nature and concentrations of herbicides chosen according to field monitoring

In laboratory:

Exposure under controlled conditions (herbicides pure or in mixture)

Measure of early and integrative stress markers

✓ **Oxidative stress enzyme activities:** Ascorbate peroxidase and gluthatione reductase

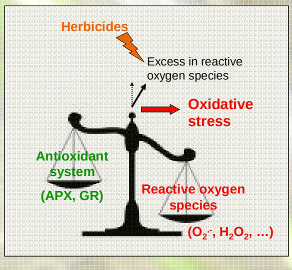

Fig. 3: Conceptual representation of the oxidative stress. When an organism is exposed to a contaminant, an imbalance in favor of reactive oxygen forms ($O_2^{.-}$, H_2O_2 ..) can take place. This excess of oxidants leads to oxidative stress, endangering the cell. To cope with this stress, the organism has developped an antioxidant system. There are particularly two key detoxifying enzymes: ascorbate peroxidase (APX) and glutathione reductase (GR). If their activity increases, the plant undergoes an oxidative stress.

✓ **Pigment composition:**

HPLC and spectrophotometer measurements (Fig. 4)

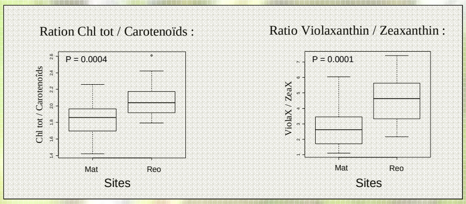

Fig. 4: Preliminary experiment showing a difference in pigment composition between eelgrasses from two sites in Arcachon Bay: Matoucaille (Mat), where eelgrasses are presumably impacted, and Reousse (Reo), where eelgrasses are in good condition. Zeaxanthin and carotenoïds are known to protect plants in stressful situations and their concentration increases compared to other pigments in these cases.

✓ **RNA / DNA ratio:**

RNA/DNA ratio reflects plant investment in protein synthesis and growth.

Assay in microplate (Fig. 5) with specific fluorescent markers (Picogreen for DNA and Ribogreen for RNA). Fluorescence is mesured with a microplate reader.

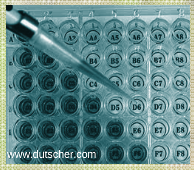

Fig. 5: Example of microplate where fluorescence is measured to determine the RNA / DNA ratio.

✓ **Photosynthetic yield:**

Assessed via fluorometry (Fig. 6)

Fig. 6: The Diving-PAM (Walz, Germany) underwater fluorometer allows to assess the effective quantum yield of photochemical energy conversion.

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