

# First approach towards the implementation of passive sampling adsorption devices for the identification of lipophilic toxins in the shellfish monitoring program in the coastal embayments of the Ebro Delta

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## INTRODUCTION

Molluscan safety monitoring programs search for control systems able to give an estimation of the concentration levels of marine toxins present in water, which could represent a risk for the health of consumers of shellfish products. In this study a deployment of passive sampling devices consisting of resin filled sachets was carried out weekly during two years in Alfacs Bay, a coastal embayment of the Ebro Delta in the NW Mediterranean, Spain. Lipophilic marine toxin concentration profiles were obtained analyzing the content of the polymeric resins after extraction and analysis by liquid chromatography coupled to a quadrupole ion trap mass spectrometer.

## MATERIAL AND METHODS

1. Packing of the previously conditioned styrene divinylbenzene resin: Diston HP-20
2. Deployment of the resin bags during 1-week close to the shellfish production area in the Alfacs Bay
3. Washing of the resin with water
4. Extraction of the resin in MeOH
5. Evaporation of the extract
6. Reconstitution
7. Injection into the LC-MS-MS (MacKenzie et al. 2004)

## RESULTS

### Spiroliodes

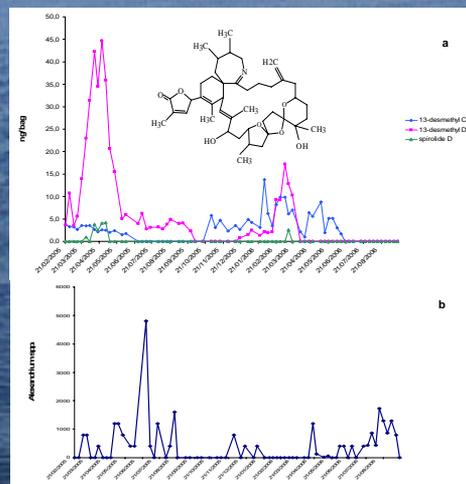


Figure 1. a) Determined concentration of spiroliodes adsorbed onto the resin bags, and b) cell/L counts of *Alexandrium* spp within the water column in the Alfacs Bay.

### Yessotoxin

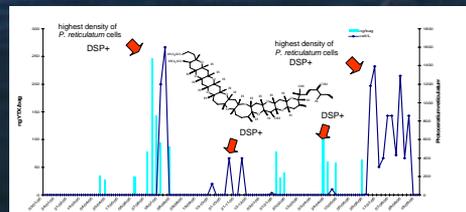


Figure 2. Determined concentration of yessotoxin adsorbed onto the resin bags, and cell/L counts of *Protoceratium reticulatum* within the water column in the Alfacs Bay.

### Gymnodimine

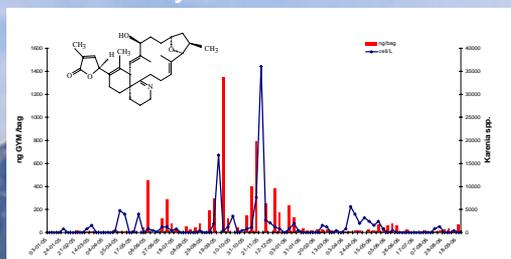


Figure 3. Determined concentration of gymnodimine adsorbed onto the resin bags, and cell/L counts of *Karenia* spp within the water column in the Alfacs Bay.

### OA, PTX-2, PTX-2sa

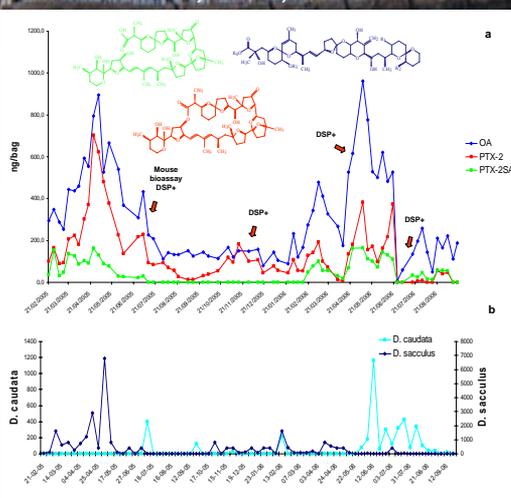


Figure 4. a) Determined concentration of okadaic acid, pectenotoxin 2 and pectenotoxin secoacid adsorbed onto the resin bags, and b) cell/L counts of *D. Caudata* and *D. sacculus* within the water column in the Alfacs Bay.

### LC-MS-MS measurements

<b>Column</b>	Hypersil BDS 3µm, 50 x 2 mm, Phenomenex	<b>Ionspray voltage</b>	5500V
<b>Mobile phase</b>	A 2 mM NH <sub>4</sub> For, 50 mM HFor B 2 mM NH <sub>4</sub> For, 50 mM HFor in 95% ACN	<b>Desolventing potential</b>	50V
<b>Flow</b>	0.2 mL/min	<b>Entrance potential</b>	10V
<b>Gradient</b>	From 5%B to 100% in 10 min, 5 min 100%B, and back to initial conditions in 3 min	<b>Cell exit potential</b>	15V
<b>Temperature</b>	20°C	<b>Collision energy</b>	55V (40 GYM)

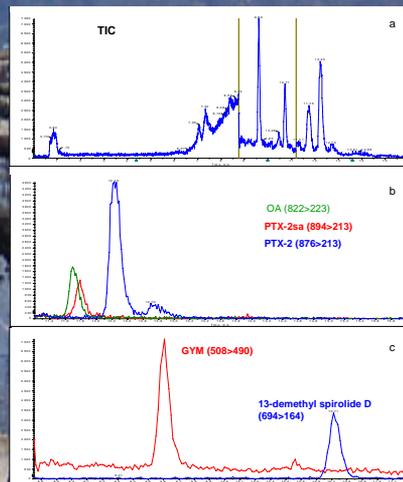


Figure 5. LC-MS-MS measurements were carried out on a ABI SCIEX-4000 QTrap mass spectrometer coupled to an HPLC Agilent model 1100 LC. Chromatograms corresponding to the resin bag deployed between 7/04/2005 and 14/04/2005. Total Ion Current (TIC), b) MRM 508-490 (GYM), 692>150, 694>150, 692>164, 694>164, 706>164, 708>164 (spiroliodes), and c) MRM 822>223 (OA, DTX2), 836>237 (DTX-1), 946>223 (OA diolester), 876>213 (PTX2), 874>213 (PTX12), 894>213 (PTX2sa), 892>213 (PTX1, 11, 12sa), 842>824 (AZA), 1160>965 (YTX).

## DISCUSSION AND CONCLUSIONS

- Adsorption rates of okadaic acid were up to 0.8-0.9 µg/bag per week during the recording of the highest density of *Dinophysis sacculus* cells (May 2005, May 2006), and up to 0.25 µg/bag per week for yessotoxin (*P. reticulatum*, July 2005). Mean yearly adsorption rates calculated for 2005 and 2006 showed values around 0.3 µg/bag per week for okadaic acid.
- OA, PTX2 and PTX-2sa profile concentrations showed the same trend in 2005 and 2006, the highest concentrations were detected in April and May.
- Results obtained showed that okadaic acid (OA) and gymnodimine (GYM) were continuously detected throughout the year, although in some cases at very low concentrations, whilst PTX2 and PTX2-seco acid were determined in 90% and 60% of the resin bags analysed, respectively.
- Good correlation between toxin concentration and phytoplankton cell counts were observed for OA, PTX2, YTX and GYM and the most putative toxin producing species.
- Further studies should be performed in order to correlate the determined concentration of spiroliodes and desmethylspiroliodes with the presence of *Alexandrium* spp within the water column, since the spiroliode producing species in the Ebro Delta has not been determined yet.
- Diarrhetic shellfish commercial closures were detected after 4-weeks of the recording of the highest OA concentration (July 2005, December 2005, July 2006). Closures are also related to high densities of *P. reticulatum* cells and high levels of yessotoxin in the embayment (July 2005, July 2006).
- The deployment of resin bags filled with styrene-divinylbenzene resin constitutes a useful tool to integrate and record toxic events related to lipophilic toxins occurring during a week. Other sorbent materials could be explored to integrate marine toxins responsible of the neurotoxic and paralytic shellfish poisoning.
- The management of the Alfacs Bay shellfish production requires the implementation of methodologies able to differentiate the group of yessotoxins from other lipophilic toxins.

**References**  
 MacKenzie, L., Beuzenberg, V., Holland, P., McNabb, P., Selwood, A. Solid phase adsorption toxin tracking (SPATT): a new monitoring tool that simulates the biotoxin contamination of filter feeding bivalves. *Toxicol* 44 (2004) 901-918.

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