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

MATERIAL AND METHODS

1. Packing of the previously conditioned styrene divinylbenzene resin: Diaion 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

The monitoring programme carries out weekly the sampling and analysis of diarrheic toxins (DSP) by the mouse bioassay (according to Yasumoto 78).

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

Spirolides

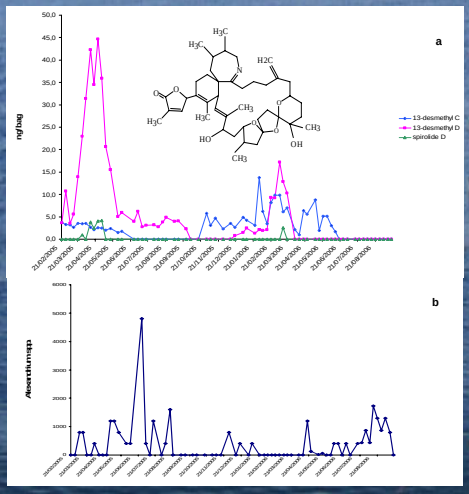

Figure 1. a) Determined concentration of spirolides adsorbed onto the resin bags, and b) cell/L counts of *Alexandrium spp* within the water column in the Alfacs Bay.

Yessotoxin

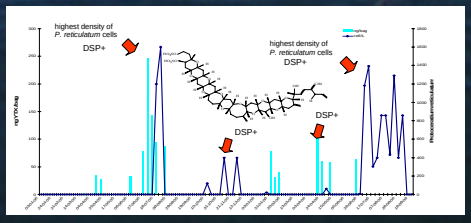

Figure 2. Determined concentration of yessotoxin adsorbed onto the resin bags, and cell/L counts of *Protoceratium reticulatum* within the water column in the Alfacs Bay.

Gymnodimine

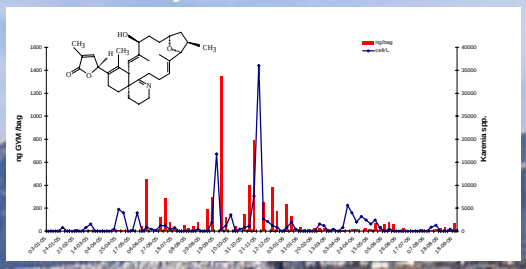

Figure 3. Determined concentration of gymnodimine adsorbed onto the resin bags, and cell/L counts of *Karenia spp* within the water column in the Alfacs Bay.

OA, PTX-2, PTX-2sa

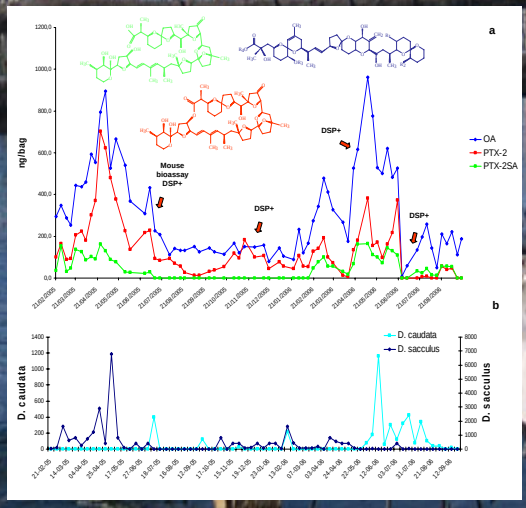

Figure 4. a) Determined concentration of okadaic acid, pectenotoxin 2 and pectenotoxin secoacid adsorbed onto the resin bags, and b) cell/L counts of *D. Caudata and D. sacculus* within the water column in the Alfacs Bay.

LC-MS-MS measurements

Column	Hypersil BDS 3µm, 50 x 2 mm, Phenomenex
Mobile phase	A 2 mM NH_4For, 50 mM HFor B 2 mM NH_4For, 50 mM HFor in 95% ACN
Flow	0.2 mL/min
Gradient	From 5%B to 100% in 10 min, 5 min 100%B, and back to initial conditions in 3 min
Temperature	20°C

Ionspray voltage	5500V
Declustering potential	50V
Entrance potential	10V
Cell exit potential	15V
Collision energy	55V (40 GYM)

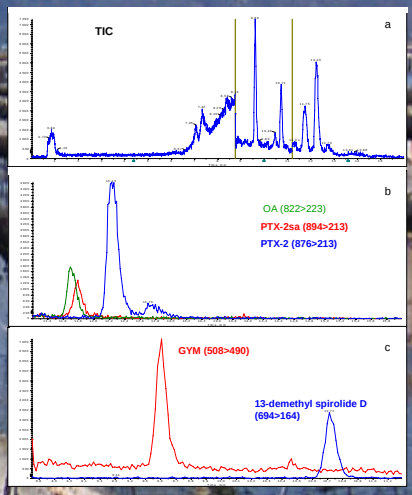

Figure 5. LC-MS-MS measurements were carried out on a ABI SCIEX-4000 QTrap mass spectrometer coupled to an HPLC Agilent model 1100 LC. Chromatograms corresponding to the resin bag deployed between 7/04/2005 and 14/04/2005. Total Ion Current (TIC), b) MRM 508>490 (GYM), 692>150, 694>150, 692>164, 694>164, 706>164, 708>164 (spirolides), 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 continuosly 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 spirolides and desmethylspirolides with the presence of *Alexandrium spp* within the water column, since the spirolide 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 neutotoxic 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. *Toxicon* 44 (2004) 901-918.

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