

# PRELIMINARY RESULTS OF RISK CHARACTERIZATION OF CIGUATERA FOOD POISONING IN EUROPE GP/EFSA/AFSCO/2015/03



L. Cebadera-Miranda<sup>1</sup>, A. Canals<sup>\*1</sup>, F. Melo<sup>2</sup>, E.V. Martínez<sup>3</sup>, D. Núñez<sup>3</sup>, C. Varela<sup>\*3</sup>, F. Martín<sup>4</sup>, M. Friedemann<sup>5</sup>, M. Oleastro<sup>6</sup>, I. Bozariis<sup>7</sup>, F. Real<sup>8</sup>, J.A. Sánchez<sup>8</sup>, N. García<sup>8</sup>, P. Reis-Costa<sup>9</sup>, N. Gouveia<sup>10</sup>, G. Papageorgiou<sup>11</sup>, A. Aligizaki<sup>12</sup>, J. Diogène<sup>\*13</sup>, D. Castro<sup>14</sup>, A. Pequeño<sup>14</sup>, P. Estevez<sup>14</sup>, O. Vilarino<sup>14</sup>, J.M. Leao<sup>14</sup>, A. Gago-Martínez<sup>\*14</sup>

<sup>1</sup>Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN); <sup>2</sup>Portuguese Authority for Food and Economic Safety (ASAE); <sup>3</sup>Institute of Health Carlos III (ISCIII); <sup>4</sup>Canary Health Service (CHS); <sup>5</sup>German Federal Institute for Risk Assessment (BfR); <sup>6</sup>Instituto Nacional de Saúde Doutor Ricardo Jorge, I.P. (INSA); <sup>7</sup>University of Thessaly; <sup>8</sup>University of las Palmas de Gran Canaria (ULPGC); <sup>9</sup>Instituto Português do Mar e da Atmosfera (IPMA); <sup>10</sup>Fisheries Department (FD), Direcção Regional das Pescas de Madeira; <sup>11</sup>General State Laboratory (GSL); Ministry of Health of Cyprus; <sup>12</sup>Aristotle University of Thessaloniki; <sup>13</sup>Institute for Research and Technology in Food and Agriculture (IRTA); <sup>14</sup>University of Vigo (UVIGO).

\*Corresponding authors  
ciguatoxinas@mscbs.es

## Background

Ciguatera food poisoning is one of the most common food-borne illnesses related to seafood consumption worldwide, mainly in tropical and subtropical areas. However autochthonous outbreaks have been reported in Spain and Portugal, specifically in Macaronesia area.

## EuroCigua Project

EuroCigua is a project co-funded by the European Food Safety Authority (EFSA) and it is implemented through a Framework Partnership Agreement (FPA). It is composed of four independently managed subprojects named Specific Agreements (SA). SA1: AECOSAN & ASAE; SA2: ISCIII, CHS, BfR and INSA; SA3: IRTA, UPLGC, U. Thessaly, IPMA, FD, GSL, U. Thessaloniki and finally SA4: UVIGO & IFREMER. EuroCigua started on June 1<sup>st</sup> 2016 and has a foreseen length of four years.

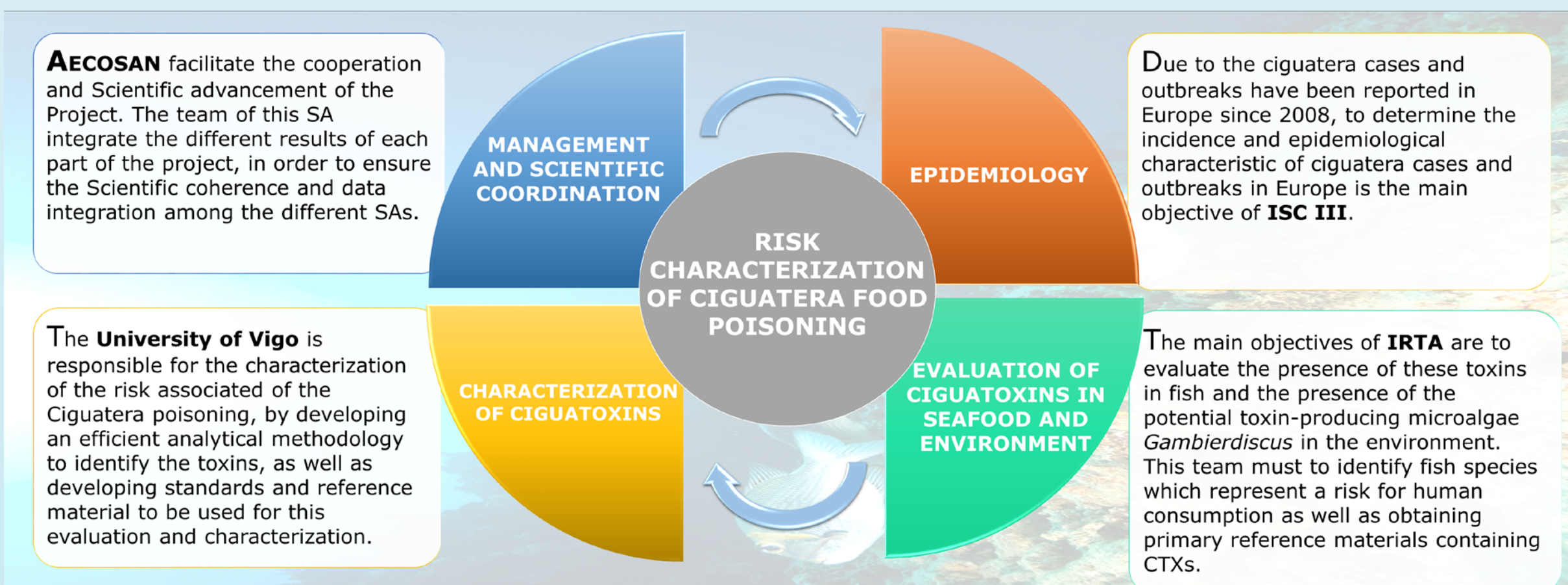


Figure 1. Organization and specific objectives of EuroCigua project.

## Objectives

The EuroCigua project focuses on characterizing the risk of ciguatera food poisoning in Europe. The main objectives are: to estimate the incidence of ciguatera in Europe; to assess the presence of ciguatoxin in food; and to develop and validate methods for the determination of ciguatoxin contaminated specimens. In this poster, an update of results is presented.

## Methods & Results

### SA No. 2: Epidemiology

An epidemiological surveillance protocol for ciguatera in the EU has been developed by SA2.

Year	Reporting country	No. of Cases	No. Hospitalized	Type of fish	Origin of fish	CTX detection
2012	Germany	23	4	<i>Lutjanus bohar</i> <i>L. argentimaculatus</i>	India	Yes
2012	Portugal	12	12	<i>Seriola</i> sp. <i>Lachnolaimus maximus</i>	Selvagem Island	Unknown
2012	Spain	10	0	<i>Seriola</i> sp.	Canary Islands	No
2012	Spain	9	0	<i>Seriola</i> sp.	Canary Islands	No
2012	Spain	4	0	<i>Seriola</i> sp.	Canary Islands	No
2012	Spain	12	0	<i>Epinephelus</i> sp.	Canary Islands	Yes
2013	Spain	16	0	<i>Epinephelus</i> sp.	Canary Islands	Yes
2014	Germany	6	1	<i>Lutjanus bohar</i> <i>L. erythropterus Pinjalo pinjalo</i>	Indonesia	Yes
2015	Portugal	7	4	<i>Epinephelus marginatus</i>	Selvagens Island	Unknown
2015	Spain	3	0	<i>Mycteroperca fusca</i>	Canary Islands	No
2015	Spain	2	0	<i>Pamatomus saltatrix</i>	Canary Islands	Yes
2015	Spain	3	0	<i>Mycteroperca fusca</i>	Canary Islands	No
2015	Germany	16	2	<i>Lutjanus bohar</i>	India	Yes
2016	Spain	2	0	<i>Epinephelus</i> sp.	Canary Islands	Yes
2016	Spain	3	0	<i>Seriola</i> sp.	Canary Islands	Yes
2016	Spain	2	0	<i>Pagrus pagrus</i>	Selvagens Island	Yes
2016	Germany	4	2	<i>Lutjanus</i> sp.	India	Yes
2017	Spain	2	0	<i>Epinephelus</i> sp. <i>Mycteroperca fusca</i>	Canary Islands	Yes
2017	Germany	15	2	<i>Lutjanus bohar</i>	Vietnam	Yes

Table 1. Epidemiological data of ciguatera outbreaks reported in Europe since 2012 until 2017

### SA No. 3:

Sampling of microalgae for the isolation of ciguatoxin (CTX) producing species of the genera *Gambierdiscus* and *Fukuyoa* has proceeded in Macaronesia (Canary Islands and Madeira) and in the Mediterranean area (Cyprus, Crete and Balearic Islands).

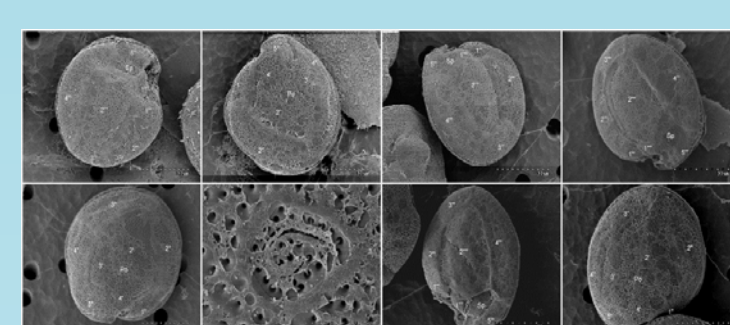


Figure 2. *Gambierdiscus australes* from Menorca, Balearic Island

After taxonomic and morphological evaluation and molecular genetics, *Gambierdiscus* species was found in all islands. Environmental data were recorded and are currently under analysis.

Species	Latin name	No. Samples	Weight (Kg)	CTX-like toxicity (level)
Amberjack	<i>Seriola</i> spp.	10	21.00 - 70.00	Low / Medium-Low / Medium / High
Black moray	<i>Muraena augusti</i>	4	0.40 - 1.03	Medium-Low / Low
Brown moray	<i>Gymnothorax unicolor</i>	1	2.72	High
Common two-banded seabream	<i>Diplodus vulgaris</i>	1	0.32	Medium
Dusky grouper	<i>Epinephelus marginatus</i> spp.	3	6.00 - 29.00	Medium-low / High
Fangtooth moray	<i>Enchelycore anatina</i>	1	1.44	Medium
Island-grouper	<i>Mycteroperca fusca</i>	4	2.50 - 8.00	Low / High
Macaronesian sharpnose-puffers	<i>Canthigaster capistratas</i>	1	0.02 - 0.03	High
Mediterranean moray	<i>Muraena helena</i>	1	0.82	Medium-low
Parrotfish	<i>Sparisoma cretense</i>	4	0.37 - 0.48	Low / Medium / High
Redporgy	<i>Pagrus pagrus</i>	1	4.00	High
Wahoo	<i>Acanthocybium solandri</i>	1	14.00	Low
White trevally	<i>Pseudocaranx dentex</i>	1	0.23	Low
Zebra seabream	<i>Diplodus cervinus cervinus</i>	1	0.68	Medium

Table 2. Positive CTX samples from the Canary Islands (May, 2016 - July, 2018). Bold green correspond to fish species with the higher number of positive CTX samples. Green shaded lines correspond to samples related to ciguatera cases & outbreaks.

In the Canary Islands, a total of 349 fish samples were analyzed for CTX detection by Neuroblastoma-2a (N2a) Assay, and 9.7 % (34 samples) were positive. The higher number of positive samples was collected during the warm season mainly in Tenerife and El Hierro (23% and 47% respectively). Among positive samples, 35% presented low levels of CTX-like toxicity.

CTX-Like TOXICITY	IC50 (mg Eq./ml)	IC80 (mg Eq./ml)
High	<20	
Medium-High	20-40	<80
Medium	20-40	>80
Medium-Low	40-80	
Low	>80	

Table 3. Toxicity ranking according to the tissue equivalents of fish muscle causing 50 or 80 % mortality in cells.

### SA No. 4:

In fish samples that tested positive by N2a Assay from the Canary Islands, Madeira and Selvagens, CTXs were confirmed using LC-MS/MS.

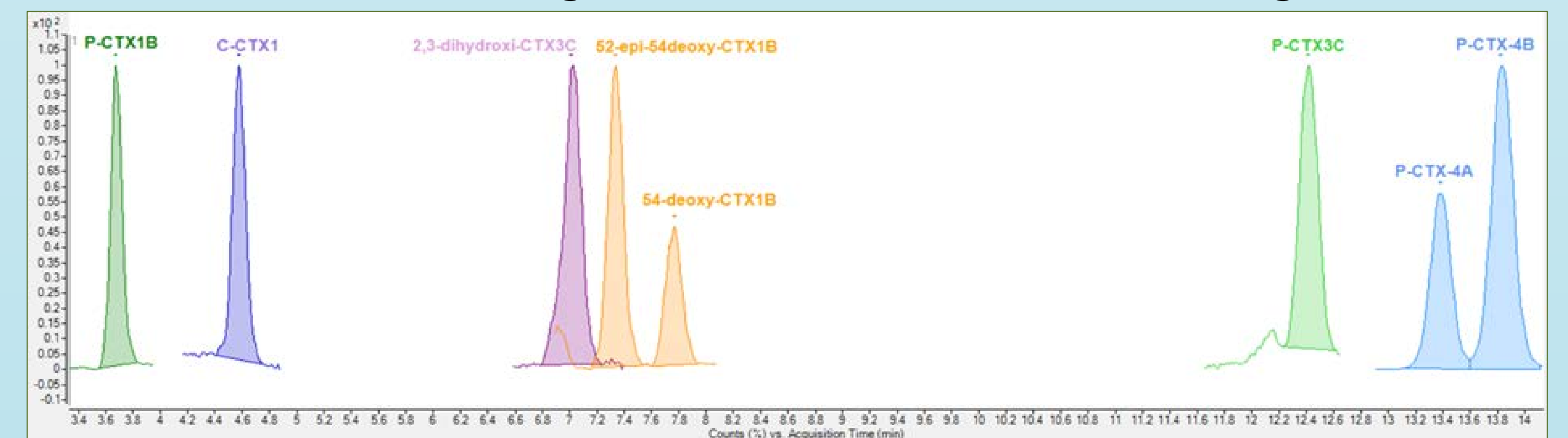


Figure 3. Standard solution used for setting up and optimization of the LC-MS/MS method.

A pure standard solution was used for setting up and optimization of the LC-MS/MS method using  $[M+Na]^+$  as precursor and product ion. This standard solution consisted on a mix of Pacific CTXs.

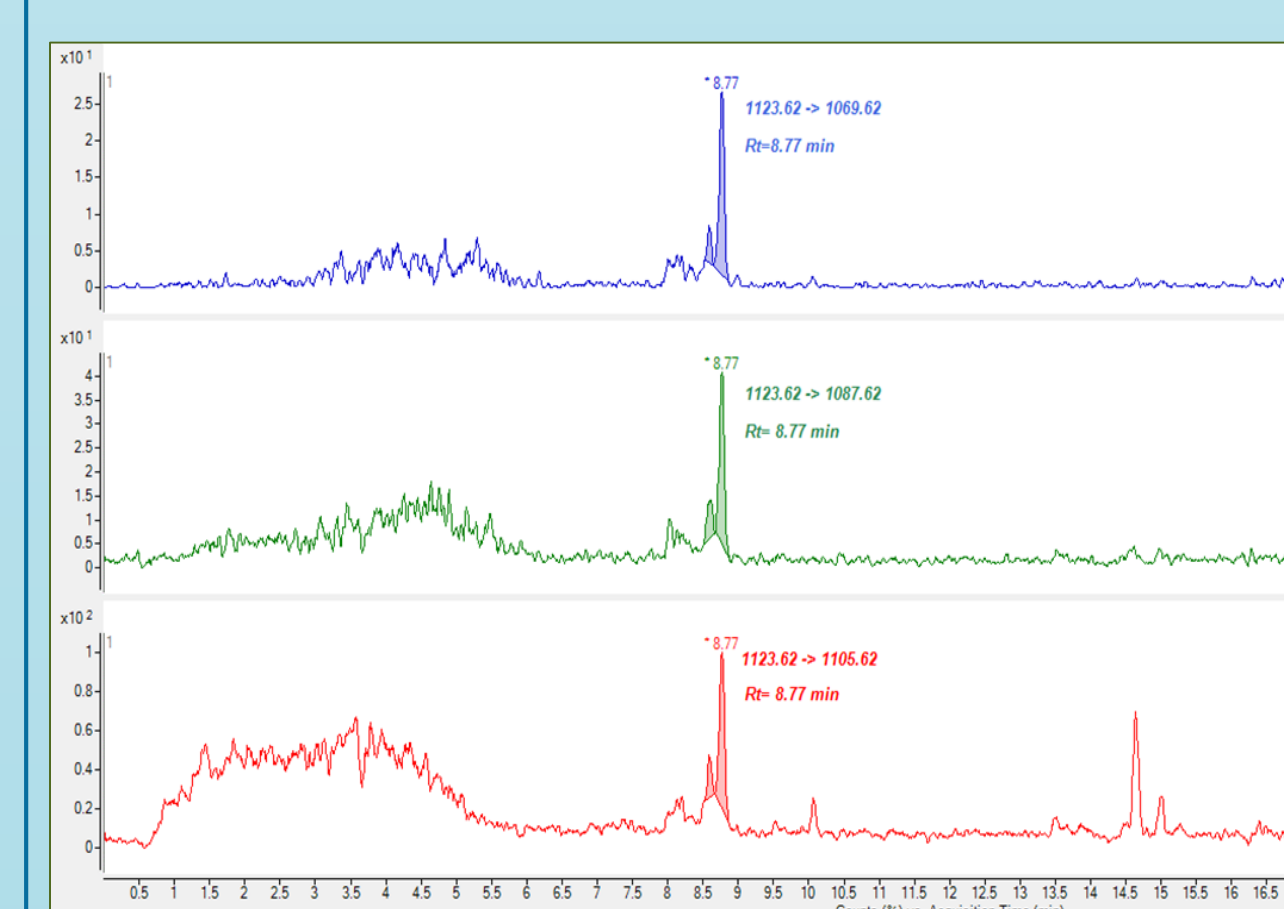


Figure 4. CTX profile of a contaminated fish from Selvagens, containing C-CTX1

The Multiple Reaction Monitoring (MRM) transitions selecting  $[M+H-nH_2O]^+$  were also used for the analysis of CTXs in contaminated fish samples from Canary Islands, Madeira and Selvagens Islands. A similar CTX profile was observed for the contaminated samples. Other CTX analogues, including C-CTX1 isomers, might be also present (confirmatory experiments are part of the undergoing research).

Additional MRM transitions using C-CTX1 standard and selecting fragments as  $[M+H-nH_2O]^+$  were also used for confirmation.

## Conclusions

From 2012 to 2017, a total of 151 cases from 19 ciguatera outbreaks have been notified in Spain, Portugal and Germany. The collection of epidemiological data from other European countries is still ongoing. *Gambierdiscus* and/or *Fukuyoa* species have been identified in Macaronesian Islands. *Gambierdiscus* was also identified in Balearic Island for the first time. The N2a Assay was standardized and implemented on fish samples. Among 349 fish samples from the Canary Islands, 9.7% were CTX-like positive. Optimization of LC-MS/MS method for the confirmation of the CTX toxicity was performed. C-CTX1 is the main responsible for the CTX contamination of fish from Canary Islands and Madeira.