



EuroCigua



**RISK CHARACTERIZATION OF CIGUATERA FOOD
POISONING IN EUROPE
(GP/EFSA/AFSCO/2015/03)**

EuroCigua

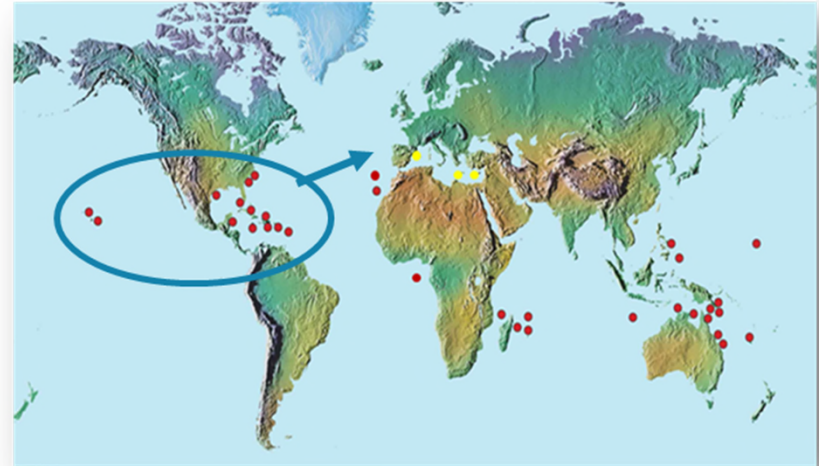
**79th MEETING OF THE EFSA
ADVISORY FORUM
3-4 March 2021**

***Ana Canals Caballero
Scientific Advisor AESAN***



Ciguatera in Europe

- In 2004 *Gambierdiscus* spp., in the waters of the Canary Islands and Madeira. First autochthonous ciguatera outbreak in Canary Islands
- 2008: autochthonous ciguatera outbreaks in Spain (Canary Islands) and in Portugal (Madeira)
- Between 2008 and 2015 continuous outbreaks and cases appeared in Canary Islands



- These new findings suggested CP is becoming an increasing risk for European countries.
- No official reference method
- No certified reference materials
- No toxicology data
- CP not well known in EU: **under-reported**



- EuroCigua co-funded by the European Food Safety Authority (EFSA) **Framework Partnership Agreement (FPA)**, coordinated by **AESAN**
- Cooperation between EFSA and **14 partners from six Member States.**
- **June 2016-January 2021**

Characterize the risk of ciguatera poisoning in Europe

Epidemiology

- Incidence
- Epidemiological characteristics

Environment

- Cell based assay
- CTX producing microalgae
- CTX in fish
- environmental data
- predictive models

Analytical methods

- LC-MS/MS
- reference materials



Epidemiology: Surveillance protocol

- Guidelines for surveillance of ciguatera at EU level from 2012 to 2019
- Case definition consensus
- Case and outbreak report forms
- List of fish previously associated with ciguatera
- Collecting Epidemiological data to implement prevention and control measures



Review

An Updated Review of Ciguatera Fish Poisoning: Clinical, Epidemiological, Environmental, and Public Health Management

Melissa A. Friedman ^{1,*}, Mercedes Fernandez ², Lorraine C. Backer ³, Robert W. Dickey ⁴, Jeffrey Bernstein ^{5,6}, Kathleen Schrank ⁵, Steven Kibler ⁷, Wendy Stephan ⁶, Matthew O. Gribble ⁸, Paul Bienfang ⁹, Robert E. Bowen ¹⁰, Stacey Degrasse ¹¹, Harold A. Flores Quintana ¹², Christopher R. Loeffler ¹², Richard Weisman ⁶, Donna Blythe ¹³, Elisa Berdalet ¹⁴, Ram Ayyar ¹⁵, Danielle Clarkson-Townsend ⁸, Karen Swajian ¹¹, Ronald Benner ¹², Tom Brewer ¹⁶ and Lora E. Fleming ¹⁷



SURVEILLANCE
PROTOCOL FOR
CIGUATERA FOOD
POISONING IN THE
EU

2017



September 29th 2020

Annex. List of fish previously associated with ciguatera

SPECIES	ENGLISH NAMES
Acanthuridae	
<i>Acanthurus achilles</i>	Redblotch surgeonfish
<i>Acanthurus ducunieri</i>	Black-spot surgeonfish
<i>Acanthurus paxillus</i>	Black surgeonfish
<i>Acanthurus lineatus</i>	Whitetail surgeonfish
<i>Acanthurus lineatus</i>	Lined surgeonfish
<i>Acanthurus maculiceps</i>	Earbar surgeonfish
<i>Acanthurus nigricans</i>	Whitehook surgeonfish
<i>Acanthurus nigricans</i>	Brown surgeonfish
<i>Acanthurus nigricans</i>	Bicolor surgeonfish
<i>Acanthurus olivaceus</i>	Orange-spot surgeonfish
<i>Acanthurus zebra</i>	Striated surgeonfish
<i>Acanthurus sandersoni</i>	Yellowfin surgeonfish
<i>Cimnochoerus flavicauda</i>	Whitetail surgeonfish
<i>Cimnochoerus zebra</i>	Striated surgeonfish
<i>Naso brevicaudatus</i>	Humpback unicornfish
<i>Naso brevicaudatus</i>	Spotted unicornfish
<i>Naso hexacanthus</i>	Sleek unicornfish
<i>Naso lituratus</i>	Orange-spine unicornfish
<i>Naso unicornis</i>	Blue-spine unicornfish
Albulidae	
<i>Albulia vulpinus</i>	Bonfish
Balistidae	
<i>Balistapus sandakanus</i>	Orange-lined triggerfish
<i>Balistes caprinus</i>	Gery triggerfish
<i>Balistes vetulus</i>	Queen triggerfish
<i>Balistodes viridicinctus</i>	Tina triggerfish
<i>Canthidermis ruffimanus</i>	Ocean triggerfish
<i>Melicolytes nigrus</i>	Black triggerfish
<i>Melicolytes viduus</i>	Pinkish triggerfish
<i>Pomadochirus fuscus</i>	Yellow-spotted triggerfish



Epidemiology: CP outbreaks/cases in Europe 2012-2019

Autochthonous outbreaks



14 outbreaks

77 cases related



2 outbreaks

19 cases related

Imported and traveler outbreaks



3 imported; 1 traveler
16 cases related



6 imported; 3 traveler
84 cases related



Conclusions

- Information on outbreaks was provided by public health/food safety authorities and the information on single cases was provided by poison centres and travel and tropical medicine units. Information was fragmented.
- The total incidence rate of the ciguatera reported cases in the EU/EEA was very low 0,0055 cases/100.000 inhabitants. The incidence rate in the Canary Islands was 0,47 cases/100.000 inhabitants. No statistically significant trend in the number of reported outbreaks was observed between 2012-2019.
- **PROBABLY UNDERREPORTED**



Conclusions

- Outbreaks due to consumption of fish from the EU/EEA (autochthonous outbreaks) were reported only by Spain (Canary Islands) and Portugal (Madeira)
- Outbreaks due to imported fish were mostly reported by Germany and France. Netherlands reported one outbreak in 2020
- The type of fish most frequently associated with ciguatera in the EU/EEA were groupers and amberjack for autochthonous fish and *Lutjanus* spp. for imported fish from India and Vietnam.

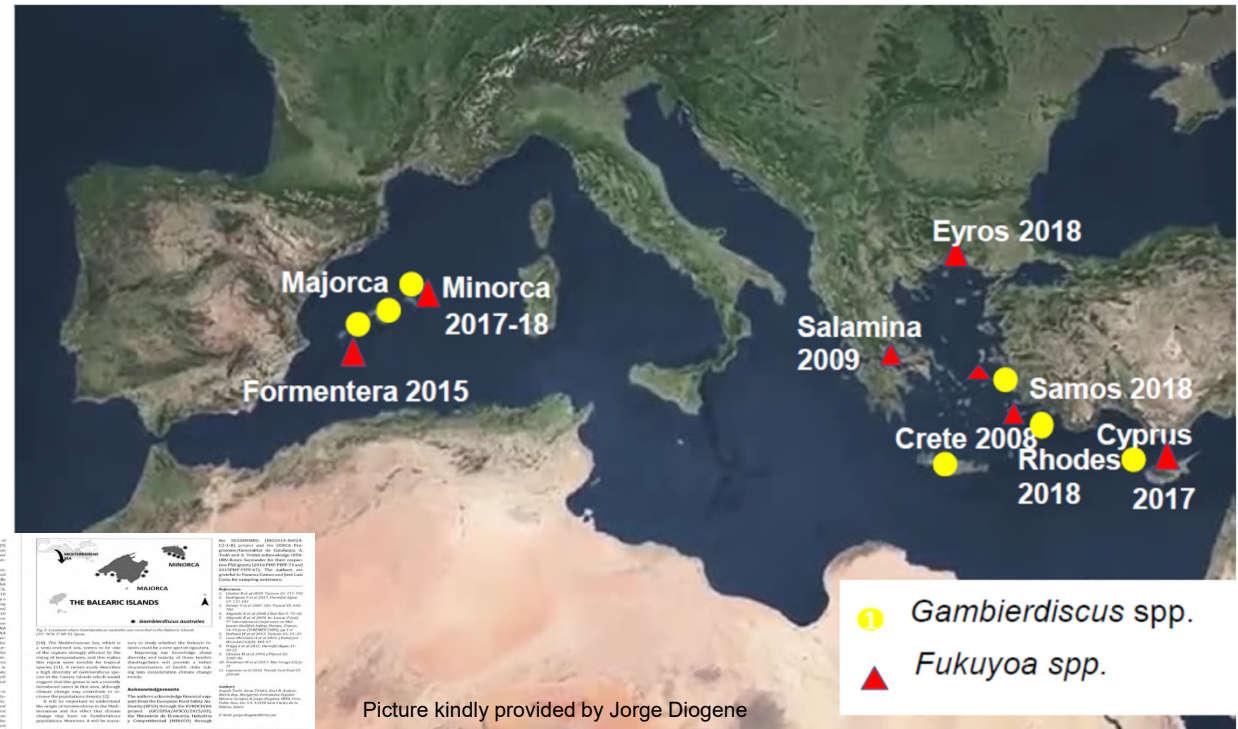


Environment: *Gambierdiscus* and/or *Fukuyoa* in Europe

Identified in:

- Canary Islands
- Madeira & Selvagens
- Crete, Cyprus, Samos and Rhodes
- Balearic Islands

Confirmation of
Gambierdiscus in
Western
Mediterranean



First report of *Gambierdiscus* in the Western Mediterranean Sea (Balearic Islands)

THE BALEARIC ISLANDS

ICBS CIEM

FORTHCOMING EVENTS

THE SEASHELL (SHELL) MEDIA

ICBS CIEM

FORTHCOMING EVENTS

Gambierdiscus spp. Strains (positive/established)

- Canary Islands: **39/93**
- Madeira & Selvagens: **1/86**
- Balearic Islands: **26/113**
- Crete, Cyprus, Samos and Rhodes: **2/195**

CTX-like toxicity(N2a)



Fish sampling in Canary Islands

In The Canary Islands ciguatera was included as mandatory notifiable disease. **Official Control Program** (Fish species selected - specific weight):

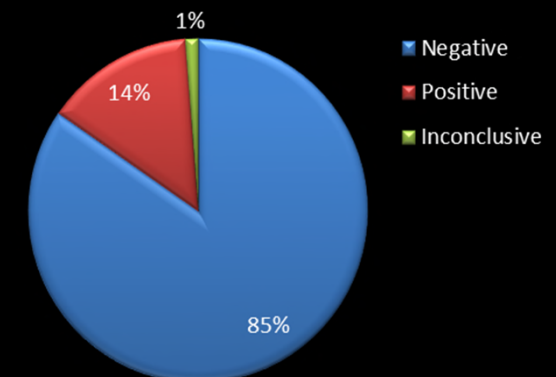
- 2018: 1128 samples (16 % positive samples)
- 2019: 839 samples (12 % positive samples)



865 Fish from Canary Islands (ULPGC):

- 747 EuroCigua sampling – Data analysis
- 118 Official Control (O.C.) – High positive samples selected for Reference materials

EuroCigua sampling (Fish ULPGC)



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CTX in fish_ Canary Islands

SPECIES	Latin name	Total	Negative	Positive	Inconcl.	Positive %	Weigh of positives (Kg)	LC-MS/MS detection
Island-grouper	<i>Mycteroperca fusca</i>	11	5	6		55	2.5-8	Yes
Tuna	<i>Thunnus sp.</i>	1	1			0		
Antoñito	<i>Dentex macrophthalmus</i>	10	10			0		
Axillary seabream	<i>Pagellus acarne</i>	1	1			0		
Yellowmouth barracuda	<i>Sphyraena viridensis</i>	17	17			0		
Blackbelly rosefish	<i>Helicolenus dactylopterus dactylopterus</i>	1	1			0		
Redporgy	<i>Pagrus pagrus</i>	28	26	2		7	1.65-4	Yes
Bogue	<i>Boops boops</i>	16	16			0		
Bonito	<i>Katsuwonus pelamis</i>	1	1			0		
Common pandora	<i>Pagellus erythrinus</i>	8	8			0		
Forkbeard	<i>Phycis phycis</i>	7	7			0		
African striped grunt	<i>Parapristipoma octolineatum</i>	20	19	1		5	0.29	Other CTX?
Mackerel	<i>Scomber colias</i>	27	26		1	0		
Blacktail comber	<i>Serranus atricauda</i>	12	12			0		
Comber	<i>Serranus cabrilla</i>	1	1			0		
Smooth-hound shark	<i>Mustelus mustelus</i>	1	1			0		
Atlantic wreckfish	<i>Polyprion americanus</i>	1	1			0		
Black seabream	<i>Spondyliosoma cantharus</i>	4	4			0		
European conger	<i>Conger conger</i>	4	4			0		
Brown meagre	<i>Sciaena umbra</i>	1	1			0		
Gilthead seabream	<i>Sparus aurata</i>	6	6			0		
Common dolphinfish	<i>Coryphaena sp.</i>	11	11			0		Other CTX?
Damselfish	<i>Chromis limbata</i>	1	1			0		
Alfonsino	<i>Beryx splendens</i>	10	10			0		
Saddled seabream	<i>Oblada melanura</i>	5	5			0		
Macaronesian sharpnose-puffer	<i>Canthigaster capistrata</i>	1		1		100	0.03	Sample N/A
Planehead filefish	<i>Stephanolepis hispidus</i>	2	2			0		
Grey triggerfish	<i>Balistes capriscus</i>	11	11			0		
Ocean triggerfish	<i>Canthidermis sufflamen</i>	1	1			0		
Striped seabream	<i>Lithognathus mormyrus</i>	3	3			0		
White trevally	<i>Pseudocaranx dentex</i>	31	25	6		19	0.23-7	*
Golden grey mullet	<i>Liza aurata</i>	1	1			0		
Seabass	<i>Dicentrarchus labrax</i>	2	2			0		

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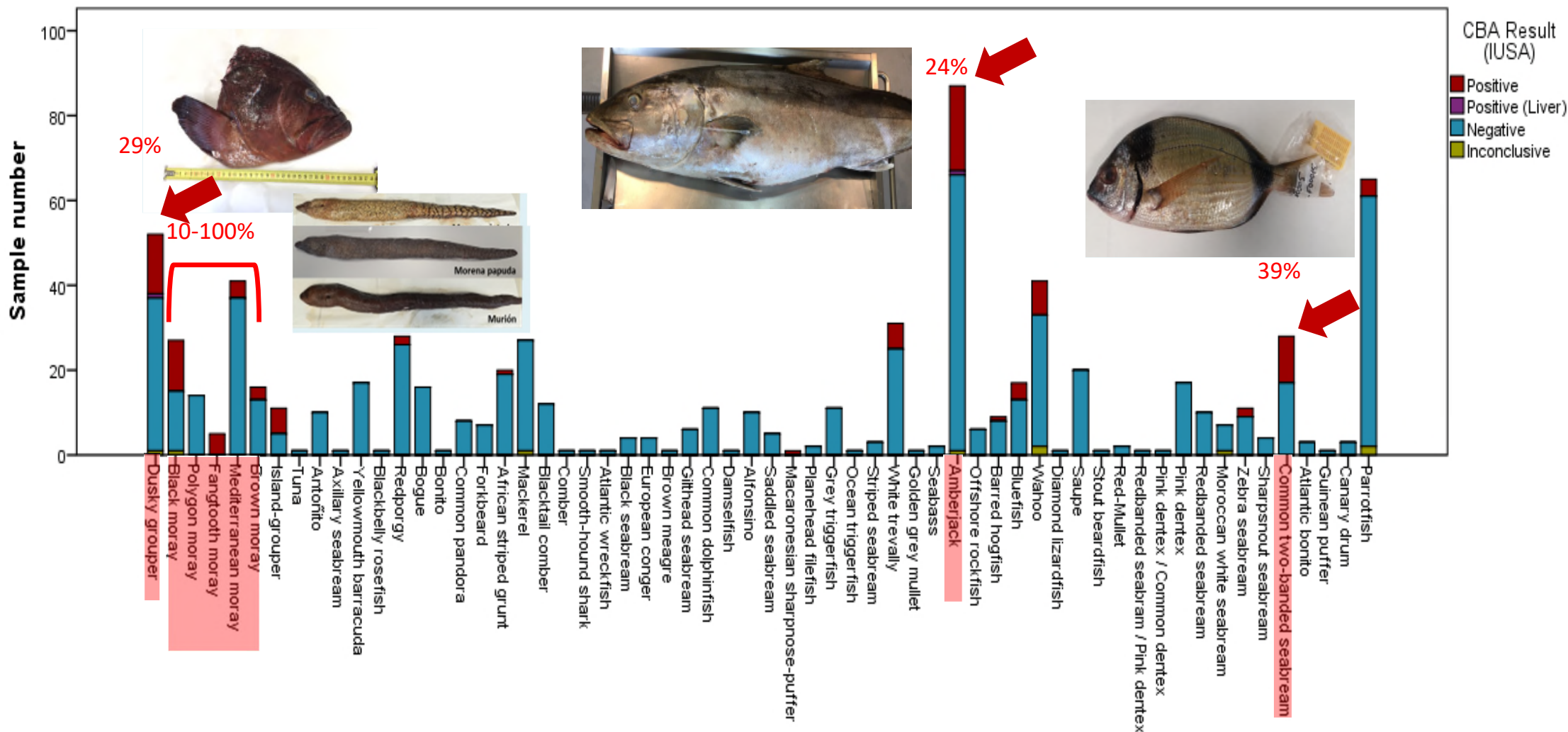
SPECIES	Latin name	Total	Negative	Positive	Inconcl.	Positive %	Weigh of positives (Kg)	LC-MS/MS detection
Amberjack*	<i>Seriola sp.</i>	87	65	21	1	24	20-70	Yes
Dusky grouper*	<i>Epinephelus marginatus*</i>	52	36	15	1	29	6-30	Yes
Black moray*	<i>Muraena augusti*</i>	27	14	12	1	44	0.40-2.81	Yes
Polygon moray	<i>Gymnothorax polygonius</i>	14	14			0		
Fangtooth moray	<i>Enchelycore anatina</i>	5		5		100	0.36-1.44	Yes
Mediterranean moray	<i>Muraena helena</i>	41	37	4		10	1.05-5	Yes
Brown moray	<i>Gymnothorax unicolor</i>	16	13	3		19	0.51-2.72	Yes
Offshore rockfish	<i>Pontinus kuhlii</i>	6	6			0		
Barred hogfish	<i>Bodianus scrofa</i>	9	8	1		11	1.56	Other CTX?
Bluefish	<i>Pomatomus saltatrix</i>	17	13	4		24	6.10-8.80	Yes
Wahoo	<i>Acanthocybium solandri</i>	41	31	8	2	20	14-30	Yes
Diamond lizardfish	<i>Synodus synodus</i>	1	1			0		
Saupe	<i>Sarpa salpa</i>	20	20			0		
Stout beardfish	<i>Polymixia nobilis</i>	1	1			0		
Red-Mullet	<i>Mullus surmuletus</i>	2	2			0		
Redbanded seabream / Pink dentex	<i>Pagrus auriga / Dentex gibbosus</i>	1	1			0		
Pink dentex / Common dentex	<i>Dentex sp.</i>	1	1			0		
Pink dentex	<i>Dentex gibbosus</i>	17	17			0		
Redbanded seabream	<i>Pagrus auriga</i>	10	10			0		
Moroccan white seabream	<i>Diplodus sargus cadenati</i>	7	6		1	0		
Zebra seabream	<i>Diplodus cervinus cervinus</i>	11	9	2		18	0.69-1.15	Yes
Sharpsnout seabream	<i>Diplodus puntazzo</i>	4	4			0		
Common two-banded seabream	<i>Diplodus vulgaris</i>	28	17	11		39	0.26-0.71	Yes
Atlantic bonito	<i>Sarda sarda</i>	3	3			0		
Guinean puffer	<i>Sphoeroides marmoratus</i>	1	1			0		
Canary drum	<i>Umbrina canariensis</i>	3	3			0		
Parrotfish	<i>Sparisoma cretense</i>	85	59	4	2	6	0.37-0.48	Other CTX?
TOTAL SAMPLES (60 SPECIES STUDIED, 17 CIGUATOXIC)		747	632	106	9	14		

RISK CHARACTERIZATION OF CIGUATERA FOOD POISONING IN EUROPE (GP/EFSA/AFSCO/2015/03) EUROCIGUA PROJECT

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17 toxic species / 60 species studied



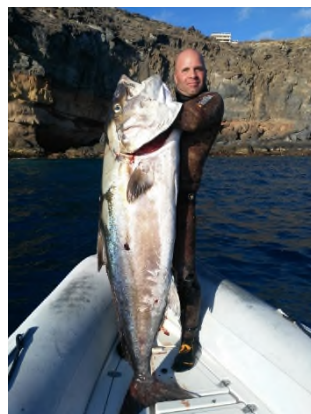
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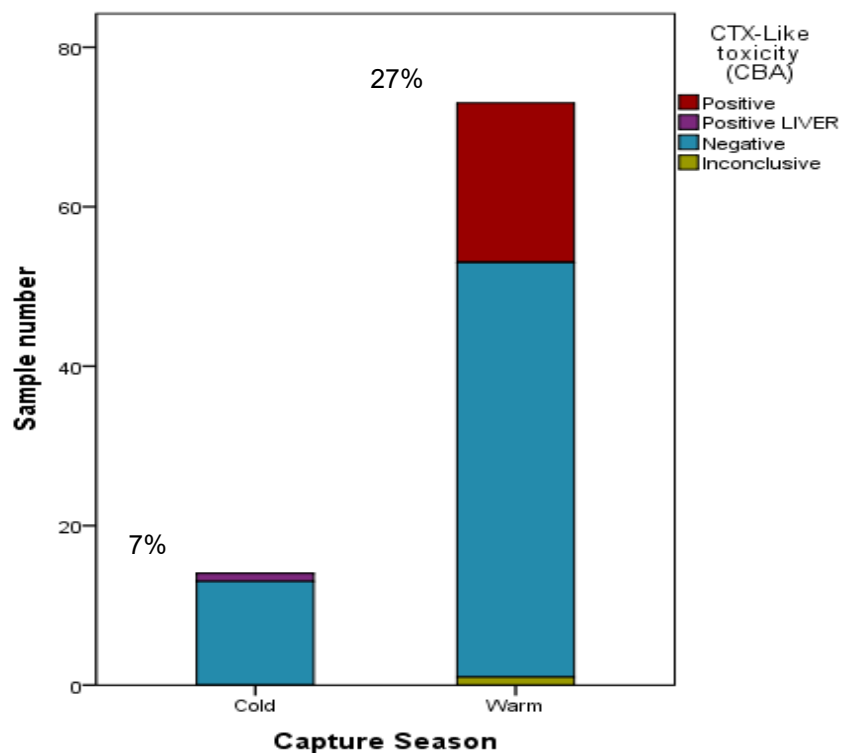


REMARKABLE FISH SPECIES

Amberjack – *Seriola* spp.



Dusky Grouper – *Epinephelus marginatus*



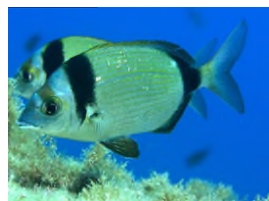
29 % Positive


Involved in CP cases in the Canary Islands, 35 people affected



Common two-banded seabream


– *Diplodus vulgaris*





39% (11/28)

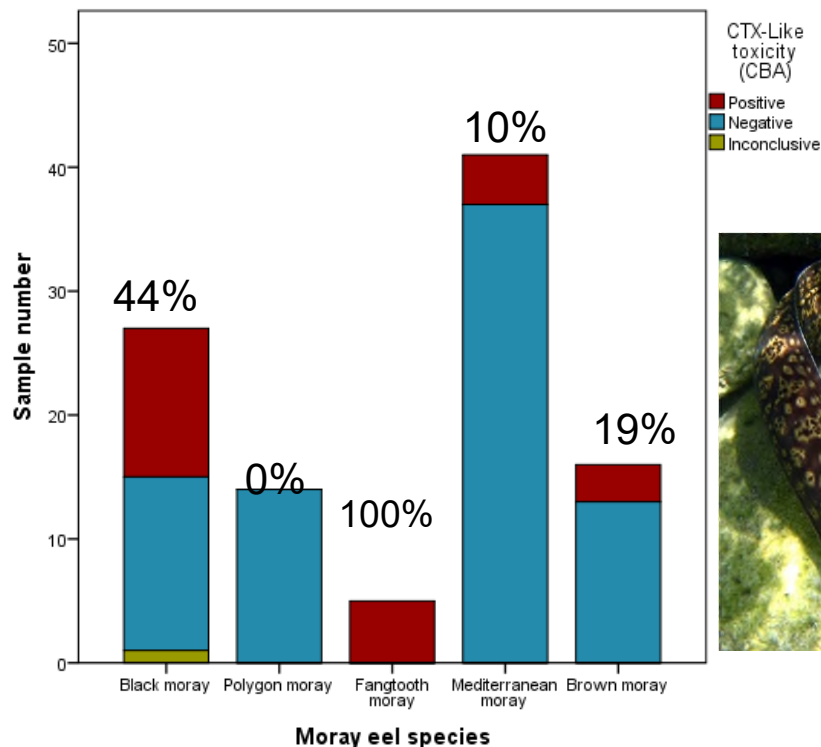
- CTX-like toxicity in *Diplodus vulgaris*
- Low (36%) and medium (64%) toxicity.



64% confirmed (7/11)

- C-CTX in *Diplodus vulgaris*
- 0,02-0,07 ppb
- Neuro-2a cytotoxicity assay Vs. LC-MS / MS:
 - Mixture of analogues not known

Moray eel



Important fish species for human consumption

Fish species	CTX-like toxicity ^a	LC-MS/MS ^b
Dusky grouper (<i>E. marginatus</i>)	0.032 ± 0.009 ppb	0.03 ppb C-CTX1
Black moray (<i>M. augusti</i>)	0.037 ± 0.015 ppb	0.05 ppb C-CTX1

No evidence of ciguatera outbreaks caused by *Diplodus vulgaris* in the Canary Islands but this species is known to be carriers of ciguatoxin (EuroCigua)



El Hierro Island



- High contamination rate:
 - 75% for seabream (*Diplodus vulgaris*) (9/11)
 - 33% for moray eels (5/15)
- Similar results have been reported previously, showing high percentages (90.9%) of CTX in dusky groupers (*Epinephelus marginatus*) (Sánchez-Henao et al., 2019)
- **An algae bloom of *G. caribaeus* occurred in October 2016 in El Hierro** (Soler-Onís et al., 2016)
- These facts considered together with previous results obtained in this island, suggest El Hierro as a ciguatoxin hot spot in the Canary Archipelago.

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CTX in fish_Madeira



	FISH SPECIES	POSITIVE	ALL FISH	Weight (kg)	Length (mm)			
	Epinephelus marginatus	1	1	19,5	970			
	Bodianus scrofa	20	20	0.77-3.01	250-530			
1 fish + by LC-MS and - by N2A	Balistes capriscus	3	7	1.966-2.64	444-500			
	Mictoperca fusca	1	1	4,533	690			
1 fish + by LC-MS and - by N2A	Serranus atricauda	6	10	0.193-0.81	250-380			
	Dentex gibbosus	1	3	8,097	846			
	Seriola dumerili	2	11	20,117-27,6	1232-1432			
	Diplodus cervinus	1	1	2,84	520			
	Sparisoma cretense	6	32	0.424-0.849	280-350			
	Sphyreanea viridensis	2	4	4.139-5.955	1060-1170			
	Seriola rivoliana	1	13	12,308	1060			
	Katsuwonus pelamis	0	9					
	Kyphosus sectator	0	2					
	Makaira nigricans	0	1			LOCATION	POSITIVE	TOTAL
	Kyphosus sectatrix	0	7			Selvagens	41	74
	Pomatus saltatrix	0	2			Madeira	3	48
	Seriola sp.	0	1			Desertas	0	5
	Seriola fasciata	0	2			Total	44	127
	Aluterus scriptus	0	1					
	Total	44	128					



CTX in fish_Macaronesia

- Among 747 samples from the Canary Islands, **14%** were CTX-like positive
- Hotspot *El Hierro*: 34%
- Among 128 samples from Madeira and Selvagens, **34%** were CTX-like positive
- Hotspot Selvagens: 54%





Evaluation of the presence/absence of CTX-like toxicity in fish

	Sampling areas	Tentative Plan	Collected fish	Weight (kg)	CTX-like toxicity (level)
Canary Islands	7	525	747 fish		CTX-like positive: 216 cases, CTX-like negative (negative): 632 cases CTX-like dubious: 17 cases
Madeira and Selvagens	3	100	128 fish (131 extracts)	0,2 – 300	CTX-like positive: 44 cases CTX-like negative (negative): 87 cases CTX-like dubious: 0 cases
Cyprus	2	70	75 (145 extracts)	0,3 – 30	CTX-like positive: 1 cases CTX-like negative (negative): 144 cases CTX-like dubious: 0 cases
Crete	2	70	70 (140 extracts)	0,6 - 12,2	CTX-like positive: 0 cases CTX-like negative (negative): 140 cases CTX-like dubious: 0 cases
Balearic Islands	2	40	36 (70 extracts)	0,7 - 3	CTX-like positive: 0 cases CTX-like negative (negative): 70 cases CTX-like dubious: 0 cases
TOTAL	16	805	1174		



Conclusions

- Several dinoflagellates: *Gambierdiscus* spp. and *Fukuyoa* spp. as the causative organisms were identified in the Macaronesia area (Canary Islands and Madeira), Crete, Cyprus, and Balearic Islands.
- Cytotoxicity assays showed CTX-like toxicity in *Gambierdiscus* spp strains from the Canary Islands, Crete and the Balearic Islands.
- Evaluation for the toxicity in fish shows CTX-like toxicity (Cell-based assay) in fish from Madeira and the Canary Islands, and no toxicity in other fish from the Mediterranean Sea including Balearic Islands

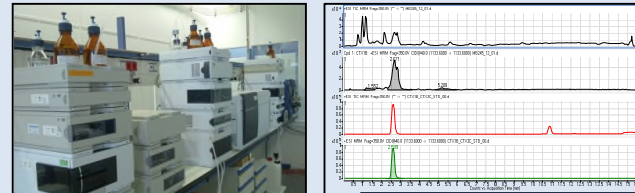


Analytical methods: CTX characterisation

SOP for LC-MS determination of CTXs



Confirmación of CTXs by LC-MS/MS and HRMS and CTX profile characterization



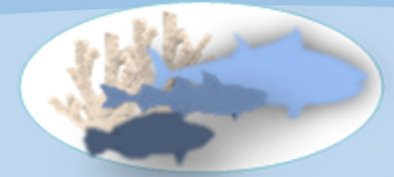
- LC-MS/MS – SOP
- C-CTX1 identified
- LC-HRMS
- CTX profile characterization
- Reference materials

Preparation of reference materials

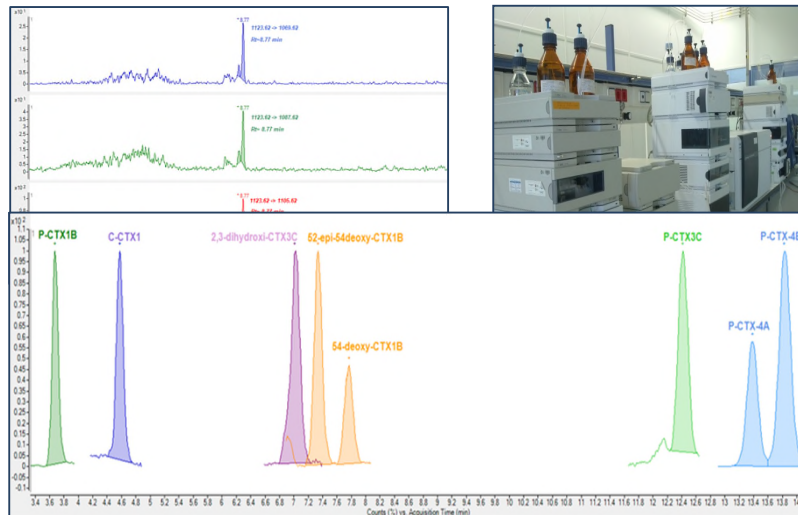


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After the screening of fish samples from Canary Islands, Madeira and Selvagens Islands, by N2a cell assay, the toxicity of CTX-like positive samples was confirmed by using the optimized LC-MS/MS



Food Chemistry
Volume 280, 15 May 2019, Pages 8-14



Implementation of liquid chromatography tandem mass spectrometry for the analysis of ciguatera fish poisoning in contaminated fish samples from Atlantic coasts

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- ^b European Union Reference Laboratory for Marine Biotoxins, CITE XVI, Campus Universitario de Vigo, 36310 Vigo, Spain
- ^c Japan Food Research Laboratories, 6-11-10 Nagayama, Tama, Tokyo 206-0025, Japan
- ^d University of Texas at Austin Marine Science Institute, Port Aransas, TX 78373, United States

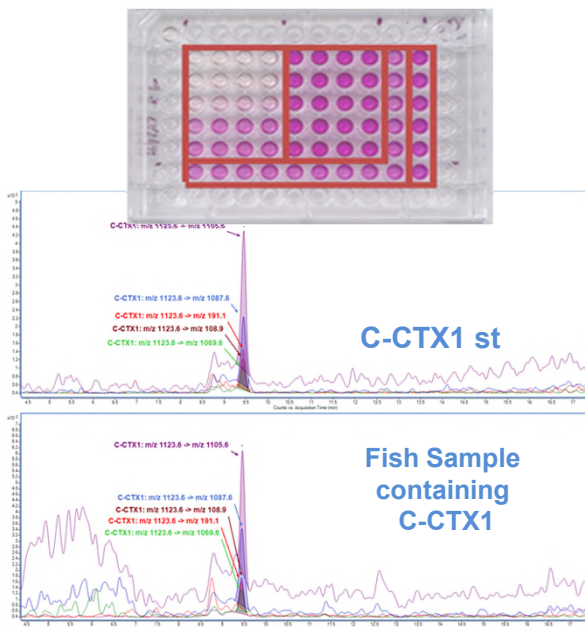
C-CTX1 is confirmed as the main responsible for the CP contamination of fish from Canary Islands and Madeira

RISK CHARACTERIZATION OF CIGUATERA FOOD POISONING IN EUROPE (GP/EFSA/AFSCO/2015/03) EUROCIGUA PROJECT

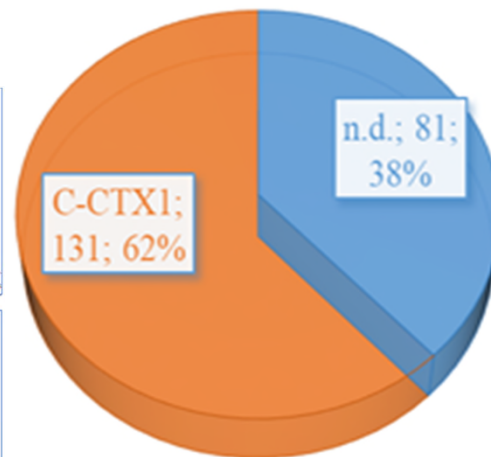
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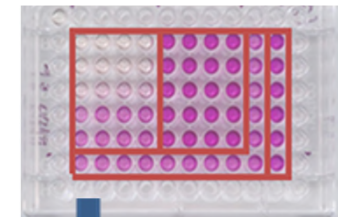
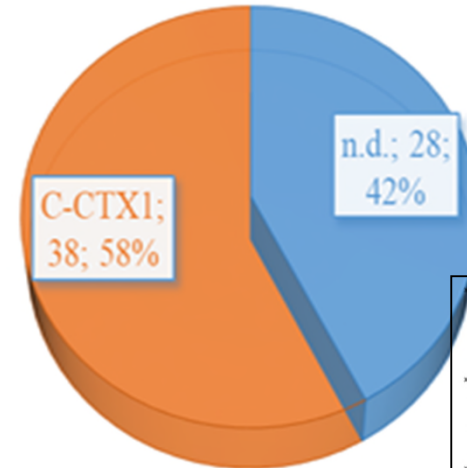
	Canary Islands	Madeira Archipelago
Total samples analysed	212	66
Max. (µg/Kg) C-CTX1	0.544	0.752
Min. (µg/Kg) C-CTX1	0.016	0.019
Average (µg/kg) C-CTX1	0.065	0.187



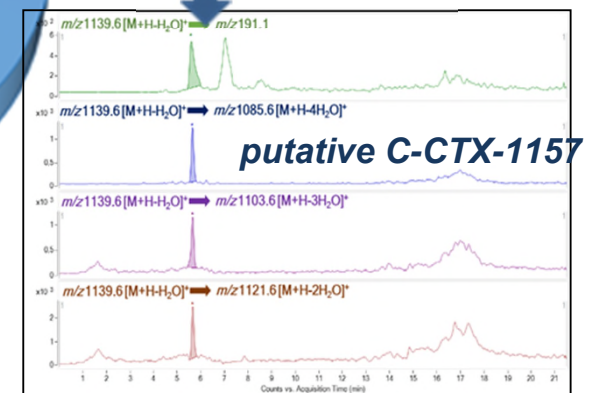
CANARY ISLANDS



MADEIRA ARCHIPELAGO



Other CTXs analogues



Levels of equivalent toxicity in fish

- 0.10 ppb C-CTX-1
- 0.01 ppb P-CTX-1



Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon



Ciguatera: A public health perspective

Robert W. Dickey*, Steven M. Plakas

U.S. FDA Center for Food Safety and Applied Nutrition, Office of Food Safety, Division of Seafood Science and Technology, Gulf Coast Seafood Laboratory, 1 Iberville Drive, Dauphin Island, AL 36528, USA

Black moray (*Muraena augusti*) from El Hierro (Canary Islands, Spain)



Reference Materials

Fish tissue reference materials autoclaved (≈ 12 kg):

Amberjack ≈ 0.26 ng C-CTX1/g

Amberjack ≈ 0.15 ng C-CTX1/g

Grouper ≈ 0.09 ng C-CTX1/g

Grouper ≈ 0.31 ng C-CTX1/g



Purified materials containing C-CTX1

Extraction and purification of ≈ 70 kg of fish tissue and liver

- Vials containing between 5-10 ng C-CTX1





Conclusions:

- An efficient **LC-MS/MS method** has been developed which allowed the characterization of C-CTX1 as the main responsible for the CP in the EU Coasts selected for this project. Further confirmation was carried out by LC-HRMS as well as using alternative tools.
- The LC-MS/MS method has been transferred to the EU REFERENCE LABORATORY FOR MARINE BIOTOXINS to make it available to the EURLMB NRLs Network



Conclusions

- The **low toxin concentration** on the samples evaluated from the selected areas, has been the main limitation that **justifies the establishment of contingency plans**, not only for the confirmation of the toxic profiles, but also for the toxin isolation and purification required for the preparation of reference materials. Neuroblastoma cell assay (N2a) played an important role on the accomplishment of this Task
- The analysis of *Gambierdiscus* sp from the areas selected did not allow to establish a correlation with the CTX profiles obtained for contaminated samples, several Maitotoxin analogues, including new ones, seem to be implicated in the toxic profile of these *Gambierdiscus* sp.

RISK CHARACTERIZATION OF CIGUATERA FOOD POISONING IN EUROPE (GP/EFSA/AFSCO/2015/03) EUROCIGUA PROJECT

EuroCigua



EUROCIGUA PUBLICATIONS

First report of *Gambierdiscus* in the Western Mediterranean Sea (Balearic Islands)

Gambierdiscus (Dinophyceae) species are benthic dinoflagellates living in marine littoral zones of circumtropical areas and have recently been described in temperate waters [1]. Some species are producers of potent neurotoxins: ciguatera (CTP) and ciguatera-like syndrome (CLS).

The present study confirms the presence of *G. australes* in the two Balearic Islands of Majorca and Minorca, and this constitutes the first report of *Gambierdiscus* genus in the western Mediterranean Sea.

...ranged from 64.1 to 90.8 μm (mean of 78.6 μm). The original description [9] described a length range of ... and a cell width of 65-84 μm . Morphological analysis will be performed using electron microscopy.

To facilitate molecular identification to species level, DNA was extracted from individual or a few cells using the ArcturusTM PicoPure Extraction Kit (Applied Biosystems, USA). Afterwards, the domain ...



Article An Attempt to Characterize the Ciguatoxin Profile in *Seriola fasciata* Causing Ciguatera Fish Poisoning in Macaronesia

Pablo Estevez¹, David Castro¹, Ana Pequeño-Valtierra¹, José M. Leao^{1,2}, Oscar Vilarinho^{1,2}, Jorge Diogène³ and Ana Gago-Martínez^{1,2,*}



Contents lists available at ScienceDirect
Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Predictive score and probability of CTX-like toxicity in fish samples from the official control of ciguatera in the Canary Islands

J. Andres Sanchez-Henao^a, Natalia García-Álvarez^{a,b}, Antonio Fernández^a, Pedro Saavedra^b, Freddy Silva Sergent^a, Daniel Padilla^a, Begoña Acosta-Hernández^a, Manuela Martel Suárez^a, Jorge Diogène^c, Fernando Real^a

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Review

Differential toxin profiles of ciguatoxins in marine organisms: Chemistry, fate and global distribution

Lucía Soliño^{a,b,*}, Pedro Reis Costa^{a,b}

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^b CCMAR - Centre of Marine Sciences, University of Algarve, Campus of Gambelas, 8005-139, Faro, Portugal



Communication

Emerging Marine Biotoxins in Seafood from European Coasts: Incidence and Analytical Challenges

Pablo Estevez¹, David Castro¹, Ana Pequeño-Valtierra¹, Jorge Giraldez¹ and Ana Gago-Martínez^{1,2,*}

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Journal of MASS SPECTROMETRY

RESEARCH ARTICLE

Design of experiments for the optimization of electrospray ionization in the LC-MS/MS analysis of ciguatoxins

Guillermo Moreiras, José Manuel Leão, Ana Gago-Martínez

Ciguatera fish poisoning outbreaks from 2012 to 2017 in Germany caused by snappers from India, Indonesia, and Vietnam

Miriam Friedemann¹

Received: 9 January 2018 / Revised: 25 September 2018 / Accepted: 16 October 2018
© Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL) 2018

MS Number: TFAC-2019-466.R1

Title: Caribbean Ciguatoxin-1 stability under strongly acidic conditions: Characterization of a new C-CTX1 methoxy congener.

Authors: Estévez, Pablo; Leao, Jose; Yasumoto, Takeshi; Dickey, Robert; Gago-Martínez, Ana

Assessment of cytotoxicity in ten strains of *Gambierdiscus australes* from Macaronesian Islands by neuro-2a cell-based assays

Laiá Reverté¹, Anna Toldrà¹, Karl B. Andree¹, Santiago Fraga², Giada de Falco¹, Mònica Campàs¹, Jorge Diogène¹



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Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aqtox

Presence of CTXs in moray eels and dusky groupers in the marine environment of the Canary Islands

Andres Sanchez-Henao^a, Natalia García-Álvarez^{b,*}, Freddy Silva Sergent^a, Pablo Estévez^b, Ana Gago-Martínez^{b,c}, Francisco Martín^d, María Ramos-Sosa^a, Antonio Fernández^e, Jorge Diogène^f, Fernando Real^g

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Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Gambierdiscus and *Fukuyoa* as potential indicators of ciguatera risk in the Balearic Islands

Àngels Tudó^{a,b}, Anna Toldrà^a, Maria Rey^a, Irene Todolí^a, Karl B. Andree^a, Margarita Fernández-Tejedor^a, Mònica Campàs^a, Francesc X. Sureda^b, Jorge Diogène^{a,*}

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Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/econvs

Rapid detection of ciguatoxins in *Gambierdiscus* and *Fukuyoa* with immunosensing tools

G. Gaiani^a, S. Leonardo^a, À. Tudó^a, A. Toldrà^a, M. Rey^a, K.B. Andree^a, T. Tsumuraya^b, M. Hiramia^b, J. Diogène^a, C.K. O'Sullivan^{c,d}, C. Alcaraz^a, M. Campàs^{a,*}

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Article

New Insights into the Occurrence and Toxin Profile of Ciguatoxins in Selvagens Islands (Madeira, Portugal)

Pedro Reis Costa¹, Pablo Estevez², David Castro², Lucía Soliño¹, Neide Gouveia³, Carolina Santos⁴, Susana Margarida Rodrigues¹, José Manuel Leao² and Ana Gago-Martínez^{2,*}



Food Chemistry

Volume 280, 15 May 2019, Pages 8–14

Implementation of liquid chromatography tandem mass spectrometry for the analysis of ciguatera fish poisoning in contaminated fish samples from Atlantic coasts

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



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Brotos de ciguatera identificados en España. 2012-2018

GP/EFSA/AFSCO/2015/03

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RISK CHARACTERIZATION OF CIGUATERA FOOD POISONING IN EUROPE (GP/EFSA/AFSCO/2015/03) EUROCIGUA PROJECT

EuroCigua



External Communication Plan for dissemination

Leaflet

EUROCIGUA
Eurocigua is a project co-funded by the European Food Safety Authority (EFSA) and 14 European organizations. The project focuses on the characterization of the risk of ciguatera food poisoning (CFP) in Europe and aims to:

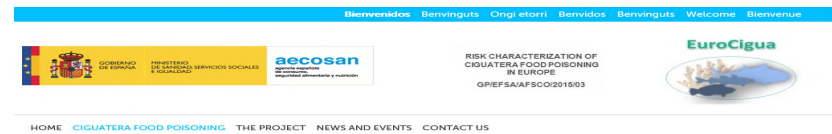
- Respond to the scarcity of standards and reference materials;
- Establish reliable methods to identify and quantify ciguatoxins in fish and microalgae;
- Understand the temporal and spatial distribution of *Gambierdiscus* spp. in EU waters;
- Evaluate the toxicity of GTX-type populations of *Gambierdiscus* spp.
- To assess the possible presence of GTX in fish, in EU waters;
- Determine the incidence and epidemiological characteristics of ciguatera cases in Europe.



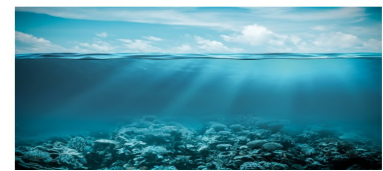
**But Remember:
Eating fish is good for your health!**



EuroCigua website



Home / Ciguatera Food Poisoning
Ciguatera Food Poisoning
f t in



Ciguatera food poisoning (CFP) is a type of food poisoning associated with the consumption of seafood with an estimate number of 20,000–500,000 poisonings per year worldwide. Ciguatera is endemic in tropical and subtropical regions of the world. Isolated outbreaks have occurred sporadically but with an increasing frequency in temperate areas such as Europe.

This seafood-borne illness is typically caused by the consumption of fish that have accumulated ciguatoxins in their flesh. Ciguatoxins are produced by benthic dinoflagellates from the genus *Gambierdiscus* spp. that represents a key aspect in studies of harmful algae in recent years due to the danger to human health.

Factsheet



BUILD UP AWARENESS

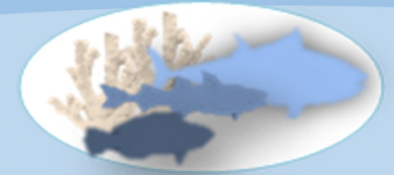
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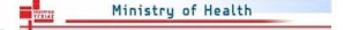
RISK CHARACTERIZATION OF CIGUATERA FOOD POISONING IN EUROPE (GP/EFSA/AFSCO/2015/03) EUROCIQUA PROJECT

EuroCigua



International EuroCigua Workshop

- network for scientific excellence



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Conclusions

- New endemic food-borne disease: emerging risk
- Surveillance protocol
- Harmonized methodologies for the extraction of toxins in microalgae and fish
- Implementation of N2a-cell based assay
- Develop methods to identify CTXs
- C-CTX1 was identified as the main responsible of CP in Macaronesia area
- Hierro and Selvagens were the main CP hotspots
- Network of scientific excellence





FUTURE

- ✓ **RAISING AWARENESS AND COLLECTING DATA OF CIGUATERA CASES AND OUTBREAKS AUTOCHTHONOUS AND IMPORTED FISH**
- ✓ **ESTABLISHMENT OF PREDICTION MODELS TO ANTICIPATE CIGUATERA EVENTS**
- ✓ **CONTINUE THE DATA COLLECTION FOR AN EFFICIENT STRATEGY FOR CIGUATERA RISK ASSESSMENT**
- ✓ **METHODOLOGICAL IMPLEMENTATION, DISSEMINATION , TRAINING AND PROFICIENCY**