1st International Electronic Conference on Toxins (IECT2021).

Contamination status of lipophilic marine toxins in commercial shellfish from Spain, Chile and South East Pacific.

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www.alertox-net.eu

ALERTOXNET

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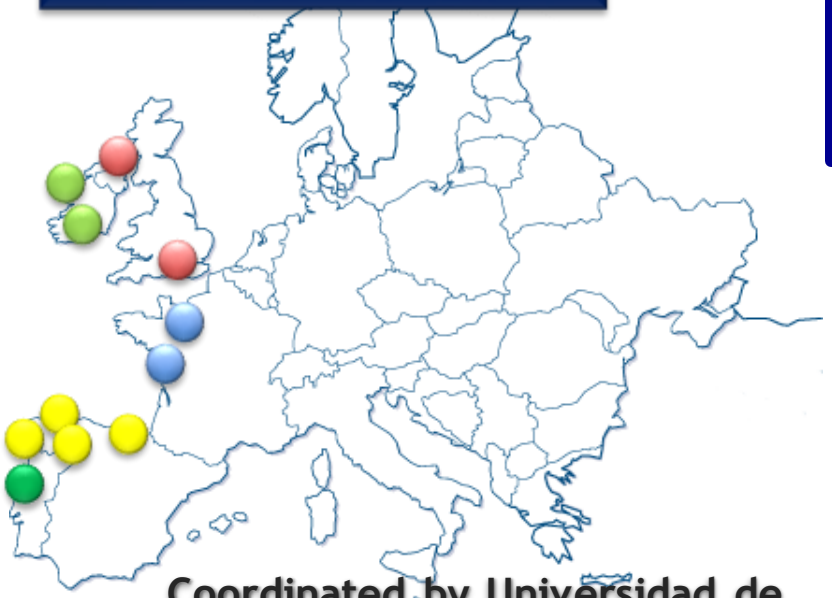
(2017-2020)

ATLANTIC AREA NETWORK FOR INNOVATIVE TOXICITY ALERT SYSTEMS FOR SAFER SEAFOOD PRODUCTS

Partnership



Coordinated by Universidad de Santiago de Compostela (USC)
(Prof. Luis Botana)

Toxicity Alert Systems
Emerging marine toxins

International Institutions:

Marine Toxins

Light Salinity
Nutriets

Natural metabolites

Toxic compounds

Structure
Mecanism of action
Biological activity

CLASIFICATION

- SOLUBILITY
  - LIPOPHILIC
  - HIDROPHILIC
  - AMPHIPHILIC

TOXIN GROUPS

OA | PTXs | AZAs | YTXs | BTXs | CTXs | CIs | STXs | DA | TTXs | MTXs | PlTXs

- OA: Diarrheic: DSP
- STXs: PSP: Paralitic
- DA: ASP: Amnesic

Group of Lipophilic Marine Toxins

LEGISLATED LIPOPHILIC TOXINS

MAXIMUN LEVELS IN SHELLFISH FOR HUMAN CONSUMPTION

Okadaic acid (OA) and dinophysistoxins (DTXs)	OA, DTX-1, DTX-2, DTX-3	**160 µg OA** eq/kg
Pectenotoxins (PTXs)	PTX-1, PTX-2	
Azaspiracids (AZAs)	AZA-1, AZA-2, AZA-3	**160 µg AZA-1** eq/kg
Yessotoxins (YTXs)	YTX, 45-OH-YTX, homoYTX, 45-homoYTX	**3.75** mg **YTX** eq/kg

LC-MS/MS reference method (2015)

EMERGING TOXINS

Cyclic imines (CI) → Spirolides (SPXs) Pinnatoxins (PnTXs) → **No oficial methods or regulatory limits** → **Included in the LC-MS/MS multitoxin methods for regulated toxins**

OBJECTIVE

To study the lipophilic toxin profile including emerging toxins in commercial molluscs in 3 locations (Galicia, Chile and South East Pacific) in order to establish a potential risk when ingested.

METHODOLOGY

ANALYSIS OF LIPOPHYLIC MARINE TOXINS IN GALICIAN COMMERCIAL MUSSELS

53 shellfish samples from 3 locations (Galicia, Chile and SouthEast Pacific).

Dec 2018 to Dec 2019

41 samples fresh mussels *Mytilus galloprovincialis:* Galicia (North West of Spain).

6 samples frozen mussels *Mytilus chilensis:* Chile.

6 samples frozen clams *Tawera gayi* and *Meretrix lyrata:* South East Pacific.

All molluscs were purchased in local markets in Lugo (Spain).

METHODOLOGY

EU-Harmonised Standard Operating Procedure **(SOP) Lipophilic marine biotoxins** in mollusk **by LC-MS/MS** *(version 5)*

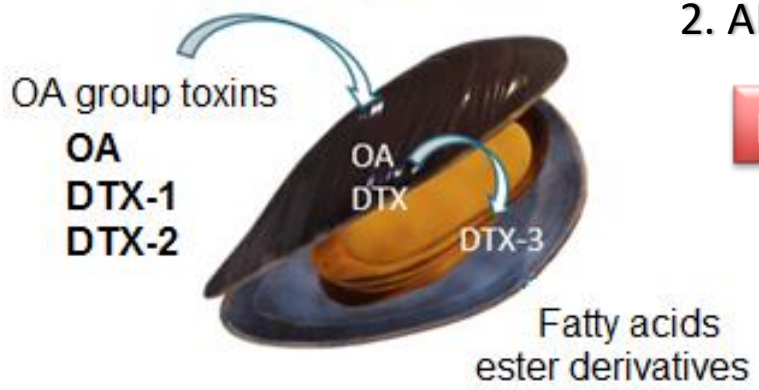
SAMPLE PREPARATION

1. LIPOPHILIC TOXIN EXTRACTION

2 g of **mussel homogenate** → **2 extractions with 100% MeOH**

Metanolic supernatants → **Aliquot** → **LC-MS/MS** → OA (free) PTXs, AZAs, YTXs

Transformation



2. ALKALINE HYDROLYSIS (Total contect of OA group toxins):

NaOH → 40 min, 76 °C → HCl

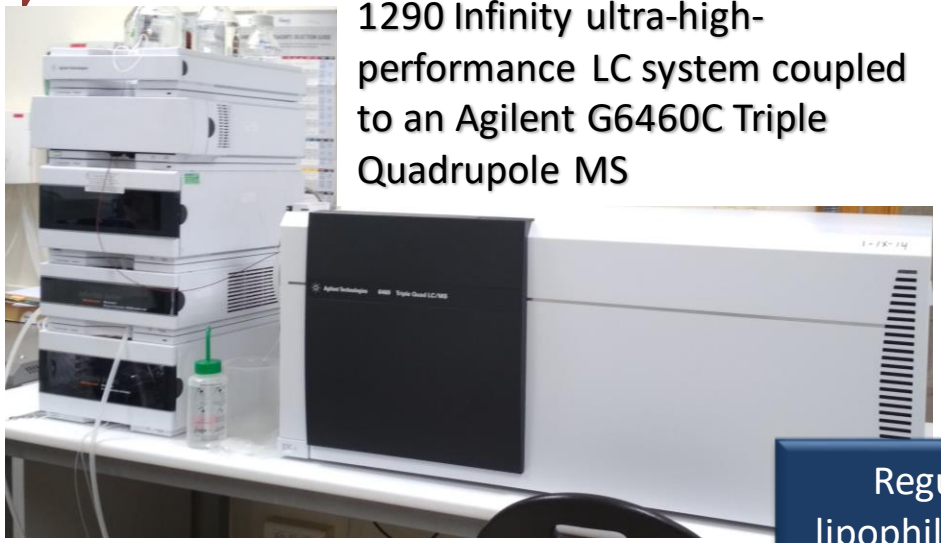
- Acylated esters of OA and/or DTXs
- OA and/or DTX-1 or DTX-2 toxins

Esterified OA group toxins

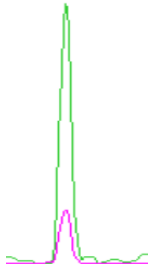
METHODOLOGY

3. ANALYSIS BY LC-MS/MS

MS detection: Multiple Reaction Monitoring (MRM) mode



1290 Infinity ultra-high-performance LC system coupled to an Agilent G6460C Triple Quadrupole MS



2 transitions compound

Regulated lipophilic marine toxins

LC separation:

Column C18 (2.7 µm, 3.0 x 150 mm)
Mobile phase:
Water
ACN
Formic acid 50 mM + Ammonium formiate 2 mM
Flow: 0.4 mL/min
Injection volumen: 5 µL

Emerging toxins CIs

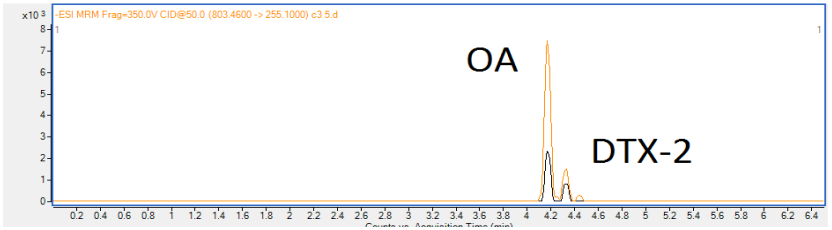
SPXs /PnTXs

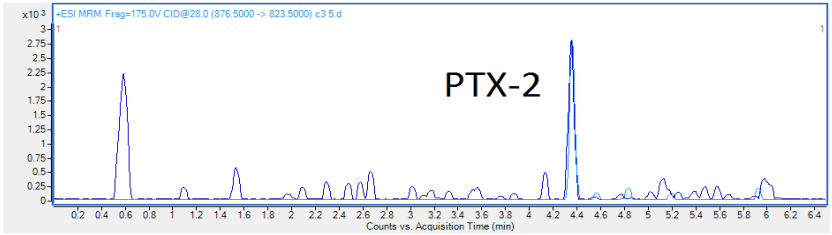
+ frequent emerging toxins in Europe

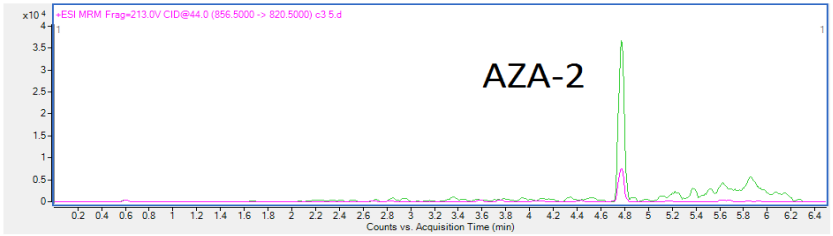
Toxins	Precursor Ion	Product Ion	Frag	CE	CAV	Polarity
45-OH-homo-YTX	1171.5	1091.5 869.5	250	40 88	4	Negative
45-OH-YTX	1157.5	1077.5 871.5	240	38 86	4	Negative
Homo-YTX	1155.48	1075.5 869.4	250	40 88	4	Negative
YTX	1141.47	1061.5 855.4	240	38 86	4	Negative
PTX-1	892.5	821.5 213.2	175	28 44	2	Positive
PTX-2	876.5	823.5 213.2	175	28 44	2	Positive
AZA-1	842.5	824.5 806.5	206	32 44	2	Positive
AZA-2	856.5	838.5 820.5	213	36 44	4	Positive
AZA-3	828.5	810.5 792.5	216	32 44	2	Positive
OA/DTX-2	803.46	113.2 255.1	350	66 50	7	Negative
DTX-1	817.5	255.1 113	350	54 70	7	Negative
SPX-13	692.45	674.4 164.1	180	42 54	4	Positive
SPX-13,19	678.44	660.4 164.1	149	30 54	4	Positive
SPX-20G	706.47	688.4 164.1	152	30 54	4	Positive
PnTX-G	694.47	458.3 164.1	149	30 54	4	Positive
PnTX-E	784.5	446.3 164.1	149	30 54	4	Positive
PnTX-D	782.48	446.3 164.1	149	30 54	4	Positive
PnTX-F	766.5	446.3 164.1	149	30 54	4	Positive
PnTX-B and C	741.47	458.3 164.1	149	30 54	4	Positive
PnTX A	712.44	458.3 164.1	149	30 54	4	Positive

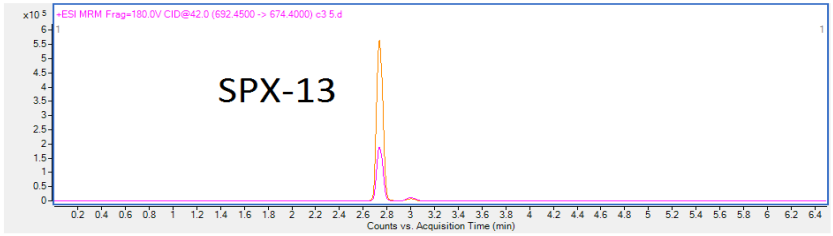
RESULTS

Toxin identification: Samples from Galicia (NorthWest Spain)

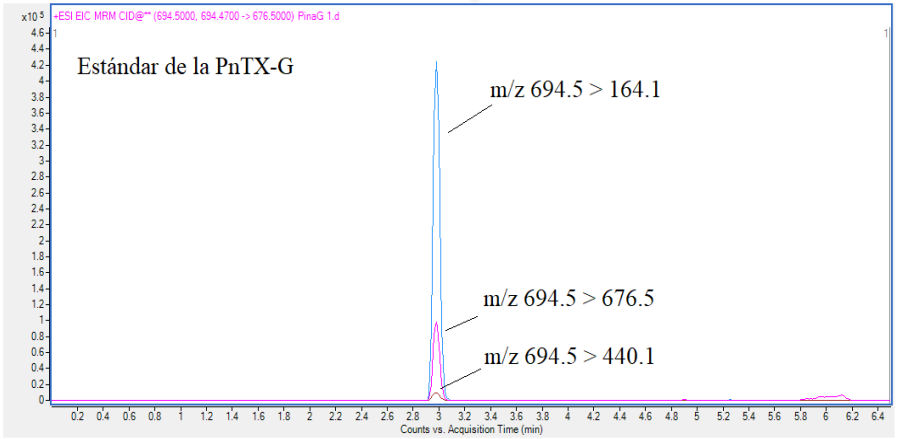


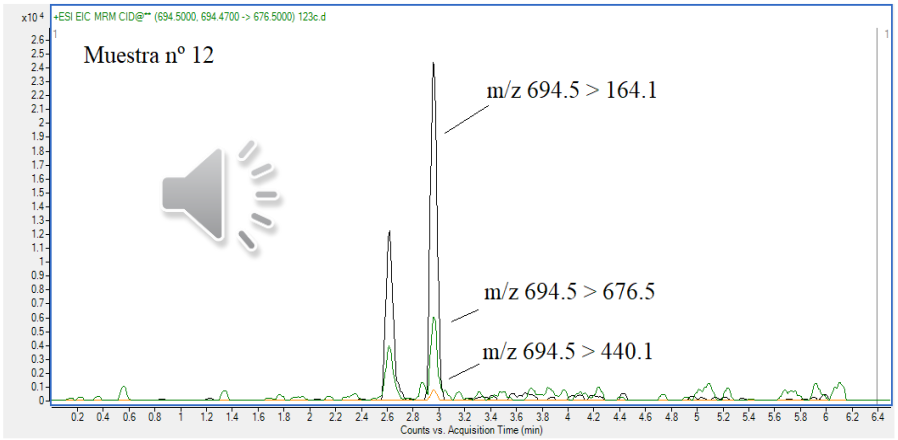






PnTX-G in Galician comercial mussels





Dynophisis spp. *Azadinium spp.* *Alexandrium spp.*

Vulcanodinium rugosum

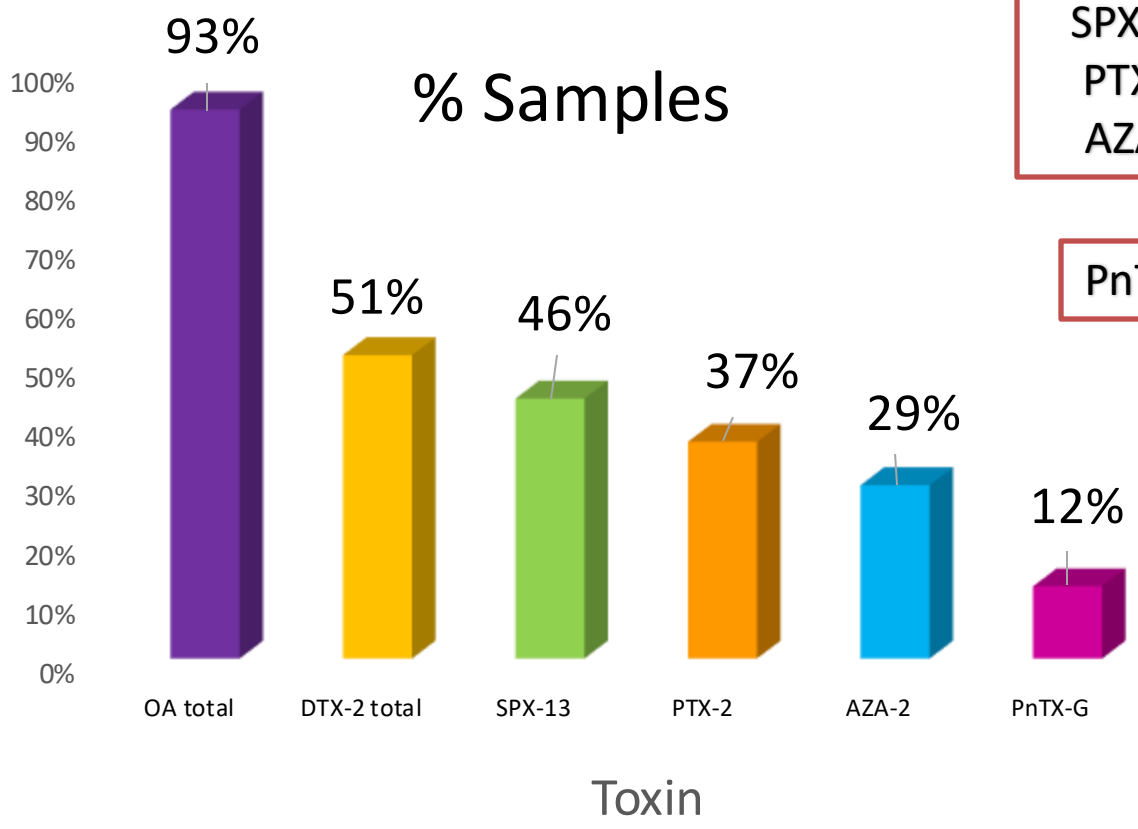
RESULTS

Toxin levels: Samples from Galia (NorthWest Spain)

OA detected in 38 samples (93%)
DTX-2 detected in 21 samples (51%)

SPX-13: 18 samples (46%)
PTX-2: 15 samples (37%)
AZA-2: 12 samples (29%)

PnTX-G in 5 samples (12%)



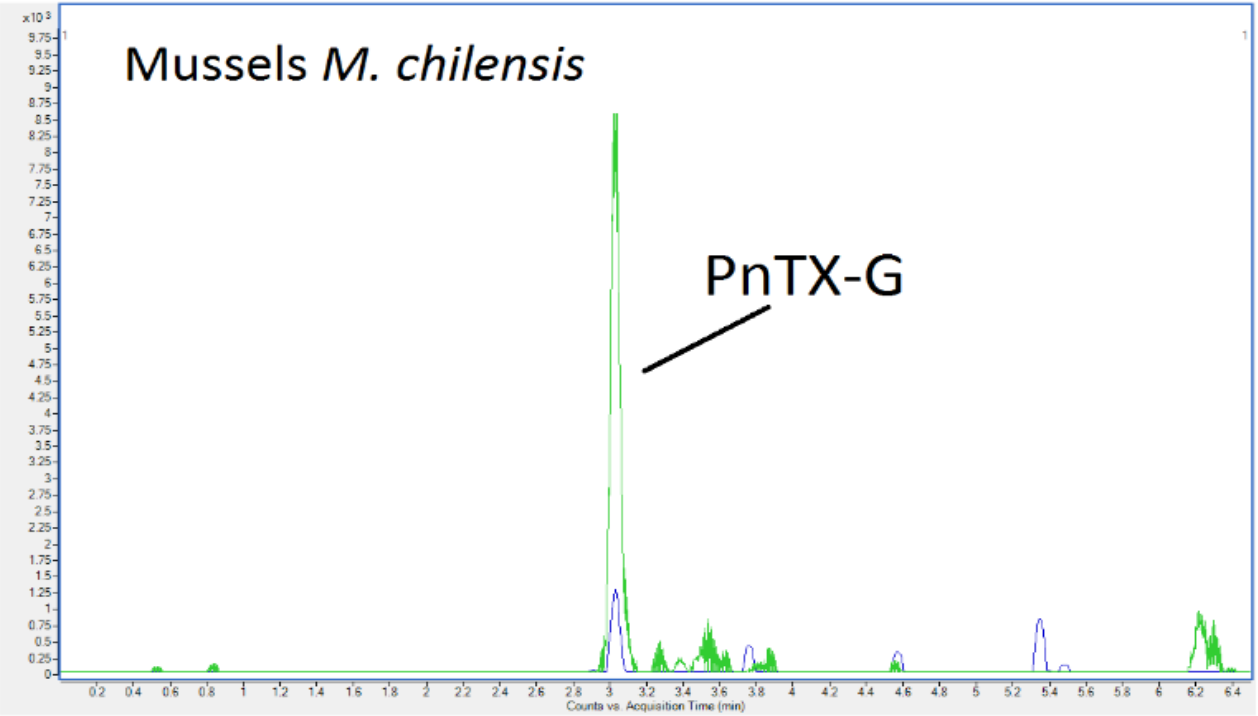
RESULTS

Toxin levels: Samples from Chile (NorthWest Spain)

Emerging PnTX-G in all samples

Levels up to 5.2 µg/kg.

First PnTX-G record in *M. chilensis*.

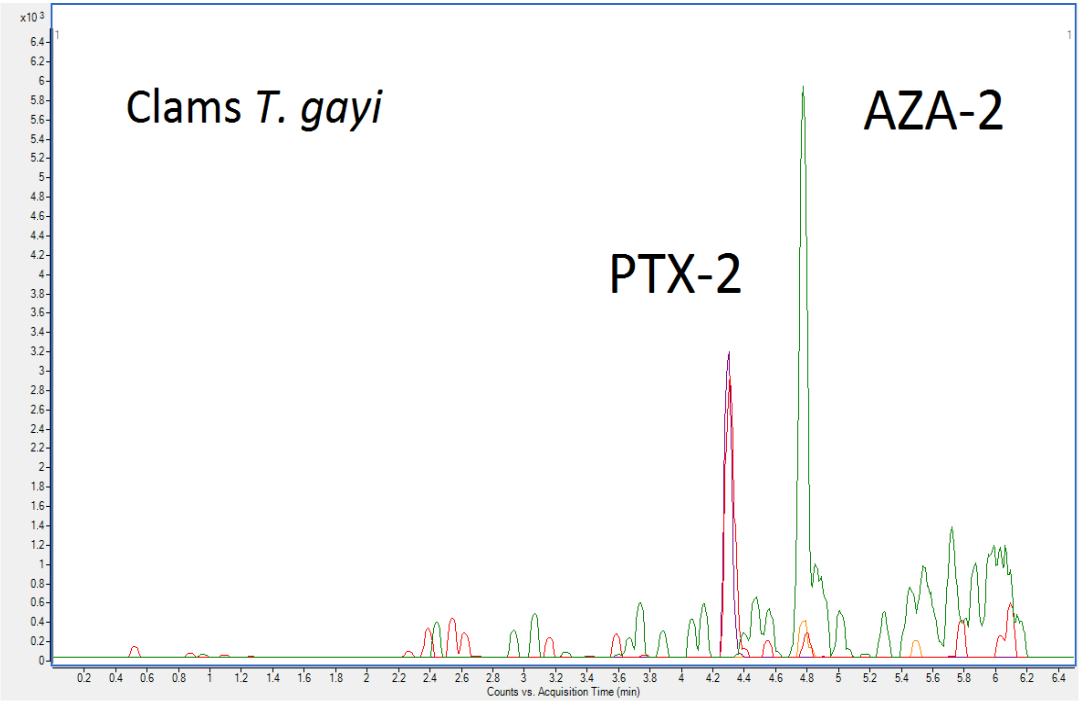


Chromatogram of PnTX-G from a mussel sample *Mytilus chilensis* from market.

RESULTS

Toxin levels: Samples from South East Pacific

Tawera gayi: AZA-2 and PTX-2 at levels up to 4.33 µg/kg and 10.88 µg/kg, respectively.



Chromatogram of PTX-2 and AZA-2 from *T. gayi* from market.

Meretrix lyrata: No lipophilic toxins were found

CONCLUSIONS

1. OA group toxins continue being the main lipophilic toxins in Galician molluscs. These toxins were detected in the 93% of the samples, followed by SPX-13, detected in the 46% of the samples.

CONCLUSIONS

2. PnTX-G is confirmed in commercial mussels from Chile for the first time. However, the low levels found (<6 µg/kg), means that there is no potential risk through mussel ingestion for the emerging toxin PnTX-G.

3. Although it does not seem to pose a potential risk through mussel consumption for the emerging toxins (SPX-13 and PnTX-G), the presence of new analogs must be considered in the shellfish safety monitoring programmes by LC-MS/MS methods.

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Thank you!!!!!