

## Supporting information

# **Cation-exchange resin applied to Paralytic shellfish toxins depuration from bivalves exposed to *Gymnodinium catenatum***

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## **Materials and methods**

*Table S1 – Average size (length x width, in cm) of mussels Mytilus edulis used in the second set of experiments. The values inside the parentheses correspond to the standard deviation.*

Tank 1	Tank 2	Tank 3	Tank 4	Tank 5	Tank 6
6.4(0.7) x 3.8(0.4)	6.2(0.7) x 4.0(0.5)	6.2(0.6) x 3.8(0.5)	6.4(0.7) x 4.0(0.5)	6.6(0.9) x 4.4(0.9)	6.4(0.6) x 3.9(0.6)

*Table S2 – Average size (length x width, in cm) of mussels Mytilus edulis used in the third set of experiments. The values inside the parentheses correspond to the standard deviation.*

Tank 1	Tank 2	Tank 3	Tank 4
6.6(0.5) x 3.5(0.5)	6.6(0.5) x 3.7(0.3)	6.6(0.4) x 3.6(0.3)	6.6(0.5) x 3.6(0.4)

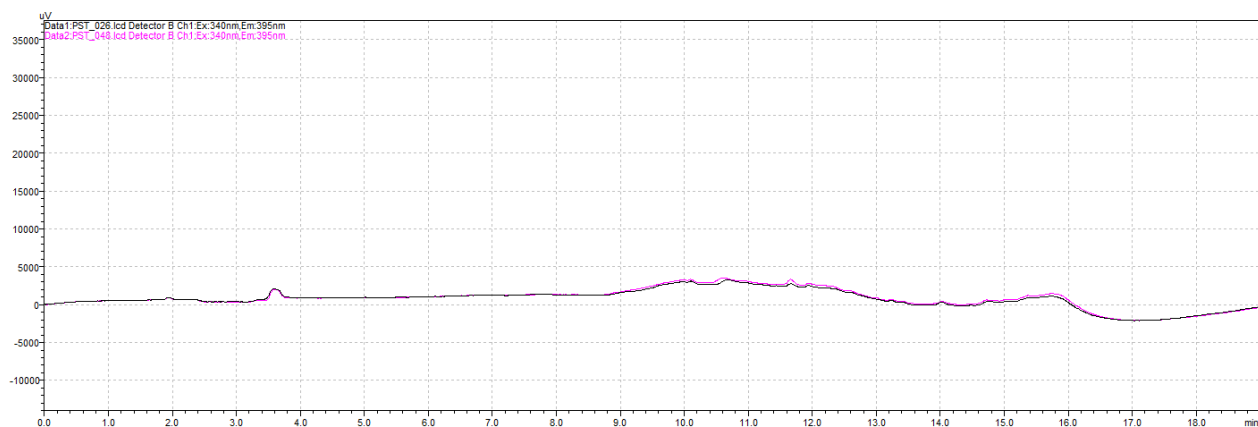


Figure S1 – Chromatograms of a chemical blank oxidized by peroxide (black) and periodate (pink)

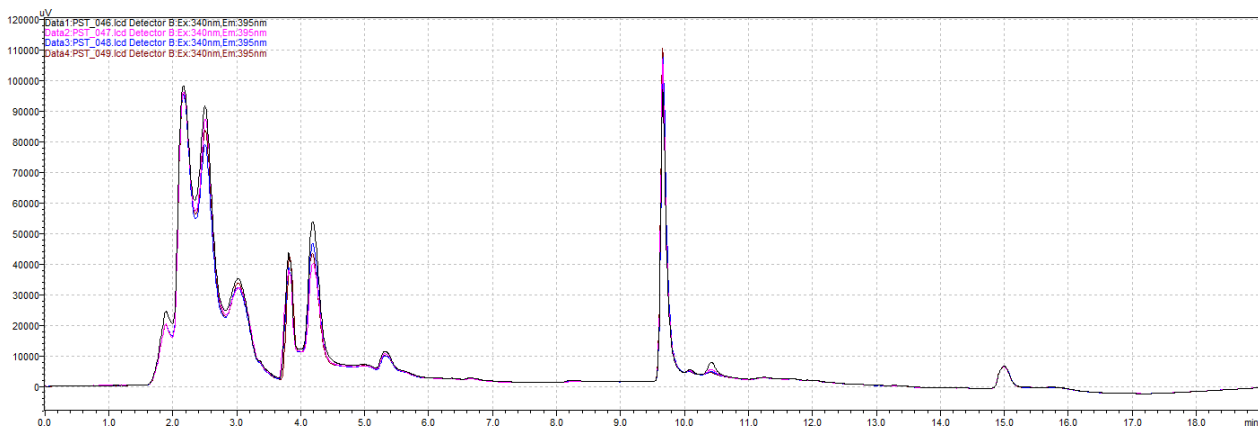


Figure S2 – Chromatograms of mussels' samples (*Mytilus edulis*) after SPE-C18, containing or not PST, not oxidized (procedure as in periodate oxidation, with matrix modifier). The products shown are naturally fluorescent co-extractives.

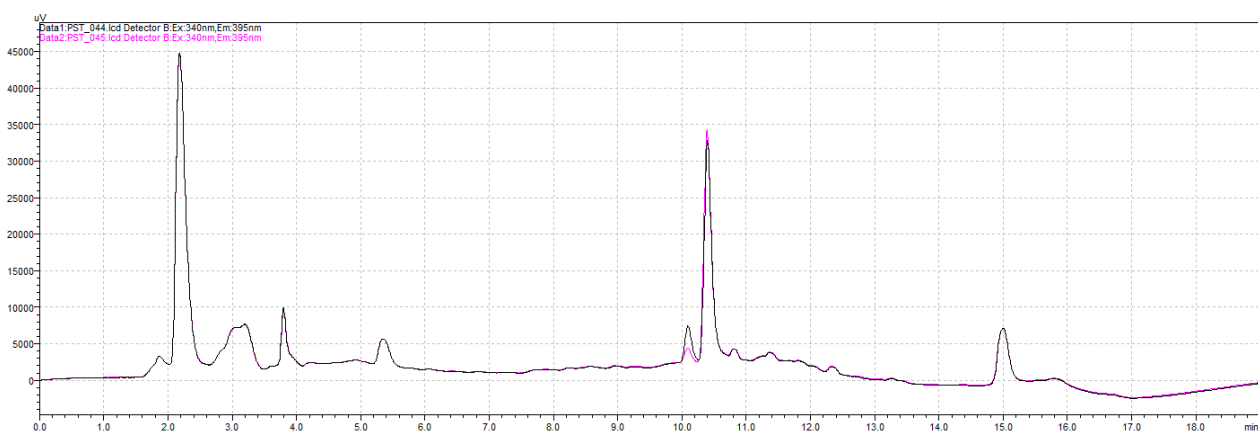


Figure S3 – Chromatograms of matrix modifier (prepared from oysters *Crassostrea gigas*), oxidised with periodate (PST-free sample).

## Results

### ➤ HPLC-FLD analysis

Table S3 – Method parameters: determination coefficient ( $R^2$ ), detection limit (LOD), quantification limit (LOQ).

Toxin	$R^2$	LOD ( $\mu\text{M}$ )	LOD ( $\mu\text{g STX.2HCl eqv/kg}$ ) *	LOQ ( $\mu\text{M}$ )	LOQ ( $\mu\text{g STX.2HCl eqv/kg}$ ) *
dcGTK2&3	$\geq 0.9998$	$\geq 0.005$	$\geq 6$	$\geq 0.02$	$\geq 21$
C1&2	$\geq 0.9999$	$\geq 0.003$	$\geq 1$	$\geq 0.01$	$\geq 3$
dcSTX	$\geq 0.9993$	$\geq 0.02$	$\geq 63$	$\geq 0.07$	$\geq 209$
GTX2&3	$\geq 0.9996$	$\geq 0.01$	$\geq 25$	$\geq 0.05$	$\geq 84$
GTX5	$\geq 0.9999$	$\geq 0.005$	$\geq 1$	$\geq 0.02$	$\geq 4$
STX	$\geq 0.9981$	$\geq 0.01$	$\geq 34$	$\geq 0.04$	$\geq 112$
GTX1&4	$\geq 0.9990$	$\geq 0.03$	$\geq 169$	$\geq 0.09$	$\geq 565$
NEO	$\geq 0.9993$	$\geq 0.02$ (0.05) †	$\geq 184$	$\geq 0.08$ (0.16) †	$\geq 615$
C3&4	$\geq 0.9994$	$\geq 0.01$ (0.03) †	$\geq 11$	$\geq 0.04$ (0.12) †	$\geq 36$
GTX6	$\geq 0.9992$	$\geq 0.02$	$\geq 13$	$\geq 0.07$	$\geq 43$
dcNEO	$\geq 0.9984$	$\geq 0.03$ (0.05) †	$\geq 97$	$\geq 0.11$ (0.18) †	$\geq 322$

† For the toxins C3&4, NEO and dcNEO, the values between parenthesis correspond to the different values obtained from calibration curves constructed without matrix modifier (data only used in samples from *G. catenatum* cultures).

\* The conversion of the values in  $\mu\text{M}$  to  $\mu\text{g STX.2HCl eqv/Kg}$  was performed according to the following equation:

$$C_i(\mu\text{g STX.2HCl eqv/Kg}) = C_i(\mu\text{M}) \times \text{TEF} \times \text{MW (g/mol)} \times \frac{V_E(\text{mL})}{m_H(\text{g})} \times D_f \quad (\text{Eq. S1})$$

Where  $C_i$  is the concentration of each toxin; TEF is the toxicity equivalence factor for each toxin, according to EFSA; MW is the molecular weight of saxitoxin dihydrochloride (372.2 g/mol);  $V_E$  is the volume after the extraction (10 mL);  $m_H$  is the mussels' homogenised tissue (5.0 g);  $D_f$  is the dilution factor for each toxin throughout the procedure.

➤ **PST clearance in bivalves:**

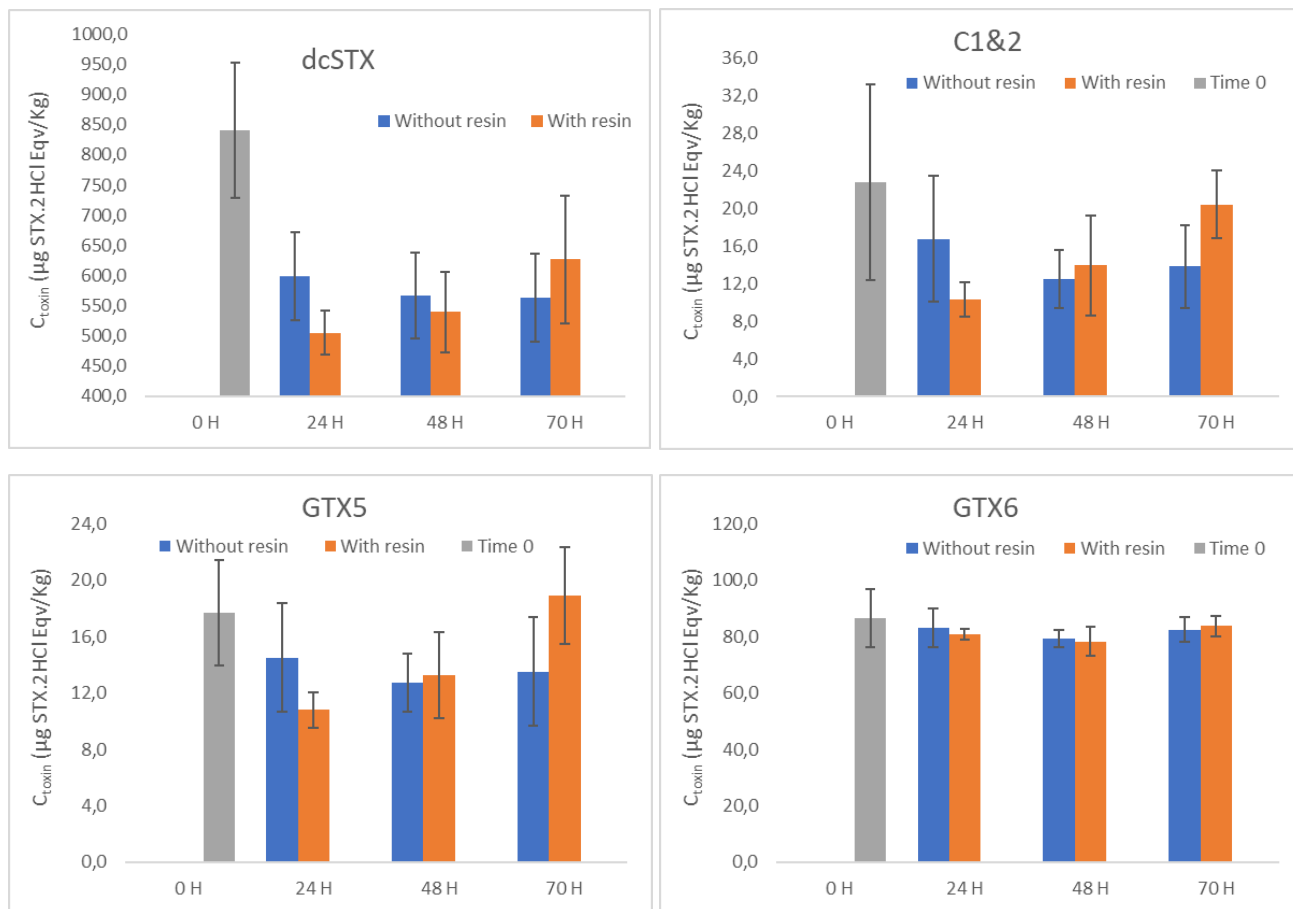
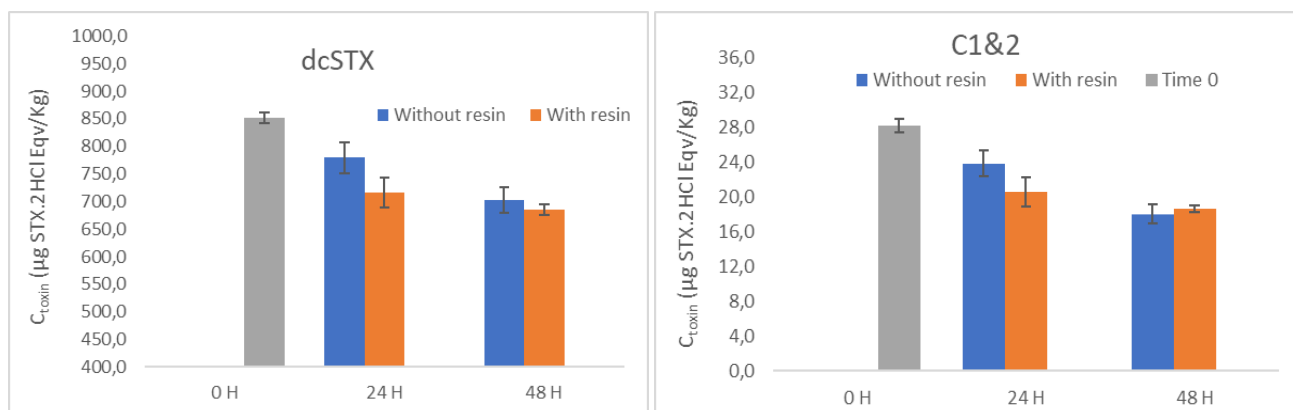


Figure S4 – Results of the PST clearance tests in live mussels (without resin and **with H-form resin**).



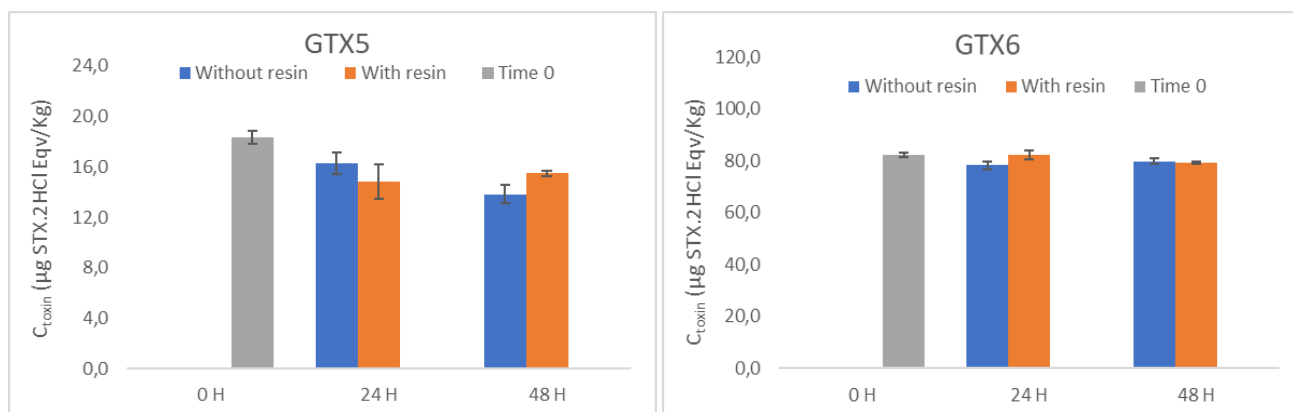


Figure S5 – Results of the PST clearance tests in live mussels (without resin and **with Na<sup>+</sup>-form resin**).

### ➤ PST concentrations

Table S4 – PST concentration, estimation in µg STX.2HCl eqv/Kg (average ± SD), in *G. catenatum* cultures, during removal studies using the H-form resin. At time 0 and 48-h control, no resin was present. Conversion from concentrations in µM were done using equation S1.

Toxin	T0 (0 h)	T1 (1 h)	T2 (3 h)	T3 (19 h)	T4 (26 h)	T5 (48 h)	C (48 h)
<b>C1,2</b>	18.2 ± 0.9	18.6 ± 0.5	17.6 ± 0.2	18.5 ± 0.9	19.3 ± 0.4	18.7 ± 0.3	18.4 ± 0.9
<b>dcSTX*</b>	249.3 ± 1.2	104.6 ± 109.4	92.0 ± 96.1	45.9 ± 83.0	ND	ND	248.6 ± 4.5
<b>GTX5</b>	6.7 ± 0.1	4.6 ± 0.3	4.7 ± 0.9	6.6 ± 0.1	4.6 ± 1.2	4.0 ± 1.5	7.1 ± 0.2
<b>GTX6</b>	42.8 ± 1.2	42.9 ± 0.5	41.9 ± 0.8	43.2 ± 1.2	42.6 ± 0.9	41.6 ± 1.2	42.7 ± 2.2

\* For the calculation of the average and standard deviation, the value of zero was assigned when the toxins were not detected.

### ➤ H-form vs. Na<sup>+</sup>-form resins

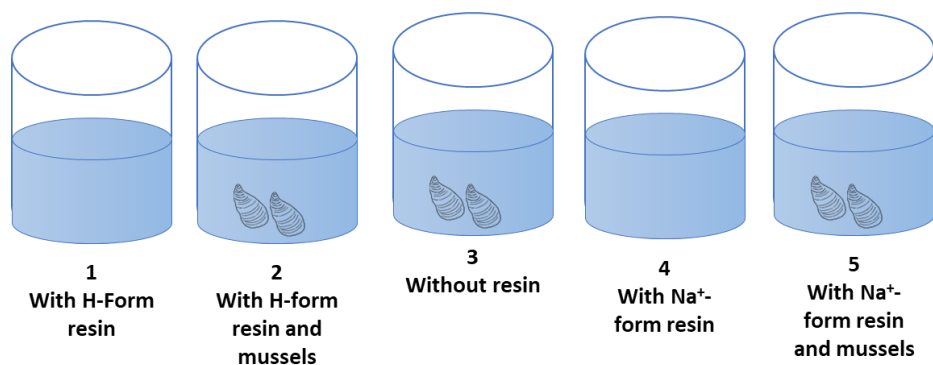


Figure S6 – Experimental design for the study of the pH-effect in in vivo experiments.