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

Seagrasses are keystone marine flowering plants... which have undergone a major worldwide decline of 7% per year since 1990 (Wayott et al., 2009)

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 developed:

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

- Eelgrass condition
  - Parameters monitored: Shoot density & leaf morphometry, Rhizome carbohydrate reserves, Stress markers
  - Correlation?
  - 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)

In laboratory:

- Exposure under controlled conditions (herbicides pure or in mixture)
  - Oxidative stress enzyme activities: Ascorbate peroxidase and glutathione reductase
  - Measure of early and integrative stress markers
    - RNA / DNA ratio:
      - RNA/DNA ratio reflects plant investment in protein synthesis and growth.
      - Assay in microplate (Fig. 5) with specific fluorescent markers (PhoGreen for DNA and RB1 green for RNA). Fluorescence is measured with a microplate reader.
  - Pigment composition:
    - HPLC and spectrophotometer measurements (Fig. 4)
      - Ratio Chlorophyll / Carotenoïdes
      - Ratio Violaxanthin / Zeaxanthin

Nature and concentrations of herbicides

In sediments:
- Punctual sampling
- Accelerated solvent extraction (ASE)

In water column:
- POCIS integrative sampling (Fig. 2)

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

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Fig. 1: The eelgrass Zostera noltii

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

Fig. 3: Conceptual representation of the oxidative stress.

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 Redousse (Reo), where eelgrasses are in good condition. Zeaxanthin and carotenoïdes are known to protect plants in stressful situations and their concentration increases compared to other pigments in these cases.

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

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

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