Accumulation, biotransformation, ways of excretion and depuration kinetics of DSP toxins in bivalves.

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Bivalves accumulate DSP toxins mainly in free forms and esterified with fatty acids (7-o-acyl derivatives, "DTX3"). In most bivalve species studied to date as the cockle *Cerastoderma edule* or several clams as *Venerupis pullastra*, *Spisula solida*, or *Ruditapes philippinarum*, 7-o-acyl derivatives soon during the depuration phase, constitute nearly 100% of the accumulated toxin. In some other species as the mussel *Mytilus galloprovincialis* or the clam *Donax trunculus*, the proportion of free toxins in the body is higher, being in the mussel typically 15% and 30% for okadaic acid and DTX2, respectively. Obviously in the first group of bivalves but also in the second one the toxins are excreted nearly completely in esterified form, as only small amounts of free forms can be found in feces (that are the elimination way).
Analysis of feces shows that the excretion of 7-o-acyl derivatives is the main way of depuration in the bivalves studied up to date. Other mechanisms and other biotransformations are also possible and, but they do not seem especially important in natural conditions.

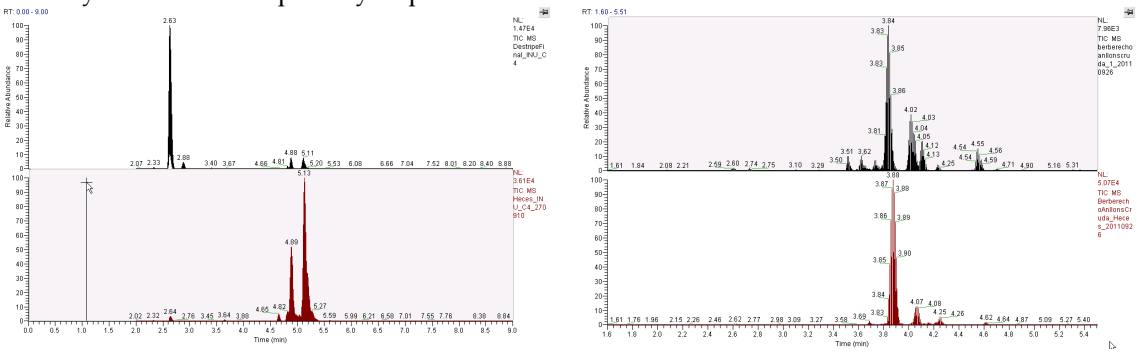

Figure 1.- Free OA (RT= 2.64 min) and esters (RT >3.8 min) in meat (upper panels) and feces (lower panels) of mussels (left panels) and cockles (right panels).

We have previously shown that the esterification of DSP toxins is an enzymatic process that takes place inside the bivalve cells (Rossignoli et al 2011), and that some genes that codify for ABC transporter proteins are upregulated in the mussel as a response to the exposure to okadaic acid. Therefore the general ways of toxin uptake or elimination could probably be those depicted in fig. 2. In a first approach, the efflux of toxins by passive diffussion could also be considered due to the relative apolarity of the 7-o-acyl derivatives

A kinetic model considering that depuration has two steps: esterification and efflux could be modeled as a Michaelis-Menten kinetics (esterification) coupled to a first order elimination kinetics (efflux by passive diffusion) or to another Michaelis-Menten kinetics (efflux by a transporter). When a passive difussion efflux is asumed OA and DTX2 should have the same rate, as they have the same polarity. In such a case, the model cannot describe the different behaviour of OA and DTX2 in mussels, suggesting that passive difussion is not the may efflux route for these toxins, and point towards membrane transporters.
A model that uses two Michaelis-Menten kinetics is able to describe correctly the behaviour of the two toxins by making the Vmax for esterification and depuration of DTX2 lower than those of OA, suggesting that both processes (especially esterification) are involved in the smaller depuration rate of DTX2 relative to OA.

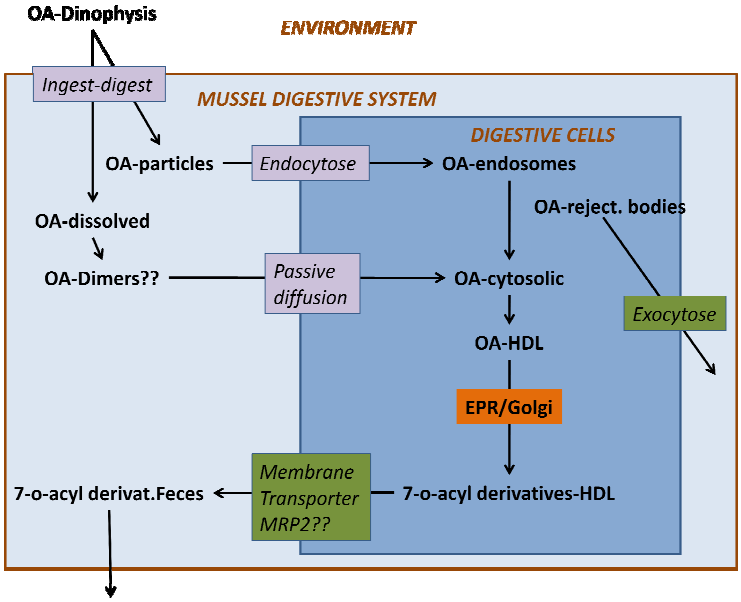

Figure 2.- Possible routes of uptake and elimination of toxins form the okadaic acid group.

Using the models with the obtained parameters to simulate the OA and DTX accumulation during 100 days of continuous exposure to an arbitrary amount of toxins (100 in each case), and 100 aditional days of depuration, suggests that the accumultion of OA in mussels stops earlier than that of DTX2 and the proportions between free and esterified toxins should differ between the uptake and the depuration phases.

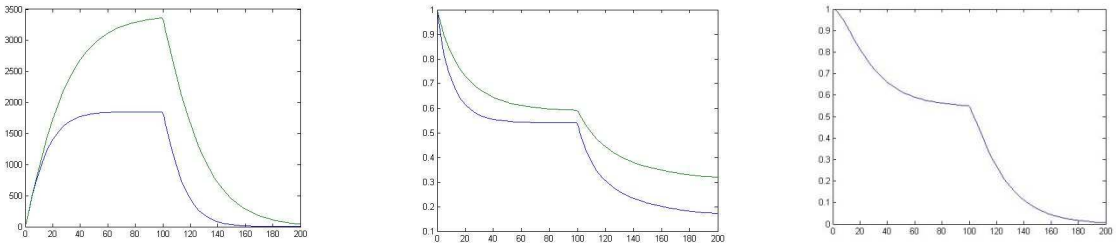

Figure 3. Time course of the simulated accumulation of OA (blue) and DTX2 (green) (left panel), proportion of the total toxin content that represent the free forms (central panel), and ratio OA/DTX2 (right panel), usin the conditions stated in the text.

When the toxic phytoplancton contains conjugated forms, a substantial increase of the free forms could be expected from their hydrolysis during digestion.

The species that accumulate mostly acyl-esters are expected to accumulate toxins to a lower extent than those that accumulate free toxins if they have the same toxin efflux rate.